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# FETAL PHYSIOLOGY AND MEDICINE
## The Basis of Perinatology

Second, Revised Edition

edited by

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# Foreword

Good clinical science is intimately dependent on developments in the basic sciences. The challenge facing everyone who works in the field of human reproduction is to encompass the understanding of these developments and to realize their significance in the future of reproductive medicine. Beard and Nathanielsz have accepted the challenge of this situation and have produced a book integrating clinical and basic sciences. The explosion of knowledge that has occurred in this field over the past 15 years has in great part been due to the basic science research of individuals who have contributed to this book. The topics include not only chapters on antenatal diagnosis, sexual differentiation, acquired immunity, and endocrine changes but also on the physiology of breathing, the control of the fetal cardiovascular system, lung maturation, fetal infections, and the effects of hypoxia on the fetal brain, and many others.

There are comprehensive reviews of fetal regulatory mechanisms such as the renin-angiotensin system, water metabolism, and fetal and placental hormone production. The clinical application of many of these studies has already assumed a major role in changing the pattern of antenatal and intrapartum practice. This is acknowledged in the chapters on antenatal fetal heart rate monitoring, the technical aspects of fetal and uterine pressure measurements, fetal acid-base balance, and the prevention of preterm delivery. The book logically concludes with a section on the transition from intrauterine to extrauterine life.

We consider it a great privilege to include *Fetal Physiology and Medicine* in the Reproductive Medicine series. The authors set themselves the difficult objective of integrating clinical and basic science and have achieved a book that is both scholarly and practical and is of great potential value to all students and practitioners of reproductive medicine.

*E. Malcolm Symonds, M.D.*

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# Preface

Fetal mortality and morbidity are problems for all countries, whatever their economic status. Abnormal development during gestation lessens the individual's potential, a potential that is the greatest resource available to the human race. Since the first edition of this book there has been a considerable increase in our knowledge of fetal development and its practical application to the delivery of fetal and maternal care. Intrauterine surgery is now being performed with encouraging results; ultrasound provides more accurate detection of a wide variety of fetal anomalies; instrumentation to monitor the fetus has improved considerably, and major advances have been made in the prevention and treatment of the many disorders affecting the preterm baby. Such advances have come as a result of years of careful, painstaking research into the fundamental processes of fetal development and are now being put to widespread use in neonatal units throughout the world.

We have tried to avoid a didactic and comprehensive review of the whole of maternofetal medicine. The individual authors addressed themselves to both the basic experimental aspects and the clinical considerations of their subject. Our aim has been to stimulate inquiry and, where necessary, to question conventional concepts. By our selection of topics, we have attempted to cover the most important areas of materno-fetal medicine and to obtain different views of the critical problems of development. The consideration of the various components of fetal metabolism discussed by Battaglia and Hay, Milner, and Mestyán and Soltész are good examples of the way in which animal experimentation has given a clear view of the interrelationship of the mother, placenta, and fetus. The information obtained by the investigations of these and other workers suggests that the days of uterine therapy for various forms of growth retardation are not far away.

This book documents many of the major advances in fetal medicine: developmental physiology, pathology, and therapy. We believe that it is only by a better understanding of the basic physiology and pathophysiology of pregnancy that the standard of maternal and fetal health care will improve.

*Richard W. Beard*

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# 1

# Antenatal Diagnosis of Congenital Structural Anomalies with Ultrasound

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## INTRODUCTION

Congenital structural anomalies (CSAs) encompass a broad spectrum of conditions, those with minimal involvement and disability (polydactyly) to those incompatible with extra-uterine life (anencephaly, renal agenesis, etc.). The etiology of CSA may be grouped into four classes: unknown (65-70%), environmental factors (10%), genetically transmitted diseases (20%), and chromosomal aberrations (3-5%) (Wilson, 1973).

Until recently the prenatal diagnosis of CSA was limited to those which could be detected during the second trimester by either biochemical or chromosomal aberrations noted in analysis of amniotic fluid obtained following amniocentesis (Sandstrom and Milunsky, 1977). With the advent of ultrasound, CSAs which grossly alter normal fetal anatomy can be screened for and detected as early as the second trimester of pregnancy (Table 1).

## ULTRASOUND

The fetus is ideal for ultrasound evaluation because it is floating in a fluid medium which provides maximum contrast with fetal tissues and, thus, optimum fetal anatomical characterization.

Initially, ultrasound consisted of a bistable white on black image which only allowed for the evaluation of gross fetal structures (Figure 1A). As the electronics became more sophisticated, the bistable image changed to one with 16 shades of gray. Structures not seen with the bistable image were observed with gray-scale ultrasound, thus allowing for a more detailed visualization of fetal anatomy (Figure 1B).

Although gray-scale ultrasound greatly improved imaging, one of the limitations was that the image was always static; that is, it did not allow for analysis of the active, moving fetus. Real-time ultrasound, which incorporates gray scale, added another dimension to ultrasound, since the still image suddenly became "alive." This made it possible to evaluate the fetus as it moved about in its intrauterine environment and to observe pulsating vascular structures such as the fetal heart, aorta, and intracranial arteries.

To accurately evaluate the fetus for CSA therefore requires (1) a static contact gray scale as well as a real-time scanner, (2) a thorough knowledge of normal fetal anatomy

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**Table 1** Diagnosis of Congenital Anomalies Made in the Second Trimester in Patients Genetically at Risk for Fetal Deformity (1976-1980)[a]

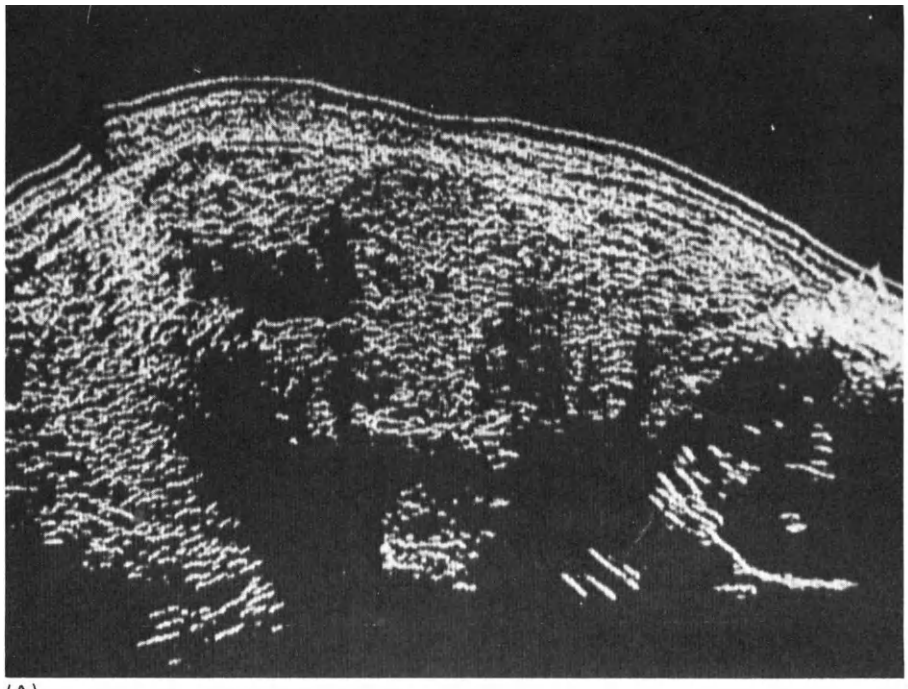
| | Positive diagnosis in those at risk |
|---|---|
| Head and spine | |
| cystic hygroma, lymphangiectasia | 1 (3) |
| facial deformities (including Pierre Robin syndrome) | 0 (3) |
| holoprosencephaly | 0 (2) |
| hydrencephalocele | 0 (1) |
| hydrocephaly (including Dandy-Walker Syndrome) | 6 (68) |
| hypertelorism | 0 (1) |
| meningomyelocele | 1 (4) |
| microcephaly (including Seckel syndrome) | 1 (7) |
| neural tube defects | |
| anencephaly in previous pregnancy | 2 (65) |
| spina bifida in previous pregnancy (including Arnold-Chiari syndrome) | 8 (92) |
| elevated alpha-fetoprotein without previous history | 11[b] (25) |
| Total | 30 (271) |
| Internal anatomy | |
| diaphragmatic hernia | 0 (10) |
| jejunal atresia | 1 (1) |
| situs inversus | 0 (1) |
| omphalocele, gastroschisis (history of elevated alpha-fetoprotein) | 5 (8) |
| infantile polycystic kidney disease | 7 (20) |
| renal agenesis (including Potter syndrome) | 1 (14) |
| renal anomalies (miscellaneous) | 0 (13) |
| Total | 14 (67) |
| Skeleton and limbs | |
| achondrogenesis | 0 (4) |
| achondroplasia | 0 (2) |
| acrocephalosyndactyly (Apert syndrome) | 0 (1) |

Table 1 (continued)

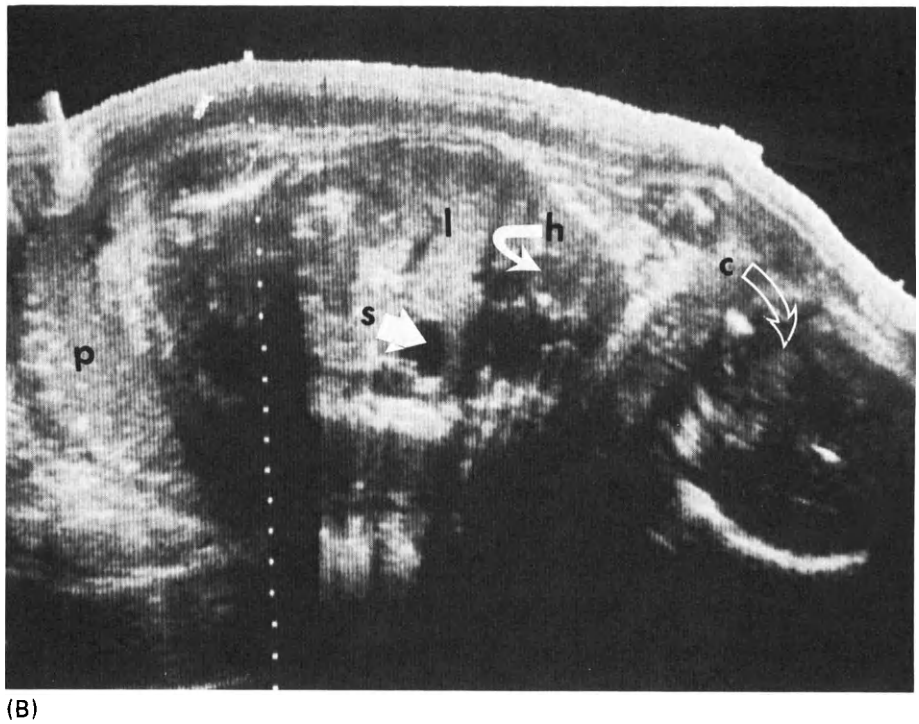
| | Positive diagnosis in those at risk |
|---|---|
| Skeleton and limbs (continued) | |
| adactyly | 0 (1) |
| asphyxiating thoracic dysplasia | 0 (2) |
| camptomelic dysplasia | 1 (1) |
| cartilage hair hypoplasia (McKusick syndrome) | 0 (2) |
| chrondroectodermal dysplasia (Ellis-van Creveld syndrome) | 1 (2) |
| diastrophic dysplasia | 1 (5) |
| ectrodactyly | 0 (2) |
| Fanconi's anemia | 0 (1) |
| osteogenesis imperfecta | 1 (13) |
| osteopetrosis | 0 (4) |
| polydactyly | 0 (2) |
| Robert syndrome | 2 (4) |
| Seckel syndrome (microcephaly, dwarfism) | 0 (1) |
| skeletal dysplasia (miscellaneous) | 1 (12) |
| spondyloepiphyseal dysplasia | 0 (1) |
| spondylothoracic dysplasia | 0 (1) |
| thanatophoric dysplasia | 0 (3) |
| thrombocytopenia with absent radii syndrome | 1 (2) |
| Total | 8 (66) |
| Miscellaneous | |
| consanguinity | 0 (2) |
| exposure to hormones | 0 (9) |
| exposure to possible teratogenic agents | 0 (20) |
| poorly defined multiple anomalies | 4 (21) |
| previous abnormal ultrasound or clinical exam (not genetic) | 5 (7) |
| Total | 9 (59) |
| Total number of women examined | 61 (463) |

[a]Figures in brackets show the number of women examined.

[b]Includes spina bifida (5), omphalocele (3), meningomyelocele (1), jejunal atresia (1), and hydrocephaly (1).



(A)




(B)

**Figure 1** Identical sagittal scans of a 26-week fetus using (A) bistable and (B) gray-scale formats (s, stomach; h, heart; c, cranium; l, liver; p, placenta).

as depicted by ultrasound, and (3) a complete understanding of the defect expected to be seen when evaluating a fetus for a specific anomaly.

The following review will focus on the use of diagnostic ultrasound in the diagnosis of the more commonly seen structural anomalies which have been studied in either the second or third trimester of pregnancy.

## DIAGNOSIS OF CONGENITAL STRUCTURAL ANOMALIES IN PREGNANCIES NOT KNOWN TO BE AT RISK

Unfortunately, CSAs are most often diagnosed at birth or shortly thereafter. This can present many problems for both the family and the physician. It is not unheard of for an obstetrician to perform a cesarean section for fetal distress occurring during labor only to find a grossly malformed fetus which dies shortly after birth.

Although some physicians advocate an ultrasonic scan for all pregnant women, this is not widely practiced worldwide. When an ultrasound scan is requested, the report usually contains information concerning the biparietal diameter, with the corresponding gestational age, the location of the placenta, the number of fetuses, and the lie of the fetus, and it may or may not indicate the relative quantity of amniotic fluid present.

Although not specific, the quantity of amniotic fluid may suggest the presence of underlying congenital anomalies. The measurement of the total intrauterine volume quantitates the contribution from the fetal, placental, and amniotic fluid compartments. By routinely quantitating the total intrauterine volume for a given gestational age, and remeasuring this parameter after a short interval, an objective evaluation of changes in amniotic fluid may be obtained (Figure 2) (DeVore and Hobbins, 1979a)

### Polyhydramnios

Between 18 and 20% of fetuses with increased amniotic fluid have congenital anomalies (Hobbins et al., 1979). In these fetuses, polyhydramnios is often thought to be secondary to an impairment of fetal swallowing, although in some syndromes the mechanisms by which excessive amounts of fluid accumulate is unknown. Examples of anomalies associated with polyhydramnios are those involving the central nervous system, the gastrointestinal system, and a few skeletal dysplasias (Table 2).

In most cases polyhydramnios is suggested ultrasonically by a total intrauterine volume greater than two standard deviations above the mean for a given gestational age; however, polyhydramnios should not be excluded if this threshold is not exceeded. Other than the total intrauterine volume, the most helpful indication of increased amniotic fluid is identification of large amounts of fluid separating the fetal small parts (Figure 3) (DeVore and Hobbins, 1979a).

### Oligohydramnios

Oligohydramnios is suggested by a total intrauterine volume two standard deviations below the mean, as well as by a scarcity of fluid in the areas of the fetal limbs, which produces an image of crowding (Figure 4). If this is noted before the twenty-eighth week of gestation in combination with symmetrical growth retardation, one should suspect CSAs and, in particular, anomalies of the urinary system (Table 3). However, after this period, nonanomalous situations such as premature rupture of the membranes, asymmetrical intrauterine growth retardation (IUGR), or post-term pregnancies may be responsible for the decreased amniotic fluid (DeVore and Hobbins, 1979a).

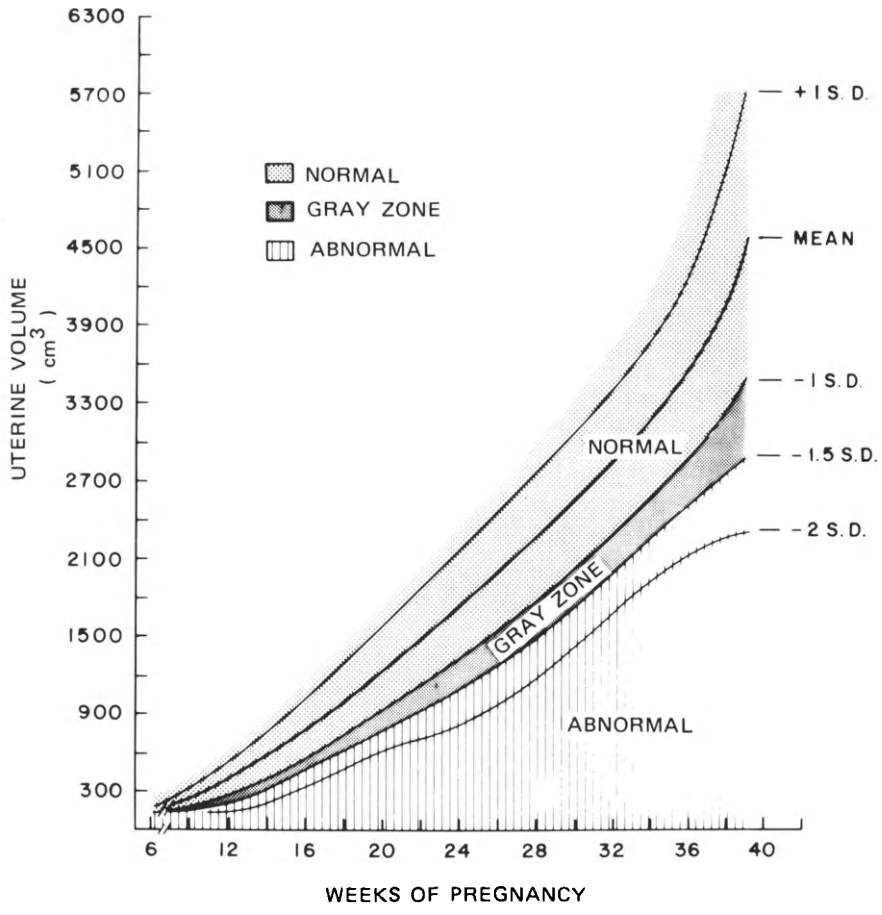



**Figure 2** Nomogram for total intrauterine volume. (From Gohari et al., 1977.)

**Table 2** Anomalies Associated with Polyhydramios and Oligohydramnios

| |
|---|
| Polyhydramnios |
| Achondrogenesis type I (lethal neonatal dwarfism) |
| Annular pancreas (intestinal obstruction due to duodenal obstruction) |
| Congenital chloride diarrhea (defective chloride-bicarbonate exchange in the distal system) |
| Chondrodysplasia punctata, Conradi-Hundermann type (skeletal dysplasia) |
| Trisomy 18 |
| CNS depression, hemorrhage, skeletal syndrome |
| Duodenal atresia or stenosis |
| Esophageal atresia |
| Lissencephaly syndrome (microcephaly) |
| Stomach atresia |
| Anencephaly |
| Oligohydramnios |
| Bilateral renal agenesis |

*Source:* Adapted from Bergsma (1979).

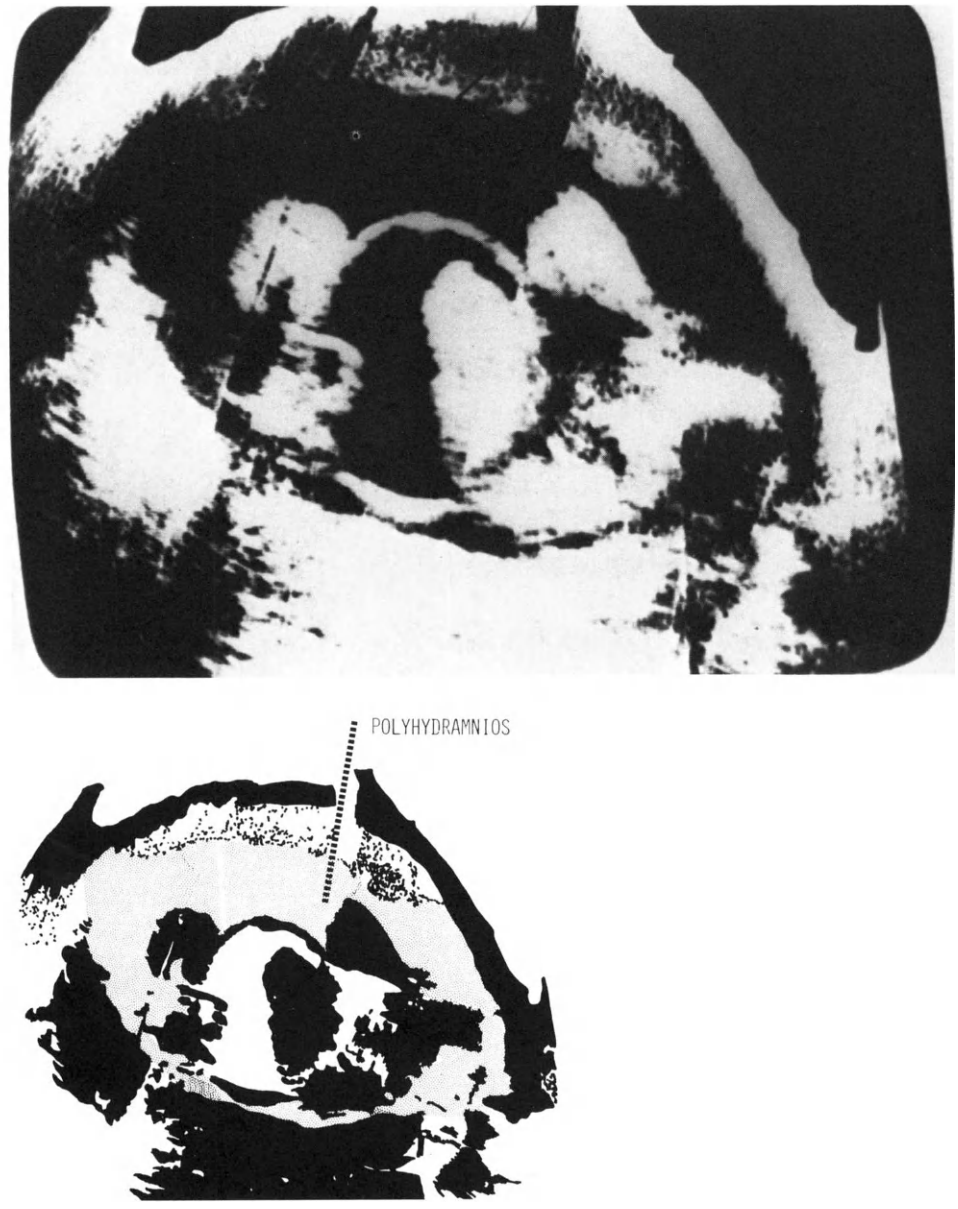



**Figure 3** Polyhydramnios (stippled area) (From DeVore and Hobbins, 1979a.)

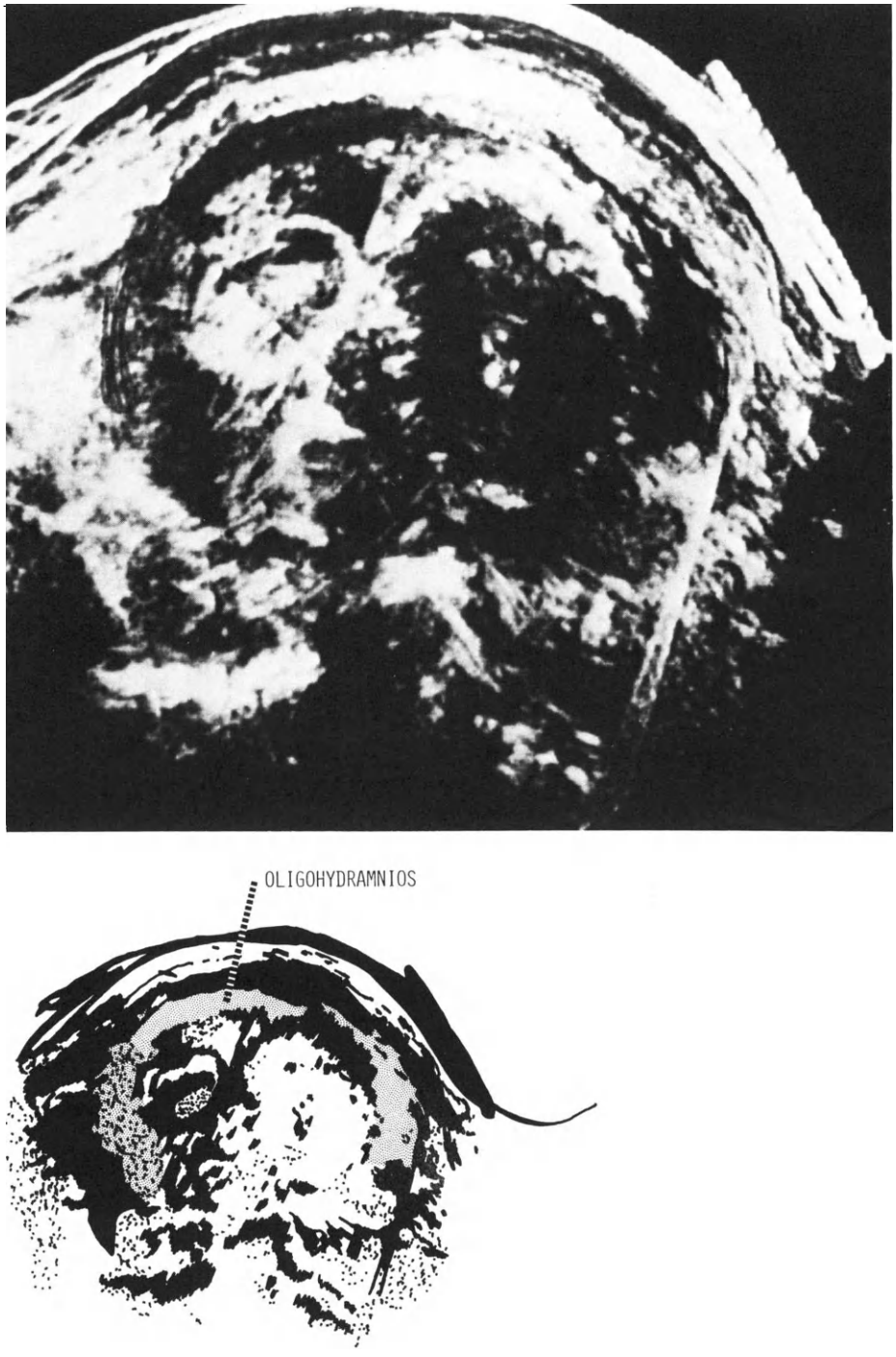



**Figure 4** Oligohydramnios (stippled area). (From DeVore and Hobbins, 1979a.)

Table 3 Data from 196 Normal Fetuses

| Menstrual age (weeks) | Lateral ventricular width (cm) | Hemispheric width (cm) | LVW/HW ratio (±2 SD) (%) |
|---|---|---|---|
| 15 | 0.75 | 1.4 | 56 (40-71) |
| 16 | 0.86 | 1.5 | 57 (45-69) |
| 17 | 0.85 | 1.5 | 52 (42-62) |
| 18 | 0.83 | 1.8 | 46 (40-52) |
| 19 | – | – | – |
| 20 | 0.82 | 1.9 | 43 (29-57) |
| 21 | 0.76 | 2.2 | 35 (27-43) |
| 22 | 0.82 | 2.6 | 32 (26-38) |
| 23 | 0.83 | 2.5 | 33 (24-42) |
| 24 | 0.83 | 2.7 | 31 (23-39) |
| 25 | 1.1 | 3.0 | 34 (26-42) |
| 26 | 0.9 | 3.0 | 30 (24-36) |
| 27 | 0.9 | 3.0 | 28 (23-34) |
| 28 | 1.1 | 3.3 | 31 (18-45) |
| 29 | 1.0 | 3.4 | 29 (22-37) |
| 30 | 1.0 | 3.4 | 30 (26-34) |
| 31 | 1.0 | 3.4 | 29 (23-36) |
| 32 | 1.1 | 3.6 | 31 (26-36) |
| 33 | 1.1 | 3.4 | 31 (25-37) |
| 34 | 1.1 | 3.8 | 28 (23-33) |
| 35 | 1.1 | 3.8 | 29 (26-31) |
| 36 | 1.1 | 3.9 | 28 (23-34) |
| 37 | 1.2 | 4.1 | 29 (24-34) |
| Term | 1.2 | 4.3 | 28 (22-33) |

*Source:* Johnson et al., 1980.

## ULTRASOUND DIAGNOSIS OF CONGENITAL STRUCTURAL ANOMALIES

### Central Nervous System

The brain and spinal cord constitute approximately 10% of major fetal anomalies. Although most defects have been described during the third trimester, the more common anomalies such as hydrocephaly and the neural tube defects (anencephaly, spina bifida, and encephalocele) can be diagnosed during the second trimester. With the advent of the ability to screen all pregnant women for fetal neural tube defects with maternal serum alpha-fetoprotein, the potential exists for over 60,000 ultrasonic evaluations of fetuses at risk each year for neural tube defects in the United States alone (U.S. Department of Health, Education, and Welfare, 1979).

### Cranium

*Normal Intracranial Anatomy* The fetal cranium can be discerned as being separate from the trunk as early as the seventh to eighth week following the last menstrual period. Beginning at approximately the fifteenth gestational week, intracranial structures can be imaged with static or real-time ultrasound.

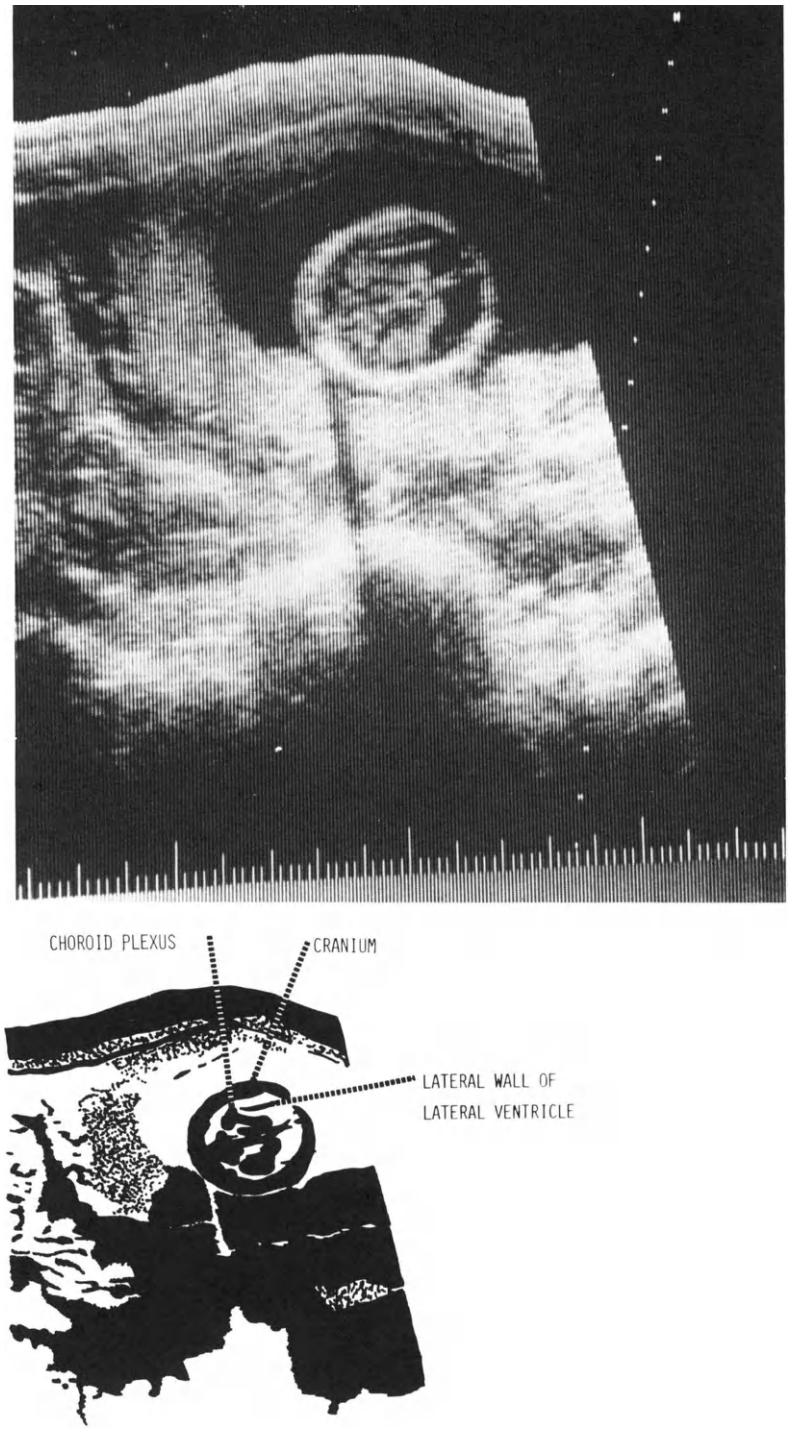



**Figure 5** Transverse scan high in the fetal head above the level of the thalamobasal ganglia complex demonstrating the lateral wall of the lateral ventricles.

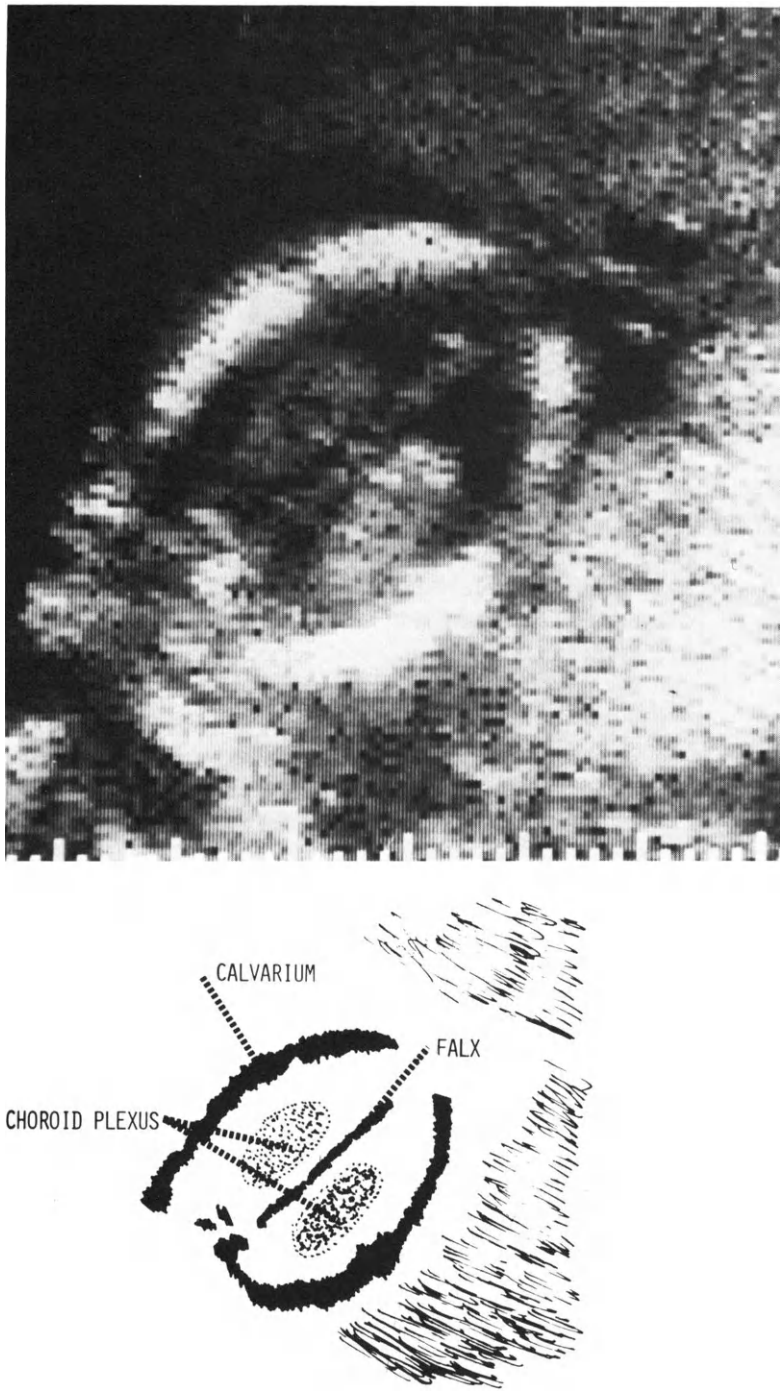



**Figure 6** Transverse scan at 17 weeks of gestation illustrating the choroid plexus, which is a normal finding at this gestational age.

Commencing with the most cephalad aspect of the fetal head, the first structures observed are the lateral margins of the bodies of the lateral ventricles which parallel the midline echo (Figure 5). In the early second trimester the lateral ventricles are often filled with the choroid plexus, which some have mistakenly identified as intracranial masses (Figure 6).

The next level includes four major structures which can be easily identified. Paired central areas represent the thalamobasal ganglia complex. The sylvian fissure, which separates the temporal from the parietal lobe of the cortex, lies at two-thirds the distance from the thalamobasal ganglia complex and appears as a dense, white linear structure in which, with real-time ultrasound, the middle cerebral artery can be seen pulsating (Figure 7).

The most caudal level contains the cerebral peduncles, which are "heart shaped," with the notch of the heart pointing anteriorly and containing the basilar artery. Strong echoes, diverging posteriorly, represent the tentorium cerebelli, which separates the cerebellum from the cerebral cortex (Figure 8).

Anterior to the thalamobasal ganglia complex and the cerebral peduncles is a rectangular structure which straddles the midline. This is the cavum septi pellucidi, which is a midline structure that connects the corpus callosum with the fornix. In the past the cavum septi pellucidi has been mistaken by ultrasonographers as the third ventricle, a structure not usually seen unless it is abnormally dilated in the fetal brain (Figure 8).

When measuring the biparietal diameter, it should be obtained at the level of the thalamobasal ganglia complex or the cerebral peduncles, since these are easily reproducible structures and are located at the widest diameter of the fetal calvarium.

*Hydrocephaly* Congenitally acquired hydrocephaly occurs in 1 out of 2000 live births. The recurrence rate of communicating hydrocephaly is less than 4%, but it can be inherited as either an X-linked recessive trait, with a 50% recurrence rate in male fetuses (aqueductal stenosis), or an autosomal recessive trait with a 25% recurrence rate (Dandy-Walker syndrome), regardless of sex (Holmes et al., 1973). If not diagnosed in utero, approximately 80% of affected newborns will be diagnosed by the first year of age. If treated, 64% will be normal and educable, while 36% will either die or suffer from mental retardation (Shulman, 1979).

Hydrocephaly results from blockage of intraventricular drainage at the level of the aqueduct of Sylvius or the fourth ventricle. As the intraventricular pressure increases, the ependymal cells, which line the ventricles, are damaged. This allows cerebrospinal fluid to accumulate within the brain parenchyma, with subsequent myelin and axonal destruction. If untreated, cerebrospinal fluid continues to accumulate and the ventricles dilate, with eventual destruction of a majority of the axons, leaving only a thin mantle of cerebral gray matter.

When evaluating a fetus for hydrocephaly it is important to realize that before the twenty-fourth week of gestation ventricular enlargement will always occur prior to an increase in the biparietal diameter (Figure 9A). Johnson et al. (1980) studied 196 normal fetal cranial sonograms from week 15 through 40 of gestation. They quantitated the ratio of the lateral ventricle width to the cerebral hemisphere width (LVW/HW) and reported that the ratio between the fifteenth and twenty-first week of gestation was greater in early pregnancy, gradually decreasing from 56 to 35% with advancing fetal maturity. This is due to the increase in size of the cerebral hemisphere relative to the lateral ventricle (Table 3). Fetuses with hydrocephaly were noted to first develop dilation of the occipital horns of the lateral ventricles, followed by an increase above the ninety-fifth percentile in the LVW/HW. This was followed by an increase in the biparietal diameter which was out of proportion to the appropriate gestational age.

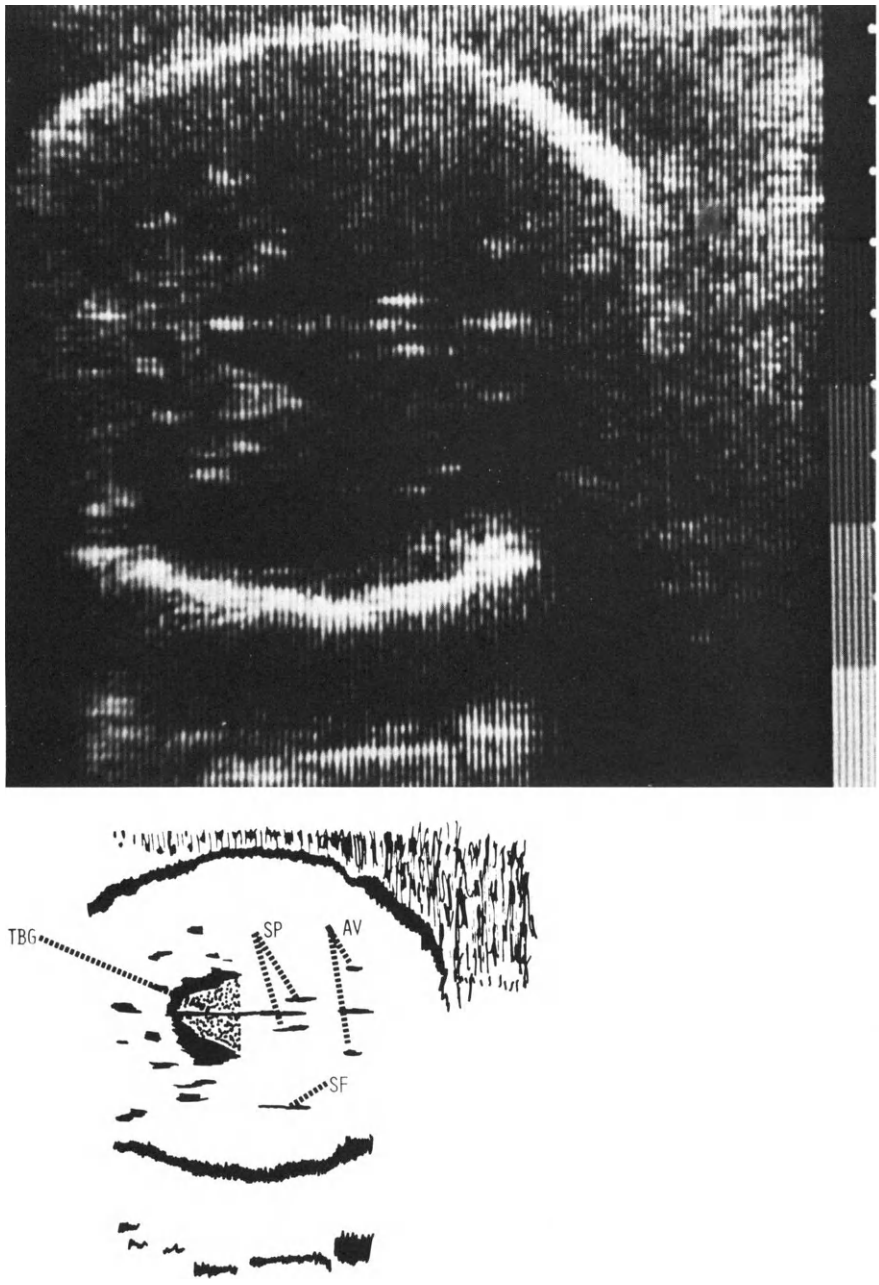
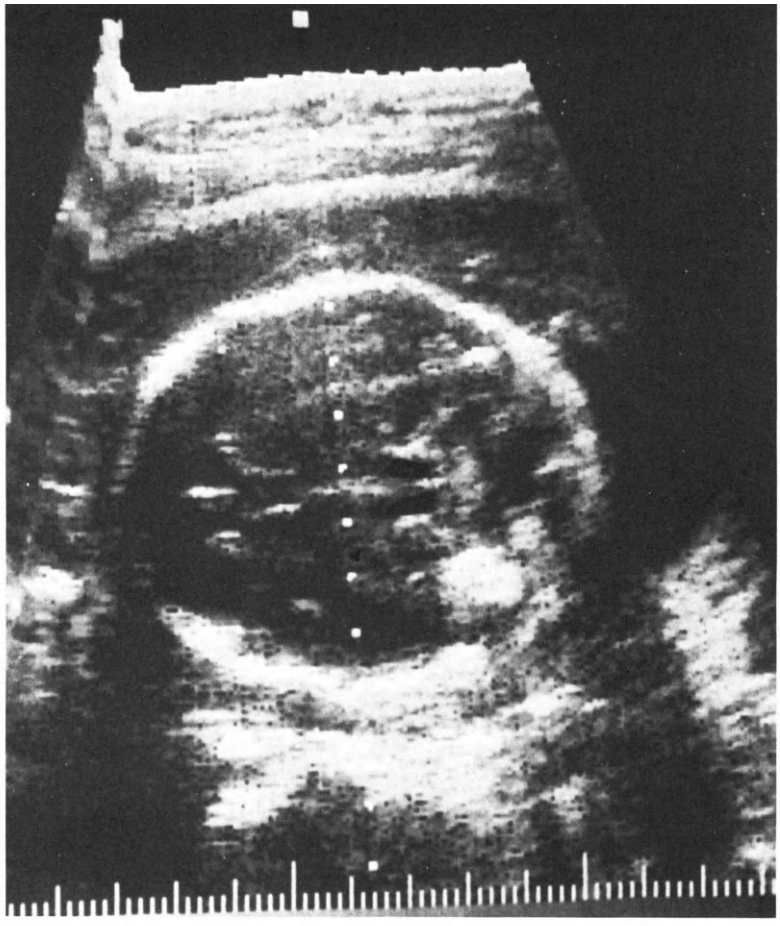
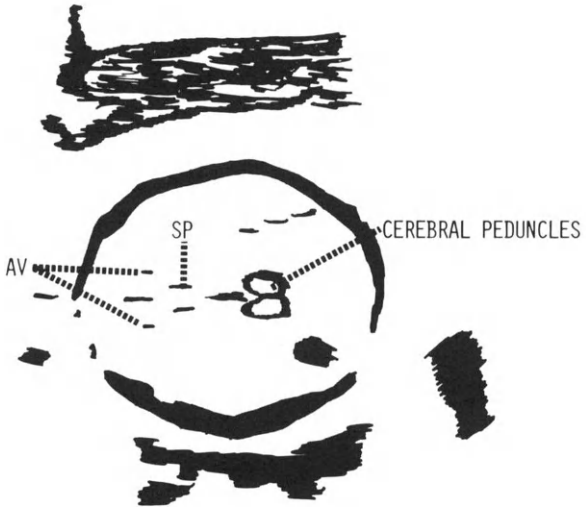



**Figure 7** Transverse scan through the level of the thalamobasal ganglia complex (TBG) demonstrating the sylvian fissure (SF), the cavum septi pellucidi (SP), and the lateral walls of the anterior horns of the ventricles (AV).




SP
CEREBRAL PEDUNCLES
AV

The diagnosis of hydrocephaly during the third trimester is not difficult once the circumference of the head has obtained abnormal proportions (Figure 9B). Because of shunting techniques, neurosurgeons are able to offer a life of reasonable quality to some infants with hydrocephaly. It is therefore imperative that the obstetrician manage the fetus with hydrocephaly in the most appropriate fashion. A potentially viable fetus may be delivered by cesarean section. In those rare cases where the chances of survival are remote, decompression of the fetal head is accomplished prior to birth. With ultrasound the head circumference as well as the cortical thickness can be quantitated. These measurements are helpful when consulting with the neurosurgeons, since different centers, depending on the above two measurements, have different survival rates following extrauterine shunting procedures. On occasion, at our institution, fetuses have been delivered before term, after utilizing corticosteroids to stimulate pulmonary maturity, in an effort to spare the fetal cortex from continuing compression.

*Anencephaly* Anencephaly is a defect of cranial development involving the frontal, parietal, and occipital bones, with subsequent necrosis of the developing cerebral hemispheres. The etiology appears to be multifactorial, having both genetic and environmental components which affect the embryo between the sixteenth and twenty-sixth postconceptual day. The incidence varies from as high as 1 out of 105 births in South Wales to 1 out of 1000 births in other parts of the world (Brock, 1976). Fetuses die either in utero or shortly after birth.

The ultrasound diagnosis can be made as early as the fifteenth week of gestation when a poorly formed cranium is noted. Unfortunately, most cases of anencephaly elude diagnosis until the third trimester, when the clinician requests an ultrasound because of polyhydramnios (Figure 10). At this time the cranial pole may lie deep within the pelvis, which makes ultrasonic evaluation difficult. In these patients a pelvic examination should be done to lift the cranial pole out of the pelvis, or an x-ray film should be taken for further evaluation.

*Encephalocele* Encephalocele occurs in 1 in 2000 live births and results from a bony defect in the skull through which a portion of the brain herniates. If it is associated with microcephaly and polycystic kidneys, one should suspect Meckel syndrome (Holmes et al., 1976; Nevin et al., 1979). The etiology of an encephalocele is thought to be multifactorial and occurs with a higher frequency in families with previous fetuses with neural tube defects. The diagnosis can be made with ultrasound by observing a saclike structure protruding from the skull (Figure 11).

*Intracranial Tumors* Although rare, intracranial tumors may appear as large, cystic, solid, echodense areas with a increased biparietal diameter. Much or all of the normal intracranial anatomy may be distorted or absent (Figure 12).

### Extracranial Structures

A variety of structures have been described which are continuous with the head but not clearly extracranial (Adam et al., 1979). Cystic hygromas, often associated with Turner

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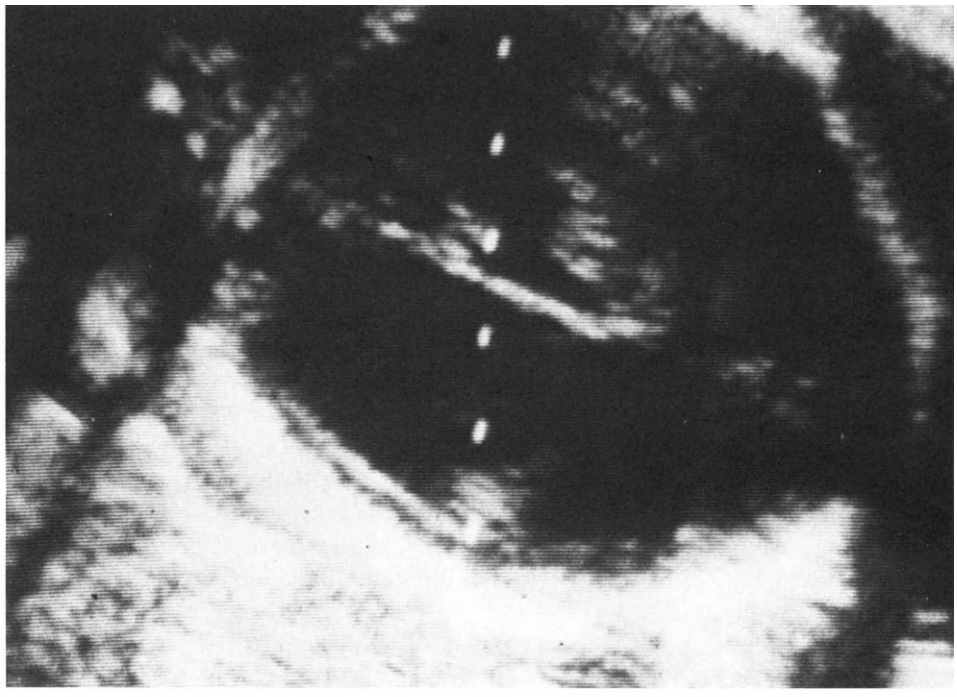
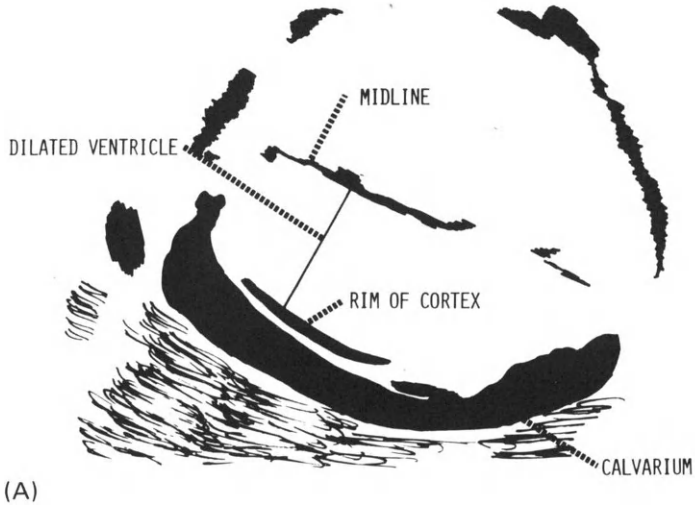
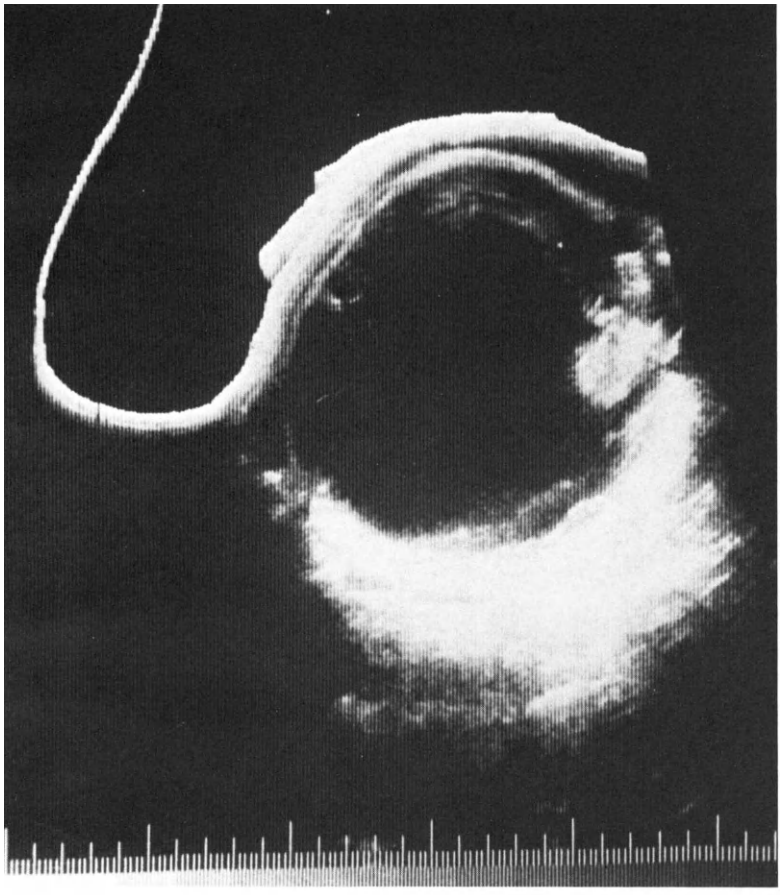
Figure 8 Transverse scan through the cerebral peduncles demonstrating the cavum septi pellucidi (SP) and the lateral walls of the anterior horns of the ventricles (AV).

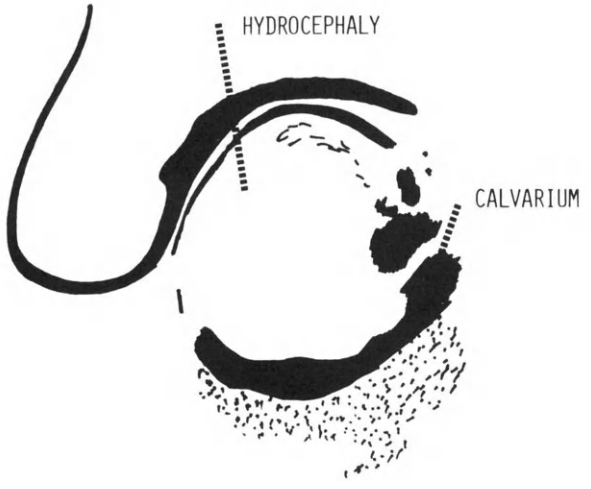





(A)

**Figure 9** Hydrocephaly. (A) At 23 weeks of gestation in which the lateral ventricles are dilated but the biparietal diameter is normal.






(B)

**Figure 9** Hydrocephaly. (B) During the third trimester with marked enlargement of the fetal head.

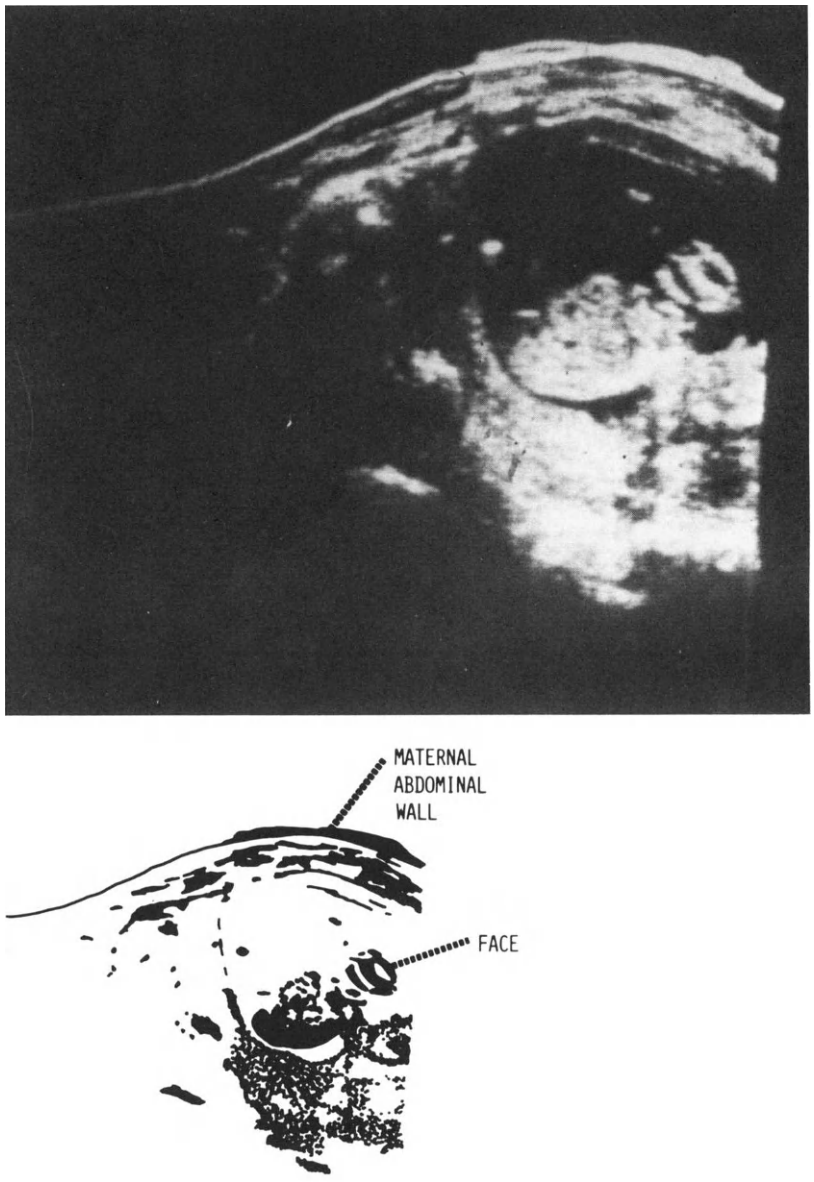



**Figure 10** Sagittal scan showing an anencephalic fetus with polyhydramnios. (From Berkowitz and Hobbins, 1982.)

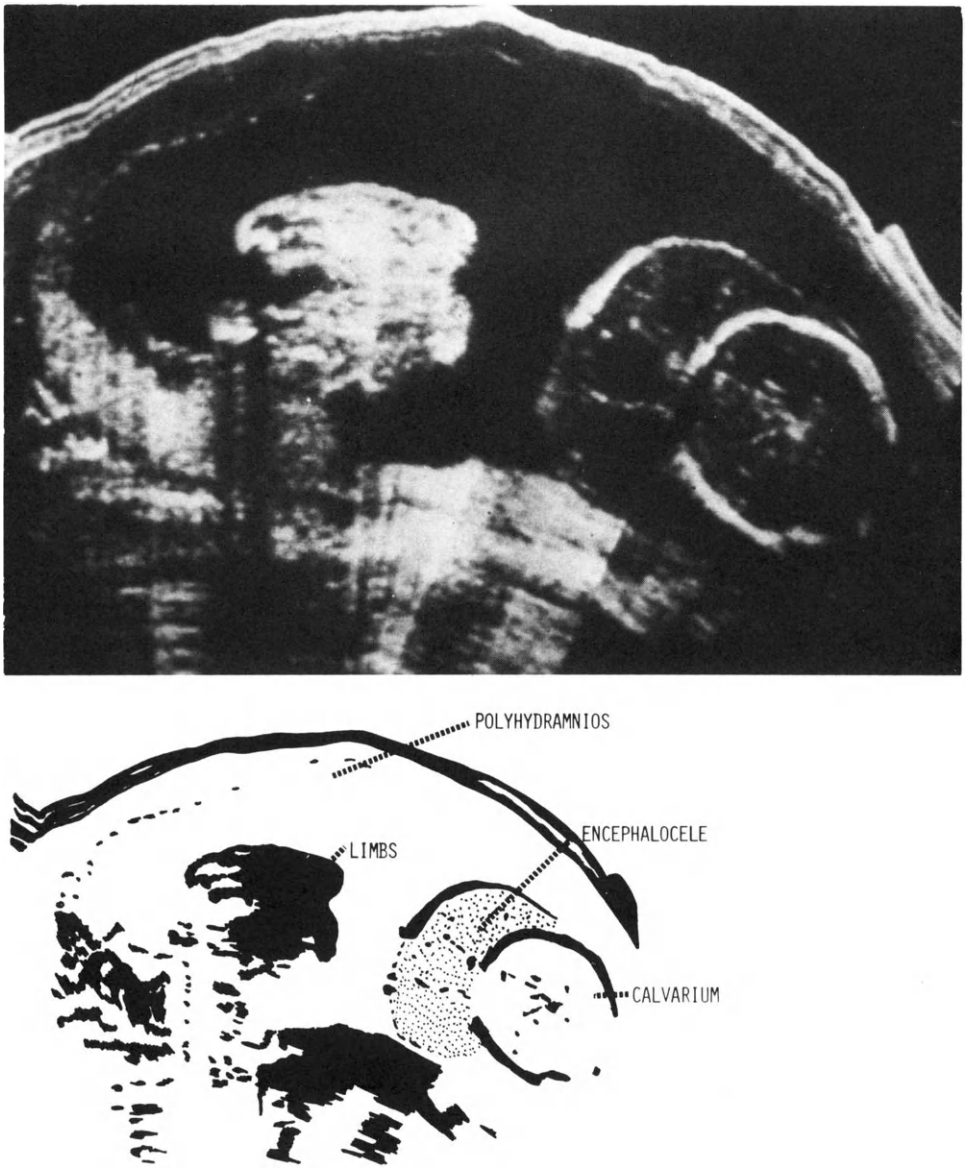



**Figure 11** Encephalocele with polyhydramnios.

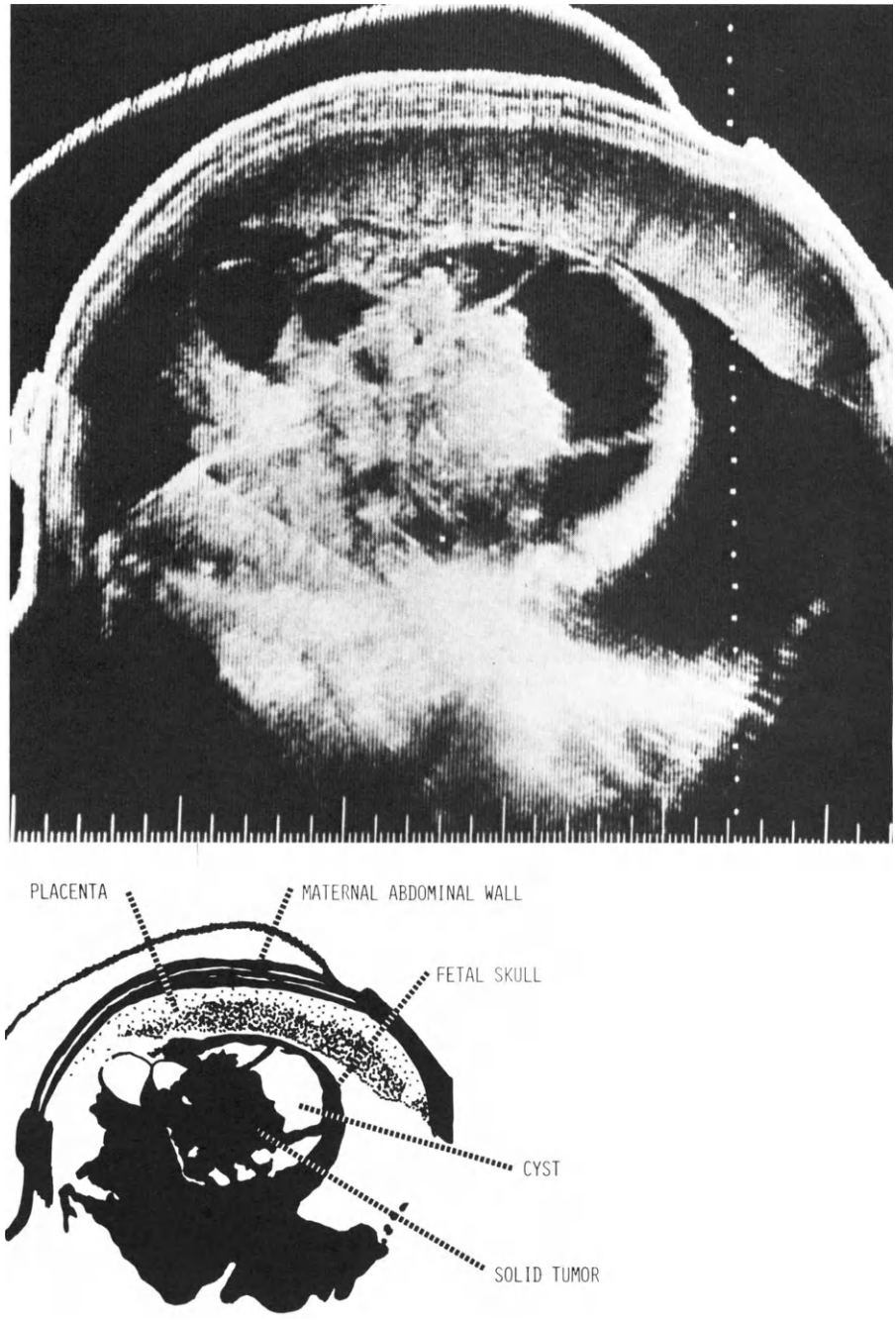



**Figure 12** Intracranial teratoma in a term fetus. (From DeVore and Hobbins, 1979a.)

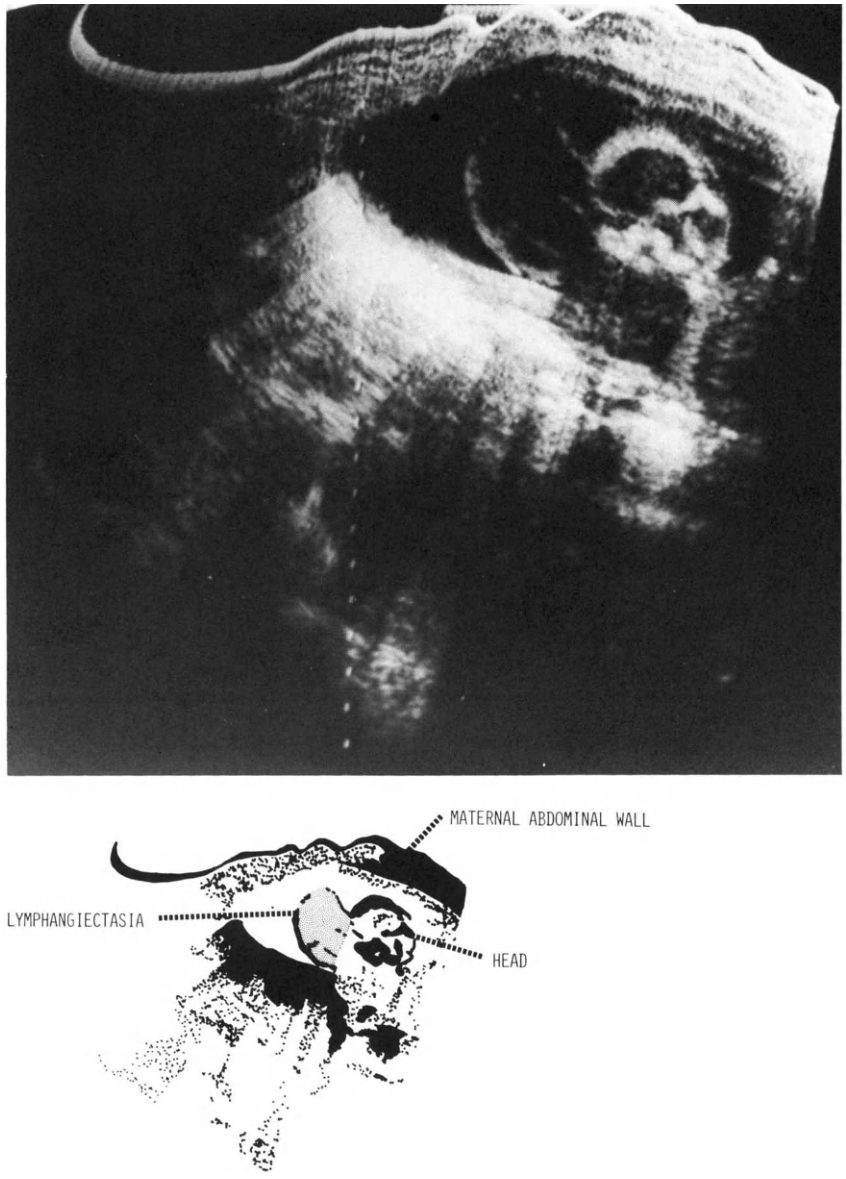



**Figure 13** Lymphangiectasia of the scalp protruding from the posterior fetal skull. (From DeVore and Hobbins, 1979a.)

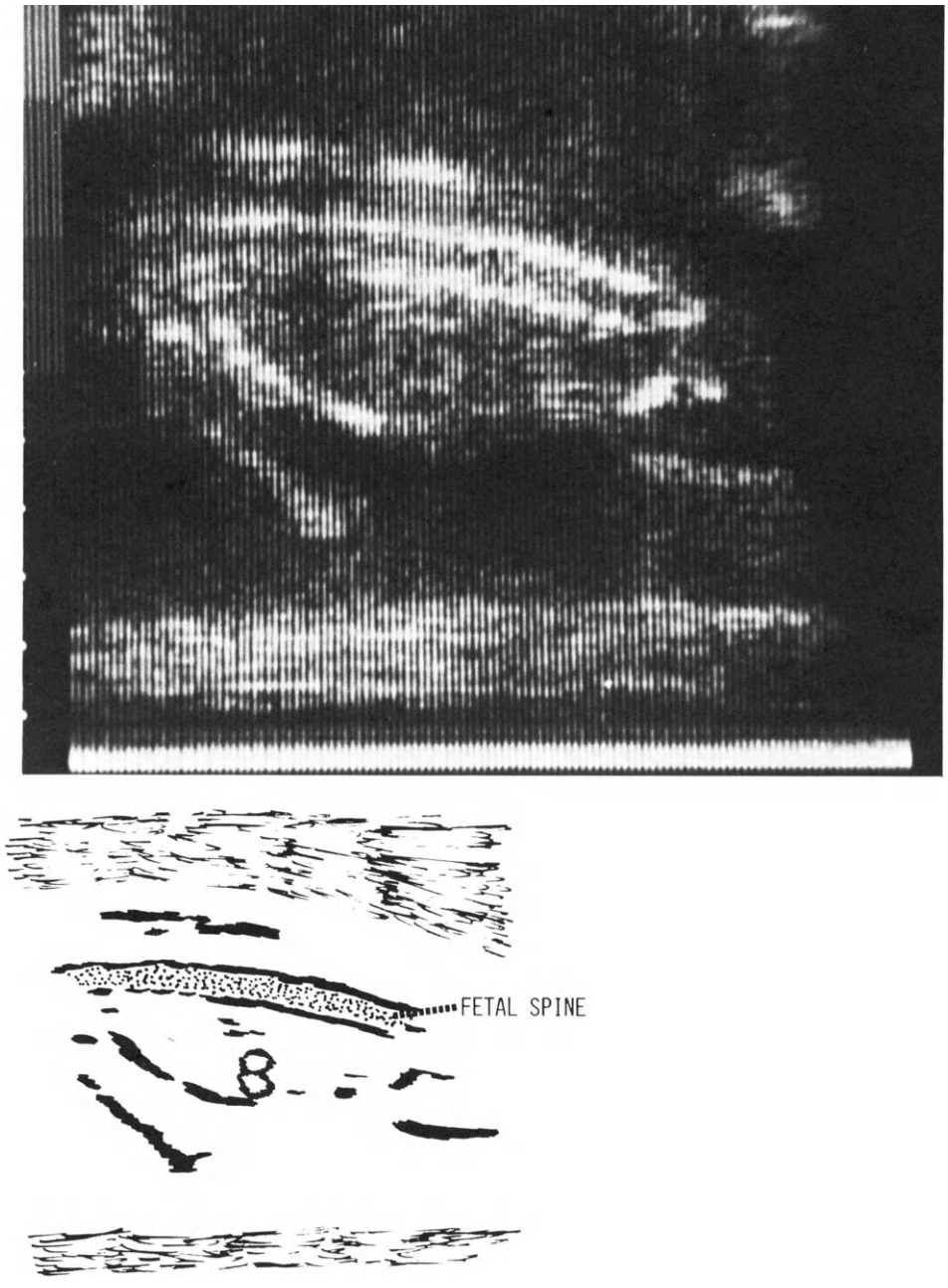



**Figure 14** Long axis of a normal fetal spine.

syndrome, and lymphangiectasia, a condition resulting in dissection of the integument away from the underlying fascia by accumulation of lymph, have been diagnosed in our ultrasound unit (Figure 13). Meningoceles can present with somewhat similar ultrasonic findings.

### Spine

*Spina Bifida* Spina bifida can be divided into two major groups, spina bifida occulta and spina bifida cystica. Spina bifida occulta is associated with a number of other congenital malformation syndromes, but in itself rarely leads to any neurological deficit. Spina bifida cystica (myelomeningocele), however, is associated with neurological deficit in over 90% of cases and occurs with a frequency varying between 1 in 200 to 1 in 500 live births in the British Isles and about 1 in 1000 live births in North America (Brock, 1976). The genetics are multifactorial, with a recurrence rate of 3-7% for both a fetus with a previously affected sibling and a fetus of an affected parent (Brock, 1976).

The most common location of spina bifida cystica is the lumbar area, in which a protrusion of meninges contains the spinal cord and nerves within a cystic sac. Neurological deficit can involve the lower extremities as well as impaired bowel and bladder function. Recurrent urinary tract infections are common and result from sphincter paralysis.

Hydrocephaly is noted in approximately 70% of fetuses with this form of spina bifida cystica. Of the fetuses born alive, 50% survive longer than 5 years; of these 25% are mentally retarded (Shulman, 1979).

The ultrasonic evaluation of spina bifida usually requires a time-consuming, careful examination of the fetal spine by an experienced investigator. Although real-time imaging is helpful, a contact static scanner provides the best image.

In fetuses with spina bifida, the spine can appear to be normal on some longitudinal cuts (Figure 14), so sagittal scanning should be performed very carefully. Spinal defects can be excluded by careful transverse scans beginning in the cervical area and by examining the full length of the fetal spine. The normal spine has the appearance of a closed circle (spinal cord) with strong circumferential echoes (spinal processes) (Figure 15). With spina bifida, however, an echogenic area is noted where the spinal processes are missing (Figure 16).

When scanning the fetus it is important that the spine be between the 9 and 12 o'clock positions, since any other position, especially the spine down, will not allow for complete evaluation because of poor penetrance or interference from other fetal structures.

*Spinal Tumor* A rare condition affecting the sacrum is a sacrococcygeal teratoma, a uniformly fatal fetal condition (DeVore and Hobbins, 1979a). The teratoma may attain huge proportions; this space-occupying tumor may produce bizarre fetal positions and configurations by extrinsic pressure (Figure 17).

## Chest

### Heart

Unlike the adult or pediatric subject, who can lie in the supine or left lateral position for m-mode or real-time echocardiographic examination, the fetus is constantly moving. Investigators have utilized either the m-mode or real time to evaluate the fetal heart for congenital anomalies. Kleinman et al. (1980) reported diagnosing a hypoplastic right

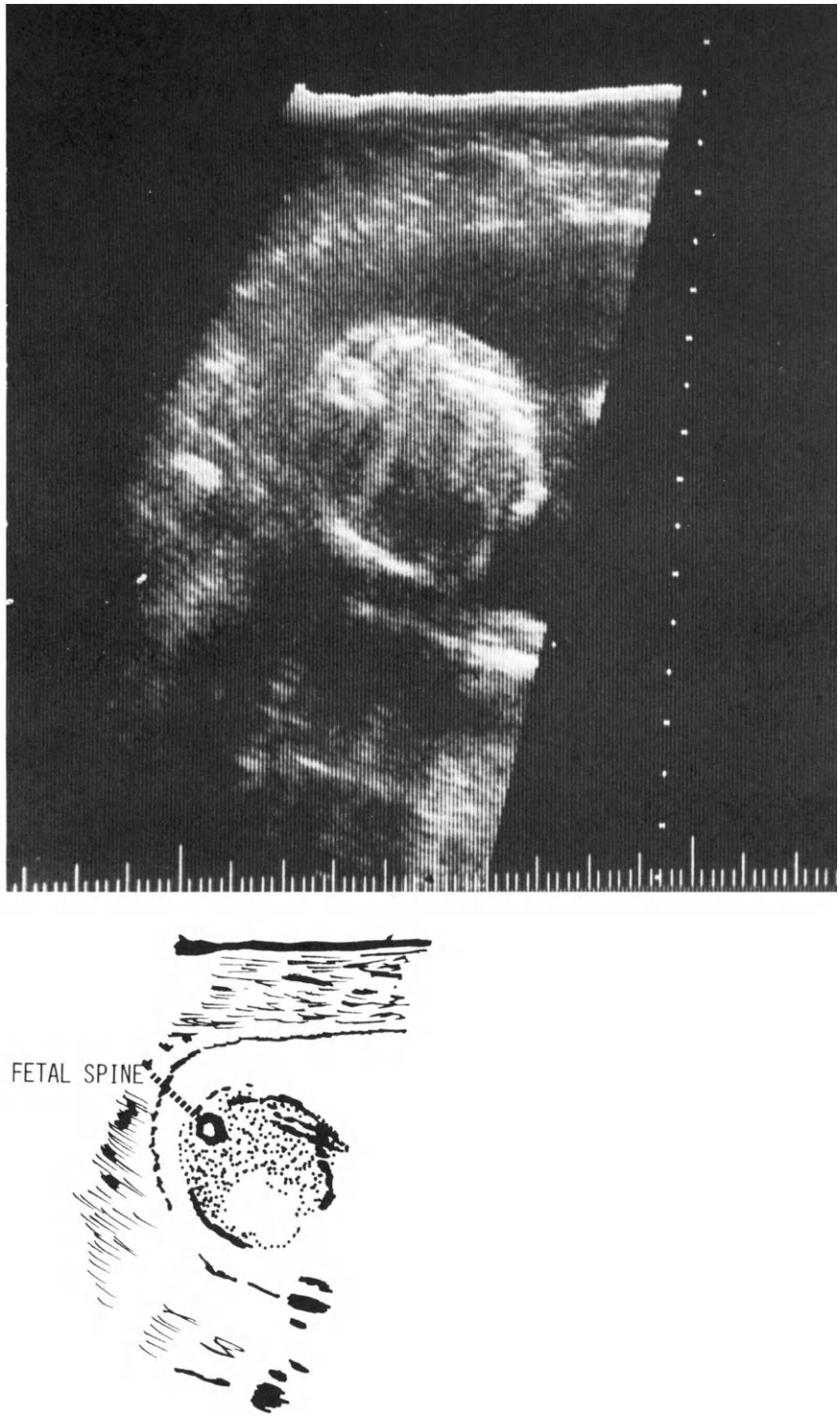



**Figure 15** Transverse scan of a normal fetal spine.

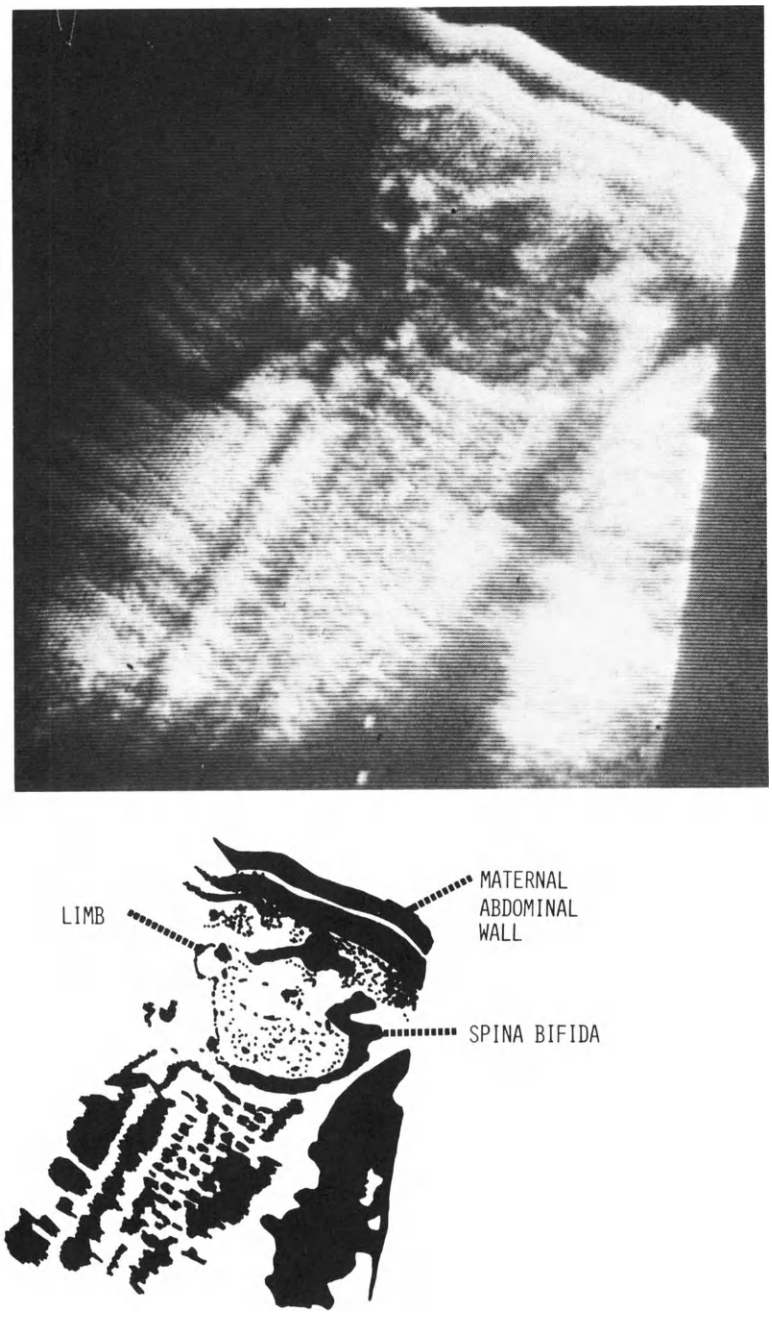



**Figure 16** Transverse scan of a fetal spine demonstrating the V-shaped defect of spina bifida. (From DeVore and Hobbins, 1979a.)

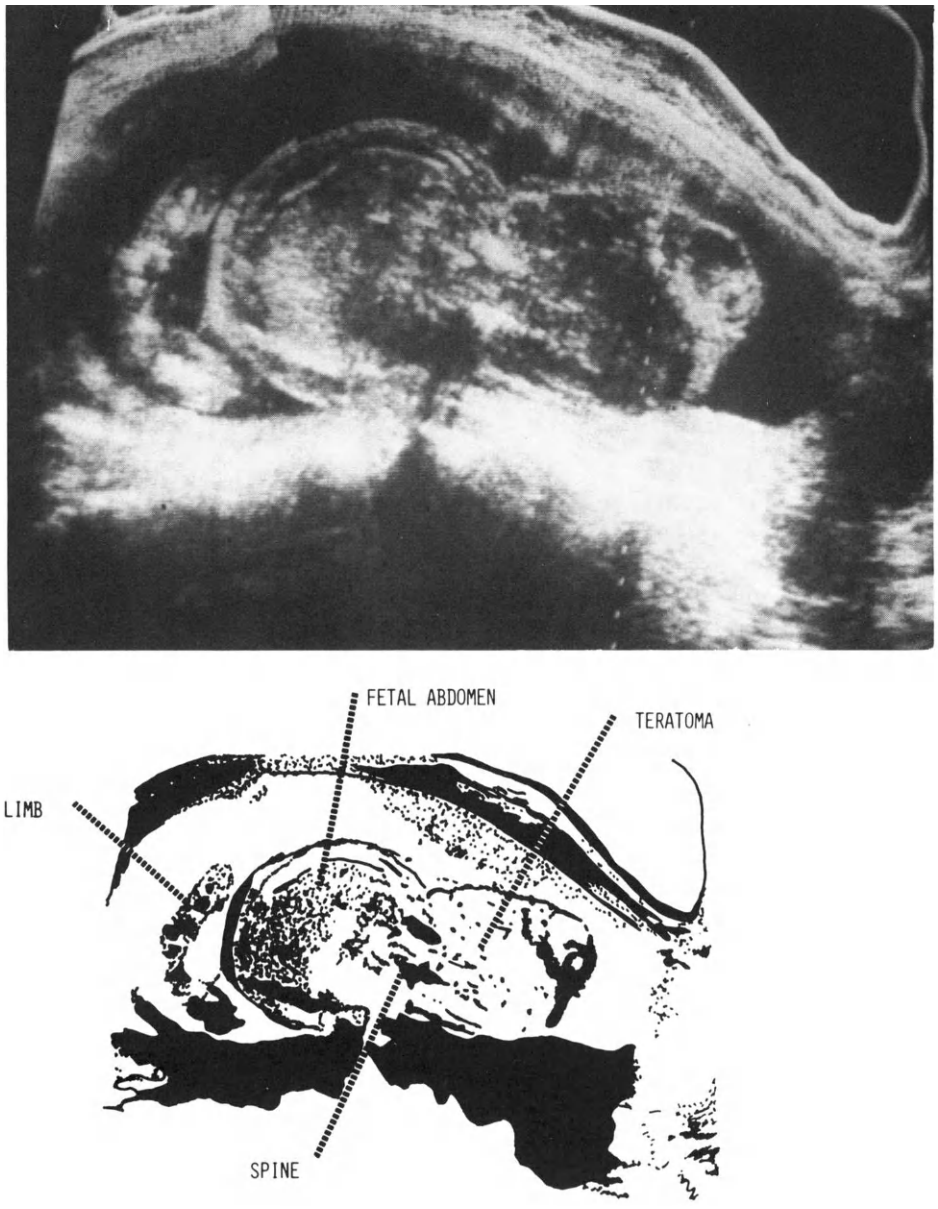



**Figure 17** Transverse scan of a fetal spine at the level of a sacrococcygeal teratoma. (From DeVore and Hobbins, 1979a).

ventricle at 34 weeks of gestation using the m mode, and a large ventricular septal defect in a 28-week fetus with suspected Down syndrome using real time. Allan et al. (1980) studied 200 fetal hearts with real time and correlated the position of the fetus with the real-time fetal heart image to accurately identify the anatomical structures of the heart.

Recently we have utilized an ATL* real-time sector scanner with a movable m-mode cursor which allows the fetal heart to be evaluated simultaneously with real-time and m-mode ultrasound. This allows precise m-mode orientation with real-time imaging and corrects for errors due to changes of fetal position during the course of an examination. Figure 18 is an example using this technique in which an interventricular septal tumor compressed the left ventricle and invaded the right ventricular cavity of a 27-week fetus. The information obtained with the m-mode identified the echolucent area seen below the mass in the left ventricle with real time as the ventricular wall, thus differentiating it from a pericardial effusion (DeVore et al., 1981).

### Intrathoracic Anomalies

Although rare, intrathoracic anomalies have been screened for and diagnosed with ultrasound. We have evaluated seven women during the second trimester of pregnancy whose fetuses were at risk for abnormal thoracic anomalies (thoracochondrodystrophy, asphyxiating thoracic dysplasia, and pulmonary hypoplasia). All were normal and the patients subsequently delivered normal fetuses. Defoort and Thiery (1978) reported a case of congenital chylothorax in which fluid was visualized within the pleural cavity.

The easiest to diagnose and the most frequently reported intrathoracic defect is the diaphragmatic hernia. Diaphragmatic hernias can be anatomically classified as either the rare retrosternal defect, which is infrequently associated with neonatal pulmonary compromise, and the posterolateral diaphragmatic defect, which occurs in 1 out of 2200 live births. The posterolateral diaphragmatic hernia involves the left side of the diaphragm nine times more frequently than the right and is secondary to an insult which inhibits or delays normal migration of the gut and closure of the diaphragm between the eighth and twelfth weeks of embryogenesis (Bergsma, 1979; Crane, 1979).

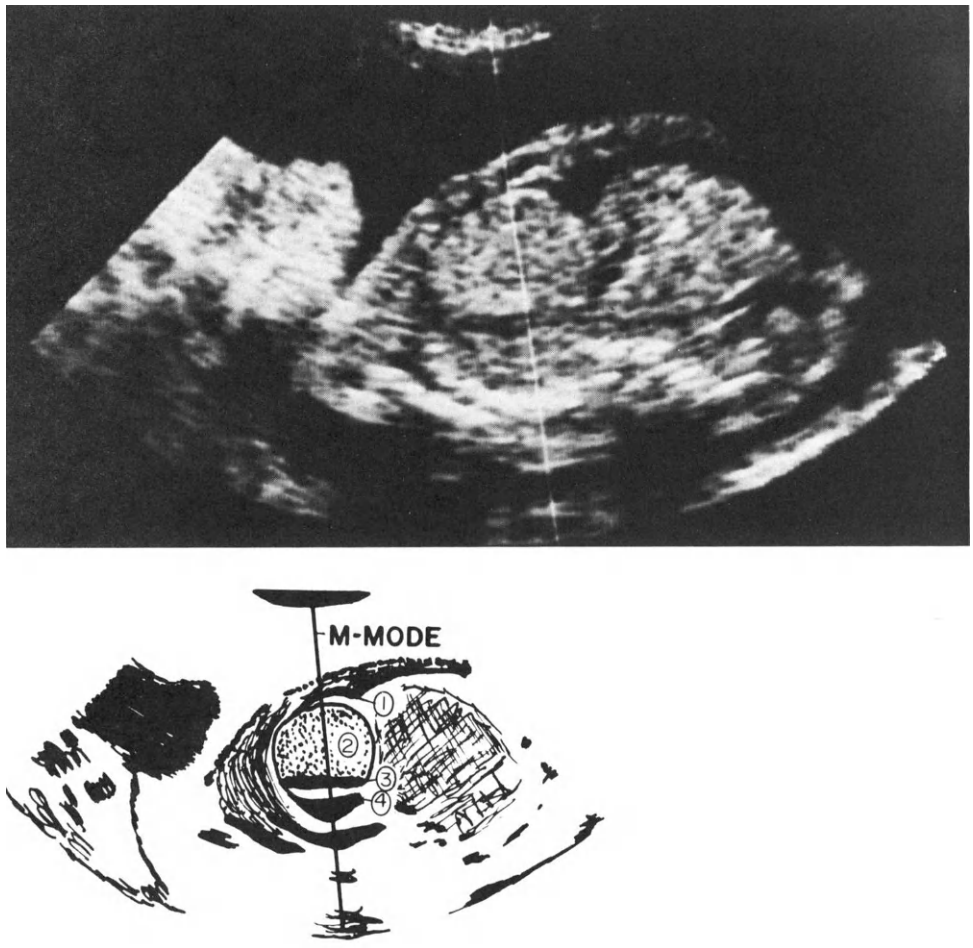
A total of 90% of stillborn fetuses and 20% of live-born infants with diaphragmatic hernias have major associated anomalies which involve the central nervous, cardiovascular, genitourinary, and gastrointestinal systems.

Unfortunately, most newborns with posterolateral defects are not diagnosed prior to birth. Only after they develop cyanosis, dyspnea, pneumothorax, pneumomediastinum, hypoxia, or acidosis or even die, is the diagnosis suspected. Owing to the delay in diagnosis, there is an increased morbidity and mortality postnatally.

If the defect is diagnosed prior to birth, then the appropriate neonatal care with eventual surgery can be carried out with minimal risk or compromise to the newborn. Figure 19 illustrates a diaphragmatic hernia in a fetus of a mother with class D diabetes, which was repaired shortly after birth. The fetus also had situs inversus, congenital heart anomalies, and duodenal atresia.

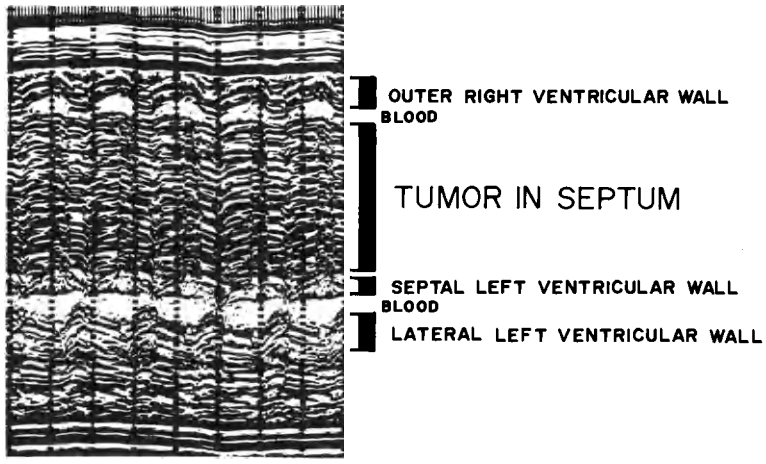
Once the in utero diagnosis of a diaphragmatic hernia has been made, and congenital anomalies screened for, the clinician should consider three congenital syndromes which have as part of their spectrum of defects herniation of abdominal viscera through the diaphragm: Beckwith-Wiedman syndrome, a rare disease associated with multiple endocrine anomalies; the fetal hydantoin syndrome, which can occur in as many as 10%

*Advanced Technology Laboratories, Inc., Bellevue, Washington.




(A)

**Figure 18** Ultrasound. (A) Transverse cut through fetal heart demonstrating the right ventricle (1), a tumor (2), and the left ventricle (3,4), using real-time ultrasound.




(B)

**Figure 18** Ultrasound. (B) An m-mode scan of the heart in 18(A), demonstrating the right ventricular wall with no endocardium secondary to invasion by rhabdomyoma. The left ventricular cavity is seen with the tumor mass impinging on the endocardium of the septal side of the left ventricle.

of fetuses exposed to diphenylhydantoin (phenytoin); and the posterior nose atresia syndrome, which occurs in 1 out of 5000 births (Bergsma, 1979).

Figure 20 illustrates a posterior intrathoracic cyst of enteric origin, which is a rare defect and looks similar to a diaphragmatic hernia.

## Gastrointestinal Tract

There are over 250 syndromes which have associated gastrointestinal (GI) anomalies (Bergsma, 1979). These anomalies can be broadly separated into two major categories: (1) those that alter normal anatomy and (2) those which affect physiological function.

Evaluation of GI anomalies of the structural type can be screened for and diagnosed with ultrasound. In most fetuses with GI anomalies, the diagnosis is suspected when polyhydramnios presents because of the inability of the fetus to swallow amniotic fluid.

### Normal Anatomy

Unlike the adult, in which the bowel contains gas and feces which make ultrasound evaluation impossible, the fetal GI system contains fluid. This allows for easy ultrasonic evaluation. The normal fetal esophagus is not imaged because, as in the adult, it is a collapsed structure. The fetal stomach, however, is easily observed as early as the fifteenth gestational week. It is located in the left upper quadrant under the diaphragm and is anechoic (Figure 21). Loops of bowel can also be imaged, but it is currently very difficult to differentiate small from large bowel (Figure 22).

### Esophagus

Two of the most common anomalies are esophageal atresia with and without concomitant tracheal fistulization. The incidence of both is 1 in 3000 live births and they are associated with other anomalies in over 50% of cases. The etiology is unknown, but the insult is thought to occur during the fourth embryonic week of development (Holder and Ashcraft, 1966). The recurrence rate is apparently not increased in siblings.

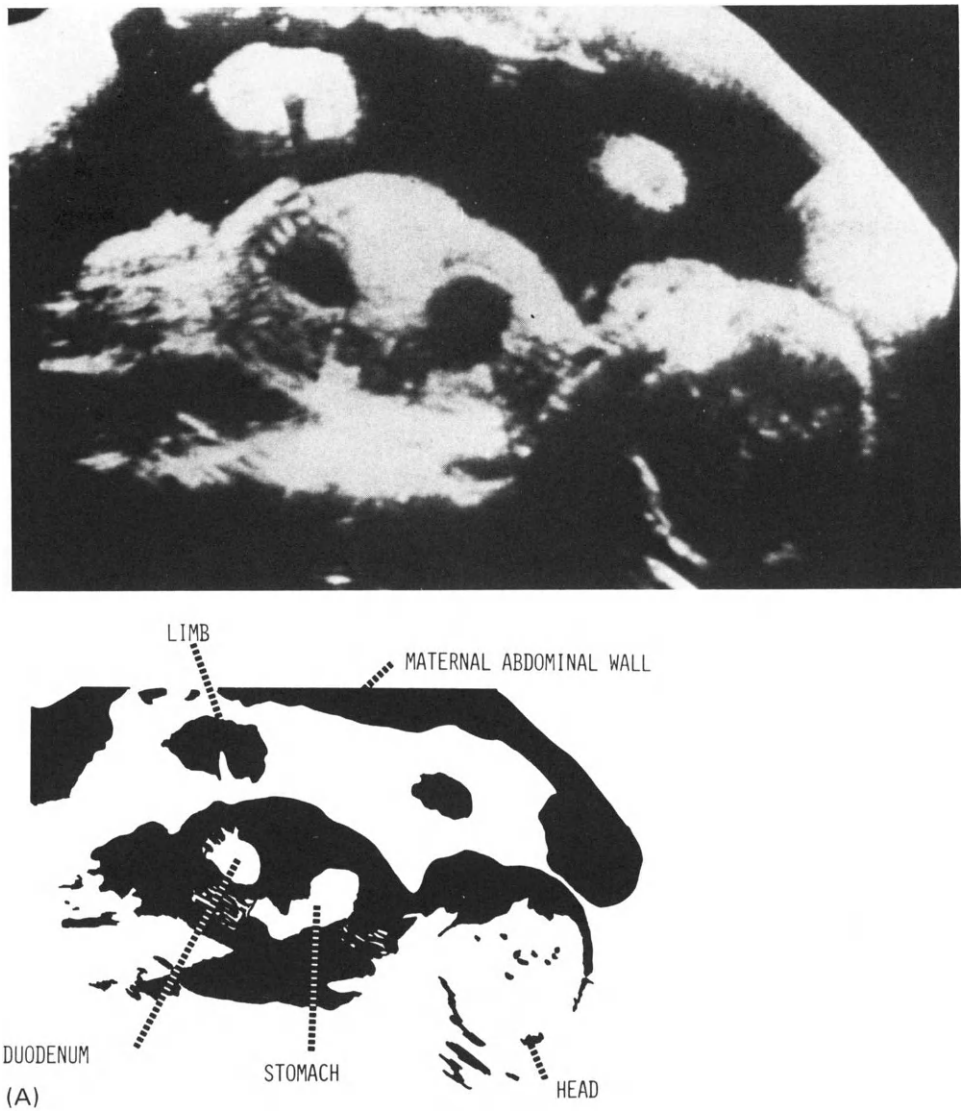



**Figure 19** Fetal diaphragmatic hernia. (A) Sagittal scan demonstrating the stomach in the fetal chest.

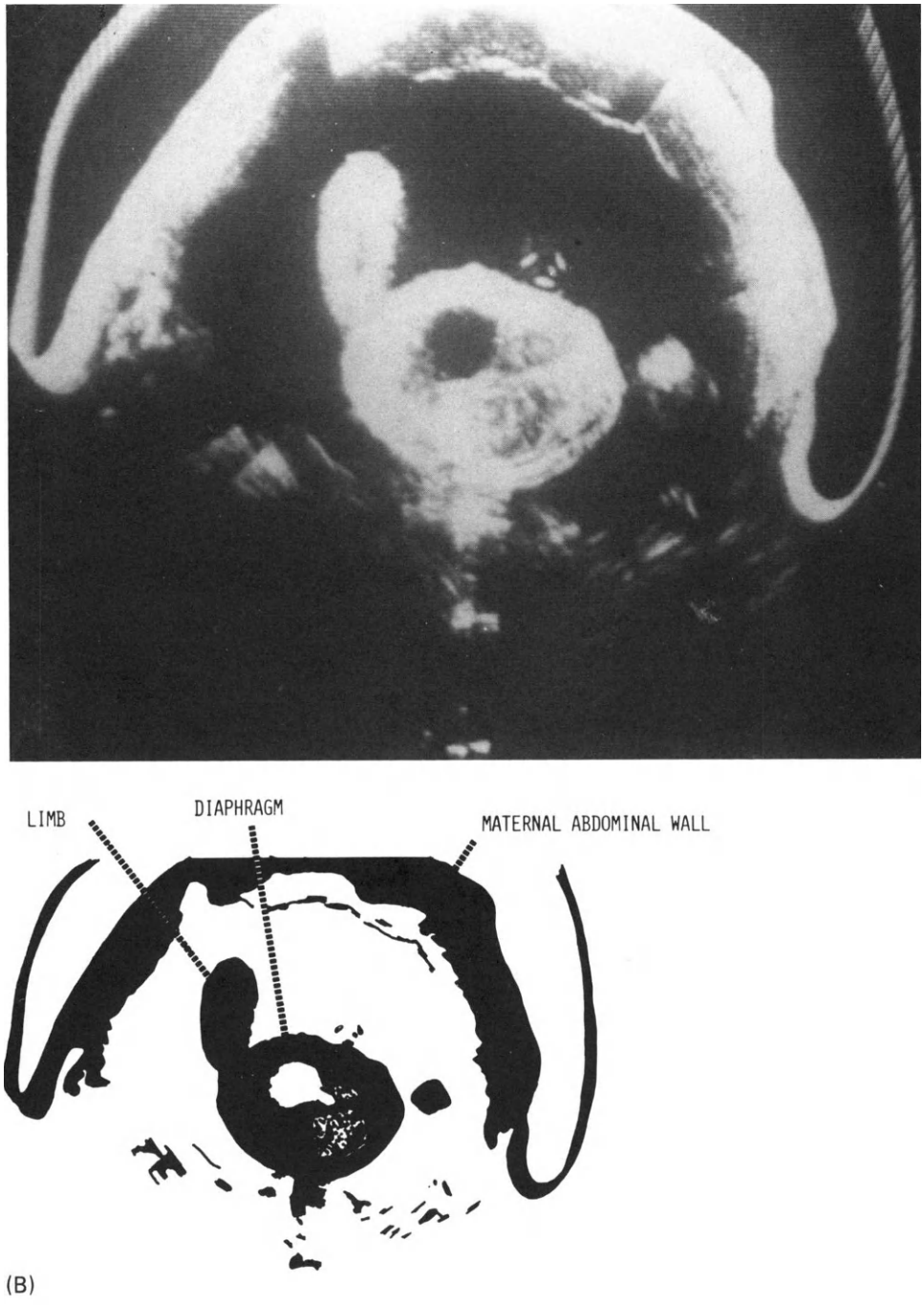



**Figure 19** Transverse scan. (B) Taken above the fetal diaphragm with the stomach within the chest. (From DeVore and Hobbins, 1979a.)

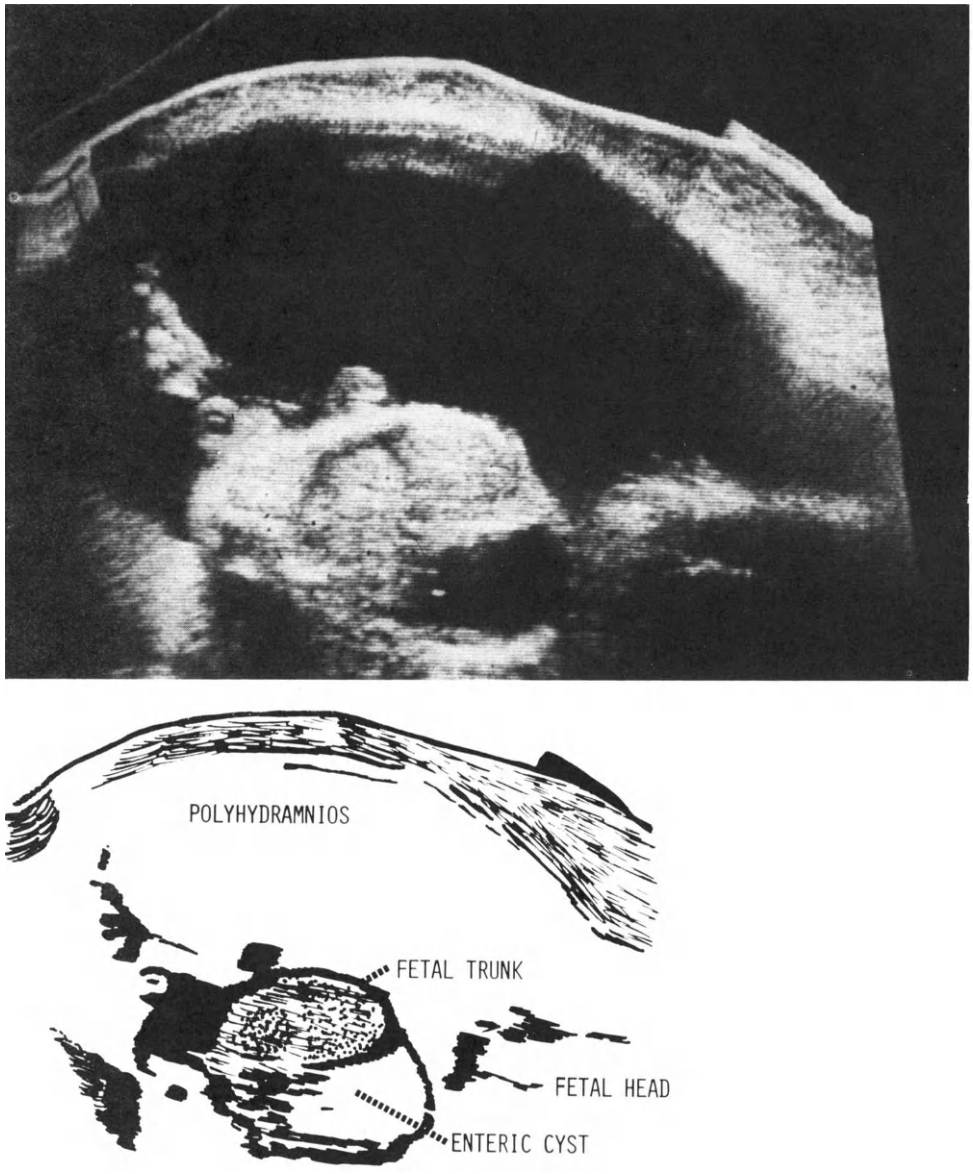



**Figure 20** Sagittal scan through a fetal trunk with an enteric cyst above the diaphragm.

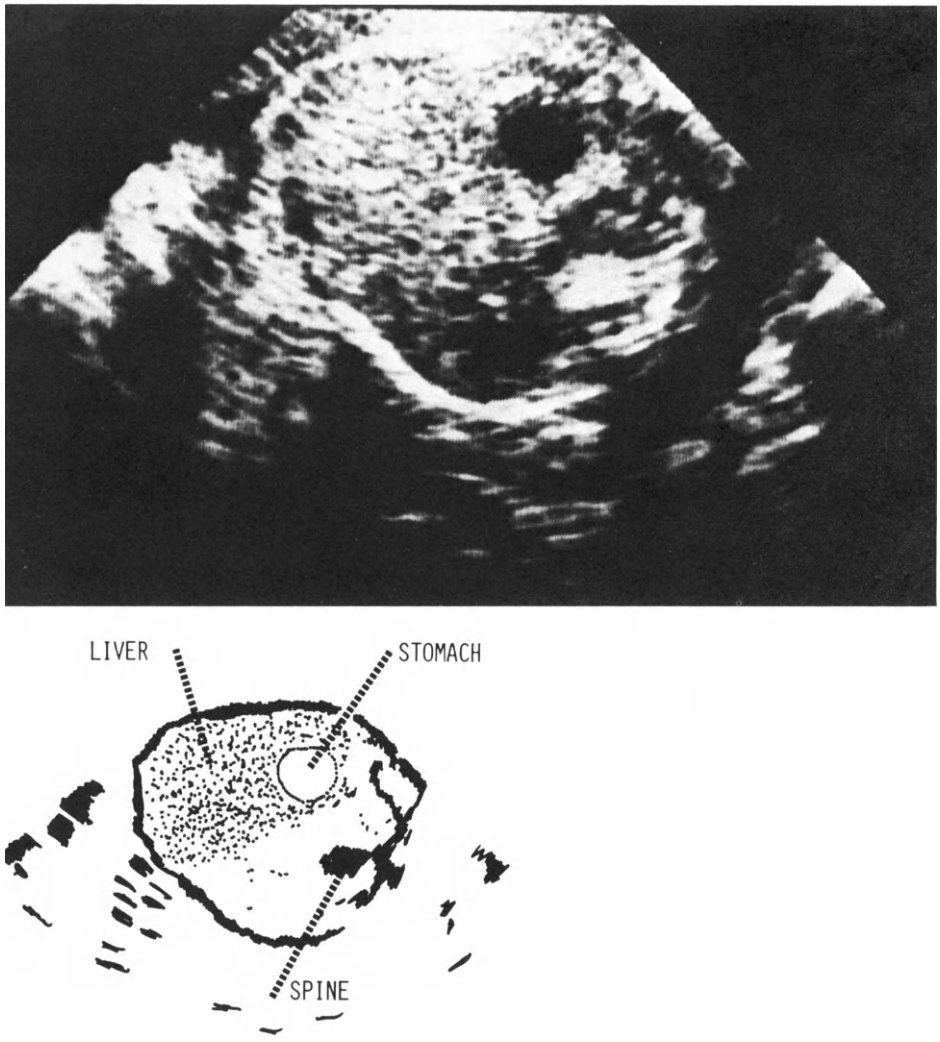



**Figure 21** Transverse scan through a normal fetal stomach.

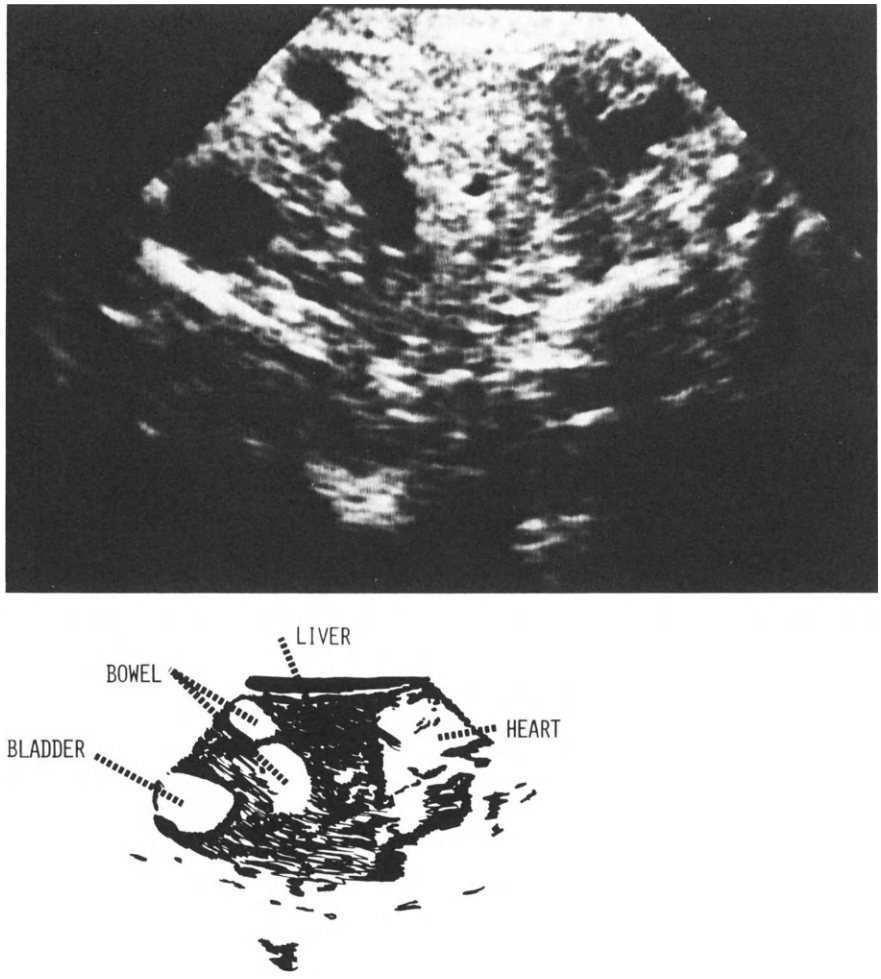



**Figure 22** Sagittal scan through a fetal trunk demonstrating the fetal bladder, normal bowel, and the fetal heart.

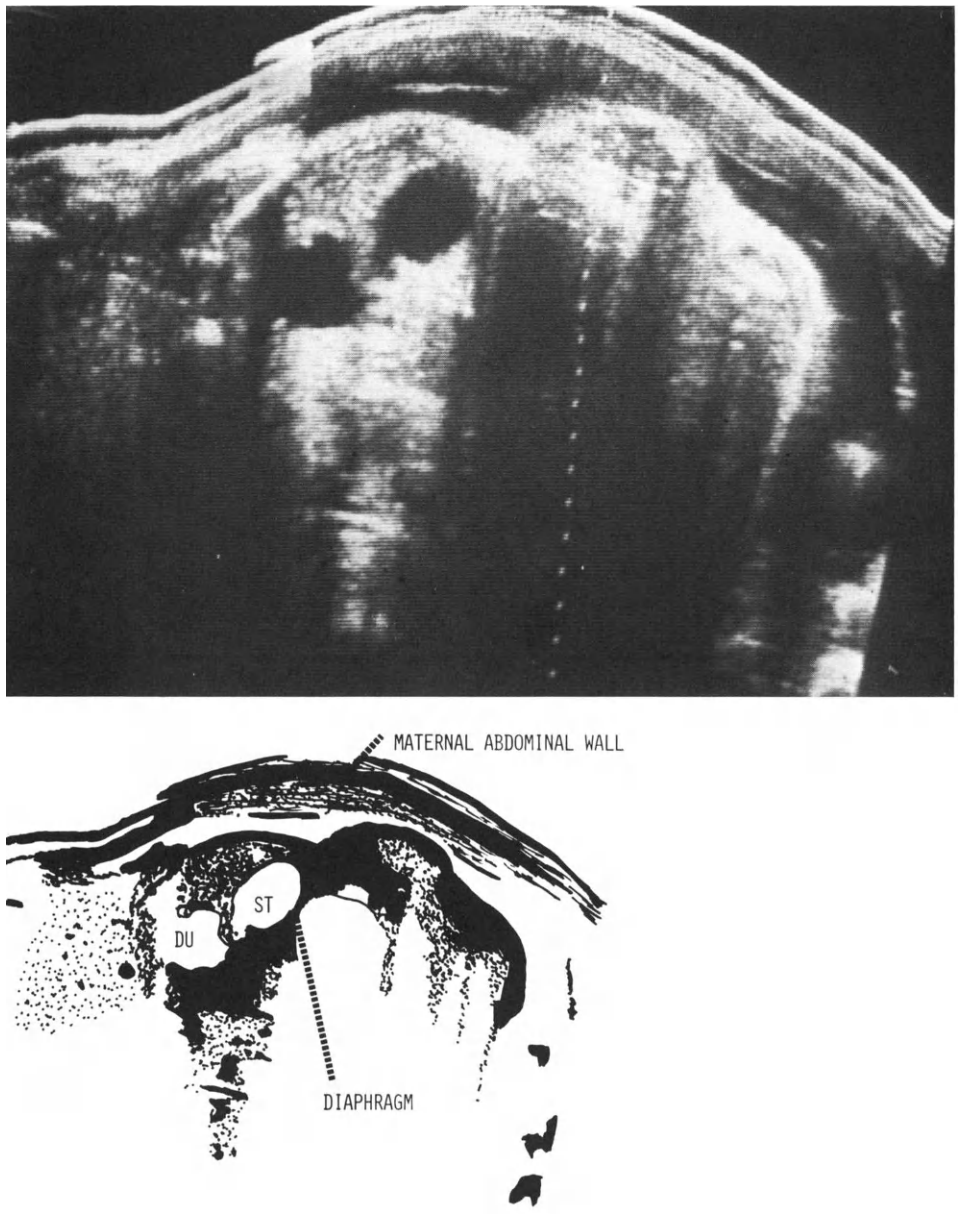



**Figure 23** Sagittal scan demonstrating a "double bubble" secondary to duodenal atresia (ST, stomach; DU, duodenum). (From Hobbins et al., 1979.)

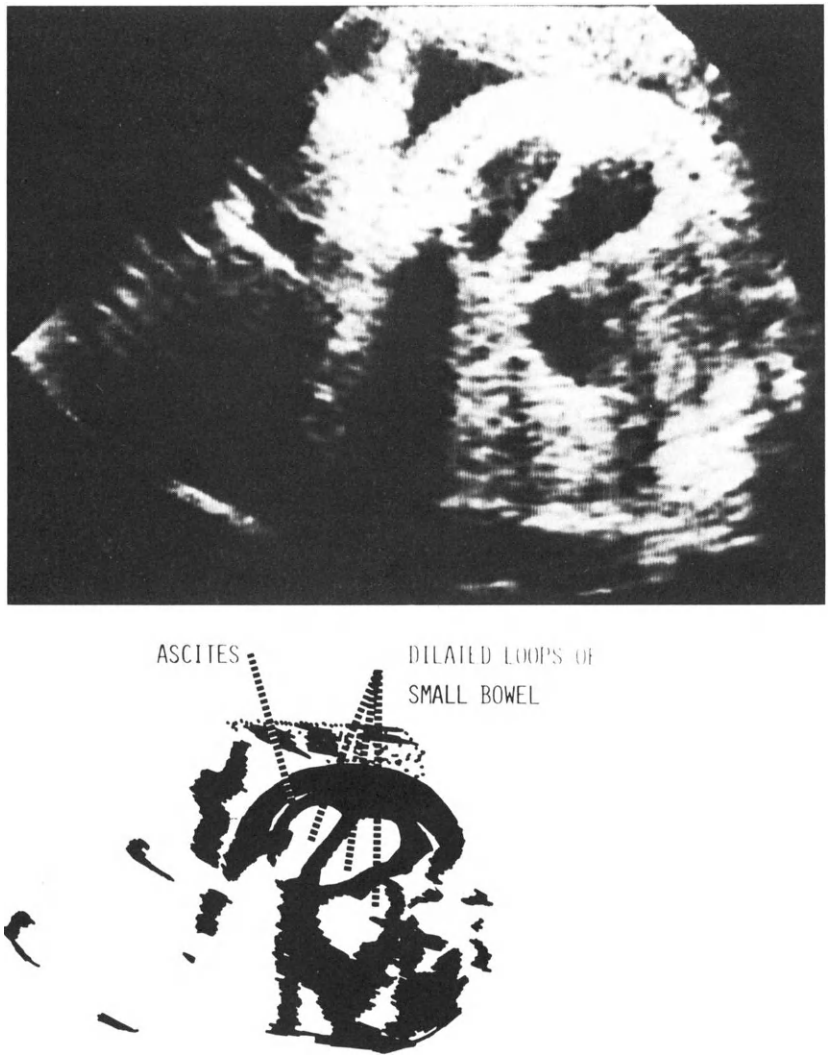



**Figure 24** Transverse scan through a fetal abdomen demonstrating meconium peritonitis with fetal ascites and dilated loops of bowel.

Although the esophagus cannot be imaged in the normal fetus, one might expect with esophageal atresia that the inability to image the stomach with ultrasound would strongly suggest the diagnosis. However, Duenhoelter et al. (1976) reported a case of duodenal atresia with concomitant esophageal atresia in which the stomach was noted to be filled with fluid and enlarged. This would suggest that even in the presence of esophageal atresia, the stomach secretes fluid into its lumen and does not require fetal swallowing for imaging with ultrasound.

### Stomach

Duodenal atresia occurs in 1 out of 10,000 live births and is due to one of three complications occurring during embryonic development: incomplete recanalization of the duodenum, a vascular accident, or an annular pancreas. Associated anomalies occur in 48% of cases; 30% have trisomy 21, 22% malrotation of the colon, and 20% congenital heart disease (Fonkalsrud, 1979). Of the mothers with fetuses with duodenal atresia, 45% have polyhydramnios.

The diagnosis can be made in utero with ultrasound by demonstrating the classic "double bubble" (Figure 23), which is seen on the plain abdominal roentgenogram following birth. In the absence of other life-threatening congenital anomalies, these infants do quite well if surgical correction of the defect proceeds promtply following birth before complications resulting from aspiration occur (DeVore and Hobbins, 1979b; Boychuk et al., 1978; Houlton et al., 1974; Lees et al., 1978).

### Bowel

Bowel obstruction, regardless of the etiology, appears as dilated "rings" which have a similar appearance on a flat plate roentgenogram of the fetal abdomen postnatally (Figure 24). If the obstruction occurs proximal to the distal ileum, polyhydramnios is often present. Unlike duodenal atresia, where there is a classic ultrasound finding, bowel obstruction can result from atresia, stenosis, adhesions, volvulus, malrotation, meconium peritonitis, and a number of other developmental problems. This precludes determining the exact etiology prior to exploratory surgery; however, it does alert the physician to deliver the fetus at an institution which has the resources for pediatric newborn intensive care, as well as skilled pediatric surgeons.

When bowel dilation is suspected, the fetus should be scanned at a minimum of once a week. We have followed three pregnancies in which bowel obstruction was suspected, but further evaluation following delivery did not demonstrate any problem. The syndromes reported thus far in the literature which have been associated with bowel dilation are meconium ileus (DeVore and Hobbins, 1979b), "apple peel" atresia of the small bowel (Fletman et al., 1980; Nikapota and Loman, 1979), jejunal atresia (Lee and Warren, 1977), megacystis-microcolon-intestinal hypoperistalsis syndrome (Vezina et al., 1979), and intestinal aganglionosis involving the colon and distal ileum (Wrobleski and Wesselhoept, 1979).

### Abdominal Wall Defects

Gastroschisis occurs in 1 out of every 20,000-30,000 live births and results from an abdominal wall defect, usually involving the right side of the midline, leaving the umbilicus intact. The defect is small, between 3 and 5 cm. The abdominal viscera herniate through the defect and may be covered with an exudate. There may be associated adhesions, atresia secondary to vascular accidents in utero, or malrotation

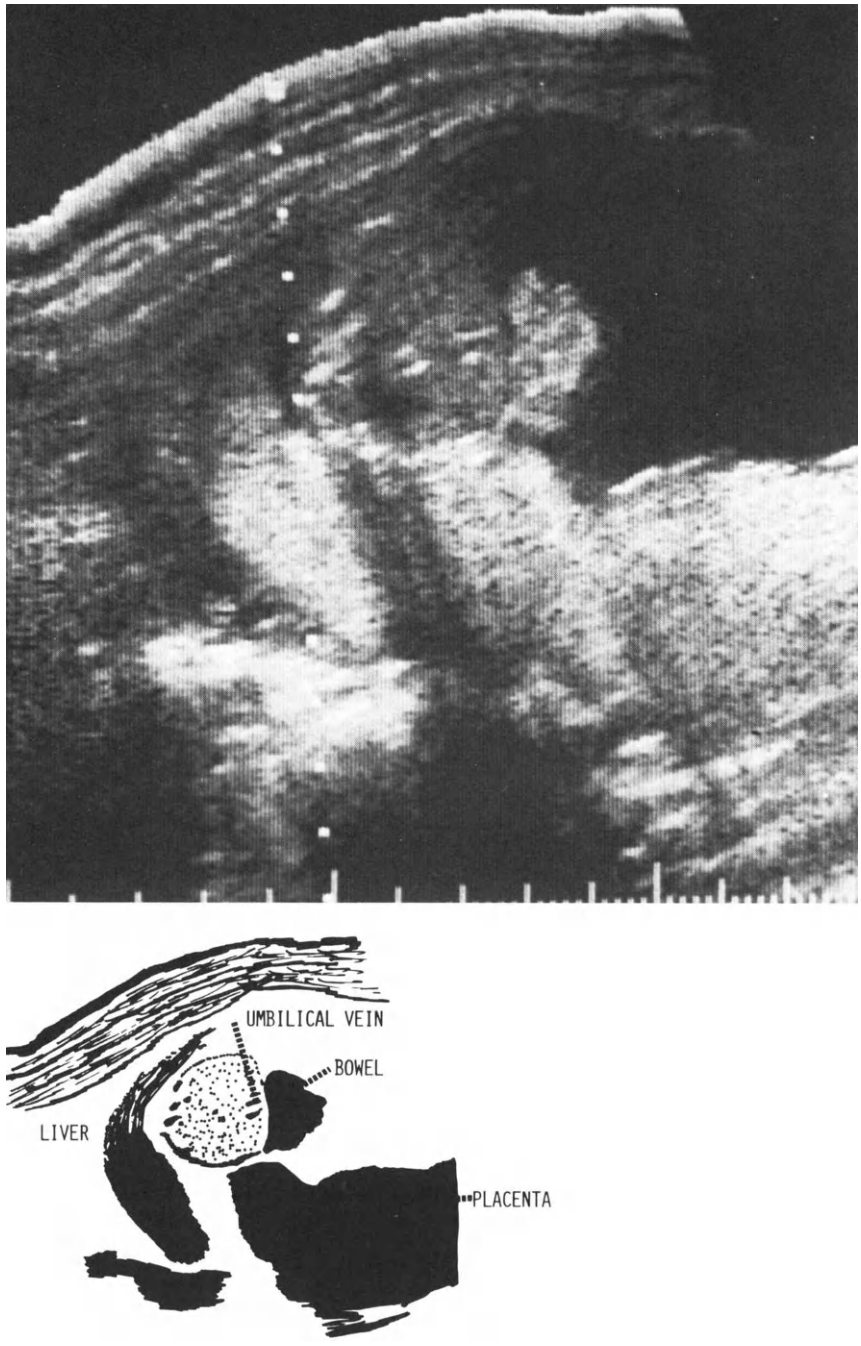



**Figure 25** Transverse scan through a fetal abdomen with bowel protruding from an abdominal wall defect.

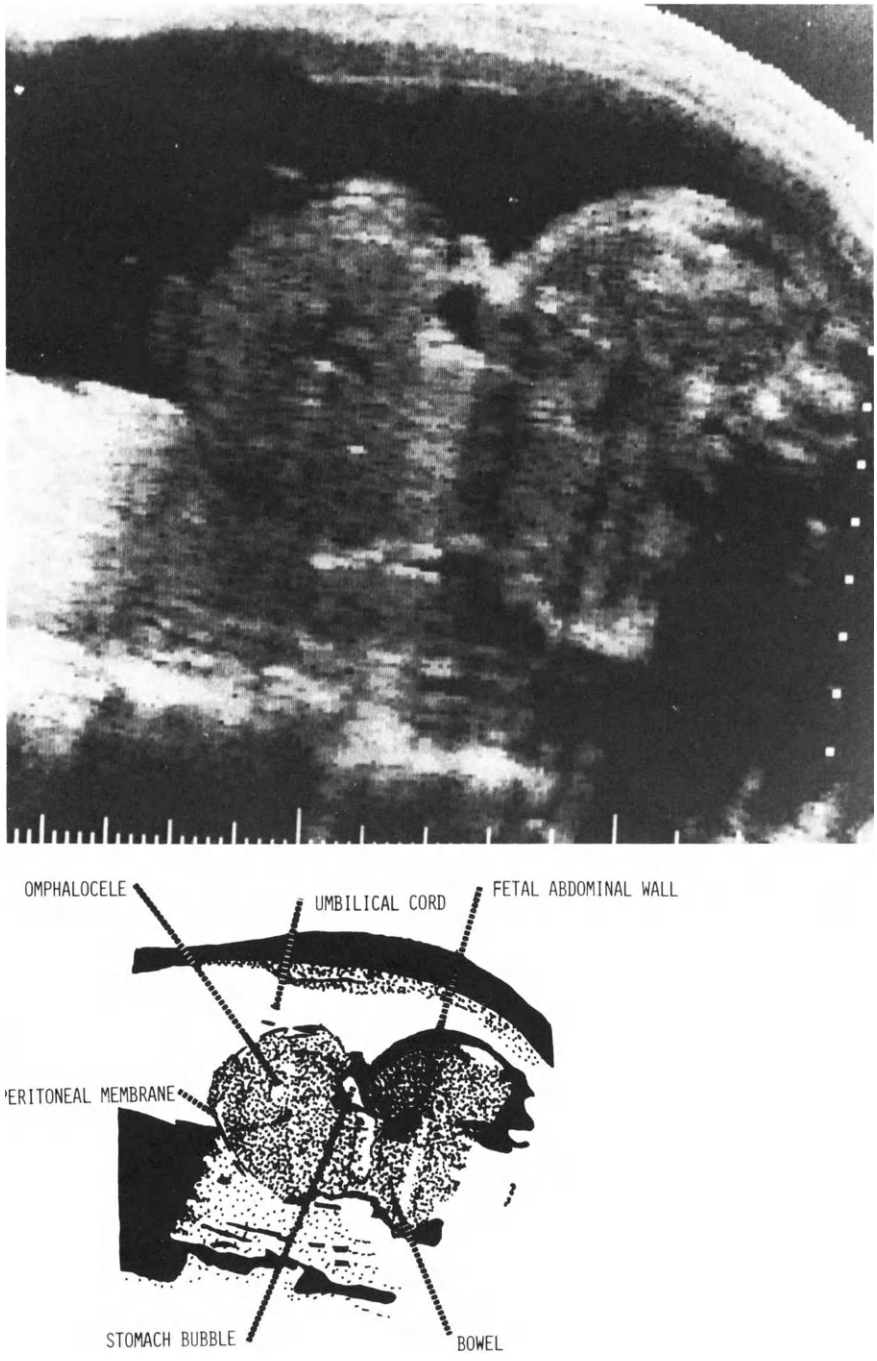



**Figure 26** Omphalocele protruding from a fetal abdomen covered by a membrane. (From DeVore and Hobbins, 1979a.)

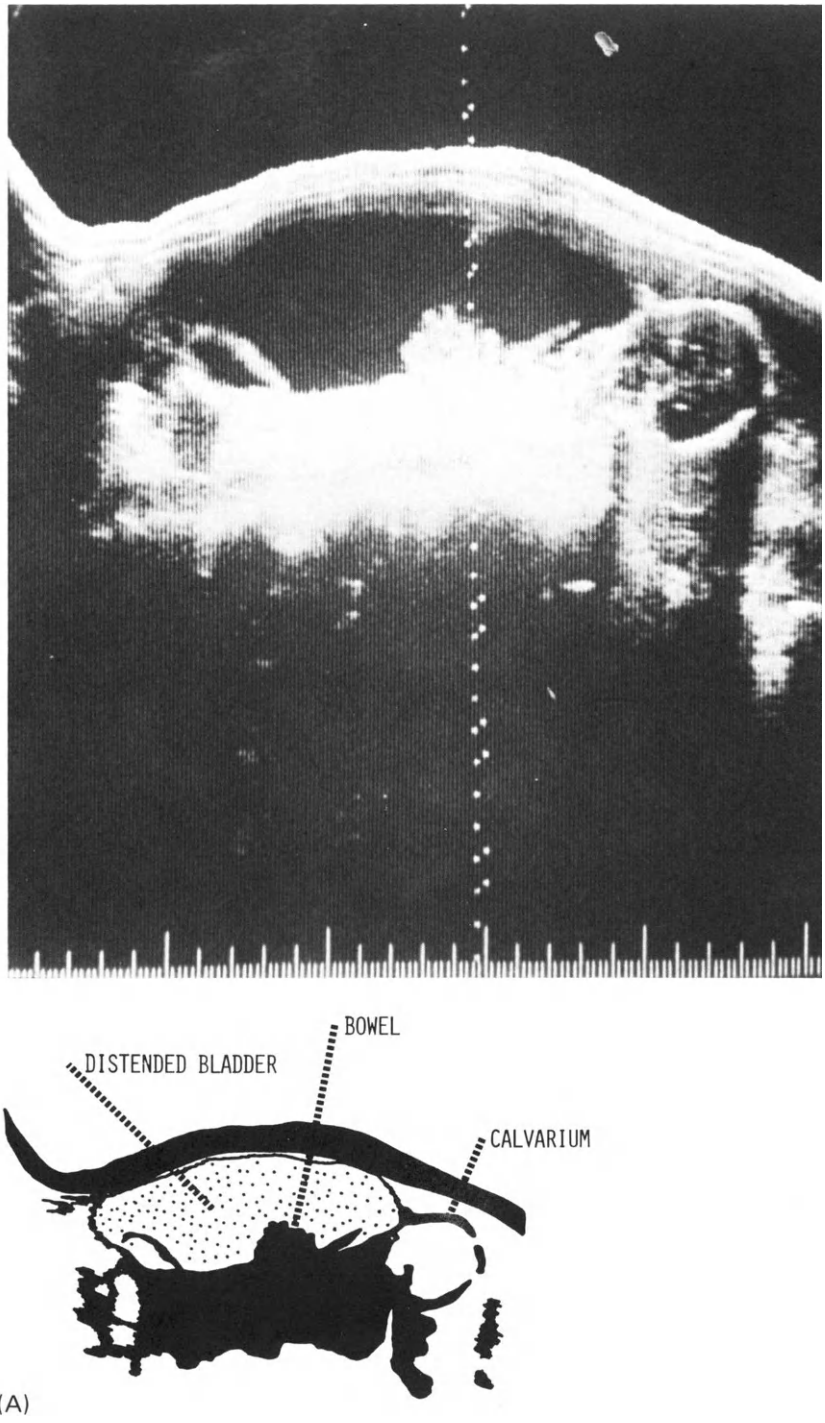



Figure 27 Saggital scan. (A) A dilated fetal bladder.

(B)

**Figure 27** Autopsy showing dilated fetal bladder. (B) The bowel was compressed up against the diaphragm.

of the gut (Figure 25). Surgery, the treatment of choice, has decreased the mortality rate to 20-30% (Giulian and Alvear, 1978).

Omphalocele, as opposed to gastroschisis, results from a defect in the umbilical ring through which bowel, liver, and spleen can herniate. The incidence is 1 in 6000 live births. The defect, covered by membranes derived from Wharton's jelly, can be only a few centimeters in length or can involve the entire abdominal wall (Figure 26). The prenatal ultrasonic diagnosis of an omphalocele allows the obstetrician to deliver the fetus by cesarean section, and thus decrease the risk of rupturing the sac during labor and delivery.

Unlike gastroschisis, fetuses with an omphalocele have a higher risk of associated anomalies of the cardiovascular system (16-20%), genitourinary system (40%), and the central nervous system (4%) (Pickett, 1979). Complications involving the gastrointestinal system can also present, such as atresia of the bowel (secondary to vascular compromise), incomplete rotation of the intestine, and occasionally abnormal fixation of the liver. The defect can be repaired surgically. The mortality rate varies between 20 and 30% and is due to infection, inanition, or unrelated congenital anomalies.

## Genitourinary System

There are approximately 200 anomalies of the genitourinary system (Bergsma, 1979). Proper evaluation of a particular disease requires a proper understanding of the disease and its presentation in utero. With current ultrasound imaging, it is possible to evaluate the fetal kidneys, dilated ureters or urethra, the bladder, as well as the external genitalia.

### Bladder

The bladder should be easily seen by 20 weeks of gestation. Its identification precludes the diagnosis of nonfunctioning polycystic kidneys or renal agenesis. Failure to demonstrate this structure (Figure 22) is not pathognomonic of the absence of kidney function, because the fetus may have recently voided. The bladder, however, should be seen at some time during an examination, especially if the mother is given furosemide (Wladimiroff, 1975), which crosses the placenta and precipitates a prompt fetal diuresis in fetuses of more than 20 weeks gestation.

If the bladder appears to be dilated, one should suspect a complete or partial obstruction of the urethra. Figure 27A illustrates a case of a posterior urethral obstruction in a female fetus which resulted in dilation of the ureters as well as secondary damage to both kidneys. At autopsy the bladder was massively distended and filled the entire abdominal cavity, compressing the viscera against the diaphragm (Figure 27B).

### Kidneys

Fetal kidneys appear as two circular structures on either side of the spine at the level of the umbilicus (Figure 28). The size of the fetal kidney relative to the fetal abdomen has recently been quantitated and a nomogram constructed comparing the abdominal circumference to the mean kidney circumference (Table 4) (Grannum et al., 1980). This is useful in evaluating patients at risk for Potter syndrome, infantile polycystic kidney disease, or multicystic kidney disease. Potter syndrome, or bilateral renal agenesis, varies in incidence from 0.1 to 0.3% and results from a failure of embryo genesis of the genitourinary system which includes complete absence of the kidneys. The recurrence rate is unknown; however, it has recurred in families at risk (Kaffe et al., 1977). Besides

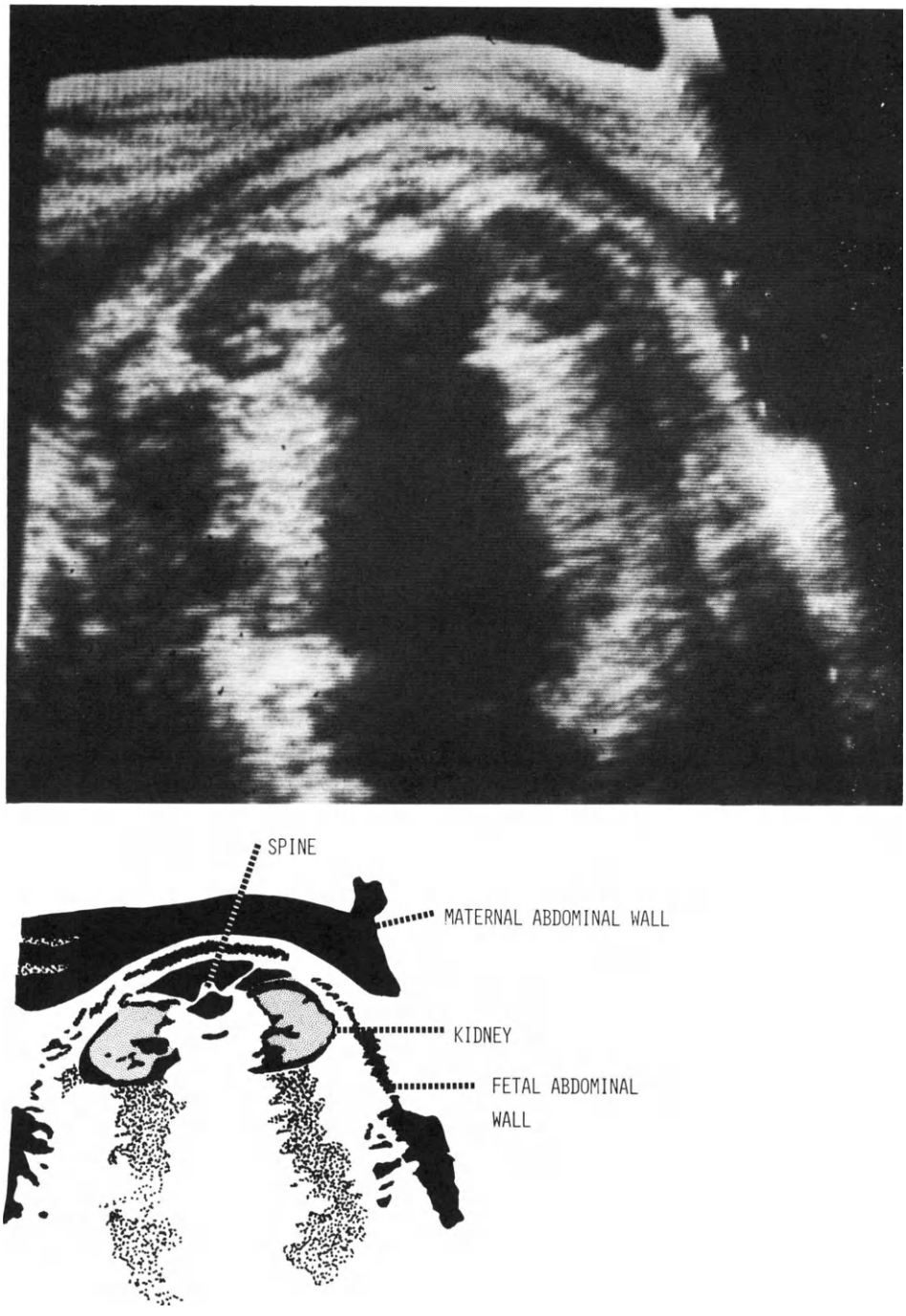



**Figure 28** Transverse scan demonstrating normal fetal kidneys (stippled area). (From DeVore and Hobbins, 1979a.)

Table 4 Mean Ratios of Fetal Kidney Circumference to Abdominal Circumference and the Standard Deviations

| | Gestational age (weeks) | | | | | |
|---|---|---|---|---|---|---|
| | <16 (*N* = 9) | 17-20 (*N* = 8) | 21-25 (*N* = 7) | 26-30 (*N* = 11) | 31-35 (*N* = 19) | 36-40 (*N* = 25) |
| Mean | 0.28 | 0.30 | 0.30 | 0.29 | 0.28 | 0.27 |
| Standard deviation | 0.02 | 0.03 | 0.02 | 0.02 | 0.03 | 0.04 |

*Source:* Adapted from Grannum et al. (1980).

renal agenesis, characteristic facial features, pulmonary hypoplasia, oligohydramnios, and intrauterine growth retardation have commonly been observed. The stillborn rate is 40%, with almost all newborns dying within the first 4 hr of birth.

The diagnosis is suspected in cases of severe oligohydramnios and absence of visualization of the bladder and kidneys (Keirse and Meerman, 1978). When the diagnosis is made, decisions on obstetric management should take into account that the condition is invariably fatal in the perinatal period.

Infantile polycystic disease (Potter type 1 dysplastic kidneys) (Potter and Craig, 1975) is a lethal autosomal recessive disease with a 25% risk of recurrence in subsequent siblings at risk. The fetal kidneys appear large and cystic (increased kidney circumference/abdominal circumference, KC/AC) with absence of filling of the bladder, oligohydramnios, and pulmonary hypoplasia. Because of the 25% risk of recurrence, it is important that fetuses of those families at risk for the disease be evaluated during the second trimester. Our experience has demonstrated that serial examinations of the fetus are important. In one case the initial ultrasound examination at 18 weeks of gestation demonstrated normal kidneys with a normal KC/AC. At week 23, however, the kidneys were markedly enlarged and cystic, with an increased KC/AC, and the bladder failed to fill after maternal ingestion of furosemide (Figure 29). Therefore serial evaluations of fetuses at risk for infantile polycystic kidney disease are essential, since a "normal examination" at 18 or 20 weeks of gestation does not exclude the diagnosis.

Polycystic kidney disease is a rare, sporadic, lethal disorder that is associated with large cysts which can encompass much of the abdominal cavity (Figure 30).

### Skeletal System

A number of congenital and acquired structural anomalies affect the fetal limbs. These include certain forms of short-limbed dysplasia, recurring in 2% of cases, and others (Table 5).

#### Normal Limb Length Measurements

It is possible to measure limb length and individual bone length in the fetus at risk for a skeletal dysplasia. These measurements, however, require considerable experience on the part of the examiner because tangential sections through the limb will produce measurements that are spuriously short. Hobbins et al. (1981) reviewed their results of 57 examinations of normal fetuses between weeks 16 and 27 of gestation. The mean in vivo femur length increased linearly from 30.7 mm at 16 weeks to 50.4 mm at 27.5

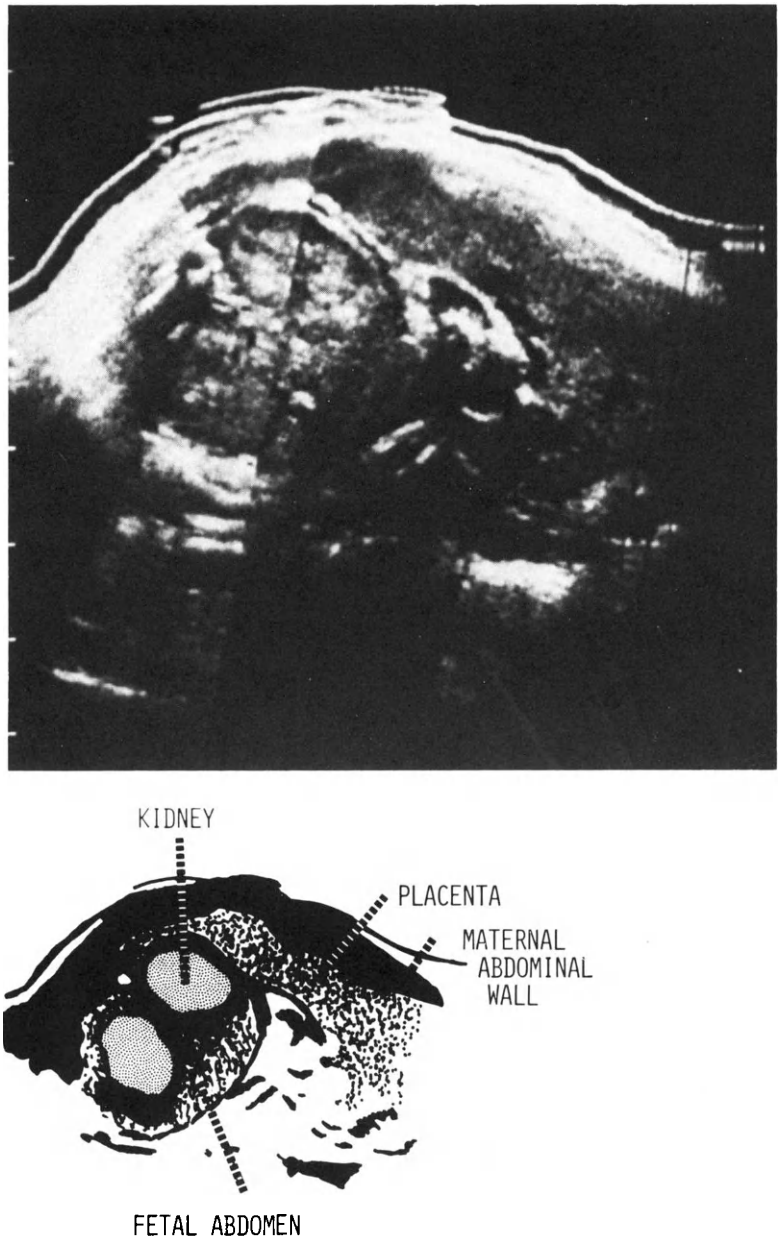



**Figure 29** Transverse scan demonstrating infantile polycystic kidneys (stippled area). (From DeVore and Hobbins, 1979a.)

**Table 5** Expected Ultrasound Findings in Skeletal Dysplasias

| Condition | Inheritance[a] | Head | Chest and body | Spine | Limbs |
|---|---|---|---|---|---|
| Achondrogenesis I (Parenti-Fraccaro) | AR | Thin, poorly mineralized skull | Short chest and body | Low-level echoes from vertebral bodies | Marked symmetrical reduction |
| Achondrogenesis II (Langer-Saldino) | AR | Increased head to body | Short, barrel-shaped thorax | Low-level echoes from vertebral bodies | Symmetrical reduction, straight |
| Achondroplasia | AD | Increased HC, BPD, and head to body ratio; bulging forehead | | | Short limbs, especially femora and humeri |
| Asphyxiating thoracic dysplasia | AR | | Long, narrow chest with very short ribs | | Variable shortening; hexadactyly (occasional) |
| Camptomelic dysplasias | NK | | | | |
| long-limbed | NK | Increased HC, BPD, and head to body ratio; micrognathia (profile) | Small, narrow thorax | Perhaps some flattening of the spine | Long, thin, and definitely bowed |
| short-limbed | NK | | | | Short, broad, angulated |
| short-limbed with craniosynostosis | NK | Increased HC, BPD, and head to body ratio; micrognathia (profile) | | | Short, broad, angulated |
| Chondroectodermal dysplasia (Ellis-van Creveld syndrome) | AR | | Long, narrow chest; congenital heart disease (occasional) | | Shortening of both distal segments; hexadactyly |
| Diastrophic dysplasia | AR | | | | Short with contractures—"hitchhiker" thumbs |

| | | | | | |
|---|---|---|---|---|---|
| Hypophosphatasia | AR | Very thin, with mineralization, sometimes collapsed | Thin, poorly visualized ribs | Thin, poorly visualized | Short, thin, ribbonlike; fractures |
| Langer mesomelic dysplasia | AR | Micrognathia | | | Severe middle segment shortening (forearms, legs) |
| Osteogenesis imperfecta | | | | | |
| type I (blue sclerae) | AD | Normal size | | | Perhaps mild bowing |
| type II (blue sclerae) lethal variety | AR | Thin, often collapsed cranium | Rib fractures | Fractures ? | Short, broad, and angulated with fractures; femurs especially tend to be broad with marked bowing |
| type III (normal sclerae) | AR | Thin, but not as marked as type II | Occasional rib fractures | | Fractures possible, broad bones mild bowing, slightly bowed |
| type IV (normal sclerae) | AD | Normal size | Occasional rib fractures | | Bowing and occasional fractures |
| Short rib-polydactyly syndromes | | | | | |
| type I (Saldino-Noonan) | AR | | Narrow thorax, protuberant abdomen | Flat vertebrae | Very short, polydactyly |
| type II (Majewski) | AR | | Narrow thorax, protuberant abdomen | | Moderate shortening, polydactyly |
| Spondyloepiphyseal dysplasia congenita (Spranger-Wiedemann) | AD | | Short, barrel-shaped chest | | Proximal shortening |
| Thanatophoric dysplasia | NK | Increased head circumference, biparietal diameter, and head to body ratio; prominent forehead | Narrow, pear-shaped thorax, protuberant abdomen | Marked flattening of vertebrae | Very short and bowed |

*Source*: From Sillence et al. (1978).

[a]AR = autosomal recessive; AD = autosomal dominant; NK = not known.

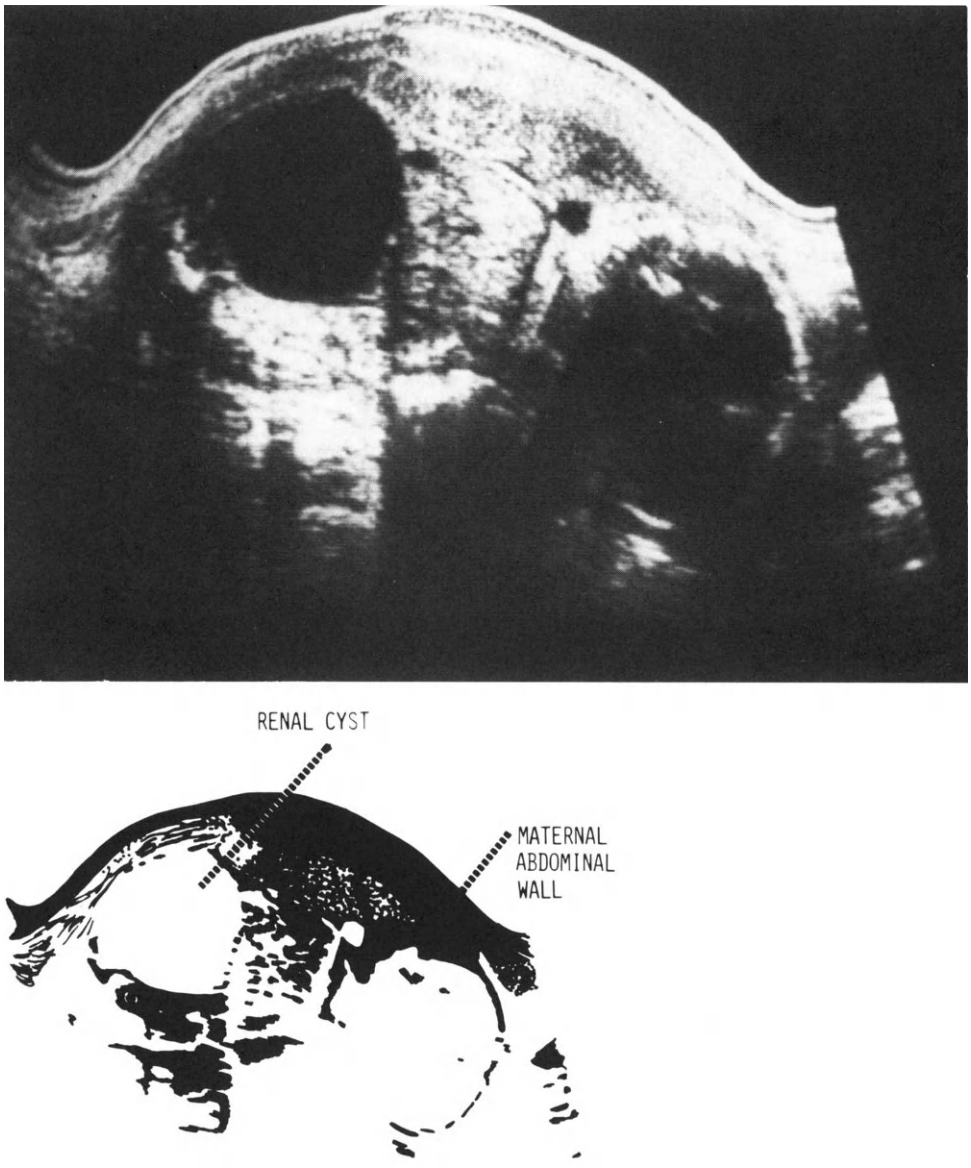



**Figure 30** Sagittal scan demonstrating a large multicystic kidney. (From DeVore and Hobbins, 1979a.)

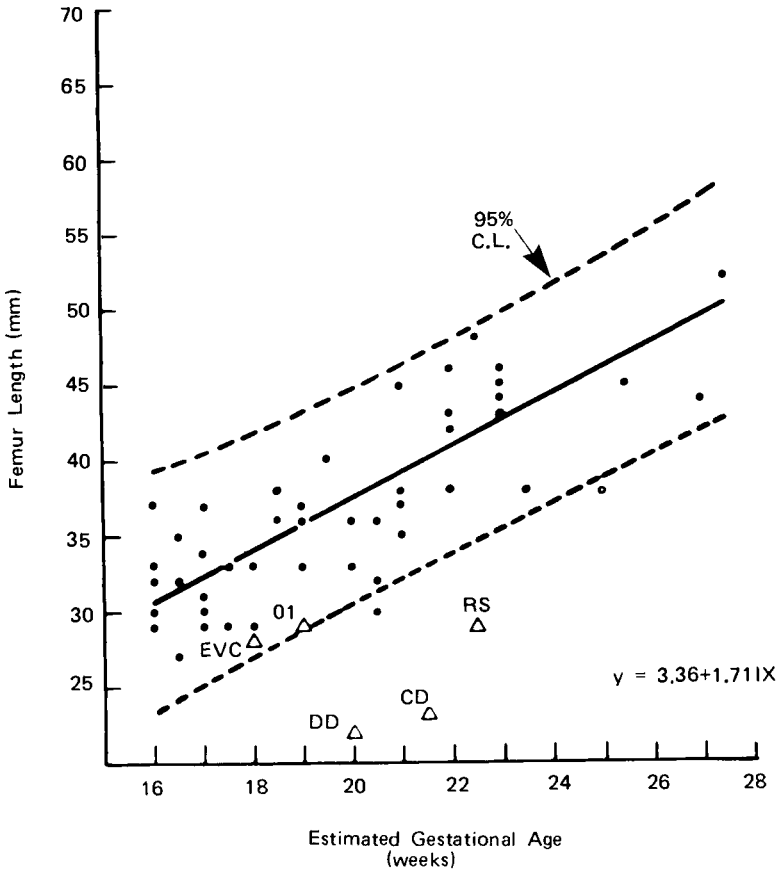



**Figure 31** Femur length of normal fetuses in utero measured by ultrasound. Values from fetuses with skeletal dysplasia are plotted on a normal curve (EVC, chondro-ectodermal dysplasia or Ellis-van Creveld syndrome; OI, osteogenesis imperfecta; DD, diastrophic dysplasia; CD, camptomelic dysplasia; RS, Robert syndrome). (From Hobbins et al., 1981.)

weeks, with an average weekly increase of 1.7 mm (Figure 31). The femur length was measured in all 57 cases (Figure 32). The humerus, however, was only successfully measured in 15 of the 57 examinations and appeared to grow somewhat more rapidly than the femur. The mean length ranged from 28.0 mm at 16 weeks to 44.0 mm at 23.5 weeks (2.1 mm per week).

### Examination of Fetuses with Skeletal Dysplasia

In 1977 Mahoney and Hobbins first reported the in utero diagnosis of Ellis-van Creveld syndrome. With ultrasound, the femur length was quantitated, and with fetoscopy, an extra digit was observed. They compared the femur length with that of 22 unaffected abortuses aged 16-22 weeks. However, when they later compared measured limb lengths of the affected fetuses with the ultrasound image length from abortuses,

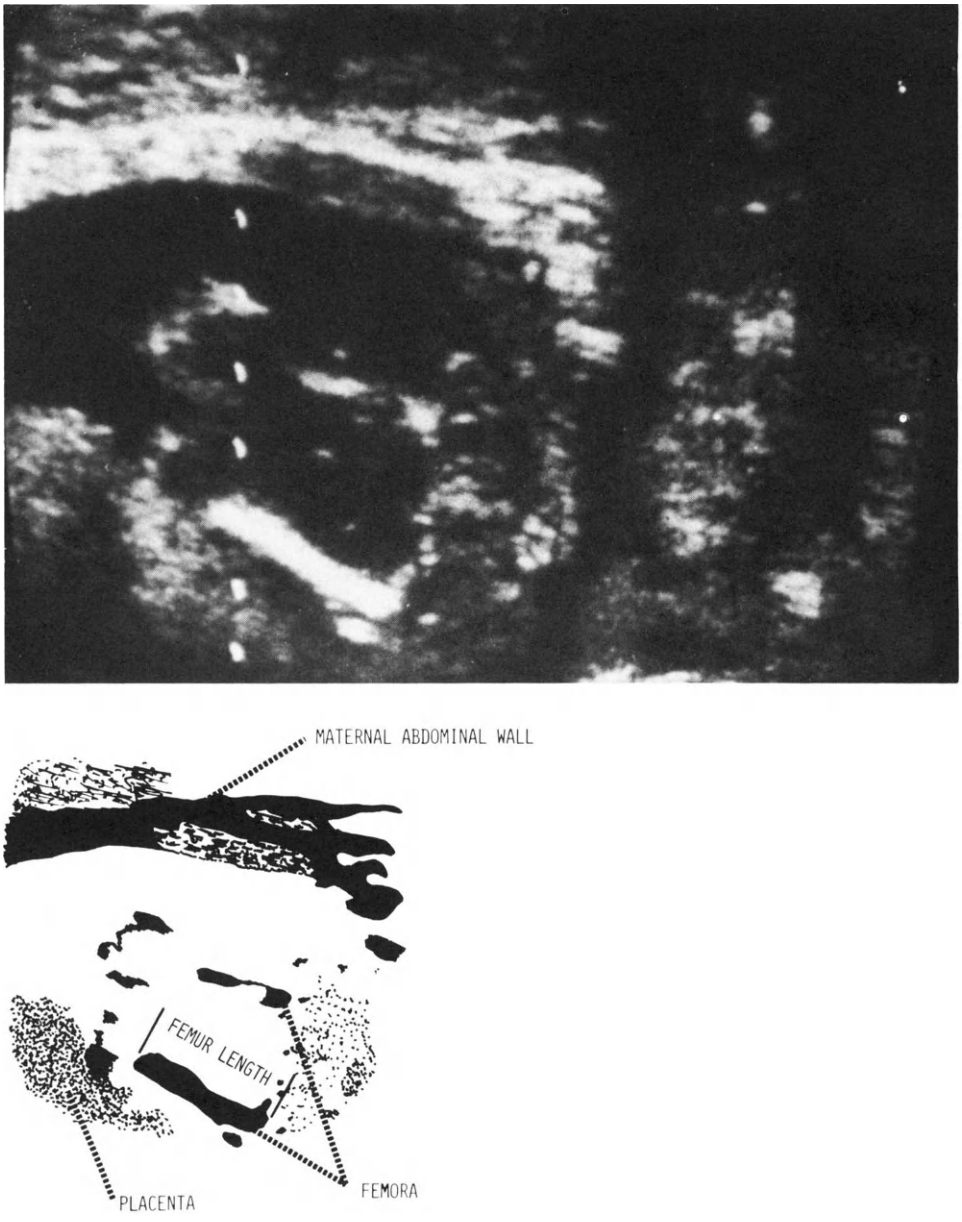



Figure 32 Normal fetal femur length. (From DeVore and Hobbins, 1979a.)

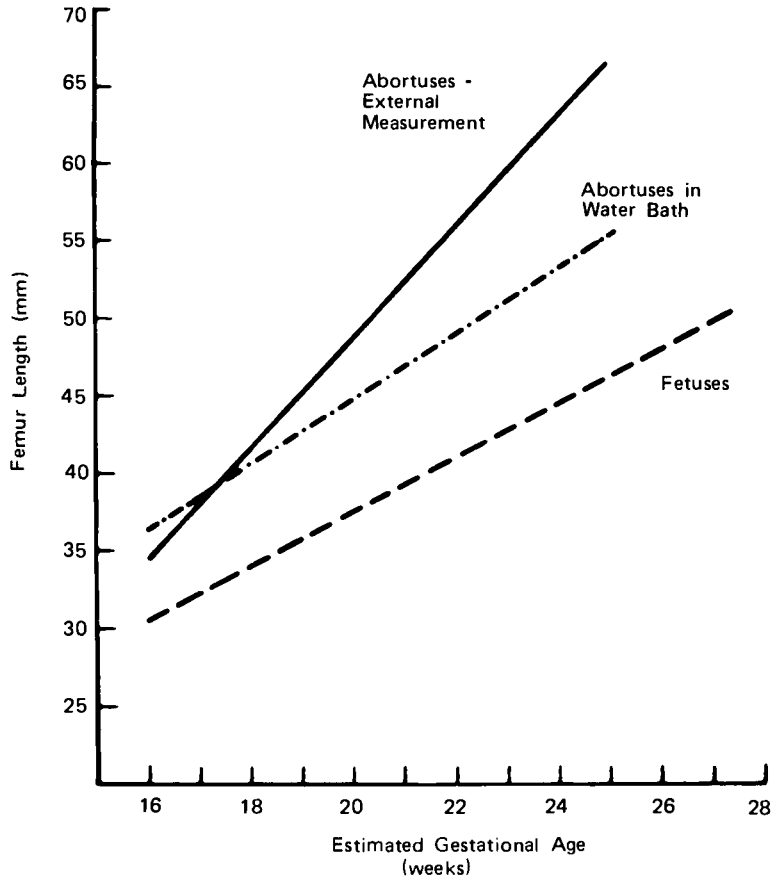



**Figure 33** Linear curves for femur length derived from external measurements of abortuses, ultrasound measurements of abortuses in a water bath, and ultrasound measurements of fetuses in utero. (From Hobbins et al., 1981.)

they noted that the ultrasound limb length was as much as 32% shorter than the actual limb length measured in abortuses of the same gestational age. They postulated that this difference was because the ultrasound image contained the ossified component and the area not imaged consisted of the cartilaginous component. However, although there were differences between in vivo and in vitro measurements, these paralleled each other when the abortuses' limbs were measured by ultrasound in a water bath (Figure 33).

Using the normal in vivo measurements, Hobbins and co-workers were able to diagnose five fetuses with skeletal dysplasias (Figure 34).

It is important to realize that of the three reported nomograms of limb lengths (Hobbins et al., 1981; Filly et al, 1981; Queenan et al., 1980), there are differences in the normal measurements. Therefore patients who are at risk for skeletal dysplasias should be compared with the nomogram which best fits the population from which they come. This can only be ascertained by each institution constructing its own nomogram from a normal population or using the nomogram which more closely represents its own population of patients.

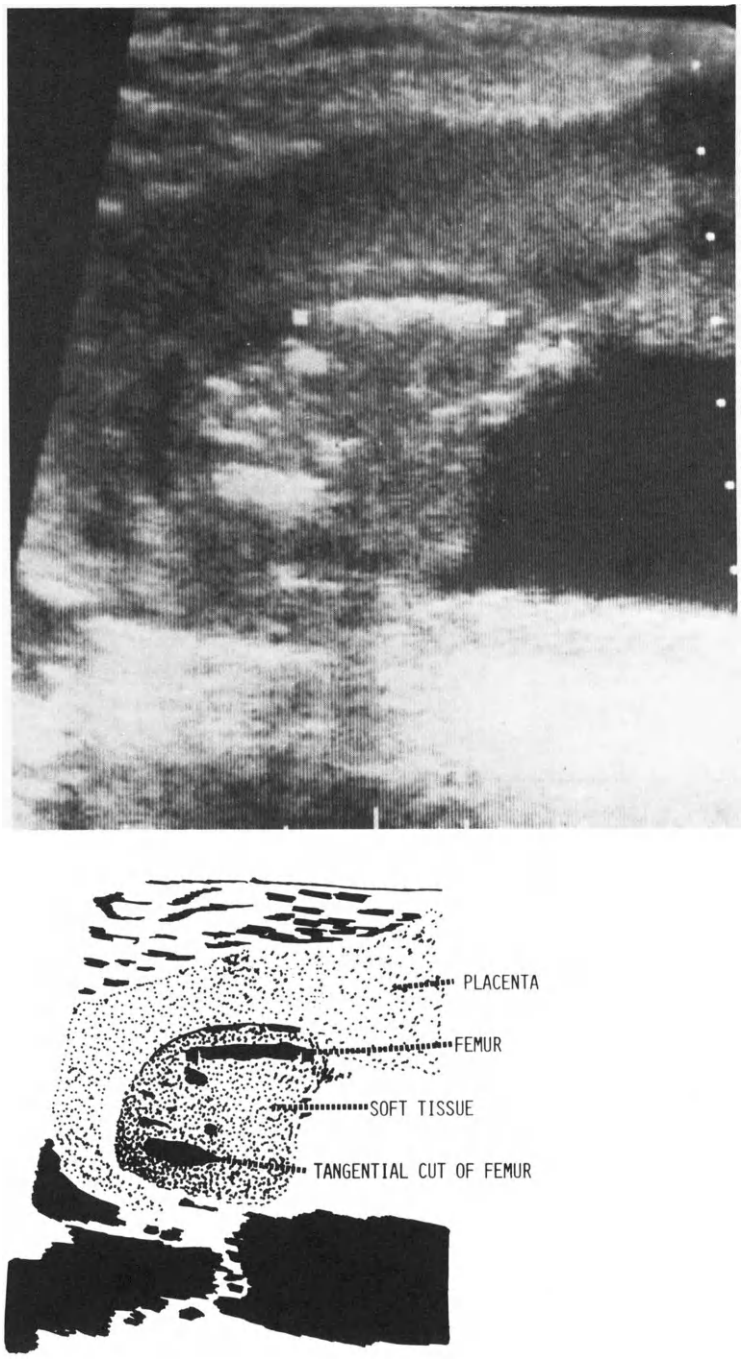



**Figure 34** Ultrasound image at 20 weeks of gestation of a long bone of a fetus with diastrophic dysplasia. Two white squares mark the ends of the bone, which measure 22 mm. (From Hobbins et al., 1981.)

## CONCLUSION

Many couples who have given birth to a child with congenital malformations have chosen not to reproduce for fear of having another child with similar birth defects. With the advent of diagnostic ultrasound, many pregnancies at risk for congenital structural anomalies can be accurately evaluated and the anomaly either diagnosed or excluded. If excluded, the pregnancy can continue with the assurance that the chance of giving birth to an affected fetus is quite low.

Those congenital anomalies amenable to surgical repair following birth have also been diagnosed with ultrasound. This allows for optimal care of the newborn at a center equipped with a newborn intensive care unit as well as pediatric surgeons.

At the present time, prenatal diagnostic ultrasound screening of congenital anomalies should not be undertaken by the novice, since interpretation of the images can be misleading. For example, the diagnosis of hydrocephaly has been entertained when in fact the image was taken in the wrong plane, giving the false impression of a markedly enlarged head. Those interested in acquiring the necessary skills should image as many structures (kidneys, bladder, stomach, limbs, head) and generate their own nomograms for the patients they serve. When a patient then presents for prenatal evaluation, the necessary skills have already been acquired for obtaining the proper images for adequate and skillful evaluation.

## ACKNOWLEDGMENT

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# 2

# Sexual Differentiation

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## INTRODUCTION

Although the genetic blueprint for mammalian sexual differentiation is established at the time of fertilization, the initial development of male and female embryos is identical. The first evidence of sexual differentiation in human embryogenesis is the appearance of the spermatogenic cords in the fetal testis at approximately 6 weeks of development. Thereafter differentiation of the male and female phenotypes is rapid and largely complete by the thirteenth week of gestation, although certain structural and functional aspects of sexual development are not finished until puberty. The pioneering work of Jost (1953, 1972) established that sexual differentiation is an ordered and sequential process: Chromosomal (genetic) sex, established at the time of fertilization, directs the development of the indifferent gonad into a testis or ovary; the differentiated gonad then determines phenotypic sexual development (Figure 1). The presence of the Y chromosome in the male dictates the development of a testis, and the secretions of the testis impose male development on the phenotypically indifferent fetus. Absence of a Y chromosome results in development of an ovary and a female phenotype. Thus a central concept in mammalian sexual differentiation is that the male is the induced phenotype, whereas the female develops as the passive consequence of the lack of male determinants.

Perhaps no other aspect of embryological development has been characterized in as great a depth as sexual differentiation (Wilson et al., 1981b). This is the result of the fact that normal sexual development, although essential for the reproductive capacity and survival of species, is not essential for the life of individuals. Therefore subjects with abnormal sexual development survive and usually come to the attention of physicians. Studies of individuals with abnormal sexual development have provided insight into the differentiation of the gonads, the mechanisms by which the gonads dictate phenotypic sexual development, and the molecular processes by which gonadal hormones act within target cells. Abnormal sexual development may be due to environmental factors, chromosomal nondisjunction, or single-gene mutations. The analysis of single-gene mutations in man (Wilson and Goldstein, 1975) and animals (Bardin et al., 1973) has been particularly informative in defining molecular and genetic determinants involved in normal sexual differentiation.

Although the events in sexual differentiation are similar in all mammalian species, this chapter will focus on the sequence in the human and integrate information from other species when necessary. We will first describe the anatomic events in male and

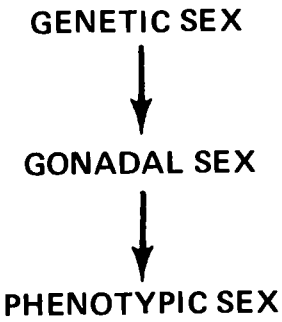


Figure 1 Central dogma of sexual differentiation.

female development and then summarize the current understanding of the mechanisms by which this development is regulated.

## ANATOMICAL EVENTS IN SEXUAL DIFFERENTIATION

The relation between gestational age of the human embryo and the anatomic development of the male and female phenotypes is schematically shown in Figure 2.

### Establishment of Chromosomal Sex

Chromosomal sex is established the moment a sperm fertilizes an ovum. In mammals the XX sex chromosome composition is characteristic of females, and the XY complement occurs in the male. On the basis of work done in a variety of species, it has been generally assumed that the Y chromosome contains genetic determinants essential for male development; and in the absence of the male-inductive influence of the Y chromosome, female development ensues as the result of the expression of genes located on the X chromosomes and on the autosomes (Stern, 1961). Analyses of clinical disorders that result from nondisjunctions of either the X or Y chromosomes have substantiated the fundamental validity of this view. For example, regardless of the number of X chromosomes (as in 47,XXY or 48,XXXY individuals), one Y chromosome is sufficient for testicular differentiation and the development of a predominantly male phenotype [although males with additional X chromosomes are usually infertile (Ferguson-Smith, 1961)]. Furthermore, in man and the mouse, the XO individual has a female phenotype (Ford et al., 1959; Russell et al., 1959; Welshons and Russell, 1959). Thus in mammals the principal and highly conserved function of the Y chromosome is to serve as the repository for male-determining genes.

However, it is now clear from the study of other forms of abnormal sexual development that genes necessary for normal male development are not confined to the Y chromosome. Indeed, genes essential for normal male development are located on the X chromosome (Lyon and Hawkes, 1970; Meyer et al., 1975; Ohno, 1979), and genes essential to the development of both the male and female phenotypes are located on the autosomes (Wilson and Goldstein, 1975). While certain of these genes play a role only in the secondary events of sexual differentiation (for example, those that code for enzymes required for steroid hormone biosynthesis and for the receptor proteins that enable a tissue to respond to a hormonal stimulus), others are essential for the

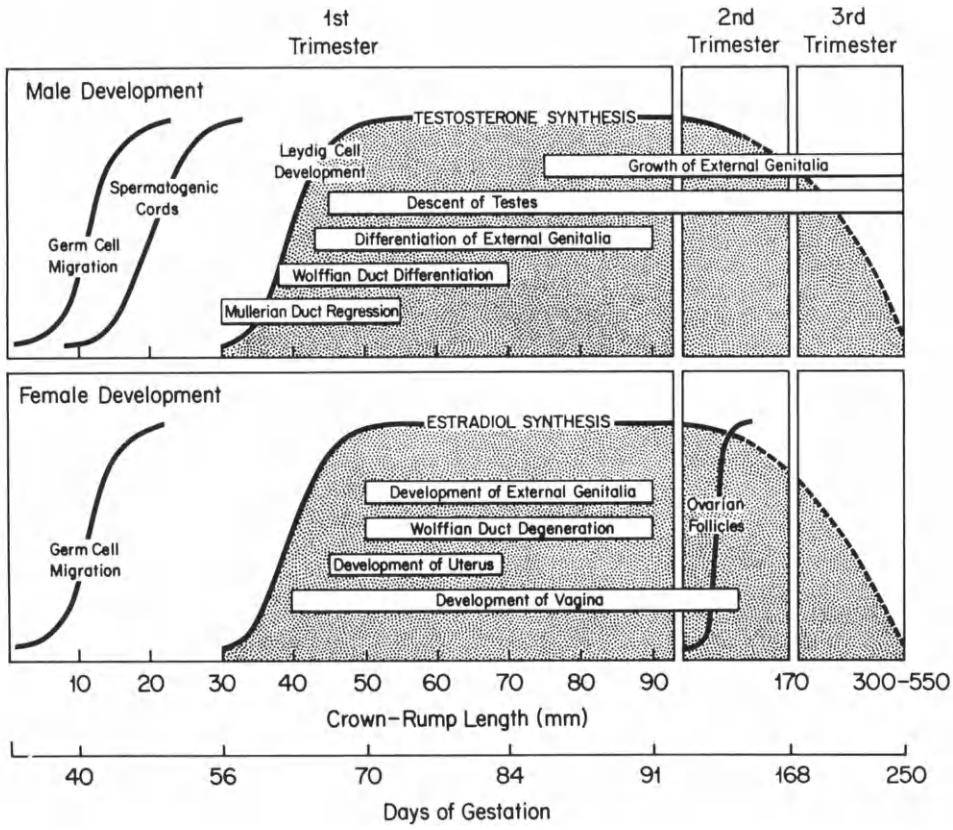


**Figure 2** The temporal relation between gonadal development, the onset of endocrine function of the gonads, and the anatomic differentiation of the internal and external genitalia of the human fetus. (From Wilson et al., 1981a.)

differentiation of the gonads themselves. For example, there are at least seven sibships with familial 46,XX pure gonadal dysgenesis (defined by the presence of streak gonads despite a normal female karyotype) in which the pattern of inheritance is most consistent with autosomal recessive transmission, implying that at least one autosomal gene is essential for normal ovarian development (Josso et al., 1963; Simpson et al., 1971). Furthermore, several pedigrees of familial 46,XY pure gonadal dysgenesis (a disorder in which genetic men differentiate as women with streak gonads) have been identified in which the mutation involves an X-linked gene (Chemke et al., 1970; Cohen and Shaw, 1965; Espiner et al., 1970; Sternberg et al., 1968). Thus the genetic determinants of normal gonadal differentiation and development of the male and female phenotypes are complex (Simpson et al., 1981) and cannot be explained by the composition of the sex chromosomes alone.

### Establishment of Gonadal Sex

The ovaries and testes are composed of three principal cell types: (1) germ cells, which originate outside the embryo proper in the endoderm of the yolk sac, (2) supporting cells

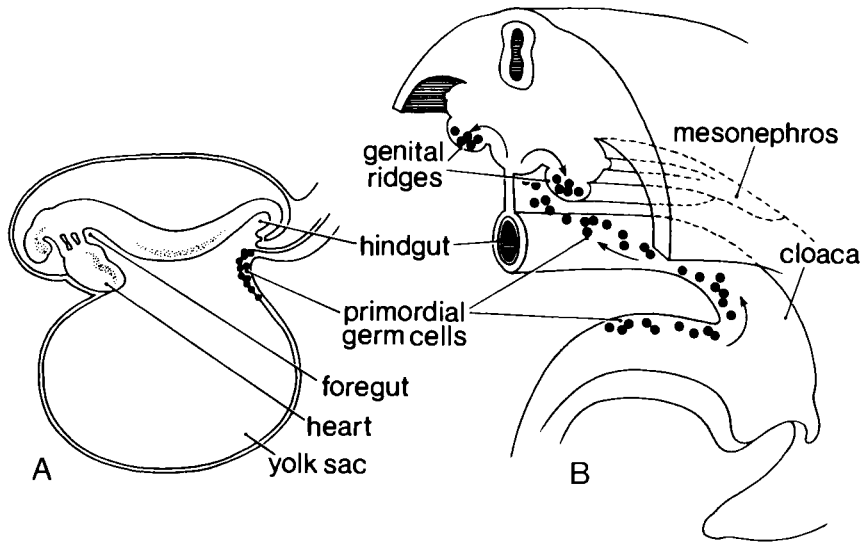


**Figure 3** (A) Schematic drawing of a 3-week-old embryo showing the site of origin of the germ cells in the wall of the yolk sac. (B) Migratory pathway of the primordial germ cells from the yolk sac entoderm to the dorsal mesentery of the gonadal ridge. (From Wilson, 1979.)

derived from the coelomic epithelium of the gonadal ridge and that differentiate either into the Sertoli cells of the testis or granulosa cells of the ovary, and (3) stromal (interstitial) cells derived from the mesenchyme of the gonadal ridge.

The primordial germ cells, recognizable because of their large size, high alkaline phosphatase activity, and high glycogen content, have been identified in the 4.5-day-old human blastocyst (Hertig et al., 1956; McKay et al., 1953). Prior to day 23 of human gestation these cells are located in the dorsal and caudal portions of the yolk sac entoderm (Figure 3A). Thereafter they migrate by amoeboid movement into the gut entoderm and mesoderm of the mesentery, eventually ending up in the coelomic epithelium of the gonadal ridges (Figure 3B) (Witschi, 1948). The germ cells replicate several times during their migration, so that more are found in the gonadal ridge than were originally present in the yolk sac (Mintz and Russell, 1957). The nature of the forces that entice the primordial germ cells to the gonadal ridges are unknown. After reaching the gonadal ridge, the germ cells, with adhering epithelial cells, infiltrate the underlying mesenchyme. This process culminates in the formation of the indifferent gonadal blastema containing the three basic cell types of the gonad and is completed by 5-6 weeks of gestation in human embryos (Figure 2). The primordial germ cells that fail to reach the gonadal ridge either degenerate or differentiate into other cell types. The somatic cells of the gonad can undergo partial organization into the type of gonad specified by the genotype even if the germ cells are prevented from migrating to the genital ridge (Merchant, 1975; McCarrey and Abbott, 1978). Thus some determinants for gonadal development are programmed into the cells of the gonadal ridge.

Sexual dimorphism of the human gonad first becomes apparent with the appearance of seminiferous cords in the fetal testis between 6 and 7 weeks of gestation. Histological

development has not been recognized in the fetal ovary until the sixth month of gestation, when primitive granulosa cells organize around the dividing oocytes to form the primary ovarian follicle (Gillman, 1948).

Although it is clear that the Y chromosome directs testicular development in mammals, the specific determinants and the nature and mechanism of action of the factor(s) involved remain unclear.

Recently a working model for the mechanism by which the Y chromosome causes differentiation of the testis has been constructed from studies of a male-specific, cell-surface, histocompatibility antigen (H-Y antigen) (Silvers and Wachtel, 1977; Ohno, 1978). This model is based upon a variety of types of evidence. The H-Y antigen demonstrates phylogenetic and evolutionary conservation, and its presence in many mammalian species is correlated with the development of the testis (Wachtel et al., 1975; Wachtel, 1981). The exact genetic programming for the H-Y antigen has not been established; the structural gene that specifies the antigen may be located on an autosome with positive regulation exerted by loci on the Y and negative regulatory control exerted by loci on the X (Wolf, 1981). Evidence that the H-Y antigen actually induces testicular development was obtained in rodent gonads (Ohno et al., 1978; Zenzes et al., 1978). When enzymatically dissociated testicular cells were incubated in rotation culture, they spontaneously reassociated into tubules characteristic of testes. If the dissociated cells were incubated with an antibody to the H-Y antigen (thus blocking the function of the antigen), they did not undergo testicular reorganization. Two types of cell-surface macromolecules are believed to be involved in the H-Y antigen-mediated differentiation of testicular cells: a membrane anchorage site for the H-Y antigen and a cell-surface receptor to which the antigen binds (Beutler et al., 1978; Müller et al., 1978, 1979).

Despite the explanatory potential of this model for understanding testicular differentiation, it is now clear that several male-specific antigens exist and that no invariable relation exists between the presence of a given antigen and development of a testis (Silvers et al., 1982). Consequently, until these various antigens and their anchorage sites are characterized in detail, it will not be possible to establish with certainty whether any is in fact the testis inducer.

Regardless of the mechanisms involved in the transformation of the indifferent gonad into an ovary or a testis, it is through the action of the gonads as endocrine organs that phenotypic differentiation is accomplished and that the full reproductive potential of the individual is attained. In the human fetus, the gonads begin to synthesize their characteristic hormones at 8-10 weeks of gestational age (Figure 4); at this time the fetal testis acquires the enzymatic capacity to synthesize testosterone (Siiteri and Wilson, 1974), and the fetal ovary acquires the enzymatic capacity to form estrogens (George and Wilson, 1978a).

### Establishment of Phenotypic Sex

The development of the urogenital tracts of both sexes is identical for approximately the first 2 months of gestation in the human embryo. During this so-called "indifferent" stage of sexual development, the urogenital tract consists of two components: (1) two duct systems (wolffian and müllerian) that are derived from the mesonephros and that constitute the anlagen of the internal organs of accessory reproduction and the upper vagina (Figure 5); and (2) the urogenital sinus and tubercle, which are the anlagen of the external genitalia (Figure 6). It is only after the onset of gonadal endocrine function

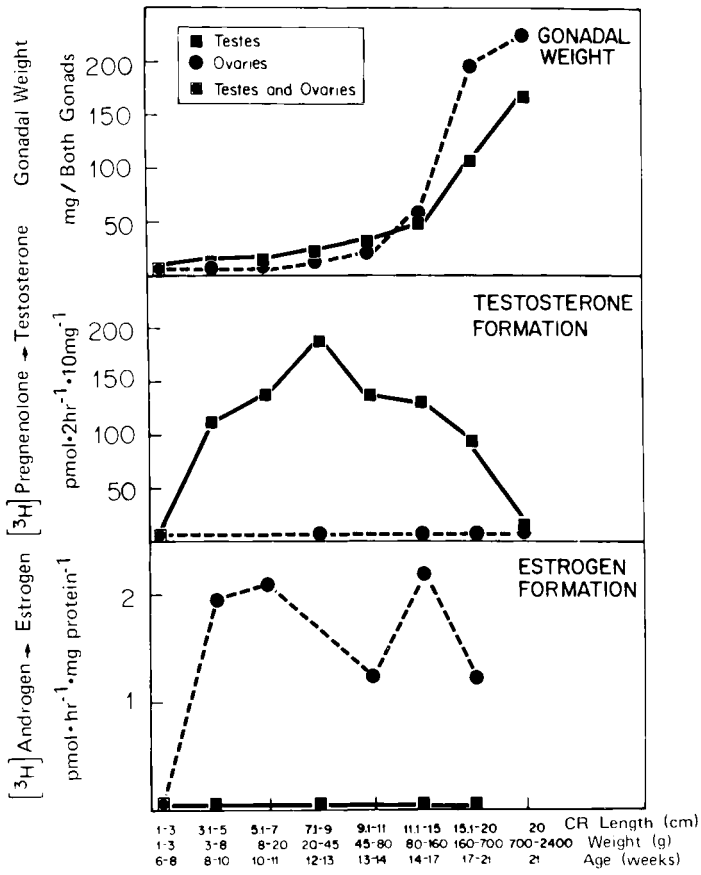


**Figure 4** Enzymatic differentiation of the human fetal gonad. (Adapted from Siiteri and Wilson, 1974, and George and Wilson, 1978a.)

that anatomic and physiological development diverge to result in the male and female phenotypes.

### Male Development

Development of the male urogenital tract begins shortly after the formation of the spermatogenic cords in the fetal testis. The initial event (occurring between 55 and 60 days of gestation) is the onset of regression of the müllerian ducts, and as a result of this only minor remnants of the müllerian duct (the oophoron) can be detected in the paratesticular fascia of the adult male.

Growth and differentiation of the mesonephric or wolffian duct in the male embryo begins shortly after the appearance of testicular Leydig cells and the onset of testosterone synthesis by the fetal testis at 8 weeks of gestational age. The upper portion of the wolffian duct, consisting of mesonephric tubules, is connected to the seminiferous tubules of the testis to form the rete testis. The portion immediately caudal becomes elongated and convoluted to form the epididymis, and the central portion of the duct develops a thick muscular coat to become the vas deferens. At its termination in the urogenital sinus the mesonephric duct becomes dilated to form an ampulla and gives

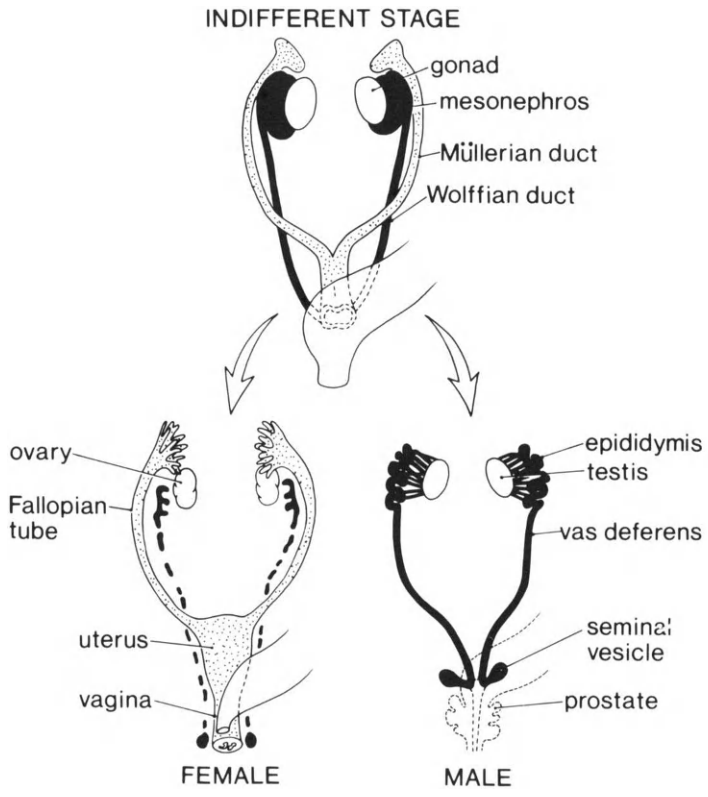


**Figure 5** Formation of the internal genitalia in male and female embryos. (From Wilson, 1979.)

rise to the seminal vesicle. The ejaculatory duct develops from a short segment of the wolffian duct between the seminal vesicle and urethra. Thus in the male the principal internal accessory organs of reproduction are derived from the wolffian duct system and form a conduit for the nurture and export of sperm.

The termination of the mesonephric ducts in the urogenital sinus divides the sinus into upper and lower portions. The upper portion develops into the upper urethra, and the lower portion differentiates into the prostate and the membranous urethra. The prostate arises as a series of entodermal buds that appear in the lining of the primitive urethra in fetuses at approximately 10-11 weeks of age (Lowsley, 1912; Bengmark, 1958; Kellokumpu-Lehtinen et al., 1980).

Development of the male external genitalia (Figure 6) commences shortly after virilization of the wolffian duct and urogenital sinus. At about 10 weeks of gestational development the genital tubercle and folds elongate, and the urethral folds begin to fuse over the urethral groove, first posteriorly and then anteriorly. This fusion joins the two urogenital swellings into a single structure, the scrotum, and results in the formation of the penile urethra (Figure 6). The anatomic development of the internal and external genital tract is completed by about 90 days of gestation. Two events in male phenotypic development take place later, namely, descent of the testes and growth of the male genitalia (Figure 2).

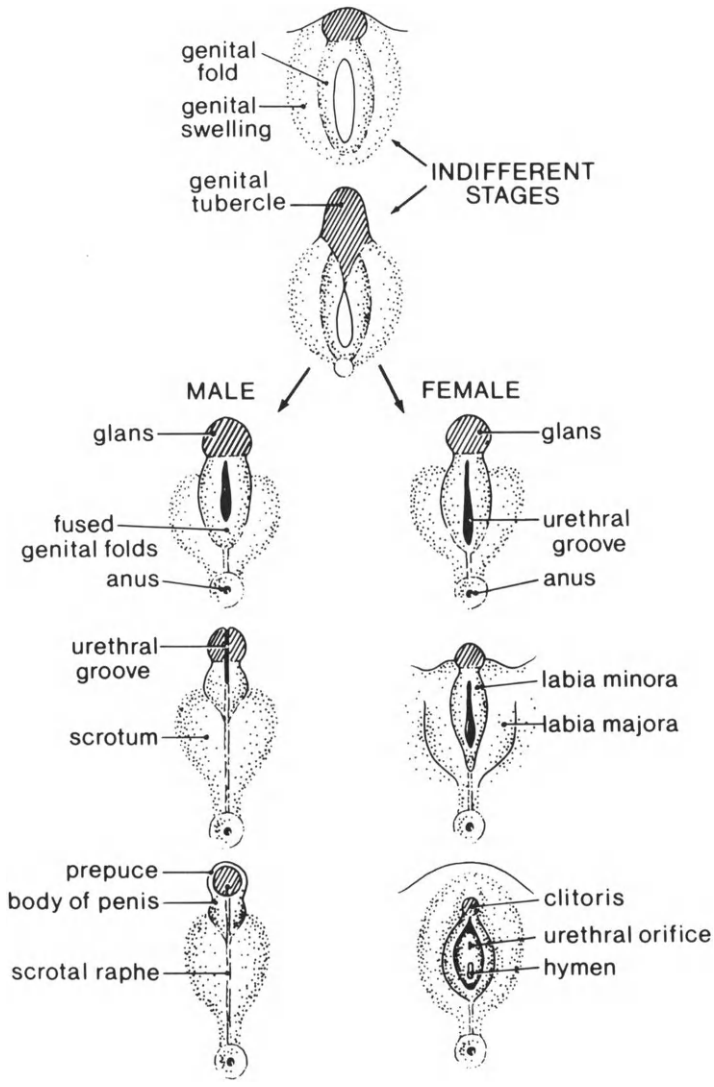


**Figure 6** Formation of the external genitalia in male and female embryos. (From Wilson, 1979.)

At the time the male urethra is completed (around day 75), no differential growth of the external genitalia has occurred, so that the size of the phallus is similar in male and female embryos. During the latter phases of embryogenesis the external genitalia of the male increase in size and by the time of birth are much larger in the male than in the female. Both the differentiation and the subsequent growth of the male external genitalia are androgen dependent.

Testicular descent begins shortly after the definitive testis is formed at 6 weeks of gestation and is often not completed until after birth (Gier and Marion, 1969). The first phase of testicular descent consists of movement of the testis from its site of origin on the genital ridge to the inguinal ring on the lower abdominal wall and is termed transabdominal movement. Actual descent of the testis through the inguinal ring and into

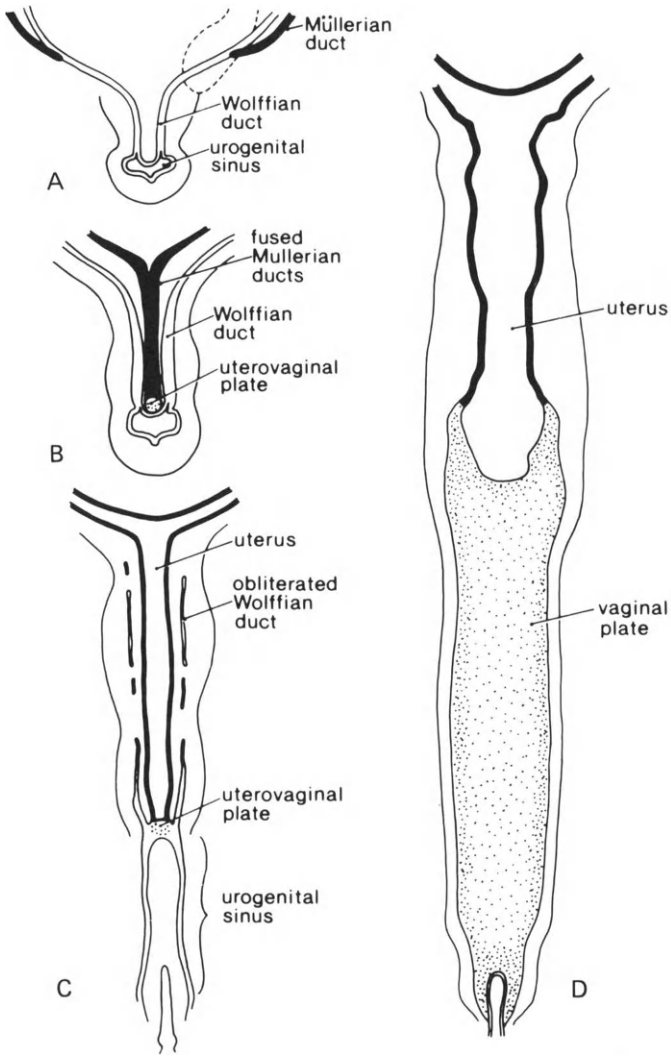


**Figure 7** Embryogenesis of the uterus and vagina showing approximate gestational stages of development: (A) 7 weeks, (B) 8½ weeks, (C) 11 weeks, and (D) 5 months. (From Wilson, 1979.)

the scrotum does not take place until after the seventh month of gestation. At a minimum this complicated process probably involves (1) disappearance of the cephalic end of the mesonephros, (2) contraction of the distal end of the mesonephros, (3) increase in intra-abdominal pressure due to organ growth and movement of the gut into the abdomen, and (4) passage of the testis through the inguinal canal and into the scrotum. In the rat, the process appears to be gonadotropin dependent and mediated by androgen (Rajfer and Walsh, 1977). In this regard it is interesting that failure of testicular descent (cryptorchidism) is associated with several disorders of sexual development in man, including persistent müllerian duct syndrome, hypothalamic hypogonadism (Kallman's syndrome), pituitary aplasia, defects in testosterone or dihydrotestosterone formation, and androgen resistance (Rajfer and Walsh, 1977).

### Female Development

Development of the internal genital tract of the female is characterized by regression of the wolffian ducts and by differentiation of the müllerian ducts into the fallopian tubes and uterus (Figure 5). At 8 weeks of development the müllerian ducts terminate in the urogenital sinus at the uterovaginal plate (Figure 7). The uterus is formed by the fusion of the caudal portions of the müllerian ducts at the uterovaginal plate. A temporary septum between the two lumina eventually disappears so that the uterus contains a single cavity lined by cuboidal epithelium (Figure 7).

Both the urogenital sinus and the müllerian ducts contribute to the embryogenesis of the vagina. The fusion of the caudal end of the müllerian duct to the urogenital sinus is followed by epithelial proliferation of the cells of the uterovaginal plate at approximately 9 weeks of development (Figure 7C,D). Thereafter the vaginal plate elongates, and after 4 months of gestational age becomes canalized to form the definitive vagina. The extent to which the cells of the vaginal plate are derived from müllerian ducts as compared to urogenital sinus has never been resolved (Bulmer, 1957).

Development of the female external genitalia (Figure 6) begins during the seventh week of intrauterine life. The genital tubercle bends caudally, and the posterior portions of the genital swellings fuse to form the posterior fourchette. The lateral portions of the labial swellings enlarge to form the labia majora. The urethral folds flanking the urogenital orifice do not fuse but persist as the labia minora. Thus, in contrast to the male in which the phallic and pelvic portions of the urogenital sinus are enclosed by fusion of the genital swellings, most of the urogenital sinus of the female remains exposed on the surface as a cleft into which the vagina and urethra open.

### Breast Development

Breast development in mammals occurs along the mammary line, which is well developed in humans at the 7-mm stage of embryonic development. In contrast to species with multiple pairs of mammary glands, the mammary line in human embryos shortens and condenses to a single bud so that only one functional pair of mammary glands develop. Between 6 and 8 weeks of development, the epithelial bud assumes a globular shape. Little change occurs until the fifth month of development, when secondary epithelial buds appear and nipples develop. Proliferation of the ductules occurs throughout the remainder of gestation, so that by the time of birth 15-25 separate glands are present, each of which is connected to the exterior through the nipple. In some species sexual dimorphism in breast development is apparent during embryogenesis, and the excretory ducts in males regress under the influence of testosterone (Kratochwil and Schwartz, 1976). In such species the epithelial buds of the male breast are left as isolated islands in the subcutaneous tissue. However, such dimorphism has never been documented in man, and the histological development of the breast in boys and girls appears to be identical prior to the onset of female puberty (Pfaltz, 1949).

## ENDOCRINE CONTROL OF PHENOTYPIC SEXUAL DIFFERENTIATION

The central dogma of sexual differentiation (Figure 1) is that genetic sex determines gonadal sex and that gonadal sex in turn directs phenotypic differentiation. This formulation evolved from the pioneering work of Jost (1953, 1972), who demonstrated that castration of rabbit embryos of either sex prior to phenotypic sexual differentiation resulted in female development. As a consequence of this type of experiment and of

studies involving either the transplantation of fetal gonads or hormone administration to castrated fetuses, it was established that in mammals the induced phenotype is male and that secretions from the fetal testis are necessary for male phenotypic development. In contrast, development of the female urogenital tract does not require secretions from the fetal ovaries, since female phenotypic development occurs in the absence of gonads. Jost also deduced that two substances from the fetal testis are essential for male development: a protein hormone (müllerian inhibiting substance) that acts to inhibit development of the müllerian duct and an androgenic steroid responsible for virilization of the wolffian duct, urogenital sinus, and external genitalia.

Müllerian inhibiting substance is an incompletely characterized hormone with a molecular weight of approximately 35,000 and is formed by the Sertoli cells of the tubules (Donahoe et al., 1977; Picard et al., 1978). Since müllerian duct regression begins shortly after formation of the spermatogenic tubules (Blanchard and Josso, 1974) and before the development of the Leydig cells and the onset of testosterone secretion by the testis, the formation of müllerian inhibiting substance is probably the initial endocrine function of the fetal testis. This factor is thought to act locally to suppress müllerian duct development rather than as a circulating hormone, since the fetal testis of the true hermaphrodite inhibits the development of the adjacent fallopian tube but does not prevent development of the contralateral müllerian duct (van Niekerk, 1974). The concept that müllerian duct regression is an active process in male development is supported by study of the persistent müllerian duct syndrome (Sloan and Walsh, 1976). In this disorder, genetic and phenotypic males who have normal male wolffian duct-derived structures also have fallopian tubes and a uterus. Family studies suggest that the disorder is due either to an autosomal or X-linked gene defect. The exact nature of the defect is uncertain but must reside either in a failure of production of müllerian inhibiting substance by the fetal testis or a failure of the tissue to respond to the hormone.

The onset of testosterone synthesis in the fetal testis occurs after the differentiation of the spermatogenic tubules and concomitant with the development of the Leydig cells (reviewed by Gondos, 1980). The principal androgen synthesized by the fetal testes of rabbit and man is testosterone (Siiteri and Wilson, 1974; Wilson and Siiteri, 1973). Testosterone has two vital functions in male embryonic development. First, it is probably required for spermatogenic tubule maturation and function; second, as a circulating hormone it is responsible for virilization of the fetus.

Although many questions about the regulation of testosterone secretion by the fetal testis are not resolved, there is now ample genetic proof that its formation is essential for male phenotypic development (Wilson and Goldstein, 1975). Five enzymatic defects have been described that result in inadequate testosterone synthesis and incomplete virilization of the male embryo (Bongiovanni, 1978; Griffin and Wilson, 1978; Wilson, 1978). Each of these enzymes (or enzyme complexes) involves a discrete biochemical step in the conversion of cholesterol to testosterone, and deficiency of any of the enzymes at the critical time in embryogenesis has profound consequences on sexual development (Griffin and Wilson, 1978).

The enzymatic differentiation of the fetal gonads that underlies the onset of endocrine function has been characterized in detail in the rabbit embryo. The pathway by which steroid hormones are synthesized from cholesterol is schematically summarized in Figure 8. At the time of onset of estradiol synthesis in the ovary and of testosterone synthesis in the testis there are only a few differences between the two sexes in the

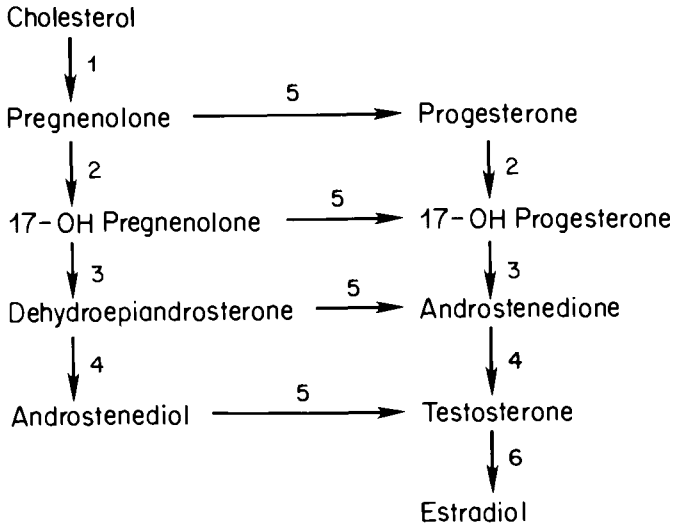


**Figure 8** Enzymatic pathway for steroid hormone synthesis from cholesterol. (1) 20,22-desmolase, (2) 17α-hydroxylase, (3) 17,20-desmolase, (4) 17β-hydroxysteroid dehydrogenase, (5) 3β-hydroxysteroid dehydrogenase-$\Delta^{4,5}$-isomerase, and (6) aromatase complex.

pathway by which cholesterol is converted to steroid hormones. In the rabbit the activity of the rate-limiting enzyme for testosterone synthesis (3β-hydroxysteroid dehydrogenase-$\Delta^{4,5}$-isomerase) is greater in testis than ovary, and the conversion of testosterone to estrogen (aromatase activity) is greater in ovary than in testis. All other enzyme activities in the steroidogenic pathway are initially equal in ovary and testis (Wilson et al., 1981a). These few enzymatic differences appear to develop independently of hormonal control and thus appear to be an inherent property of the developing ovary or testis (George et al., 1978a; George and Wilson, 1980). Thus, despite the overall complexity of the mechanisms of gonadal differentiation, differences in the activity of only a few enzymes at this time in development have profound effects on the hormones formed (and ultimately on sexual development).

The factors that regulate the rates of testosterone synthesis during the early phases of fetal development have not been elucidated. During the latter part of embryogenesis luteinizing hormone from the pituitary controls the rate of testosterone synthesis in the fetal testis, presumably by regulating the rate of conversion of cholesterol to pregnenolone as in the adult testis (Huhtaniemi et al., 1977; Abramovich et al., 1974; Ahluwalia et al., 1974); however, it is not established whether testosterone synthesis in the fetal testis is under gonadotropin control at its outset (i.e., when male phenotypic differentiation takes place). In the fetal rabbit specific gonadotropin receptors for luteinizing hormone appear in the testis simultaneously with the onset of testosterone synthesis (Catt et al., 1975). Furthermore, the differentiation of the gonadotrophs of the anterior pituitary of the fetal rabbit occurs at about the same time as development of the fetal Leydig cells and the onset of testosterone synthesis in the fetal testis (Schecter, 1970). These observations are compatible with the possibility that

testosterone synthesis is regulated from its onset by gonadotropins from the fetal pituitary and/or placenta. However, other observations suggest that the onset of endocrine function of the fetal testis may be independent of extrinsic factors and that the initial events in male phenotypic development are not dependent on pituitary or placental gonadotropins (George et al., 1978a, 1979; George and Wilson, 1980; Veyssière et al., 1980). If the latter formulation is correct, it could explain why anencephalic fetuses and individuals with hypogonadotropic hypogonadism often have microphallus and cryptorchidism but an otherwise normal male anatomy (Zondek and Zondek, 1965a,b, 1970; Walsh et al., 1979). According to this view, in these disorders the initial gonadotropin-independent phase of testosterone synthesis takes place normally, and the male phenotype is formed. However, the later gonadotropin-dependent events do not occur either because of absence of the pituitary (anencephaly) or deficient luteinizing hormone production, and consequently descent of the testes and differential growth of the male external genitalia are incomplete.

Endocrine function of the fetal ovary is less well understood. Based on histological studies, differentiation of the ovary has been thought to occur much later than that of the fetal testis (Gillman, 1948; Gondos et al., 1971). However, the fetal ovary undergoes differentiation as an endocrine organ simultaneously with the fetal testis, in that it begins to synthesize estradiol at the same time as the fetal testis begins to synthesize testosterone. Thus ovaries and testes develop as endocrine organs at approximately the same time in embryogenesis (George and Wilson, 1978a) (Figure 4).

### Mechanisms by Which Androgens Virilize the Fetus

Figure 9 depicts the current conception of how androgens act within target cells. Testosterone enters cells passively by diffusion down a concentration gradient. Once inside the cell, testosterone either binds directly to high-affinity protein receptors in the cytoplasm or is 5α-reduced to dihydrotestosterone before binding to the androgen receptor. After binding, the steroid-receptor complex is translocated to the nucleus, where it is bound to specific acceptor sites on the chromatin, thereby causing an increase in transcription of messenger RNA and in synthesis of specific proteins. Testosterone is believed to be the essential androgen for stimulation of the wolffian duct, whereas dihydrotestosterone is responsible for virilization of the external genitalia.

#### Role of Testosterone and Dihydrotestosterone

The deduction that testosterone and dihydrotestosterone perform different roles in the virilization of the male embryo was originally based on studies of androgen metabolism in rat, rabbit, guinea pig, and human embryos (Wilson and Lasnitski, 1971; Wilson, 1971; Siiteri and Wilson, 1974) and has been substantiated as the result of studies of a rare inherited human disorder originally termed pseudovaginal perineoscrotal hypospadias by Nowakowski and Lenz (1961). In this disorder, now called 5α-reductase deficiency, affected 46,XY men have normal male wolffian duct structures and lack müllerian duct derivatives. The external genitalia fail to virilize normally, and the ejaculatory ducts terminate in a blind-ending vagina. Thus the structures derived from the urogenital sinus and genital tubercle are predominantly female in character. Since defective virilization is limited to structures derived from the genital tubercle and urogenital sinus, where dihydrotestosterone rather than testosterone is believed to be the intracellular mediator of androgen action, a defect

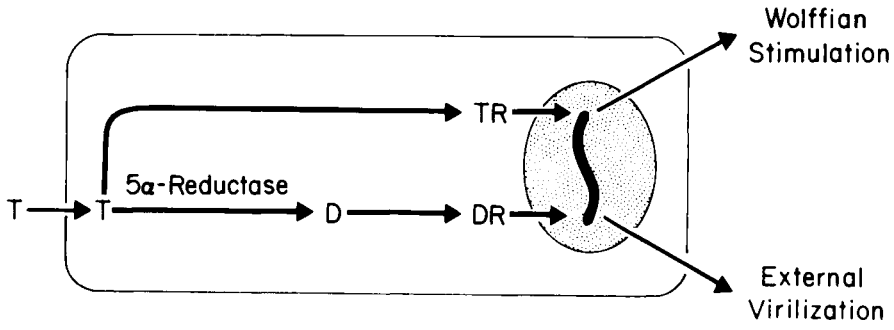


Figure 9 Schema of the molecular biology of androgen action (T, testosterone; D, dihydrotestosterone; R, cytosolic androgen receptor; TR, testosterone-receptor complex; DR, dihydrotestosterone-receptor complex).

in dihydrotestosterone formation was postulated as the cause of this disorder. Direct evidence of 5α-reductase deficiency in these patients has been documented both in vitro and in vivo (Walsh et al., 1974; Imperato-McGinley et al., 1974; Wilson, 1975; Moore et al., 1975; Peterson et al., 1977; Saenger et al., 1978; Fisher et al., 1978; Green et al., 1978).

Studies of 5α-reductase activity in fibroblasts cultured from genital skin of patients from different pedigrees have revealed genetic heterogeneity among families with clinical 5α-reductase deficiency. Most patients have a marked deficiency in the activity of 5α-reductase (Walsh et al., 1974; Imperato-McGinley et al., 1974); others form normal amounts of a structurally defective, unstable enzyme that binds testosterone normally but which has a decreased affinity for NADPH, the cofactor for the reaction (Leshin et al., 1978). Still other mutations appear to affect both steroid and cofactor binding (Imperato-McGinley et al., 1980).

It is not clear why the external genitalia of patients with 5α-reductase deficiency virilize partially at puberty. Late virilization may be due to the presence of higher levels of plasma testosterone at puberty than during embryogenesis, to the presence of some residual 5α-reductase activity in patients with this syndrome, or to some unidentified change in molecular or endocrine function with age.

### Role of the Androgen Receptor

Owing to the small amount of tissue available for study, the androgen receptor protein(s) involved in the intracellular action of steroid hormones has not been characterized directly in embryonic tissues of the male urogenital tract. However, studies in animals and humans of single-gene mutations that cause resistance to androgen action and result in development of male pseudohermaphroditism suggest that androgens act to virilize the male fetus by mechanisms fundamentally identical to those in the adult.

The first disorder of the androgen receptor to be characterized in molecular terms was the testicular feminization (*Tfm*) mutation in the mouse, an X-linked disorder in which affected males have testes and normal testosterone production but differentiate as phenotypic females (Lyon and Hawkes, 1970). Since no müllerian duct derivatives (uterus or fallopian tubes) are present in affected mice, the müllerian inhibiting function of the fetal testis remains intact. However, since such animals are profoundly

resistant to endogenous and exogenous androgens, there is total failure of all androgen-mediated aspects of male development in the wolffian duct, urogenital sinus, and external genitalia. Dihydrotestosterone formation is normal, but the androgen receptor of the cell cytosol is not detectable (Gehring et al., 1971; Bullock et al., 1971; Goldstein and Wilson, 1972). Consequently, the hormone cannot reach the nucleus of the cell and interact with the chromosomes.

Analysis of several abnormalities of the human androgen receptor has provided additional insight into the role of the androgen receptor in embryonic virilization. One such disorder is the human counterpart of the testicular feminization mutation in the mouse (Wilson and MacDonald, 1978). Patients with this disorder usually come to the attention of the physician after the onset of puberty, when they are evaluated for primary amenorrhea. However, if the testes are located within the inguinal canals in association with inguinal hernias, ascertainment may occur prior to puberty. The karotype is 46,XY, but the general habitus is female in character. Breast development at the time of expected puberty is that of a normal female and is due to increased estrogen synthesis by the testis at this time (MacDonald et al., 1979). Axillary, facial, and pubic hair are absent or scanty. The external genitalia are unambiguously female, but the vagina is short and blind ending. All internal genitalia are absent except for testes, which are located in the abdomen, along the course of the inguinal canal, or in the labia majora.

The molecular defect in some patients is similar to that in the *Tfm* mouse, in that the high-affinity androgen receptor is missing (Keenan et al., 1974, 1975; Griffin et al., 1976; Kaufman et al., 1976). Other patients have a qualitatively abnormal receptor that binds androgens normally at low temperatures but fails to bind androgen at physiologic temperature (Griffin, 1979). Thermal inactivation is reversed when the assay temperature is lowered, suggesting an alteration of the tertiary structure of the binding protein at normal body temperature. Since some patients with qualitatively abnormal receptors have androgen resistance as profound as that seen in patients with complete absence of androgen binding, it is presumed that the structural abnormality prevents normal function of the receptor.

Another type of mutation of the human androgen receptor appears to cause a less severe defect in the protein. Families with partial androgen resistance have been described by Reifenstein (1947), Rosewater et al. (1965), Gilbert-Dreyfus et al. (1957), Lubs et al. (1959), Walker et al. (1970), Gardo and Papp (1974), and Wilson et al. (1974). Each of these syndromes probably represent various manifestations of a single X-linked mutation and can be termed Reifenstein syndrome. Affected individuals have abnormalities ranging from men with infertility due to absence of sperm production through more severe defects such as abnormal development of the penis (hypospadias) to individuals with a female phenotype and blind-ending vaginas. The common phenotype is a man with hypospadias, azoospermia, and gynecomastia. Androgen receptor levels in fibroblasts cultured from patients with this disorder are about half normal (Griffin and Wilson, 1980) and constitute a mixture of subtle qualitative and quantitative abnormalities of the receptor protein (Griffin, 1979; Griffin and Durrant, 1982). The partial (and variable) virilization that occurs in this disorder is probably mediated by the residual androgen receptor. The most subtle manifestation of this partial defect in the androgen receptor is infertility due to absence or profound deficiency of sperm in otherwise normal men (Aiman et al., 1979); this latter disorder may prove to be the most common abnormality of the androgen receptor.

Our understanding of the process by which the male fetus is virilized during embryogenesis has been greatly aided by the study of these various single-gene defects that cause abnormal sexual development. Female embryos have the same androgen receptor system and the same ability to respond to androgens as male embryos, and female embryos become virilized when exposed to androgens. The administration of androgens to pregnant rats and mice results in female offspring with both male and female urogenital tracts (Schultz and Wilson, 1974). Causes of abnormal androgen exposure in human embryos include androgen ingestion by the mother, maternal tumors that secrete androgen, and congenital adrenal hyperplasia in female infants. The most common cause of congenital adrenal hyperplasia is an autosomal recessive mutation that results in a defect in the 21-hydroxylase enzyme (Bongiovanni, 1978). Synthesis of cortisol is decreased, leading to a compensatory increase in adrenocorticotropin secretion by the pituitary, which in turn leads to an increase in adrenal androgen secretion. The adrenal androgens then act to virilize the genitalia in affected females. Thus the differences in anatomic development between males and females depend on differences in the hormonal signals themselves and not to differences in the receptors for the hormones.

### Role of the Embryonic Mesenchyme in Androgen Action

Although we now have considerable insight into the mechanism by which androgens act to virilize the male, many fundamental issues in the embryogenesis of the genital tract are still poorly understood. For example, it is not known how certain tissues acquire the capacity to grow and differentiate in response to androgens while other tissues do not. In a series of studies involving the recombination of embryonic mesenchyme and epithelium from control and urogenital tissues, Cunha and his colleagues have established that the machinery that allows the tissue to respond to androgen first appears in the mesenchyme rather than in the epithelium of the urogenital tract (Cunha et al., 1981). The recombination of embryonic nongenital skin epithelium and urogenital sinus mesenchyme results in the induction of prostate formation when the recombinants are grafted into the anterior chamber of the eye of male mice, whereas recombination of urogenital sinus epithelium with skin mesenchyme does not (Cunha, 1972). Furthermore, urogenital sinus epithelium from *Tfm* mice that lack the androgen receptor develops androgen-dependent prostatic buds when recombined with normal urogenital sinus mesenchyme. In contrast, the combination of *Tfm* mesenchyme with normal epithelium does not result in prostatic development (Cunha and Lung, 1978). The mesenchyme is also the androgen target tissue in testosterone-mediated regression of the mammary bud in fetal male rodents (Kratochwil and Schwartz, 1976; Dürnberger and Kratochwil, 1980; Drews and Drews, 1977). The process by which mesenchyme in certain areas of the body acquires the ability to respond to androgen stimulation and the mechanisms by which the appropriate signal is transferred from mesenchyme to epithelium will have to be clarified in order to understand how androgens interact with the myriad of genetic determinants to cause phenotypic sexual differentiation.

### Role of Estrogens in Embryonic Development

In contrast to the established role of androgen in male development, little is known about the role of estrogen in female phenotypic development. No mutations have been identified that result in either deficient estrogen synthesis or resistance to estrogen action. Several studies suggest that estrogen action may be essential for implantation of the blastocyst and therefore for the survival of the embryo (Bhatt and Bullock, 1974;

Dickmann and Dey, 1976; Dickmann et al., 1977). In the rabbit embryo, estrogen synthesis is temporarily activated in both male and female embryos just prior to implantation (George and Wilson, 1978b). If mutations occur that either prevent the synthesis or inhibit the action of estrogens, they may interfere with the normal implantation process and thus prove lethal for the embryo.

Later in embryogenesis estradiol formation is initiated in the fetal ovary at the same time that testosterone synthesis commences in the fetal testis (Siiteri and Wilson, 1974; George and Wilson, 1978a; George et al., 1978b). Since the onset of estradiol synthesis occurs in the fetal ovary before any definitive histological changes are apparent, it is possible that histological differentiation of the tissue may be mediated in part by a local action of estradiol analogous to the role that testosterone is believed to play in testicular maturation. Although a role for estrogen in the initial phases of female phenotypic differentiation seems unlikely, estrogens, along with progestins, may be involved in the growth and maturation of the internal genital tract of the female during the latter stages of fetal development, even if not required for its differentiation.

## SUMMARY

Although genes located on the autosomes as well as on the sex chromosomes contribute to the establishment of genetic sex, determinants on the Y chromosome are paramount in inducing differentiation of the indifferent gonadal primordia in the male embryo into testes. In the absence of the Y chromosome, the indifferent gonad develops into an ovary. The function of the embryonic gonads as endocrine organs determines the development of phenotypic sex, and specifically the secretion by the fetal testis of two hormones—müllerian regression factor and testosterone—imposes male development on the indifferent fetus. Testosterone and its metabolite dihydrotestosterone act to virilize the male fetus via the same receptor machinery that mediates androgen action in the postnatal state. This receptor machinery is present in both male and female embryos. Consequently, normal phenotypic development is determined solely by the presence (in males) or absence (in females) of the specific hormonal signals at the critical time in embryonic development. The onset of testosterone synthesis at the appropriate time is the result of only one or two enzymatic differences between the testis and the ovary. The characterization of several single-gene defects in man and animals that result in various forms of abnormal sexual development has aided the elucidation of the genetic, molecular, and endocrine aspects of sexual differentiation.

Nevertheless, many fundamental issues in sexual differentiation are still poorly understood. For example, it is not known how the germ cells find their way to the gonadal ridges from their site of origin in the yolk sac or how histological differentiation of the gonads controls the endocrine function of the fetal gonads. The process by which embryonic tissues acquire the ability to respond to a hormonal stimulus and consequently initiate anatomic and functional development also remains an enigma. Ultimately, these fundamental problems of embryology will have to be resolved to understand the overall program by which the myriad of genetic and regulatory factors interact to cause development of the sexual phenotypes.

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# 3

# Ontogeny of Acquired Immunity and Maternofetal Immunological Interactions

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## INTRODUCTION

As an essential factor in the ability of the individual to preserve the integrity of the body, the immune system must be functional from the moment of birth. This is achieved during fetal life not only by the development of specialized organs and the differentiation of immunologically active cells, but also by the temporary acquisition of maternally derived immunity. The intimate association of the fetus with maternal tissues in the sheltered intrauterine environment also brings with it a serious problem. The fetus inherits from the father genetic characteristics that are foreign to the mother and hence presents her with an antigenic challenge that should be capable of eliciting immunological rejection. This chapter analyzes the complex and changing immunological interrelationships that are involved in the maintenance of the fetus as an intrauterine allograft and in the establishment of the immune defense mechanisms of the newborn. Although the evidence presented relates as far as possible to studies in man, it is frequently necessary to draw upon observations from experimental animals.

## DEVELOPMENT OF THE IMMUNE RESPONSE

For many years it was claimed that the human fetus was not capable of producing specific antibodies and that the immunological defenses of the newborn were based exclusively upon the presence of maternal antibodies which had crossed the human placenta. However, studies carried out during the last decade have demonstrated that although maternal antibodies play a very important role in protecting the human newborn against bacterial and viral infections, the maturation of both humoral (antibody mediated) and cellular (cell mediated) immune responses starts at an early stage of development in the human fetus and in the fetuses of other mammals (Sterzl and Silverstein, 1967; Adinolfi and Wood, 1969; Lawton and Cooper, 1973, 1980).

It has also been shown that other plasma proteins involved in the immunological mechanisms of protection against bacterial and viral infections, such as the components of complement, lysozyme and interferon, start to be produced at an early stage of fetal development (Adinolfi, 1972, 1977, 1981a,b).

## Organization of the Lymphoid System

In the first half of this century, a bitter controversy divided scientists investigating the fetal origin of lymphocytes in the thymus. Based exclusively on morphological studies, the hypotheses put forward maintained that thymocytes were derived either from cells intrinsic to the organ or from migrating cells. In the early 1960s, after a short period of prominence of the first theory (Auerbach, 1967), it became clear that, at least in rodents and birds, lymphocytes originate from yolk sac stem cells which migrate into the thymus, liver, bone marrow, and spleen (Metcalf and Moore, 1971; Owen, 1973, 1977).

In mammals and birds, the yolk sac (Figure 1) is the source of stem cells from which germinal cells and hemopoietic precursors are derived (Owen, 1977; Le Douarin, 1977). Perhaps one should begin to discuss the origin of lymphocytes by asking why two stem-cell lines, which play such an important role in the survival of the individual and the species, are located outside the embryo. The reason might be that erythropoietic, myeloid, and lymphoid cells, as well as spermatozoa, require precursors for most or all of the life of the individual. The isolation of these stem cells in the yolk sac during the early stages of embryonic development, when rapid differentiation occurs, may prevent a premature alteration of their genetic potentialities by the humoral inductive factors released by the embryo.

The differentiation of the stem cells into specific cell types is likely to depend upon interaction with the inductive tissues in which these cells are proliferating. The release and relocation of cells permit new interactions between emerging, diversified cell types and result in the production of stable cell lines capable of clonal proliferation. Depending upon the inducing influence of the environmental tissues, the stem cells may differentiate along one or another of the hemopoietic lines: into red cells, granulocytes, monocytes, megakaryocytes, or lymphoid cells (Yoffey and Courtice, 1970; Metcalf and Moore, 1971; Owen, 1973, 1977).

Many aspects of the regulation of lymphopoiesis and of the regulation of the diversification of lymphocytes await further clarification. The general view is that in the bone marrow of adult individuals there is a heterogeneous population of

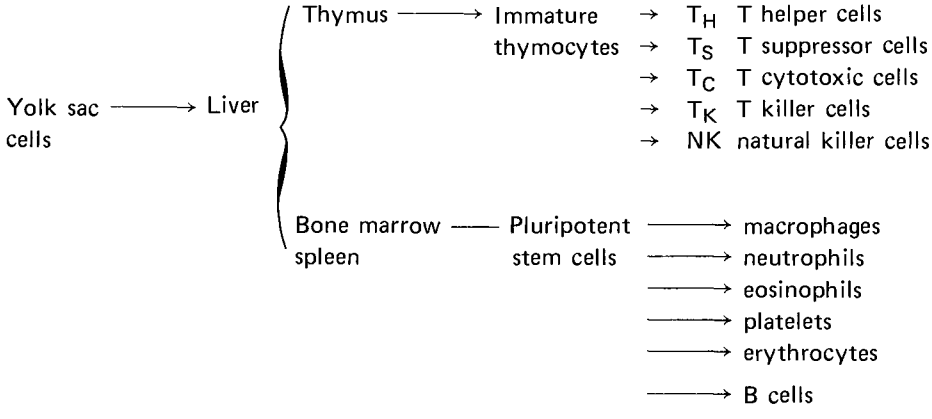


Figure 1 Migration and differentiation of yolk sac cells.

pluripotent stem cells, some of which are already more restricted in the type of cells they can produce than are other stem cells.

### Properties of T and B Cells

Migration of stem cells into the thymus will lead to the proliferation of several types of thymocytes (T cells) with characteristic immunological properties and expressing specific surface antigens. Thymocytes are capable of prolonged recirculation, are endowed with immunological memory, and are mainly involved in the cell-mediated immune response. These cells are characterized by the presence of specific membrane antigens, have a high degree of radioresistance, and respond in vitro to phytohemagglutinin stimulation and to mitomycin-treated or irradiated allogeneic cells (Miller and Osoba, 1967; Mitchison, 1971; Katz and Benacerraf, 1972; Hong, 1980; Reinherz and Schlossman, 1981).

Although T lymphocytes in peripheral organs are derived from the thymus, there are substantial differences between thymus and peripheral T cells in terms of their functions and cell-surface antigens. Thus only a minority of thymus lymphocytes have been shown to be immunologically responsive, and this population, like the peripheral T cells, is less sensitive to the cytolytic effects of corticosteroids than the majority of the T cells.

These and other studies reviewed by Chess and Schlossman (1977) and Boyse and Old (1978) have shown that T cells differentiate into various subpopulations, each endowed with characteristic surface markers and biological properties, such as helper or suppressor functions (Table 1).

The T helper cell ($T_H$) subpopulation has its major role in the cell-cell cooperation which leads to the recognition and processing of the antigen and the synthesis of antibodies by the B cells. T suppressor cells ($T_S$) were first thought to be responsible for the inhibition of immunoresponses in patients with common variable immunodeficiency (Waldmann et al., 1974), but have since been shown to be present in normal individuals and to regulate the level of antibody response. T killer cells ($T_K$) are involved in the cytotoxic phase of graft rejection and also play a major role in the immune defenses against viral infections. Within each of these subpopulations, subclasses of T cells have been identified (e.g., $T_{S1}$ and $T_{S2}$), each endowed with unique functions and characterized by typical markers. Antigen stimulation leads to the induction of several subpopulations, both of helper and suppressor cells. The primary and secondary responses are functionally dependent upon the interaction of these subpopulations of cells.

A major component of natural cell-mediated cytotoxicity in man and rodents is associated with a particular subpopulation of lymphocytes which have been termed natural killer (NK) cells (Herberman et al., 1979). Although they reside in the T-cell lineage, NK cells differ from other lymphocytes. Of great interest is their presence in nude mice lacking the thymus. The low incidence of tumors in these mutant mice has been specifically attributed to the presence of NK cells. Natural killer cells share certain typical T-cell markers, such as Thy-1 antigen and reactivity with T-cell-specific antisera, but their reactivity is weak. They also express receptors for Fc of IgG, but have no membrane-bound immunoglobulin, rather like T suppressor cells. They are capable of killing certain target cells and can bind specifically to target-cell monolayers, but their antigen-binding site probably differs in type from either the immunoglobulin variable region or the T-lymphocyte antigen receptor. Effector NK cells are induced to

Table 1 Membrane Markers of Human Lymphocytes[a]

| Methods of detection | Cells[b] $T_H$ | $T_S$ | B |
|---|---|---|---|
| Antisera | | | |
| anti-TLA | + | – | – |
| anti-BLA | – | – | + |
| anti-Ig | – | – | + |
| monoclonal | | | |
| anti-$T_4$ | + | – | – |
| anti-$T_5$ | – | + | – |
| anti-$T_8$ | – | + | – |
| anti-Ia | – | – | + |
| Viruses | | | |
| Epstein-Barr virus | – | – | + |
| measles | + | | – |
| Erythrocyte rosettes | | | |
| sheep | + | | – |
| mouse | – | | + |
| Complement | | | |
| C3b | – | – | + |
| C4b | – | – | + |
| Immunoglobulins | | | |
| Fc IgG | – | + | + |
| Fc IgM | + | – | – |
| Bacteria | | | |
| *Brucella melitensis* | – | – | + |
| Lectins | | | |
| phytohemagglutinins | + | + | – |

[a]Some examples of markers to distinguish T and B cells. Conventional immune sera react specifically with T (TLA) or B (BLA) antigens. Monoclonal antibodies can recognize subpopulations of T and B cells. Bacteria interact with specific carbohydrates present on the cell membranes. Phytohemagglutinins stimulate T cells preferentially.
[b]$T_H$, T helper cell; $T_S$, T suppressor cell.

differentiate from precursors by interferons, particularly IF$\alpha$ and interleukin II (IL2); interferon also renders normal cells resistant to NK attack, while virus-infected and tumor target cells remain NK susceptible (Amagai et al., 1980; Minato et al., 1980). Natural killer cells are therefore important in the early host response to virus infections and their action is probably central to the effect of interferons which has been reported on cancers of various types.

Juxtaposed to the T cells stands the system of the antibody-producing cells, or bone marrow-derived B cells. In fully differentiated form, these are cells capable of producing and secreting immunoglobulin molecules (Katz and Benacerraf, 1972; Warner, 1974).

Studies of human myeloma proteins derived from single clones of cells, together with the analysis of isolated antibodies and immunofluorescence techniques, have shown that each antibody-producing cell (plasma cell) synthesizes only one class or subclass of immunoglobulin (Pernis, 1967; Natvig and Kunkel, 1973). This restriction is extended

Table 2 Human Immunoglobulins

| Classes | | Heavy chains | Light chains | Other chains[a] | Some physiological properties |
|---|---|---|---|---|---|
| IgG | IgG1, IgG2, IgG3, IgG4 | $\gamma$ | $\kappa$ or $\lambda$ | – | Placental transfer |
| IgA | IgA1, IgA2 | $\alpha$ | $\kappa$ or $\lambda$ | J and SC | In external secretion |
| IgM | | $\mu$ | $\kappa$ or $\lambda$ | J | Early immune response |
| IgD | | $\delta$ | $\kappa$ or $\lambda$ | – | Unknown |
| IgE | | $\epsilon$ | $\kappa$ or $\lambda$ | – | Reaginic activity, mast-cell fixation |

[a]J, "joining" chain in IgA and IgM; SC, secretory piece in IgA only.

to the products of allelic genes. An exception to this rule is represented by the expression on the cell surface of both IgM and IgD molecules with the same type of light chain and variable region. Multiple expression of immunoglobulin isotypes is common on the surface of B lymphocytes which have not yet encountered antigen.

## Classes of Immunoglobulins

Five different classes of immunoglobulins have been identified in man on the basis of the discrete physicochemical properties and antigenic specificities associated with the polypeptide chains forming these molecules (Cohen, 1971; Natvig and Kunkel, 1973; Porter, 1973). In order of their relative concentrations in serum, these five classes are IgG, IgA, IgM, IgD, and IgE (Table 2). By virtue of subtle antigenic differences, four subclasses of IgG (IgG1, IgG2, IgG3, and IgG4) and two subclasses of IgA (IgA1 and IgA2) have been recognized to date.

The basic structure of each Ig molecule is the result of the fusion of two heavy (H) polypeptide chains with two light (L) chains; both H and L chains are formed by a variable (VL or VH) region and a constant (CL or CH) region. The antibody-combining sites of the molecules result from the interaction of two VL and VH domains which contain hypervariable regions responsible for the great heterogeneity of the immunoglobulins (Kabat, 1980) (Figure 2). The arrangement of the V and C domains is brought together by a series of joining (J) peptides and this enhances the heterogeneity of antibody specificities (Figure 3).

*IgG* About 80% of circulating antibody is made up of this class of immunoglobulin, which has a molecular weight near 150,000; IgG antibodies can activate complement, promote opsonization, and participate in the antibody-dependent cytolytic reactions. After initial antigenic challenge, the immune response is usually associated with the production of IgM; upon subsequent antigen challenges the response is usually a predominantly IgG response.

*IgM* These molecules, with a coefficient of sedimentation of 19S, form about 8-10% of the total Ig in serum. They are excellent agglutinating antibodies and react readily with the first component of complement. Certain antigens induce a persistent IgM response, such as antibodies to polysaccharide antigens, ABO blood groups, the Wassermann and heterophile antibodies, and the antibodies to endotoxins of gram-negative organisms. Naturally occurring IgM monomers (7S) are present in cord serum (Perchalski et al., 1968) and may be found in high concentrations in the sera of patients

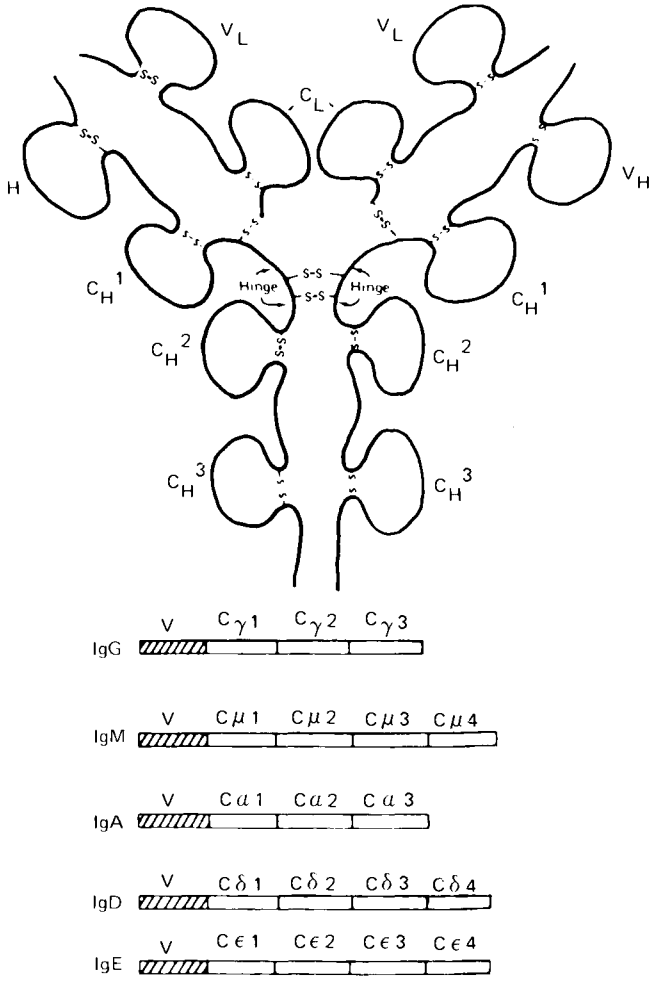


**Figure 2** Diagrammatic representation of the structure of human IgG molecules showing the interaction between two identical heavy polypeptide chains and two light chains. Each chain is formed by the fusion of a variable domain (VH or VL) and a set of constant (Cγ1, Cγ2, etc.) domains. The two variable regions form the antibody-combining site. The H chains of the five major classes of Ig vary with regard to the properties and number of C domains.

with lupus erythematosus, rheumatoid arthritis, or certain immune deficiencies such as ataxia-telangiectasia. IgM monomers appear to be the predominant form of this class of Ig present on the cell surface.

*IgA* Isoagglutinins, brucella, diphtheria, and poliomyelitis antibodies are examples of responses which are primarily IgA. These molecules are the most important antibodies in secretions; they are mainly present as dimers (11S) containing two additional polypeptide chains, the J chain and the secretory component. Colostrum contains bacterial IgA antibodies which, in combination with lysozyme, lactoferrin, and macrophages, play an important role in the protection of the newborn.

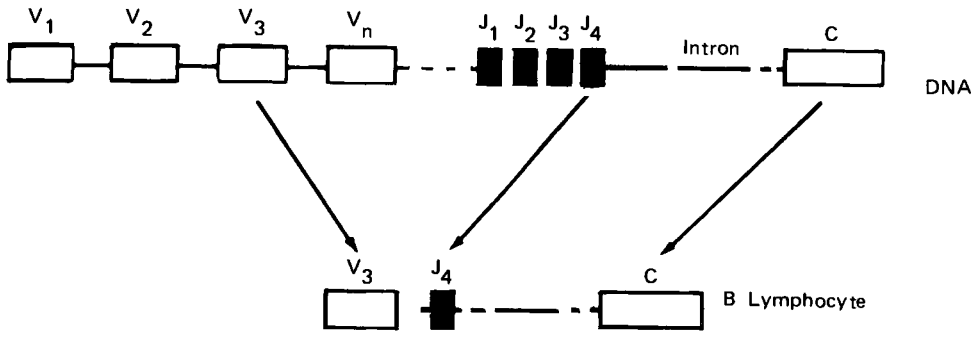


Figure 3 (Top) Arrangement of the V, J, and C genes in the chromosome and (bottom) fusion of the corresponding peptides. The great heterogeneity of the Ig molecules results from the fusion of one peptide synthesized by a variable (VH) gene and a peptide corresponding to the constant (C) domains. The V and C domains are linked by the product of one of the J (joining) genes.

*IgD* An important clue to the biological role of IgD is the observation that these molecules, present in low concentration in serum, are expressed on a high percentage of B cells, particularly during fetal and perinatal life (Rowe et al., 1973). Nearly all lymphocytes expressing IgD molecules on the surface (sIgD) also stain for IgM. This has led to the suggestion that IgD serves as an antigen receptor and plays an important role in initial antibody response, particularly during development.

## Transfer of Immunoglobulins Across the Placenta

The route and degree to which the various classes of immunoglobulins are transferred to the offspring vary among different species of mammals (Table 3) (Brambell, 1970; Wild, 1973; Hemmings, 1974).

Not all types of maternal Ig are transferred into the fetal circulation. There is evidence that maternofetal transfer is not related to the number or thickness of the placental membranes, but depends upon the capacity of the cells forming such membranes to allow a percentage of proteins having undergone endocytosis to be transferred across without being degraded (Brambell, 1970; Wild, 1973).

Studies on the transfer of plasma proteins, reviewed by Gitlin (1974), have shown that diffusion is most likely the process whereby albumin reaches the fetal circulation from the maternal plasma, whereas active transport is involved in the transfer of IgG molecules.

The concentration of a maternal plasma protein in the fetal blood is not a simple expression of its transfer rate; it depends upon several factors, such as the concentration of the protein in the maternal blood, the rate of its degradation in the fetus, the diffusion of the protein from the plasma into the fetal interstitial fluids, and the transfer of the same protein back to the maternal circulation. If the rates of degradation of two proteins are different in the mother, the fetus, or both, their concentration in maternal and fetal blood will be different even if their transfer is identical.

In man, transmission of immunoglobulins occurs exclusively by way of the chorioallantoic placenta (Brambell, 1970). Selection is perhaps one of the most remarkable features of this transfer; in fact, only IgG molecules readily cross the placental barrier,

Table 3 Time and Route of Transmission of Passive Immunity in Different Species

| Species | Transmission | | Route | |
|---|---|---|---|---|
| | Prenatal | Postnatal | Prenatal | Postnatal |
| Man | +++ | 0 | Placenta | – |
| Monkey | +++ | 0 | Placenta | – |
| Rabbit | +++ | 0 | Yolk sac (+placenta?) | – |
| Guinea pig | +++ | 0 | Yolk sac | – |
| Rat, mouse | + | ++ | Yolk sac | Gut |
| Dog, cat | – | ++ | Unknown | Gut |
| Horse, pig | 0 | +++ | – | Gut |
| Ruminants | 0 | +++ | – | Gut |

*Source*: From Brambell, 1970.

while other classes of maternal immunoglobulins either are not transferred or only cross the placenta in small quantities (Wiener and Berlin, 1947; Vahlquist, 1958; Hitzig, 1959; Freda, 1962).

Maternal IgG molecules are first detectable in the fetal blood after 10 weeks gestation: at this stage of development maternal anti-Rh antibodies have been found in human Rh-negative fetuses (Mollison, 1967). At the end of normal gestation, the mean level of IgG in new born sera is slightly higher than that in the corresponding maternal blood. Variations in the total levels of IgG in the mother are usually reflected in the concentration of this class of proteins in cord blood. The levels of IgG2 are slightly lower in cord sera than in the corresponding maternal samples. However, this subclass of IgG has been detected in fetal blood at 11 weeks, together with IgG1 and IgG3. On the other hand, IgG4 molecules have been detected occasionally after 14 weeks and consistently after 19 weeks (Schur et al., 1973).

Evidence that the major part of IgG present in fetal and newborn blood is derived from the maternal blood has been confirmed by studies of the genetic markers of these proteins in pairs of maternal and cord samples (Grubb, 1970). In fact, the IgG molecules present in the newborn carry Gm factors similar to those present in the corresponding maternal serum, irrespective of the genotype of the infants (Table 4). During the first months of

Table 4 Gm(a) at Birth and at 1 Year of Age[a]

| Mother | Infant | |
|---|---|---|
| | At birth | At 1 year |
| Gm(a+) | Gm(a+) | Gm(a+) |
| Gm(a+) | Gm(a+) | Gm(a–) |
| Gm(a–) | Gm(a–) | Gm(a–) |
| Gm(a–) | Gm(a–) | Gm(a+) |

[a]At birth the phenotype of the child resembles that of the mother. The maternal IgG molecules are slowly replaced by the infant IgG and at about 1 year of age the serum contains the product of the child's Gm genes.

life the maternal IgG molecules are replaced gradually by similar immunoglobulins produced by the infants; at about 1 year of age, the IgG molecules will express exclusively the infant's own Gm markers.

The mechanisms involved in the active transfer of IgG across the human placenta are not yet known. The complexity of the problem is exemplified by the contradictory results of studies of the transfer of two fragments (Fab and Fc) obtained by enzymatic digestion of IgG molecules. Brambell et al. (1960) have studied the transfer of labeled Fab and Fc fragments of rabbit IgG by measuring the amount of radioactive fragments in the fetuses 24 hr after inoculation into the uterine lumen of pregnant rabbits. They observed that while the concentration of the Fc fragment in the fetal serum was $\frac{1}{4}$ of that attained using intact IgG molecules, the levels of the Fab fragments were only $\frac{1}{6}$-$\frac{1}{10}$ those of the Fc fragments. These results suggest that the transport of IgG molecules across the placenta was mediated through a receptor on the Fc fragment; however, these findings could not be confirmed by Gitlin and collaborators (Gitlin et al., 1964; Gitlin, 1974), who injected labeled IgG fragments into pregnant women. In fact, the half-lives of the two fragments proved to be different from each other, the half-life of the Fc fragment being about 4 days and that of the Fab only 0.3 days. When the transfer of the fragments was reevaluated taking into consideration their degradation, it became apparent that they were transferred with identical rates. However, further studies have clarified the role of Fc as a placenta receptor site of IgG. The presence of the receptor has recently been demonstrated on the surface of the trophoblast at 10 weeks and at term (Jenkinson et al., 1976).

According to Gitlin (1974), the rate of active transport of human IgG varies in the course of normal gestation. Between 6 and 16 weeks of pregnancy the concentrations of IgG range between 100 and 200 mg/100 ml. After 22 weeks of gestation, however, the transfer of maternal IgG increases, and concentrations similar to those detectable in maternal sera are reached at 26 weeks. This increase in permeability seems selective for IgG, since there is no concomitant increase in the passage of other maternal plasma proteins. The observed increased transfer of alpha-fetoprotein from the fetal circulation into the maternal circulation toward the end of gestation appears to confirm that the permeability of the placenta varies during pregnancy.

In sera from normal infants born at term, specific maternal antibodies associated with IgM and IgA cannot be detected and IgD molecules are either absent or present at low levels. Specific maternal IgE reaginic antibodies are not detectable in the corresponding cord blood. There is, in fact, good evidence that the IgM, IgA, and IgE present in newborn blood are produced by the fetus during life in utero.

### Ontogeny of the Cellular Immune Response

It is generally accepted that yolk sac stem cells appear in the mouse around the seventh day, and in man around the third week. They migrate into the mouse embryo around the tenth day and appear in the thymus as large basophilic cells on the eleventh day (Moore and Owen, 1967). The time of migration in man is not known, but the fetal liver starts to be erythropoietic at about 4-5 weeks. Lymphocytes appear in the human fetal liver after 2 weeks, and in the thymus after the tenth week.

Yolk sac cells do not express T-cell markers or immunoglobulins (Figure 4) (Owen, 1977). The basophilic stem cells, which are seen in the fetal mouse thymus and which are precursors of the T cells, express characteristic T-cell markers such as the Thy-1, TL, and Lyt antigens. The amount of Thy-1 antigen on peripheral T cells decreases

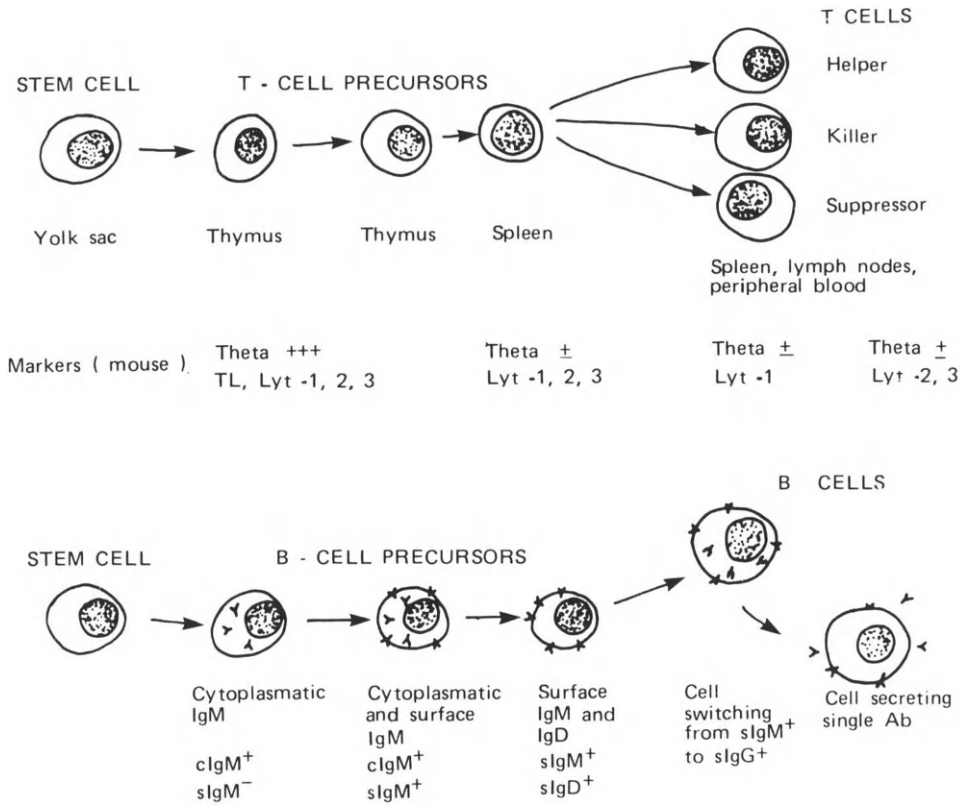


**Figure 4** The differentiation of T and B cells with the expression of their specific markers (see text).

and the TL antigen disappears entirely (Owen, 1973, 1977; D'Eustachio et al., 1977). In the mouse, T cells can be seen outside the thymus around the time of birth and have been detected in the spleen 3 days before delivery, and in the lymph nodes and Peyer's patches a few days after birth.

The T cells found in the spleen of the neonatal mouse express all three Lyt markers (Lyt-1,2,3$^+$) (Cantor and Boyse, 1977). During the first 3 weeks after delivery the proportion of Lyt-1,2,3$^+$ cells gradually declines and cells having only Lyt-1 antigen (Lyt-1$^+$) or Lyt-2 and 3 (Lyt-2,3$^+$) appear. Around this period immunoresponsiveness begins to develop and evidence has been produced that this is the result of the appearance of T helper cells Lyt-1$^+$; the Lyt-2,3$^+$ have, instead, suppressor effects (Hirst et al., 1975; Feldmann et al., 1975) (Figure 4).

Thomas and co-workers have found that if conventional Thy-1.1 antiserum is absorbed with neonatal spleen cells, it retains 60% of its activity against adult T lymphocytes (Thomas et al., 1978). They found that the unabsorbed antiserum contained at least two anti-T-cell specificities: one found on young (and possibly also on older) T lymphocytes—those less than 2 weeks post-thymic—and the other confined to the older post-thymic T-cell population. When the authors separated T lymphocytes into their two populations, based upon this reactivity, they were able to show that both populations could express all normal T-cell subset functions, but that the

younger T-cell population was capable of at least five passive transfers of graft-versus-host reactivity, two more transfers than is described for "whole" T-cell populations. This clearly marks a functional differentiation antigen for post-thymic T lymphocytes in the mouse.

Neonatal mice have few or no cells responsive to phytohemagglutinin (PHA) and concanavalin (ConA); the number of cells responsive to con A increases sharply a few days after birth, reaching a plateau at about 3 weeks. These cells are Lyt-$1^+$ and are therefore T helper cells (Spear and Edelman, 1974).

The development sequence of T cells in human fetuses has not yet been fully elucidated, but the response of lymphocytes to PHA or to allogeneic stimuli has been used by various investigators to detect the onset of the functional development of T cells in the fetus. Thymocytes have been found to respond to PHA after 10 weeks of gestation (Pegrum et al., 1968; Papiernik, 1970a,b; August et al., 1971; Ceppellini et al., 1971; Pegrum, 1971; Prindull, 1974; Stites et al., 1974). At this stage of development a well-demarcated thymic cortex and medulla are present. Phytohemagglutinin responsiveness of spleen and peripheral lymphocytes follows that in the thymus in time; this is consistent with the supposed thymic origin of these cells.

The formation of "rosettes" between human lymphocytes and sheep red cells has been recognized as a property of the thymus-derived cells. In a study of 13 fetuses, a maximum of 65% rosette-forming cells (RFCs) was found in the thymus, with a poor correlation between incidence and fetal age. Only a small proportion of RFCs was found in fetal bone marrow and spleen (Stites et al., 1974). The results of these studies are consistent with the notion that RFCs originate from the thymus and gradually migrate to peripheral blood during embryogenesis.

Using in vitro tests of thymocytes, spleen cells, and peripheral lymphocytes, a tendency for an increase in PHA-induced DNA synthesis with age has been noted. The highest reaction occurs near 19 weeks and thereafter the responsiveness declines.

The ability to respond to allogeneic stimuli by the mixed lymphocyte reaction was first detected using thymic cells from 12.5-week fetuses (Stites et al., 1972, 1974; Toivanen et al., 1978). Human fetal thymus and spleen cells injected under the kidney capsule of cyclophosphamide-treated rats have also been shown to induce a xenogeneic graft-versus-host reaction (Asantila et al., 1973).

Only a few studies of the specific binding of antigen by T cells during fetal life have been carried out. Dwyer and MacKay (1970) have shown that thymuses of human fetuses between 20 and 22 weeks old contain cells capable of binding radioiodine-labeled flagellin. The number of antigen-binding thymocytes was higher in fetal thymus than in postnatal and adult thymus.

The studies described so far have dealt with the maturation of T-cell recognition; little is still known about the development of T effector cells during ontogeny. Non-specific PHA-induced cytotoxicity against chicken red cells has been shown using cord lymphocytes (Stites et al., 1972).

The most striking difference between infant and adult blood lymphocytes in their cytotoxic activity against homologous target cells has been observed by Campbell et al. (1974). Antibody-dependent cytotoxicity (K-cell activity) was readily detected using cord blood cells, though it was lower than that of adult cells; PHA-induced cytotoxicity was very low in all cord samples.

To study the acquisition of immunologically specific effector cells, Toivanen et al. (1978) used the cell-mediated lympholysis test and measured the $^{51}Cr$ release from

specific and third-party target cells by the sensitized cells. The results indicate that the capacity for cell-mediated lympholysis is fully developed at the time of birth; it was not observed in fetuses less than 20 weeks old and was first detected in a premature baby born at 28 weeks.

Using monoclonal antibodies against the T-cell antigens, it appears that in adult individuals the earliest lymphoid cells within the thymus lack mature T-cell determinants (e.g., T4, T5, T6, and T8); instead, 10% of the early thymocytes are reactive with anti-T9 and anti-T10 and acquire thymocyte-distinct determinants, T4, T5, and T6. With further maturation, thymocytes lose T6 and start to react weakly with anti-T1 and anti-T3, while the full subsets of T4 and T5/T8$^+$ cells are well defined. Outside the thymus, the T cells mature to T1$^+$, T3$^+$, and T4$^+$ helper cells and T5/T8$^+$ cytotoxic suppressor cells. The T4 determinant is expressed in approximately 55-65% of peripheral T cells, whereas T5/T8 are present in about 20-30% (Reinherz and Schlossman, 1981). Preliminary results suggest that the T4$^+$ subset is analogous to the murine Lyt-1$^+$2 subset, while the T5/T8$^+$ cells mediated the cytotoxic and suppressor functions of the Lyt-2$^+$3$^+$ subset; in fact, there is also good evidence to indicate that the T5 and Lyt-2,3 antigens are structurally similar.

The distribution of T3, T4, and T8-positive cells in fetal and cord samples is still under investigation. According to Yachie et al., (1981), the population of E rosetting lymphocytes in cord blood shows a higher ratio of T4- to T8-positive cells than in adults. In spite of this, there is a profound and paradoxical suppressive effect mediated by the T4-positive cells tested by a functional assay. However, this may simply be a reflection of the small number of mononuclear cells which are E in cord blood and the functional immaturity of T helper cells (Anderson et al., 1981).

It is of great interest that alterations of T-cell maturation seem to be associated with specific immunodeficiencies; thus it appears that patients with combined immunodeficiency may have thymocytes blocked at an early stage of maturation (T9$^+$ and T10$^+$), while in a small number of patients with acquired agammaglobulinemia there is an excess of T5$^+$ cells. Deficiency of T5$^+$ cells, on the other hand, has often been observed in association with naturally occurring autoimmune diseases.

### The Prethymic Immunocompetent Liver Cells

As shown in Figure 1, yolk sac stem cells seed into the fetal liver and it is in this organ that the first immunocompetent cells appear. In fact, liver cells from 7- to 10-week-old human fetuses, without an identifiable thymus, show a strong reaction when confronted with mitomycin C-treated allogeneic cells (Stites et al., 1974). Liver cells from such young fetuses are also capable of inducing graft-versus-host reactions when transplanted into immunodeficient children, indicating the presence of prethymic T-cell precursors. The enigma of the existence in fetal liver of cells capable of an immune response in the absence of a mature thymus was solved when it was demonstrated that these cells respond *in toto* to allogeneic stimuli; that is, when cells responding to a first stimulus are eliminated, the remaining cells cannot respond to a different stimulant (Toivanen et al., 1978). The properties of these fetal liver cells have not yet been fully identified; the mixed lymphocyte reaction is inhibited by anti-$\beta$2 microglobulin, suggesting a T-cell-like nature, yet they do not express T-cell markers and are not stimulated by PHA. The present findings support the suggestion

that the prethymic liver cells have no specific recognitive properties, but proliferate in response to factors released by the mitomycin-treated cells.

At this early stage of development, the liver also contains cells which produce immunoglobulins. In fetuses 7.5 weeks old, approximately 0.1% of the liver cells contain cytoplasmatic IgM ($cIgM^+$). These are called pre-B-cells. In fetuses 9.5-12.5 weeks old, the first cells expressing surface IgM ($sIgM^+$) appear; the incidence of these cells increases to 2-6% between 12 and 16.5 weeks and they are called "baby" B cells. Cells carrying surface IgD ($sIgD^+$) have first been detected in fetuses 12 weeks old; at about 14-15 weeks of age, 50% of $sIgM^+$ cells also carry IgD molecules on the membranes (Lawton and Cooper, 1980). Pre-B-cells divide rapidly and it is possible that at this stage the genes coding for the antigen-binding site, V genes, undergo recombination and/or somatic mutation. The occurrence of kappa-chain V-gene clusters, whose copies are highly similar within but rather less similar between clusters, would favor recombination. The "baby" B cell is easily killed by low concentrations of antigen and it seems as though they are probably the targets of anti-idiotype antibody, as the modulating influence for the preservation of "new" idiotypes.

### Synthesis of Immunoglobulins by the Fetus

The concentration of IgG estimated in infants bled at intervals soon after birth appears to decrease during the first 3 months of life. For many years this phenomenon was interpreted as evidence for the slow catabolism of the maternal IgG molecules, which were not replaced by similar proteins produced by the infants. However, as early as 1959, Trevorrow showed that if the dilution of serum proteins were taken into account, the amount of immunoglobulins during the first months of life would be relatively constant. On the basis of these observations, Trevorrow suggested that immunoglobulins are synthesized during life in utero in the course of normal pregnancy.

The low number of plasma cells in normal human fetuses, even during the last months of gestation, seems to be the effect of the absence of environmental stimulation rather than the cause of an inefficient antibody response. In fact, following intrauterine infections, human fetuses may respond to antigenic stimulation by the proliferation of plasma cells after the sixth month of gestation. Both congenital syphilis and toxoplasmosis have been found to be associated with infiltration of plasma cells into various fetal tissues (Pund and Von Haam, 1957; Silverstein and Lukes, 1962).

Early infection may not cause sufficient damage to result in the death of the fetus. The immunological immaturity of the fetus may allow the infective agent to persist in the tissues; however, this does not necessarily imply that the fetus becomes tolerant to the invading organism (Silverstein, 1972).

Following intrauterine infections, specific antibodies and high levels of IgM have been detected in cord sera. High levels of IgM have been observed in sera from infants with congenital rubella, cytomegalic inclusion disease, or infections of *Toxoplasma gondii* (Alford, 1965; McCracken and Shinefield, 1965; Remington and Miller, 1966; Alford et al., 1967; Adinolfi, 1981a). The correlation between intrauterine infection and high levels of IgM has been repeatedly observed and long sustained antigenic stimulation during fetal life has been found to affect the synthesis of IgG during infancy (Soothill et al., 1966). For instance, low levels of IgG have been detected during the first 12 months in infants with congenital rubella and high levels of IgM at birth.

Some of these infants showed a high susceptibility to infection. IgM antibodies have also been detected in normal newborns; these antibodies are directed against red cell and occasionally bacterial antigens (Adinolfi, 1981a). It is of interest that immunoglobulin molecules that behave as 7S proteins, as judged by gel filtration, but which are antigenically related to IgM have been detected in human cord blood (Perchalski et al., 1968); 7S IgM has also been detected in lower vertebrates (Clem and Leslie, 1969).

In vitro cultures of fetal tissues in the presence of labeled amino acids and the analysis of the culture fluids by immunoelectrophoresis and autoradiography have confirmed that the human fetus is capable of producing IgG and IgM after the twelfth week of gestation. Spleen and lymph nodes are the main sites of synthesis (van Furth et al., 1965; Gitlin and Biasucci, 1969). Immunofluorescent staining of fetal spleen has demonstrated that medium-size and large lymphoid cells, as well as plasma cells, secrete IgM and IgG molecules.

The number of B cells in cord blood bearing surface Ig has been investigated by Fröland and Natvig (1971); the mean value for cells containing IgM was 9.7% and that for IgG 7.9%. It is of interest that the dominant subclass of IgG expressed on cord lymphocytes was IgG2. No cells positive for IgA were detected in peripheral newborn blood. In fact, only low levels of IgA have been detected in the serum of normal neonates; however, high values of IgA have been detected in infants with congenital infections and those previously transfused during life in utero (Stiehm and Fudenberg, 1966; Hobbs et al., 1968).

IgD immunoglobulins are usually absent or are present only in low concentrations in sera from normal human newborns. However, these molecules, present on the surface of 3.5% of B cells in normal adults, have been detected in as many as 18% of the lymphocytes from cord blood, usually in association with IgM molecules (Rowe et al., 1973).

IgE globulins are present in sera from normal newborns. The concentrations in paired cord and maternal blood samples are not correlated and specific maternal reaginic antibodies are not present in the newborn blood. In vitro cultures of fetal tissues have confirmed that IgE is synthesized during life in utero.

The evidence that the human fetus is capable of producing antibodies at an early stage of development is in agreement with studies on the ontogeny of acquired immunity in other species. Synthesis of antibodies during life in utero has been observed in the monkey, lamb, cow, and guinea pig (Solomon, 1971). In the lamb there is evidence that immunological competence to various antigens does not arise simultaneously, but as a stepwise maturation of the ability to respond to different antigens at different stages of development (Silverstein and Predergast, 1970; Silverstein, 1972). A diagram of the comprehensive ontogeny of the immune system is shown in Figure 5.

### Ontogeny of Complement, Lysozyme, and Lactoferrin

Complement, lysozyme, and lactoferrin have been recognized to play an important role in the mechanisms of defense against bacterial and viral infections in association with the cellular and humoral immune response (Miller, 1980). Since the beginning of this century the ontogeny of complement has been the object of several investigations both in human newborns and in experimental animals (Adinolfi, 1972, 1981b; Colten, 1972; Ballow, 1977). However, only during the last two decades has clear evidence of the

| Week | | | |
|---|---|---|---|
| 3 - | - Yolk sac haemopoiesis | | |
| 4 - | - Liver hemopoiesis | | - C1 inhibitor produced by liver cells |
| 5 - | - Lymphocytes in liver (1%) | | |
| 6 - | - Thymus rudiment appears | | |
| 7 - | - Spleen rudiment appears<br>- thymus becomes lymphopoietic | - Liver cells: one-way MLR positive, PHA-cell unresponsive; E rosette positive | - C3, C4, and C5 synthesis begins |
| 8 - | - Lymphocytes in blood | | |
| 9 - | - Active lymphopoiesis in thymus | - 0.1% Liver cells cIgM$^+$<br>- E rosette cells in thymus (90%) | - LZM in serum |
| 10 - | | - sIgM$^+$ liver cells<br>- Peripheral lymphocytes PHA-positive | |
| 11 - | | | |
| 12 - | - Lymphocytes in blood near 1000/mm$^3$ | - Peripheral lymphocytes sIgM$^+$, sIgD$^+$<br>- sIgG$^+$ cells in peripheral blood | - C7 and C9 synthesis. LZM produced by alveolar macrophages. Synthesis of lactoferrin in liver, spleen and thymus |
| 13 - | | | |
| 14 - | | | |
| 15 - | | | |
| 16 - | | - Lipopolysaccharide B stimulates fetal lymphocytes | |
| 17 - | | | |
| 18 - | | | - All components of C in fetal sera |
| 19 - | | | |

**Figure 5** The comprehensive ontogeny of the human immune system (see text).

onset and site of synthesis during fetal life of various components of complement (C), lysozyme, and lactoferrin been obtained, mainly as a reult of the introduction of new methods for the isolation and identification of the plasma proteins forming the C system, the use of short-term cultures of fetal tissues in the presence of labeled amino acids, and the discovery of the genetic polymorphism of several components of C.

At present, more than 20 components have been shown to interact in the complex mechanism of activation of the C system (Table 5) (Müller-Eberhard, 1972; Lachmann, 1979).

Most of the distinct plasma proteins which form the C system are present in blood in an inactive form. The interaction of C system with antigen-antibody complexes (Ag-Ab) or directly with bacterial polysaccharides results in the sequential activation of the various components and the formation of multimolecular structures, some of which adhere on the surface of biological membranes and are responsible for the elimination of foreign materials from the body.

Table 5 Some Properties of Human Components of C and Their Levels in Sera from Normal Adults

| Protein | Levels in serum (mg/100 ml) | Electrophoretic mobility | Molecular weight | Major fragments |
|---|---|---|---|---|
| Classical components | | | | |
| C1q | 18 | γ2 | 400,000 | |
| C1r | 10 | β | 180,000 | |
| C1s | 11 | α | 86,000 | |
| C2 | 2.5 | β1 | 117,000 | C2a,C2b |
| C3 | 130 | β2 | 180,000 | C3a,C3b,C3c, C3d |
| C4 | 43-64 | β1 | 206,000 | C4a,C4b |
| C5 | 8 | β1 | 180,000 | C5a,C5b |
| C6 | 7.5 | β2 | 110,000 | |
| C7 | 5.5 | β2 | 95,000 | |
| C8 | 8 | γ1 | 163,000 | |
| C9 | 23 | α | 79,000 | |
| Alternative pathway initiating factor (IF) | 2-5 | γ1 | | |
| properdin (P) | 20 | γ2 | 150,000 | |
| factor B (C3 pro-activator, C3PA) | 14-22 | α | 93,000 | |
| Alternative pathway factor D (C3 proactivator convertase) | 0.1-0.5 | α2 | 24,000 | |
| Regulatory proteins | | | | |
| C1 inhibitor (C1-INH) | 18 | α2 | 105,000 | |
| C3b inactivator (C3b-INA; KAF anaphylatoxin inactivator | 4 | α | 300,000 | |
| β1H (factor H) | 13.3 | β | 150,000 | |

Recent studies have shown that C fulfills various functions besides acting on the bacterial membrane. In fact, C may also produce activation of specialized cell properties, such as an increased vascular permeability, the release of histamine from mast cells and platelets, the contraction of smooth muscle, and the enhancement of phagocytosis.

A unique property of C proteins is their inherent ability to undergo transition from soluble molecules to membrane constituents through the generation of binding regions. In fact, cleavage of a component of C usually results in the formation of a minor fragment capable, for a short period of time, of binding to an appropriate receptor.

Operationally the activation of the C system has been divided into two pathways (Figure 6). The first or *classical pathway,* mediated by Ag-Ab complexes, has been

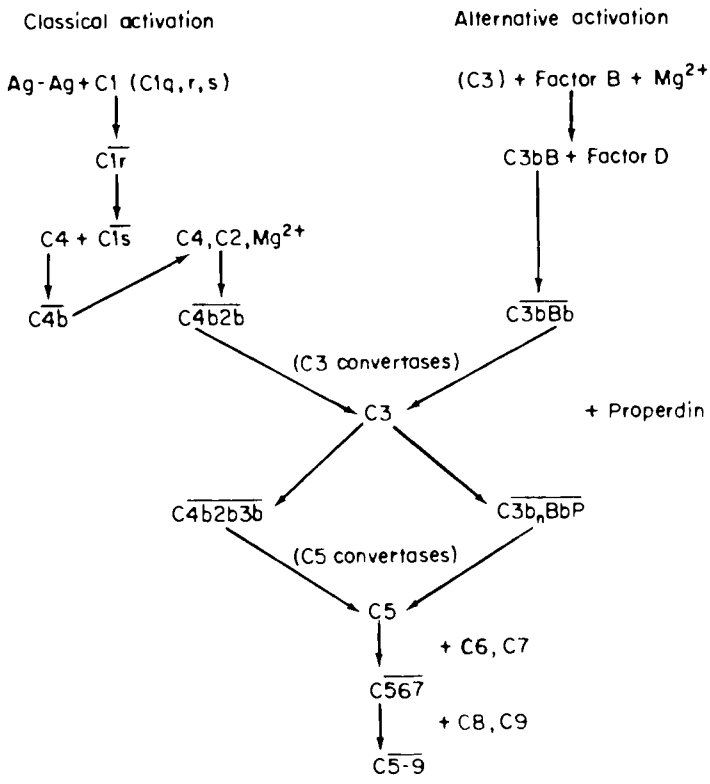


Figure 6 Classic and alternative pathways of complement activation. The classic activation starts with the interaction between C1 and Ag-Ab complexes or C-reactive protein; it involves the cleavage of C4 and C2. The alternative pathway is regulated by the interaction between the initiating factor (IF), factor B, factor D, properdin (P), and C3. The activation of both pathways results in the cleavage of C3, the formation of C5 convertases, and the interaction of the late components of C.

grouped into three different units: the recognition, activation, and membrane attack systems (Müller-Eberhard, 1972). The *alternative pathway* activated by IgA or naturally occurring polysaccharides and lipopolysaccharides has come to light following the discovery by Pillemer et al. (1954) of a nonspecific resistance to infections mediated by properdin, a normal serum protein. The activation of both pathways results in the cleavage of C3 and the formation of C5 convertases, which cleave C5 and activate the late components of C.

In addition, the C system includes "regulatory" proteins such as C1 inhibitor (Table 5). These proteins play an important role in the biological control of C activation, since the deficiency of any of them is usually associated with severe clinical disorders (Lachmann, 1979).

Comprehensive and critical reviews of the ontogeny of C have been published in recent years (Adinolfi, 1972, 1977, 1981b; Rosen, 1974; Colten, 1974; Ruddy, 1974; Ballow, 1977). Here we plan to summarize the most important results.

Studies on the ontogeny of human C have shown that the mean level of total C activity in newborn sera is about half that detected in maternal blood (Adinolfi, 1972;

Rosen, 1974). The introduction of the radial diffusion technique and of sensitive hemolytic tests for the estimation of single components of C have made it possible to evaluate the levels of these proteins in fetal and newborn sera. C3 and C4 have been detected in sera from human fetuses after 14 weeks and occasionally in 9- to 10-week-old fetuses. C1, C3 activator, C5, C7, and C9 have also been detected at an early stage of fetal development (Adinolfi, 1972, 1975, 1981b; Adinolfi and Beck, 1975) (Table 6).

The estimation of the levels of C3, C4, C6, and C7 and C3 activator in paired maternal and cord blood samples has shown that the mean concentration of these proteins in newborn samples is about half the values detected in maternal or adult sera. The only exception is for C9, which is present in cord blood in concentrations of 10-25% of the mean in samples from normal adult subjects (Figure 7) (Adinolfi and Beck, 1975).

Direct evidence that C1, C3, C4, and C5 are produced during fetal life has been obtained by incubating fetal tissues in media containing labeled amino acids (Adinolfi et al., 1968; Colten et al., 1968; Gitlin and Biasucci, 1969; Adinolfi, 1972; Köhler, 1973). Analysis of the culture fluids for the presence of specific newly synthesized components of C, either using hemolytic tests or by autoradiography of the immunoelectrophoretic plates, has shown that C3 was produced in the liver cultures from fetuses more than 14 weeks old; in addition, hemolytically active, de novo synthesized C3 has been isolated from the supernatants of fetal liver cultures. Similarly, synthesis of C4 has been demonstrated in liver tissue cultures obtained from fetuses more than 8 weeks old (Table 6).

Human peritoneal and alveolar cells from fetuses more than 14 weeks old have been shown to produce C3 and C4 in vitro. These findings are in agreement with evidence that these components of C are produced by liver, lung, and peritoneal cells from adult monkeys, rats, rabbits, and guinea pigs. In man and experimental animals, macrophages collected from adult tissues seem to be capable of in vitro synthesis of C4 and C2. When human fetal liver macrophages were separated from other hepatic cells on a discontinuous albumin gradient, C4 was found to be produced by a fraction rich in macrophages (Colten, 1974).

The type of cells involved in the synthesis of C5 during fetal life is not yet known. In vitro cultures suggest that human C5 is produced mainly in fetal liver and spleen,

**Table 6** Site of Synthesis of Human Components of C During Fetal Life

| Component | Main tissue | Age (weeks)[a] |
|---|---|---|
| C1 | Intestinal epithelium | 19 |
| C1q | Spleen | 14 |
| C2 | Liver, macrophages | 8 |
| C4 | Liver, macrophages | 8 |
| C3 | Liver cells | 8 |
| C5 | Liver | 9 |
| | Spleen, liver | 8-14 |
| C6 | Unknown | – |
| C7 | Liver | 14 |
| C8 | Unknown | – |
| C9 | Liver | 20 |
| C1 inhibitor | Liver | 4 |

[a]Early detection using in vitro cultures.

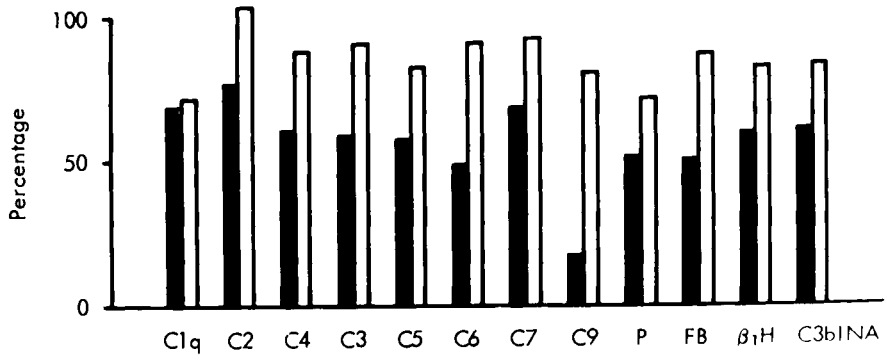


Figure 7 Levels of complement components in sera from newborns at birth (filled columns) and at six months of age (open columns) expressed as a percentage of the levels in adults.

but there is some evidence of C5 biosynthesis by fetal colon, lung, thymus, placenta, and peritoneal, and bone marrow cells in culture (Köhler, 1973; Colten, 1974).

In vitro synthesis of the first component of C1 was observed by Colten et al. (1968) using tissues obtained from fetuses more than 19 weeks old. Isolated fragments of the small intestine and colon were found to be capable of in vitro production of hemolytically active C1. No significant synthesis was observed in the culture fluids of fetal liver, lung, kidney, thymus, spleen, and stomach.

Gitlin and Biasucci (1969) have also investigated the synthesis of C1 inhibitor by the autoradiographic techniques. Newly produced C1 inhibitor was detected in the culture fluid of liver tissue from 4-week-old human fetuses. Early production of C1 inhibitor in fetal liver has been confirmed by Colten (1972), who noticed that the rate of synthesis of C1 inhibitor in an 11-week-old fetus appeared to be similar to that observed in normal adult subjects.

Lysozyme (LZM) is also produced at an early stage of fetal development; the enzyme has been detected in sera from fetuses more than 9 weeks old and all cord samples tested; levels similar to those detected in normal adults are reached at about 18 weeks of gestation (Glynn et al., 1970; Adinolfi, 1972). Using an immunoperoxidase technique, LZM has been detected in the alveolar macrophages of lung in human fetuses, as well as in fetuses of experimental animals (Klockars et al., 1974).

In 1970, Glynn and collaborators measured the levels of LZM in sera from 66 normal full-term newborns and their mothers and found that the mean concentrations of the enzyme, measured by the lysis of *Micrococcus lysodeikticus*, were 9.65 μg/ml in maternal sera and 12.59 μg/ml in newborn samples. The difference of the two means was statistically significant. When the individual concentrations of LZM in pairs of maternal and newborn sera were compared, no correlation between the values was observed; in 14 cases the concentration of LZM in cord serum was at least twice that observed in the corresponding maternal serum. Lysozyme was detected in three out of nine fetuses between 9 and 12 weeks old, and in seven out of eight fetuses from 21 to 24 weeks (Adinolfi, 1972). Evidence for the fetal synthesis of the enzyme was obtained by showing intracellular activity of LZM in leukocytes from human newborns (Adinolfi, 1972).

Using the immunoperoxidase method, major changes in the distribution of LZM have been shown to occur during fetal life in man (Klockars et al., 1974). In the youngest fetus studied, 10 weeks old, LZM was observed in macrophages and monocytes (Figure 5).

Lactoferrin is a protein present in colostrum and milk from many species (Masson and Heremans, 1971); its function is that of inhibiting the growth of certain microorganisms by chelating iron (Bullen et al., 1972). Human lactoferrin has a molecular weight near 75,000 and it appears to consist of a single polypeptide chain, since no change in the molecular weight is observed when the reduced and alkylated protein is investigated in 6M urea.

Recent studies suggest that lactoferrin present in the specific granules of mature granulocytes may play an important role in the physiological regulation of granulopoiesis (Broxmeyer et al., 1978).

The site of synthesis of lactoferrin during fetal life has been investigated using an immunoperoxidase method. Lactoferrin is already present in fetuses 12 weeks old in liver, spleen, thymus, and lung (Figure 5). The distribution of lactoferrin is similar to that of LZM; however, of great interest is the observation that in young fetuses lactoferrin is present in pancreas and in the thymus, in Hassall's corpuscles (Reitamo et al., 1981).

## MATERNOFETAL IMMUNOLOGICAL INTERACTIONS

Pregnancy is a process in which one or more members of a species develop, from the fusion of a single sperm with an ovum, in intimate physiological contact with a female of the same species. In outbred populations, the fetus and mother are usually histoincompatible. The phenomenon represents the only natural histoincompatible graft to occur in man and the success of the fetus as a homograft is not fully understood (Beer and Billingham, 1971; Scott et al., 1973; Edwards and Coombs, 1974; Gill and Repetti, 1979).

In the absence of any modification of normal immune responsiveness, the recognition of the unshared paternal haplotype, expressed by the fetus (Searle et al., 1975) and the mother's response to it, might be expected to compromise pregnancy (Billington, 1975a). Since this does not usually occur, maternal unresponsiveness might be due to nonspecific immunosuppression or the expected maternal response to paternally derived antigens may be modified to neutral or conceivably beneficial ends.

### Historical Review

The possibility of exchanging tissues between humans and/or animals is an ancient concept. The Egyptians tried infusions of animal blood into men, without recorded success, around 1500 B.C. (Grapow, 1935, 1936). Hindus of the Tilemaker cast practiced grafting during ritual trepanning around 100 B.C. (Haas, 1935). John Hunter transplanted the spurs and testes of cocks and human teeth in the eighteenth century (Paget, 1897) and the first modern blood transfusion was carried out by James Blundell in 1829 for postpartum hemorrhage (Cartwright, 1967).

The first transplantation study of mammalian gestation was published by Walter Heape in 1890. He transferred blastocysts removed from a Belgian hare to the uterus of a pregnant Angora rabbit, which subsequently delivered a mixed litter of leverets

and rabbits. This xenogeneic experiment demonstrated the relative indifference of mothers to fetal transplantation antigens at a time when such molecules were unknown.

Studies on the immune response in pregnancy over the succeeding 50 years were handicapped by lack of knowledge of transplantation immunology (Medawar, 1954). The pioneering work of Medawar, Brent, and Billingham in the early 1950s (Billingham et al., 1953) established the genetic basis of transplantation reactions (Bach, 1976). A summary of the present situation is that within each species there are clusters of transplantation loci (Graff and Bailey, 1976) [in the mouse, 30 autosomal groups, 1 X linked and 1 Y linked (Klein, 1975)], but that for each species a single cluster of loci, known as the major histocompatibility complex (MHC), is especially potent (Hindemann, 1970) [the H-2 complex on the murine seventeenth chromosome and the HLA complex on the human sixth chromosome (Ceppellini and Van Rood, 1974)]. At each locus within the MHC, one of many alleles may be expressed (Bodmer, 1972), although whether they are true alleles or whether all possible genes are present at each locus in each member of a species and expression is controlled in some complex way is not clear (Bodmer, 1975). Genes within the MHC control not only transplantation reactions (Thorsby, 1974) but also immune responsiveness (McDevitt and Benacerraf, 1969), mixed lymphocyte reactions (Bach et al., 1972), cell-mediated lympholysis target antigens (Nabholz et al., 1974), and complement components (Lachmann et al., 1975). It seems likely that the products of these genes are of importance in the immune response of pregnancy (Beer and Billingham, 1971, 1974, 1976, 1977) and that they are involved in maternofetal interactions.

At the same rate as the unraveling of the genetics of transplantation immunology, studies of immune interactions between mother and fetus were carried out. Initially, examination of blood group reactivity was undertaken to search for the induction of fetal tolerance, but this failed to demonstrate any effect (Owen et al., 1954). In contrast, many workers have studied the possible sites and mechanisms of blocks in the immune response which might protect the fetus until delivery (Billingham, 1968). However, the uterus is not an immunologically privileged site, since immune responses can be elicited within it and skin and parathyroid allografts will grow there only on a fetus (Beer et al., 1977; Poppa et al., 1964). Other approaches include searches for immunosuppressive factors, investigation of placental structure and function, the analysis of seminal antigens, and the investigation of the role of MHC antigens. The idea that conventional immune responses are blocked in pregnancy has been superseded by the view that we ought to consider the antigens that might be significant, the immune response to them, their implication for fetal survival, and their genetic basis. The rest of this chapter will be devoted to this approach to maternofetal immunobiology.

## Paternal and Fetal Antigens

### Seminal Antigens

Exposure to seminal antigens occurs during sexual intercourse. Although it has been suggested that bacterial cross-reactivity may be an explanation for priming against such antigens (Sarkar, 1974), there is no evidence to support this hypothesis, other than that based upon the expected incidence of shared determinants. Although the antigens present in seminal fluid have not been fully characterized, they include those produced by the MHC, HLA in man and H-2 in the mouse (Talwar, 1980; Tung, 1980). Small amounts of these antigens have been detected on spermatozoa and they are assumed

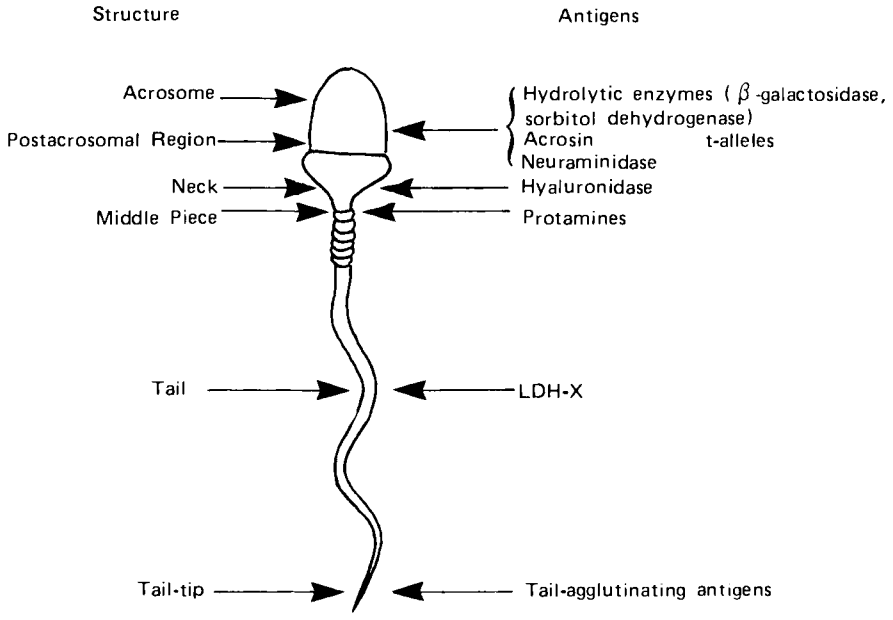


Figure 8 Localization of some spermatozoal antigens. (Adapted from Talwar, 1980.) LDH-X is the X-chromosome encoded isotype of lactic acid dehydrogenase.

to be passively adsorbed. Lactoferrin is also present in seminal fluid and is presumably similarly adsorbed onto spermatozoa (Rümke, 1982). Anaphylaxis following sexual intercourse has been attributed to pathological hypersensitivity to seminal antigens, but the sensitizing factors have not been fully characterized (Boettcher et al., 1977). The structure of spermatozoa, together with the localization of the antigens found on their surface, is shown in Figure 8. Immune responses to acrosin (Zaneveld et al., 1973), sorbitol dehydrogenase (Bishop et al., 1967), the corona-dispersing enzyme (Menge, 1971), and neuraminidase (Srivastava et al., 1970) have been experimentally induced; however, there is no evidence that naturally occurring allo-antigenic responses occur to them. Naturally occurring antibodies directed against presumed idiotypic variants of hyaluronidase have been found in infertile women, suggesting that it may be a significant antigen in vivo (Metz, 1973). The X-chromosome-encoded isotype of lactic acid dehydrogenase (LDH-X) is an organ- and cell-specific molecule, potentially both iso- and autoantigenic (Goldberg, 1975). It is found on the spermatozoal midpiece, but although immune responses can be generated against it, in experimental animals, naturally occurring immune responses have not been discovered. The spermatozoal acrosome carries β-galactosidase and the amount of this enzyme has been shown to be related to the presence of lethal alleles at the *t* locus in the mouse (Shur et al., 1979a,b). The gene product of *t* alleles are expressed on the acrosomal surface and can have marked effects upon reproductive performance (Hamilton et al., 1979; Klein and Hammerberg, 1977). Although no complex analogous to *t* has been described in man, there is good indirect evidence, particularly relating to the cross-reactivity of anti-F-9 (an antiserum directed against the normal allele of murine *t*) with spermatozoa from man and other species, to support its presence (Jacob, 1977). There are other molecular species present on the spermatozoal

surface and recent studies on membrane chemistry have defined two glycoprotein peaks which are as yet unidentified. Preliminary studies by M. Hjort (personal communications, 1981) on the chemical nature of antigens found on the spermatozoal surface may allow a more precise indentification of their correspondence with those present on cells of the early embryo.

It has been suspected for many years that seminal plasma exerts a protective effect against female immunization, since in experimental animals antibodies against sperm antigens are readily produced following introduction of washed sperm into the genital tract. Several investigators have also demonstrated immunosuppressive effects of seminal plasma in vitro and have attributed this action to various distinct factors (Stites and Erickson, 1975; Prakash et al., 1976; Anderson and Tarter, 1982).

### Fetal Antigens

Although, strictly speaking, antigens present upon the zona pellucida are maternal in origin, it is appropriate to mention them at this point. Several distinct glycoprotein and carbohydrate peaks have been defined by two-dimensional gel electrophoresis (Gwatkin, 1979) and antisera to these determinants have been raised which are capable of inhibiting fertilization (Dudkiewicz et al., 1976). Although naturally occurring antibody against the zona pellucida has been found (Shivers and Dunbar, 1977), research has been limited by the very small amount of material that can be obtained for experimental purposes. Several antigens have been shown to occur on cells of the early embryo and certain teratocarcinomata. The excellent review by Jacob (1977) describes their serological distribution, but, with the exception of *t*-complex products, they have undergone little chemical analysis. Antigens of the MHC (specifically H-2 in the mouse) are expressed on cells of the blastocyst, whereas non-H-2 antigens are found on the cleaving ovum (Goodfellow et al., 1976; Webb et al., 1977). There is evidence for the expression of paternal HLA antigens before implantation, followed by their marked reduction or disappearance at implantation (Searle et al., 1975; Johnson, 1975). Although the developing embryo continues to express HLA (McIntyre and Faulk, 1979a,b), there has been a prolonged debate as to whether paternal HLA specificities are expressed upon the outer membrane of the syncytiotrophoblast (Whyte and Loke, 1979; Faulk et al., 1977, 1978). Although most mouse placental cells in culture express H-2, a small number remain negative and it is postulated that these are derived from the syncytiotrophoblast (Goodfellow et al., 1976; Searle et al., 1974). There is , on the maternal side of this membrane, a "hyaline" substance, the Nitabuch zone, which has been found to contain immunoglobulin, complement, and albumin as well as HLA (Bradbury et al., 1970). This layer made the interpretation of immunofluorescence studies very difficult. Although large amounts of HLA antigens can be extracted from placenta, and small amounts are synthesized in vitro, careful observations have been unable to detect HL-A on the syncytiotrophoblast membrane (Searle and Jenkinson, 1978; Sundqvist et al., 1977) until recently. Using a very sensitive triple-sandwich technique, low amounts of paternal H-2 of sparse distribution have been found on this interface, which would act as poor targets in cell-mediated lympholysis (Chatterjee-Hasrouni and Lala, 1979). Finally, blood group antigens expressed by the fetus–such as RhD, A, B, or H, and Kell and others–may be paternally derived and capable of inducing maternal immune responses to them, although 30% of mothers (and male $RhD^-$ volunteers) are unresponsive to the RhD antigen.

## Placental Antigens

It is now clear that the maternofetal interface is of major importance in the immunological relationship between mother and fetus. It is therefore crucial to be aware of the nature of those molecules situated at the maternofetal interface. Faulk and his colleagues have defined at least two antigens, TA-1 and TA-2, which are present upon the syncytiotrophoblast surface (Faulk et al., 1978). The best characterized of these is TA-1, which is believed to consist of two polypeptide chains of 68,000 and 70,000 daltons each and, from studies carried out on lymphocyte reactivity, to have species specificity, although there is no evidence to support allovariety. TA-1 is also present upon the amniotic membrane; however, its immunosuppressive effect, reported against mixed lymphocyte reactivity (McIntyre and Faulk, 1979a,b), has not been confirmed by other workers (G. Stirrat, personal communication 1980). The application of techniques for the manufacture of monoclonal antibodies to the analysis of molecules present at the maternofetal interface has given interesting results. Sunderland et al. (1981) have found three distinct molecular species, the best studied of which is released into the maternal bloodstream, is not present upon microvilli, and has a half-life of 15 min. Johnson et al., (1981) have found 30 non-cross-reacting determinants, of which 3 bound only to syncytiotrophoblast membrane and 1 of these to microvilli. Another monoclonal bound only to syncytiotrophoblast and lymphocyte membrane, while one bound to the former and to ovarian carcinoma cells only.

One antibody binds to placental alkaline phosphatase (PAP) and shows no cross-reactivity with bone or liver phosphatase. Placental alkaline phosphatase is an enzyme which has considerable allovariety, and the selective pressures which might be responsible for the maintainance of these allotypes is not immediately obvious. No functional biochemical explanation is known and the possibility that these allotypic determinants, when paternally derived, might be responsible for generating a necessary maternal immune response seems unlikely. If the gene coding for PAP were linked closely to another, highly polymorphic gene complex, like HLA, then the preservation of its allotypic variation might be explained in part by linkage disequilibrium, leaving the functional advantage obscure. Using a slightly different technique, P. Travers (personal communication, 1980) found nine different antigenic determinants. Although some of these molecules have been defined, the most interesting have yet to be examined in detail.

## Maternal Immune Response

As mentioned earlier, the essential integrity of the immune system in pregnancy is illustrated by the absence of significant infectious disease susceptibility. However, slight changes in the activity of autoimmune disease suggest that subtle control mechanisms may be altered in their settings. The maternal response to paternal and fetal antigens may eventually give us the explanations for these observations, but as yet responses to only a few have been studied. Historically, the earliest responses detected were against blood group antigens. The response to Rh antigens, especially D, has been well described, as has the prenatal production of isohemagglutinins to antigens of the ABO group. Occasionally, other fetal blood group antigens may elicit responses (Mollison, 1967). Responses to Gm groups have been described in pregnancy: Most mothers make antibody to unshared Gm groups and fetal responses to maternal Gm groups have been found. The maternal antibody response to antigens of the HLA system was first

discovered in sera from multiparous women and subsequently found in up to 40% of such women (Ceppellini and Van Rood, 1974). Although such antibodies might be considered potentially harmful, their common induction suggests otherwise. The production of such antibodies demonstrates the integrity of the afferent, central, and efferent limbs of the maternal immune response and strengthens the importance of placental interposition as a factor in fetal survival. The detection of cell-mediated responses to paternal and fetal antigens has proved to be a much more complex exercise. As usual, in such situations, animal models, particularly murine pregnancy, have been utilized in attempts to answer this question. Although pregnancy in the mouse is so short that it is not reasonable to lay much weight on the implications for postimplantation pregnancy, three important clues have been detected. Firstly, thymic involution occurs very early (Clarke, 1979); secondly, those lymph nodes draining the uterus hypertrophy (Ansell et al., 1978; Clarke and McDermott, 1978); and thirdly, the spleen enlarges (Gill and Repetti, 1979; Mattsson et al., 1979). This suggests that an immune response, which involves the segregation of maternal cells capable of reacting with fetal antigens, is taking place (Baines et al., 1977). Specific studies of murine maternal responses to H-2 antigens have provided three further hints: suppression of maternal antipaternal mixed lymphocyte reaction (MLR) can be detected during pregnancy (Smith, 1981), but only in certain strain combinations; secondly, antifetal cell-mediated cytotoxicity may be found under similar restrictive conditions (Zagury et al., 1979; Stern and Kahan, 1981); and thirdly, these effects map to the right of murine H-2, between S and D (Chaouat et al., 1979). Information on responses to transplantation antigens in man is more difficult to obtain and less easy to interprete. Several studies have suggested that the maternal antipaternal MLR may be decreased (Knobloch et al., 1976), but the best of the most recent work shows no alteration in maternal MLR, although effector cell function against paternal antigens was slightly reduced (Moen et al., 1980). There have been experiments in which maternal cell-mediated lympholysis (CML) against paternal antigens was found (Ceppellini et al., 1971), but the same recent study (Moen et al., 1980) did not find any increase in primed lymphocyte typing responses against paternal antigens, which ought to be a good indicator of the absence of antipaternal CML priming.

It should be stressed here that peripheral lymphocytes from normal newborns, cultured without PHA, incorporate approximately 6-10 times more radioactive thymidine into DNA than adult cells. Therefore allowance should be made for such increased synthesis in studies of allogeneic stimulation of cord lymphocytes with maternal or paternal cells and unrelated mitomycin-treated adult lymphocytes.

The MLR appears particularly suitable to investigate whether specific modifications of cellular immunity are produced in the mother against alien fetal histocompatibility antigens or in the fetus against maternal antigens (Ceppellini et al., 1971; Bonnard and Lemos, 1972; Carr et al., 1974).

There is good agreement that, after calculation of the stimulation ratios, in many instances cord lymphocytes are less reactive against allogeneic stimuli from unrelated adult cells. According to Ceppellini and his collaborators (1971), maternal lymphocytes are a poorer stimulus than unrelated cells. Carr et al. (1974) have observed that when the kinetics of stimulation by maternal and adult unrelated cells are compared, the results do not suggest any specific unresponsiveness of fetal lymphocytes toward maternal histocompatibility antigens. However, according to Carr et al. (1974), the kinetic reactions of maternal lymphocytes in the MLR have the appearance

of the usual allogeneic responses and the results do not suggest specific unresponsiveness toward fetal histocompatibility antigens either. Although stimulation with related mitomycin-treated cord lymphocytes is usually poorer than against unrelated cells, this is not unexpected, since at least one-half of the histocompatibility antigens are similar in related mothers and newborns. It has also been suggested by Ceppellini et al., (1971) that maternal lymphocytes, collected at time of delivery, are often less responsive to the corresponding newborn lymphocytes than to histocompatibility antigens from unrelated adults. In a careful study, Birkeland and Kristoffersen (1977) have investigated cellular immunity during pregnancy using various lymphocyte tests and rosette techniques for the detection of T and B cells. No pregnancy-related changes were found in the numbers of T or B cells, no major changes in PHA, pokeweed mitogen, or MLC responses, but a reversible depression of the response to purified protein derivative in the second half of pregnancy.

Although the results of the studies so far published show some discrepancies, they clearly suggest that, although marked immunological depression of maternal lymphocytes with respect to the related fetal histocompatibility antigens cannot be demonstrated in human pregnancies, this immune deficiency is not essential to fetal survival. The lack of the ability to detect suppression of maternal immune responses to paternal alloantigens in pregnancy and the ability of mothers to respond to third-party allogeneic cells normally (Moen et al., 1980) suggest that a failure to express a damaging antifetal response would be more likely to be one affecting the central or efferent limb of the immune response. Recent observation of the increase in homozygosity at the HLA A and B locus in preeclamptic toxemia (PET) (Redman et al., 1978) and of HLA antigen sharing in couples with a maternal history of recurrent abortion (Taylor and Faulk, 1981; Beer, 1980) lend support to the idea that a vigorous maternal immune response may actually promote gestation. A balanced judgment might be that maternal immune responses against paternal antigens may improve placental function and therefore, indirectly, fetal health and survival.

## Immunological Disorders of Pregnancy

As far as the immunophysiology of human pregnancy is concerned, observations of various disorders of human pregnancy may yield information which has a bearing upon immunological processes in normal pregnancy. This approach is analogous to that originally advocated and followed by Good and Zak (1956) in the study of disorders of the immune system as probes for the analysis of the normal immune system. Some published information, for example, on PET, is contradictory and based upon alternative pathological processes.

### Spontaneous Abortion

Early studies suggested that abortion might be associated with heightened maternal antipaternal immune reactivity (Larsen and Galask, 1978), but it has been shown that a significant proportion of early abortions have abnormal genetic conformations with definite chromosomal abnormalities (Boué et al., 1974). The most intriguing recent work has shown that a proportion of those women who suffer recurrent unexplained abortion have an excess of shared HLA specificities with their husbands (Taylor and Faulk, 1981; Beer et al., 1982; Schachter et al., 1979; Komlos and Halbrecht, 1979). These data were obtained from patients in whom other definable causes of recurrent abortion, such as maternal anatomic abnormalities, had been excluded and in whom abortion had

occurred in the first trimester. Several groups have jumped to the conclusion that this must mean that such women are unable to make an appropriate immune response to paternal transplantation antigens and they were therefore given multiple blood transfusions (Taylor and Faulk, 1981) or white cell infusions from their husbands (Beer et al., 1982) with some reported success.

The possibility that a beneficial maternal anti-fetal immune response might be induced by blood or white cell transfusion has tempted several other groups to begin treatment. However, three crucial requirements need to be met in future studies: firstly, mothers who might be at risk from such treatment—for example, those with a genetically determined susceptibility to systemic lupus erythematosus—should be excluded; secondly, appropriate blind controls, such as the reinfusion of autologous maternal cells, must be included; and, thirdly, observation of the immune responses induced in such women should be undertaken.

Two theoretical problems remain at the moment: Such women have between a 25 and 50% chance of carrying a fourth pregnancy to term without any treatment (Warburton and Fraser, 1964; Tho et al., 1979), and there are alternative explanations, for example, the sharing of another gene or gene complex between mother and father, such as a human analog of the *t* complex (Schachter et al., 1979) in strong linkage disequilibrium with HLA. No doubt extensive clinical trials and other observations will clarify this issue, but the importance of transplantation antigens in human pregnancy has at least been emphasized.

### Toxemia of Pregnancy

Toxemia of pregnancy is present only when the pre-eclamptic toxemia (PET) is not arrested and the affected women convulse (Jenkins, 1974). Studies of PET have demonstrated an association between homozygosity at the HLA B locus and PET, and that in patients who are homozygous, PET is particularly severe (Redman et al., 1978). It is not clear, but it seems likely, that there is an association with homozygosity at the HLA DR locus, but at least these data make PET a unique disease as far as HLA association is concerned (Gerencer et al., 1978). The immunological implications of this finding are unknown, but it is possible that pregnant women may benefit from the possession of an HLA and B (or DR) antigen that the fetus does *not* possess, in order to mount an appropriate immune response (Komlos and Halbrecht, 1979). Provided that HLA sharing between mother and father is not too extensive, an initial pregnancy affected by PET could prime mothers against weaker, possible non-MHC antigens and reduce the severity of or abolish PET in subsequent pregnancies (Feeney, 1980). If exposure to paternal antigens is a factor in preventing the condition, then the increasing rarity of PET may be explained by the lengthening interval between first sexual experience and the increasing use of contraception, allowing women to become more thoroughly immunologically exposed to paternal antigens before conception.

### Hydatidiform Moles and Choriocarcinoma

The association between these two conditions lies in the greater likelihood that hydatidiform mole may progress to choriocarcinoma (Lawler et al., 1976). Moles are also associated with a higher risk of PET than normal pregnancy and recent observations show that such tumors are usually of reduplicated male origin; in other words, when they contain two X chromosomes, *both* are of male origin and, by banding studies,

all other pairs of chromosomes in these diploid tumors are identical. The speculation that PET might be causally associated with the excess of HLA sharing between mother and fetus therefore could be operating for PET in association with hydatidiform mole also. Several early studies showed that inbreeding and blood group antigens might be factors linked to an increased risk of choriocarcinoma. Speculation that this might reflect an inability of mothers to respond immunologically was shown to be unlikely when immune responses to such tumors were demonstrated (Bagshawe, 1974). It seems more probable that these associations underlie a basic link with MHC sharing between parents, although it is still possible that a different gene or gene cluster, as suggested earlier, lies at the root of the condition.

### Graft-Versus-Host Disease

Almost the only documented cases of neonatal graft-versus-host (GVH) disease have been in newborn infants suffering from primary immunodeficiency (Parkman et al., 1974). When large numbers of lymphocytes are passively injected into the human fetus, as a consequence of intrauterine blood transfusion in the treatment of severe RhD isoimmunization, although prolonged donor lymphocyte survival has been described, few instances of GVH have occured (Gill, 1977). Furthermore, the best present evidence does not support the transfer of significant numbers of immunocompetent maternal cells into the fetus during pregnancy. Acute transfer during labor may occur, since elliptocytes have been shown to cross (Hedenstedt and Naeslund, 1946) and abortive GVH may occur in the newborn, as the rash of urticaria neonatorum is very common and is histologically similar to the rash found in GVH.

Palm (1970) made detailed studies of the outcome of back-crosses between DA and BN strains of rats, which are identical at H-1 (the rat MHC), and found a high rate of fetal and neonatal loss, together with a neonatal GVH-like disease. She thought that possibly dissimilarity at the MHC might protect against GVH mediated against minor transplantation loci. This would be an extremely unusual situation in human populations, but it might occur in isolated communities, such as the Tuareg tribe studied by Degos et al. (1974). If successful first-trimester pregnancy requires some HLA differences, then neonatal GVH (directed against minor transplantation loci) will never be seen.

### Rhesus Isoimmunization

The transplacental transfer of IgG anti-RhD antibody from an $RhD^-$ mother to her $RhD^+$ fetus can lead to severe intrauterine hemolytic anemia. Exchange and intrauterine transfusion techniques and methods of assessing the severity of the disease have reduced morbidity and mortality (Bowman, 1978), but the passive injection of anti-RhD antibody into $RhD^-$ mothers immediately postpartum has almost completely abolished the disease (Goplerud, 1977). A few mothers make antibody in their first pregnancy, but it is not clear whether this represents a group of women primed by a previous gestation, by contact with a cross-reacting antigen, or at their own birth to an RhD mother (Stern, 1979). Present opinion has inclined against this last possibility on rather inadequate epidemiological grounds: This seems a pity, since it would be a simple matter to protect those few Rhd babies born to RhD mothers by the administration of anti-RhD antibody (Stern, 1979). In the context of this section, however, RhD isoimmunization represents the commonest naturally occurring condition in which a mother makes a harmful immune response directed against a fetal antigen: ABO

incompatibility and platelet isoimmunization are other examples. As mentioned earlier, these responses show that mothers are capable of potent antifetal responses, supporting either a selective failure of pregnant women to respond to specific fetal antigens or, more plausibly, the importance of effector cell impotence, probably as a consequence of placental interposition, as the principal agencies of fetal survival.

### Congenital Malformations

Terasaki and others (1970) have been unable to find any association between congenital malformations and maternal anti-HLA antibody. However, greater than normal titers of antibody against HLA antigens have been found with increased fetal malformation (Burke and Johansen, 1974), but claims that these maternal antibodies cause fetal malformations should be discounted. The placenta acts as an effective absorption filter for such antibody (Wegmann et al., 1978) and it is at least equally likely that higher titers of anti-HLA antibody are consequent upon fetal malformation and therefore abnormal pregnancy.

### Maternal Autoimmune Diseases

Certain maternal diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, and polymyositis, are associated with immune responses directed against self-antigens. The natural history of these conditions in pregnancy might be expected to shed light upon the nature of the maternofetal immunobiological relationship, since mother and fetus are semiallogeneic with respect to each other. The interpretation of these data is fraught with difficulties, since many of these patients are being treated with immunosuppressive drugs, but most information seems to support an amelioration of autoimmune disease during pregnancy (Larsen and Galask, 1978; Fröelich et al., 1980). In SLE, the majority of patients improve, but a small minority suffer a severe exacerbation of their symptoms during pregnancy, while most suffer a reversal in the postpartum period (Garsenstein et al., 1962). In rheumatoid arthritis, mild improvement is the rule (Persellin, 1976), whereas in pregnant women with polymyositis (V. Dubowitz, personal communication 1981) or glomerulonephritis, either no change or slight improvement occurs.

The mild general improvement seen in these conditions might suggest that some immunosuppressive factor or fetal suppressor cells or their products were entering the maternal circulation, but the observation that some women with SLE actually get worse when pregnant may indicate that a more specific dislocation of deleterious autoimmune processes may be taking place. The shedding of large amounts of trophoblast with attendant antigens into the maternal circulation (Goodfellow et al., 1976) could have profound effects on such maternal immune respones, but detailed analysis will have to await the identification of these antigens.

## Other Pregnancy-Specific Immune Phenomena

### Immunosuppressive Factors

During the last few years, many papers have also been published which claimed that proteins or hormones present in maternal sera have immunoregulatory functions (Table 7) (Kasakura, 1971; Jones, 1971; Jones et al., 1973; Von Schoultz et al., 1974; Murgita and Tomasi, 1975a,b). In several instances, the initial evidence of immunosuppressive activity of the investigated factor was not confirmed by other investigators. For

Table 7 Some of the Postulated Immunosuppressor Factors in Maternal Sera

| |
|---|
| Plasma proteins |
| α-fetoprotein |
| pregnancy-associated $\alpha_2$-glycoprotein |
| pregnancy-specific $\beta_1$-glycoprotein |
| $\alpha_1$-antitrypsin (increased levels) |
| Hormones |
| chorionic gonadotrophin |
| placental lactogen |
| cortisol |
| progesterone |
| other steroids |
| Immunoglobulins |
| "blocking" antibodies, Ig eluted from placenta |

example, the early reports of a suppressive role, in vitro, of human chorionic gonadotrophin (Beling and Weksler, 1974; Han, 1974) were not confirmed when more purified preparations in which the phenol present was removed were used (Gundert et al., 1975; Caldwell et al., 1975). Following the reports of Murgita and Tomasi (1975a,b), it is often mentioned in the literature that alpha-fetoprotein (AFP) is an important factor present in maternal serum with immunoregulatory functions. Yet many have been unable to observe the in vitro suppressive effect of purified human or mouse AFP (Sheppard et al., 1976, Adinolfi, 1981c). Furthermore, an in vitro suppressive effect of purified AFP has not been observed when fetal serum or maternal serum with high levels of AFP were investigated (Tomasi, 1978; Adinolfi, 1981c). An immunosuppressive function of murine or human AFP presupposes the presence of specific receptors on T cells for the fetal protein. Although an early report (Dattwyler et al., 1975) claimed that such receptors were present in about 20% of spleen cells from adult mice, further investigations have not confirmed these findings (Adinolfi, 1982). Thus spleen cells from adult mice incubated with purified AFP labeled with $^{125}I$ do not absorb the fetal protein on their surface. Furthermore, spleen cells incubated with AFP or collected from pregnant mice do not react with antisera against AFP. $\alpha_2$-Pregnancy-associated glycoprotein ($\alpha_2$-PAG) has been claimed to play an important role in the materno-fetal relation (Beckman et al., 1974); $\alpha_2$-PAG can suppress an MLR at concentrations well below those found during pregnancy (Horne et al., 1978), but the immunological significance of this is unclear. Levels of $\alpha_2$-PAG are also raised in Hodgkin's disease and in leukemia in both males and females (Thomson and Horne, 1980), so that could be a secondary effect (Stimson, 1977; Horne and Nisbet, 1979).

## Blocking Factors

The Hellström hypothesis that the production of maternal blocking antibody might act as a protective mechanism for the fetal mouse (Hellström and Hellström, 1974) has inspired many searches for similar systems in other animal models and in man. The methodology from Hellström's original paper does not exclude other explanations, such as the presence of murine $\alpha_2$-PAG, but it is possible to demonstrate that pregnant animals can make antibody which will sometimes block T-lymphocyte-mediated cytotoxicity without a concomitant increase in antibody-dependent cytotoxicity. Although

a role for such a mechanism in early pregnancy could be of critical importance, possibly at the time of the development of the placenta, it is already clear that later in gestation blocking antibody will be one factor only in the immune response of pregnancy.

### Nonresponsiveness to Self-Antigens

The failure of normal individuals to make immune responses against self-antigens, contrasting with the occasional development of autoimmune reactions, has been the subject of a new hypothesis with each new immunological discovery. Clonal deletion, immunosuppressive factors, antibody blockade, T suppressor cells, thymic education of self- and bacterial cross-reactivity have all been presented as self-sufficient mechanisms of self-tolerance. It is worth remembering that there does not appear to be a time during fetal development when a damaging autoimmune response appears, so that explanations of self-tolerance should encompass fetal life. Mitchison (1978) has proposed a convincing model, which he has called "immunological silence." His suggestion has the merit that it includes those previous ideas in their logical places. He stated that, for unknown reasons, Ia antigens do not normally lie in association with self-antigens on the cell membrane, but that the intervention of environmental factors, such as viruses or chemicals, may cause this to occur and thus trigger an autoimmune response. This "core" mechanism is bolstered by a successive "mantle" and "crust" of T- and B-cell-idiotypic networks, respectively. Mitchison believed that the likely role of the T suppressor cell is to "damp down" a developing autoimmune reaction. His hypothesis agrees with our present knowledge and could apply to fetal as well as extrauterine life.

### The Nature of the Maternofetal Immune Interaction

After implantation, antigens present on fetal cells are exposed; implantation itself shows some of the features of delayed hypersensitivity (Marcus and Shelesnyak, 1968): Local immunization can lead to changes in the effective fertility of a single uterine horn in the rat (Beer and Billingham, 1971); but it is the maternal immune response in postimplantation pregnancy, long lasting in man, with which we are primarily concerned. Evidence presented here suggests that in outbred populations like man, maternal immune responses against both fetal and paternal antigens *are* induced and that these responses include the production of both humoral and cell-mediated effector cells. It has been pointed out that although the injection of specifically immune lymphocytes can lead to the rejection of intrauterine skin grafts which have healed, in the presence of a fetus such skin grafts do not in themselves induce a transplantation reaction (Padykula, 1976; Beer and Billingham, 1974, 1976). The possibility that the decidua is incapable of allowing the transfer of antigen to the mother, blocking the afferent limb of the immune response, has been suggested, notwithstanding the evidence already given here which shows that mothers make antifetal immune responses during pregnancy. It is possible, however, that the decidua might have such a function prior to placental development. Furthermore, a surge in the production of human chorionic gonadotrophin (Siiteri et al., 1977) coincides with an increase of up to 35-fold in decidual progesterone receptors at the time of implantation (M. Young, personal communication, 1981). These events may be related, not only to the efficient nutrition of the

the early embryo, but also to its immunological protection. However, although there is good evidence for the passage of trophoblast and other fetal antigens into the maternal circulation, there is little evidence to indicate that maternal immunocompetent cells enter the fetus.

Another important immunoregulatory aspect to be considered is the role played by the T suppressor fetal cells (Adinolfi, 1975, 1982). The presence in fetal blood of lymphocytes which can abrogate the in vitro proliferation of lymphocytes from mothers as well as normal adults was initially suggested by Olding and collaborators (Olding et al., 1974, Olding and Oldstone, 1974; Olding, 1978), following the observation that if an artificial mixture of maternal and cord lymphocytes is incubated in the presence of PHA, the majority of the dividing cells are of fetal origin. Dividing fetal cells also predominated (88-92%) in cocultures comprising lymphocytes from nonpregnant women and adult males. On the other hand, lymphocytes from one newborn did not suppress the division of lymphocytes from another baby (Olding, 1978), thus suggesting that while adult lymphocytes have receptors for the suppressive agent released by the fetal lymphocytes, the fetal cells lack it.

In a separate series of experiments, it was shown that the suppression of mitosis of adult lymphocytes by the fetal cells was not due to a cytotoxic effect (Olding, 1978). Other fetal cells besides lymphocytes have no ability to abrogate the proliferation of adult lymphocytes.

Further studies also demonstrated that whereas enriched T cells from the newborn blood strongly suppressed the maternal lymphocytes, the newborn B lymphocytes were not endowed with this property.

Evidence was also obtained suggesting that the abrogation of maternal lymphocyte proliferation was mediated by a soluble low molecular weight substance released by the activated lymphocytes from the newborns (Olding et al., 1977; Olding, 1978).

Some of these results have been confirmed by other investigators in humans (Lawler et al., 1975; Gille et al., 1977) and in mice (Wallis et al., 1976; Mosier et al., 1977).

It has not yet been demonstrated that the mitogenic suppression exhibited by fetal lymphocytes in vitro, using cocultures and PHA stimulation, operates also in vivo, nor is there full agreement about the type of T cells (T$\gamma$ or T$\mu$) endowed with this property. The physiological role of these suppressor cells is therefore still uncertain and whether the suppressor molecules cross the human placenta remains to be established. It has been suggested that one function is to inhibit the few maternal lymphocytes that may have crossed the placenta from mounting an immune response against the fetus (Olding, 1978). However, neither in man nor in mouse are T-deficient fetuses immunologically rejected by the mother. On the other hand, the restriction of neonatal GVH to a few usually immunodeficient infants and the presence of this type of fetal lymphocyte capable of suppressing the MLR maternal lymphocytes suggests that if such maternal effector cells were to cross the placenta, they would be unlikely to have any pathological effect and would be extremely hard to find. In any event, as mentioned earlier, donor lymphocytes acquired during intrauterine transfusion do not usually cause GVH. Consequently, although it is conceivable that maternal lymphocytes cross into the fetus, it is likely that this occurs rarely and at delivery. It seems that the placenta plays an important role in maternofetal immunobiological relationships by its interposition between mother and fetus (Billington, 1975b; Kirby et al., 1964). The most important plane is the outer syncytiotrophoblast membrane, together with the outer surface of the amnion, the former being in

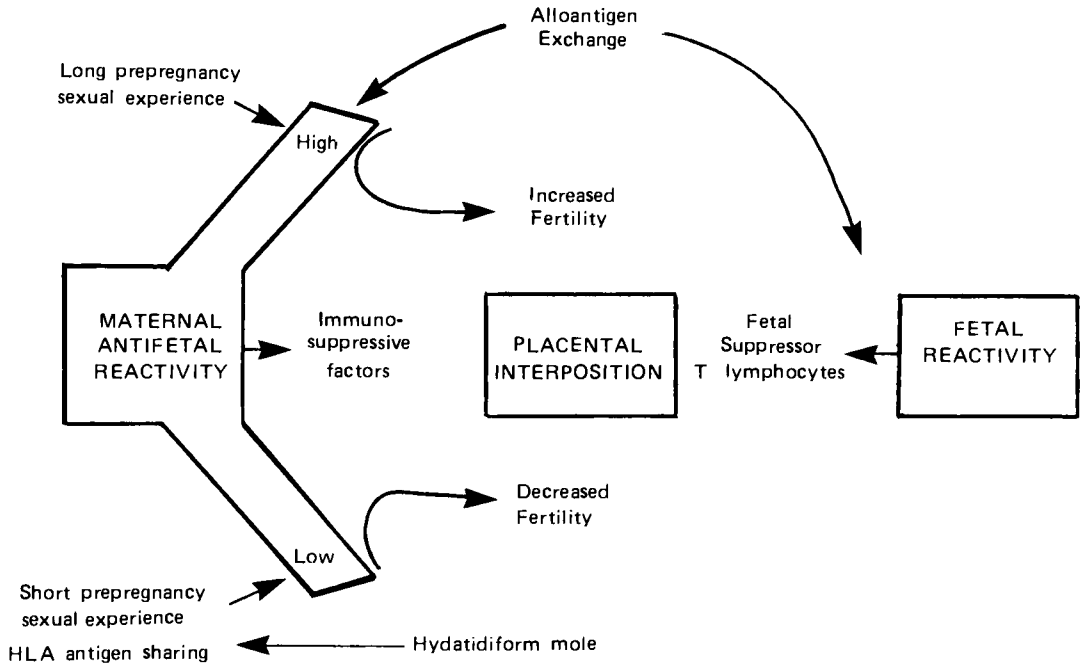


**Figure 9** Immunological interactions in pregnancy (see text).

contact with maternal blood lakes (Billington, 1975a). The syncytiotrophoblast membranes possess many frequently shed microvilli: the presence of these microvilli in the maternal circulation must help to protect the placenta from attack. There are several integral antigens which are presently under study, as described earlier, including paternal HLA antigens, but the distribution of this sparse HLA antigen is such that no effective immune response is likely to damage them. Villous stroma forms a thick layer between maternal and fetal blood, which must be crossed by any molecule or cell on its way to the fetus (Kaufmann et al., 1979). It contains large amounts of HLA antigen and can therefore "mop up" the anti-HLA antibody produced by some mothers (Wegmann et al., 1978). Large quantities of immune complexes and complement can be detected in villous stroma by immunofluorescence (Sundqvist et al., 1977), so other fetal antigens may be present, against which maternal immune responses might be mounted, allowing the stroma to act as a sink, soaking up unwanted maternal anti-fetal responses (Baines et al., 1976).

A current view of the nature of the immunological relationship between mother and fetus is shown in Figure 9, which summarizes the data presented here.

## The Genetics of Reproduction

In this section it has been suggested, either directly or by implication, that the maternal immune response is normal in pregnancy, in that it is capable of expressing activity against paternal and fetal antigens. Furthermore, there are hints that although an anti-fetal immune response is not a requirement for successful gestation, there is a direct correlation between the vigor of the immune response and fertility. This has been supported by the observation that when mother and father (and therefore fetus) share a

greater-than-normal number of HLA determinants, there is greater fetal morbidity and mortality, either with PET or by recurrent abortion.

Fertility declines markedly during the selective breeding of an inbred line from an outbred strain ("inbreeding depression"). Fertility increases again as syngeneity is established, but it always remains less than that of the original strain. The opposite phenomenon is the increase in fertility seen when two inbred lines are mated to produce an F1 generation: This is known as "heterosis" or hybrid vigor. It is not unreasonable to suggest that rather than seeing an ill-defined effect mediated by multiple genetic interaction, heterosis and inbreeding depression are the consequences, in animal models, of selection for or against allogeneity for genes of the MHC. This is not a new concept, it was originally proposed, on a slightly different basis, by Clarke and Kirby (1966). The *T/t* complex, originally discovered by Dobrovolskaia-Zavadskaia in the mouse and studied in detail by Dunn and Bennett (Klein and Hammerberg, 1977), has its principal effects upon the development of the fetal murine central nervous system. Homozygosity for lethal alleles of *t* have a profound effect upon fertility, because they lead to fetal death in utero at a stage dependant upon the *t* complementation group to which they belong. In addition, a particular *t* allele is always linked to the same H-2 haplotype, both in laboratory colonies of mice and in the wild. "New" *t* alleles are generated at a remarkable rate—roughly 1% of all identified—and, by using a cross-reactive antiserum, anti-F-9, which detects the normal allele of *t*, the gene product of *t* has been found on human cells of the male germ line (Jacob, 1977).

It can be argued that the genetic effect of the *T/t* complex is to strongly discourage homozygosity and to encourage heterozygosity and that it does so by means of its effects upon gestation. With regard to the overwhelming linkage disequilibrium it shares with H-2, if such a system were operating in man, the previously described association with infertility would be observed. Although certain defined HLA specificities are associated with and predisposed toward particular diseases, and are likely to have a causal role through the agency of immune response genes, both the polymorphism of HLA and the low population frequencies of disease-linked HLA specificities could be explained by the direct control of human *T/t* analog complex and its effect upon gestation. Should, in the near future, either this or an analogous model be unearthed, we shall be closer to an understanding of the selective processes which allow the preservation of genetic polymorphisms in general and of the immune response of pregnancy in particular.

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# 4

# Endocrine Pancreas of the Pregnant Mother, Fetus, and Newborn

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## THE MATERNAL ENDOCRINE PANCREAS

Throughout pregnancy the maternal endocrine pancreas must adapt to fulfill the metabolic requirements of the developing conceptus. The constant drain of nutrients across the placenta represents an ever-increasing challenge to maternal homeostatic mechanisms. However, during normal pregnancy, the maternal environment is kept relatively constant and the nutritional needs of the fetus are met without requiring any adaptation of the fetal secretory mechanisms prior to birth. The major metabolic substrate of the growing fetus is glucose, which crosses the placenta by facilitated diffusion (Howard and Krantz, 1967). Fetal blood glucose concentrations parallel those of the mother, whether the latter are basal levels or artificially elevated by a constant infusion or acute injection of glucose. A maternofetal gradient is, however, maintained in normal pregnancies (Patterson et al., 1967; Raivio and Teramo, 1968; Schwartz, 1968; Adam et al., 1969; Obenshain et al., 1970). The developing fetus is therefore dependent on a well-controlled maternal blood sugar level for a steady supply of glucose (de Gasparo and Hoet, 1971) by the way of a maternal glucose production which is increased by 16% during a normal pregnancy (Kalhan et al., 1979). Other nutrients of the maternal blood are also drained constantly. In general, the birth weight of infants of gestational diabetic mothers is correlated with maternal fasting plasma glucose, plasma triglycerides, and plasma alanine, serine, valine, isoleucine, and glycine (Freinkel and Metzger, 1979). It may be concluded that, besides glucose, other insulin-dependent fuels in the normal mother are important for the growing fetus and are likely to be disturbed by diabetes. This state of events may be determined by the growth of the fetal pancreas (de Gasparo et al., 1978), as well as by the somatic growth of the fetus.

Carbohydrate metabolism is challenged in all women during pregnancy. There are many reports showing that glucose tolerance tested by either an intravenous or oral load is significantly altered with advancing pregnancy (Burt, 1962; Picard et al., 1968; O'Sullivan et al., 1970; Campbell et al., 1971; Lind et al., 1973). There is no evidence to support the view that marked variation in the maternal blood sugar and hyperglycemia is of any benefit to the fetus–in fact, all the evidence is to the contrary. A group of normal mothers with no suggestion of a diabetic tendency was studied by

serial glucose determinations over a 24-hr period and a 3-hr oral glucose tolerance test. These women were shown to have no significant alteration in the diurnal profiles of plasma glucose concentrations with advancing pregnancy (Gillmer et al., 1975). The only indication of any glucose intolerance was a depression of preprandial levels and an elevation of postprandial levels, which has been confirmed by Freinkel (1980). From these results it can be concluded that the majority of women have a sufficient margin of glucose homeostasis to maintain stable blood sugar levels throughout pregnancy. However, some women with normal blood sugar concentrations when nonpregnant develop a variable degree of intolerance that deteriorates with advancing pregnancy. The reasons for the development of this intolerance will be discussed below.

There is general agreement that the basal insulin release and the insulin secretion challenged by a meal or a glucose load are greatly increased during pregnancy. This hypersecretion of insulin increases progressively from early to late pregnancy (Leake and Burt, 1962; Spellacy and Goetz, 1963, Bleicher et al., 1964; Spellacy, 1971); in addition, insulin release will be reduced quickly during fasting. Changes in the second pancreatic hormone, glucagon, may also occur as a result of pregnancy. Glucagon concentrations will remain normal in the fasting state and will level off quickly after a meal. This dual adaptation of the maternal endocrine pancreas induces a facilitated anabolism whereby insulin will promote retention and storage of nutrients when the mother feeds herself. An accelerated catabolism is also created by which the constant drain of nutrients to the fetus will be maintained (Freinkel and Metzger, 1979). This functional adaptation of the maternal B and A cells is consistent with the heightened activity of the islets of Langerhans observed in pregnant women. The pancreases from 15 pregnant women have been studied by Rosenlöcher (1932), and an additional 5 by Van Assche et al. (1978).

In addition to the observed changes in B and A cells, there is an increase in the size and the number of blood vessels which are concentrated in the endocrine tissue of the pancreas. These features suggest an enhanced blood flow with an increased hormonal output (Van Assche and Aerts, 1975). The increased amount of islet cell tissue that occurs during pregnancy is associated with an increased absolute number of glucagon A cells, but no biological or histological information is available concerning the gut glucagon cells during pregnancy. The ratio of insulin cells to glucagon cells remains in favor of the insulin cells, thereby enhancing inhibition of glucagon secretion, which in turn would favor the insulin-induced retention and storage of dietary nutrients (Freinkel et al., 1974). The hyperinsulinism of pregnant women is consistent with the heightened activity of the B cell that has been observed in animal studies. In the rat, the endocrine pancreas of pregnancy shows significant hypertrophy of the islets of Langerhans. Both the total amount of endocrine tissue and the relative number of B cells per islet are increased (Hellman, 1960; Van Assche, 1974). The higher insulin levels during pregnancy are related to the increased islet size and the increased number of B cells (Saudek, et al., 1975).

Ultrastructural analysis on the individual B cells shows precise features of hyperactivity during pregnancy associated with increased secretion of insulin (Van Assche et al., 1979). The increased percentage of light beta granules and the increased volume of mitochondria indicate in addition an increased secretory capacity of the individual B cells, although not of the A cells (Aerts and Van Assche, 1975). The other cell types seem to undergo changes also. In the rat the distribution of pancreatic polypeptide (PP) and glucagon cells, which is unequal between the head and the tail, tends to disappear during a normal

pregnancy. The number of somatostatin cells, however, tends to diminish during normal pregnancy (Van Assche et al., 1980).

The enhanced secretory capacity of the B cell during normal pregnancy is confirmed by in vitro studies. Glucose, arginine, and theophylline are effective stimulants of insulin secretion at lower concentrations during pregnancy than in the nonpregnant state. Glucagon and leucine, however, are ineffective in increasing the insulin release from the islets of pregnant rats (Green and Taylor, 1972). The failure of glucagon to stimulate insulin secretion could be because the adenylcyclase activity in the B cell is already maximal and cannot be stimulated further (Malaisse et al., 1967). The heightened sensitivity of the membrane receptor for glucose may also be attributed to increased adenylcyclase activity (Matchinsky et al., 1971). Studies in the rat have revealed an increased glucose-insulin flux in the rat islet during pregnancy. This may be responsible for the facilitated inhibition of glucagon secretion and the lower basal blood sugar levels that have been observed in human pregnancy (Saudek et al., 1975).

Many factors are implicated in the metabolic changes and structural modifications of the endocrine pancreas as a consequence of pregnancy. Human placental lactogen (HPL) is released in vast quantities from the placenta into the maternal circulation as pregnancy proceeds (Spellacy, 1969). It has metabolic actions similar to those reported for human growth hormone and prolactin. All three hormones stimulate lipolysis and thus elevate plasma free fatty acid (FFA) concentrations. This lipolytic action could result in a resistance to the action of insulin in peripheral tissues and be indirectly responsible for the hyperinsulinism of pregnancy. However, studies on insulin-dependent diabetics during pregnancy have failed to demonstrate any correlation between the insulin requirement and the serum HPL concentration (Spellacy and Cohn, 1973). These observations suggest that the metabolic changes of pregnancy cannot be attributed solely to HPL and the involvement of other factors must be considered. Human growth hormone is suppressed during pregnancy, probably as a consequence of the HPL-induced elevation of FFA, and can therefore be excluded from consideration.

As previously stated, the inhibition of glucagon secretion by glucose is enhanced during pregnancy. Changes in this hormone therefore cannot account for the observed alterations in the peripheral actions of insulin. Although early studies reported greatly elevated levels of plasma glucocorticoids during pregnancy, subsequent studies have shown that this is mainly due to the increase in bound cortisol, which is biologically inactive. Further investigations designed to measure the levels of biologically active unbound corticoids indicate a small but significant increase in their circulating levels (Doe et al., 1969). Even this modest rise in cortisol is likely to have metabolic conseuqences similar to those attributed above to HPL.

There is evidence that steroids, such as estrogen and progesterone, may cause an insulin resistance and as a result elevate both plasma glucose and insulin concentrations in the pregnant female (Kalkhoff et al., 1975). Investigations on the metabolism of human placental tissue in vitro and in vivo have revealed an ability to remove insulin from the circulation and degrade it (Freinkel and Goodner, 1960). A continual breakdown of insulin such as this could bring about a compensatory secretion of more insulin from the maternal pancreas. Such a mechanism might therefore account for an increased secretion of insulin, but would not necessarily affect the basal plasma glucose concentration. An alternative, though unproven, explanation for the apparent insulin "resistance" of pregnancy may be that the insulin secreted is in some way less effective than in the nonpregnant state, also, changes in insulin receptors and a reduced insulin

effect on selected tissue may be held responsible for the different response. The observed decreased insulin binding to monocytes from normal pregnant women might be related to hormonal changes or may also be due to the weight increase which occurs during pregnancy (Beck-Nielsen et al., 1979).

However, the alterations of the function of the gastrointestinal system due to the pregnant state have to be taken into consideration. Several hormonal and nutritional changes occur during pregnancy which may interact to produce increased gastrointestinal growth and secretion. Specific changes in the gastrointestinal tract due to pregnancy do occur. The weight, mucosal volume, and peptic and parietal cell populations of the stroma increase steadily during pregnancy to a peak before the maximal increase in food intake, and then decline as food intake increases. The anatomic changes were attributed to the effect of work hypertrophy, but also to an observed gastrin release. The gastrointestinal growth and the gastrin levels are pituitary dependent in normal mice. Prolactin will promote growth of the duodenal mucosa (Lichtenberger and Barkte, 1979). During pregnancy, prolactin increases and is conditioned by the fed and fasting states (Reusens et al., 1979). In addition, active transport of glucose across the intestine increases during pregnancy in the rat (Larralde et al., 1966). It is therefore possible that the increase in insulin response during pregnancy is largely due to the more rapid entry of nutrients into the blood circulation following a meal. However, the regulation of the insulin secretory response is known to be dependent on gastrointestinal hormones. Further work is needed on the hormonal adaptation of the enteropancreatic axis to pregnancy to elucidate a possible role for the gastrointestinal tract in this respect.

Failure of the maternal pancreas to adapt to the homeostatic metabolism of pregnancy results in clinical or gestational diabetes. In gestational diabetes, low insulin levels after a glucose challenge or during a daily profile are associated with slightly increased blood glucose levels (de Gasparo et al., 1969a; Gillmer et al., 1975). Studies in pregnant rats with experimental diabetes have shown that the endocrine pancreas is no longer able to meet the increased insulin requirements of pregnancy (Van Assche et al., 1979). The lower percentage of insulin B cells in pregnant diabetic rats compared with pregnant nondiabetic rats is not unexpected.

The percentage of other cell types is increased. The increase is relative for the PP cells and the somatostatin cells, whereas it is absolute for the glucagon cells. The decreased ratio of the amount of insulin cells to that of glucagon cells fits with the catabolic condition of severe ketotic diabetes and pregnancy. Indeed, the consequences of the catabolic condition of the mother may often be fetal growth retardation (Van Assche et al., 1980). The relation of the latter to maternal adaptation to pregnancy requires further investigation. However, failure of the maternal control mechanisms may be hazardous to the unborn child, as work in sheep has demonstrated that hyperglycemia may be fatal to the slightly hypoxic fetus (Shelley et al., 1975). It has also been suggested by Beischer et al. (1977) that the glucose homeostasis of the mother fails in relation with intrauterine growth retardation. In addition, the basic defect of this condition may be situated at the prostacyclin level (J. Vermylen and F. A. Van Assche, unpublished data 1981), which may be related to vascular complications of maturity onset diabetes (D. Pyke, personal communication 1980).

Nevertheless, it seems reasonable to state that the hyperglycemia and associated metabolic changes of pregnant women, whose insulin secretion fails to adapt and in whom the insulin resistance is not adequately overcome, will prematurely challenge or alter the maturation of the secretory mechanisms of the fetal endocrine pancreas.

## THE FETAL ENTEROPANCREATIC AXIS

### Gastrointestinal Endocrine Cell System

The ontogeny of the largest endocrine gland of the body—the gastrointestinal cell system—is only partially understood and too little attention has been paid to this system during fetal life (Larsson et al., 1977). Gastrointestinal endocrine cells (gastrin, secretin) develop very early in human fetuses (week #10) and rat fetuses (day 16) and even before a definite intestinal lumen is established. This suggests that these endocrine cells participate in the regulation of growth and morphogenesis of the gastrointestinal tract (Larsson et al., 1977; Larsson, 1980). These endocrine cell types are very numerous during fetal and early neonatal life and in certain areas (antrum and duodenum) they are even much more frequently present during fetal life than during adult life (Larsson and Jorgensen, 1978; Larsson et al., 1976).

Certain endocrine cells (glucagon, somatostatin, PP) have a permanent scattered distribution in the gastroduodenopancreatic region; for other hormones this dispersed distribution is transitory, occurring during a restricted fetal period. This is the case for gastrin in the rat fetus (Larsson et al., 1977). Some fetal duodenal endocrine cells [gastrin, cholecystokinin (CCK), secretin] have the capacity to store and probably synthesize several distinct biologically active molecules, of which, generally, only one remains in adult life. This indicates that the endocrine cells of the fetal duodenum do not form a differentiated cell population. It is also evident that trans- or redifferentiation (gastrin-cholecystokinin) exists in fetal duodenal endocrine cells (Larsson and Jorgensen, 1978). Fetal (rat and human) gastrointestinal endocrine cells (gastrin, CCK, and secretin cells) produce 5-hydroxytryptamine, which is not the case in adults. These findings suggest that the fetal endocrine cells in the gastrointestinal tract may be vital to the development and differentiation of the gastrointestinal tract and the endocrine pancreas (Larsson, 1980). This concept has already been put forward by Johnson (1976). The recently discovered C-terminal gastrin-CCK tetrapeptide seems to stimulate the endocrine pancreas. Gastrin cells produce peptides other than gastrin, which may have an effect on the function and growth of the gastrointestinal system and of the B cell.

### The Fetal Endocrine Pancreas

#### Structural Development

Various histological staining techniques have been used to differentiate the various cell types of the endocrine pancreas. The histochemical basis of this technique is still obscure. Better classification can be obtained with the aid of electron microscopy and immunocytochemistry. Differentiated B cells have been observed in the human fetus as early as 10 weeks of gestation, and A cells slightly earlier, from the ninth week (Like and Orci, 1972). Later in fetal life (16 weeks) four hormone-containing cells can be recognized in the human endocrine pancreas: insulin B cells, glucagon A cells, somatostatin D cells, and PP cells (Van Assche et al., 1976) (Figure 1).

In the human fetus at least one other cell type is present: This cell type is usually located close to nerve fibers (Aerts et al., 1980). This was already observed by Laguesse (1906), who indicated that the nervous system closely follows the distribution of the vascular bed into the endocrine pancreas and becomes independent in the islet in the form of small ganglia. It is more developed in the early phases of fetal life and tends to degenerate progressively. In the rat fetal endocrine pancreas, gastrin cells are

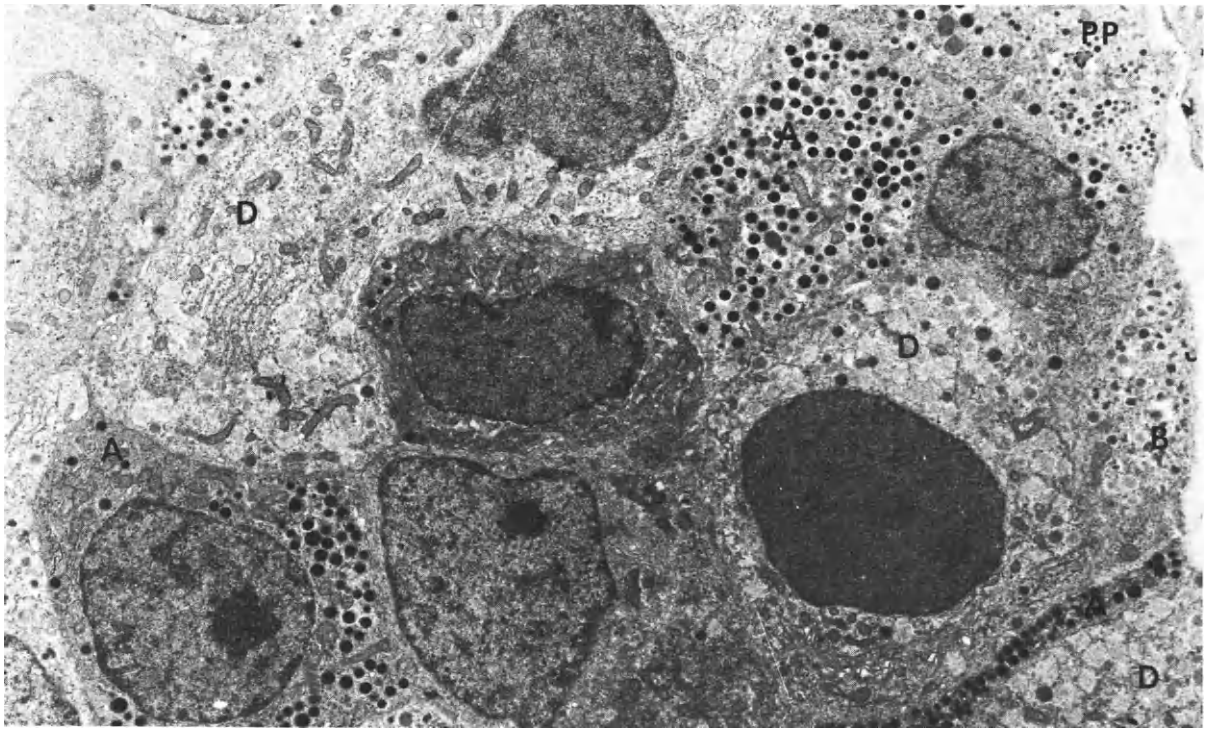



**Figure 1** Endocrine cells in the islet of a human fetus at 24 weeks of gestation: B, A, D, and PP cells (X 5400).

observed (Larsson et al., 1977), but not in the human fetal endocrine pancreas analyzed from 16 weeks until term (Aerts et al., 1980). The different cells are located in mantle islets, (B cells in the center), scattered in the exocrine parenchyma, or situated in small buds originating from the epithelium of minute ducts (Van Assche et al., 1976; Larsson et al., 1977). This was confirmed by an in vitro observation indicating that the primary pancreatic ducts cells can differentiate in a B cell and presumably in other endocrine cells (Dudek et al., 1980). Orci et al. (1976) have reported in the normal human fetus, as well as in fetuses of other species, an irregular distribution of the PP and glucagon cells, the proportion of PP cells being higher in the islets of the head of the pancreas than in the islets of the tail, whereas the reverse distribution is the case for glucagon cells. In addition, a PP-rich zone is present in the dorsal part of the head of the pancreas. The variability of the islet population has been confirmed, but its functional significance is not known as yet (Rahier et al., 1981) (Figure 2).

### Functional Development

Insulin is present in the human fetal pancreas from 8 to 9 weeks of fetal life, indicating that synthesis and storage of the hormone begins at an early stage in intrauterine life (Adam et al., 1969; Grillo and Shima, 1966). Concentration in the pancreas increases with advancing age. Fetuses of fairly well controlled insulin-dependent diabetics have greater pancreatic insulin concentrations than control fetuses of comparable gestational age (Steinke and Driscoll, 1965; Rastogi et al., 1970). Insulin has been measured in fetal plasma from the twelfth week of gestation (Adam et al., 1969; Obenshain et al., 1970). This insulin is entirely of fetal origin, since the fetus is impermeable to insulin or the amount transferred is physiologically insignificant (Adam et al., 1969).

More information is available on the fetal pancreas in the rat. Insulin secretion occurs throughout intrauterine life, but it is debatable if the B cell is functionally mature enough to increase its secretion above a basal level at the time insulin first appears in the cell. It is not known if the endocrine cells which appear transiently in the gastrointestinal tract have a secretory function beyond basal hormonal secretion during fetal life.

Basal insulin secretion in the normal fetus is unresponsive to secretagogue challenge early in fetal life. The fetal endocrine rat pancreas does not adapt its insulin secretion to a glucose load given to the mother or to a fast imposed on the mother at 19.5 days, but by 21.5 days it responds appropriately, suggesting rapid maturation of the system (B. Reusens and J. J. Hoet, unpublished observations, 1980). The role of amino acids in the growth of the beta cells, insulin accumulation, and the precocious development of the glucose secretory response has been determined by in vitro studies. In particular, amino acids stimulate the growth of the B cells, whereas glucose may become the trigger for insulin secretion by the fetal B cell of the rat, fully differentiated at 20 days (de Gasparo et al., 1978). This is the time that the gastrointestinal tract has matured and that the villi and the brush border cells which are responsible for the absorptive processes are fully developed (Hoet and Reusens, 1976; Reusens et al., 1980) (Table 1 and Figure 3). In the gut of the chick, glucose transport, uptake, and utilization appear to be inactive up to 3 days before hatching, but achieve maximal capacity just before hatching. Active sugar transport in anaerobic conditions is a feature of the intestine of newborn of other species too. It therefore seems likely that the gastrointestinal tract already acquires an absorptive function for glucose shortly before birth (Bogner et al., 1966; Hoet and Reusens, 1976). This is also true for other nutrients.

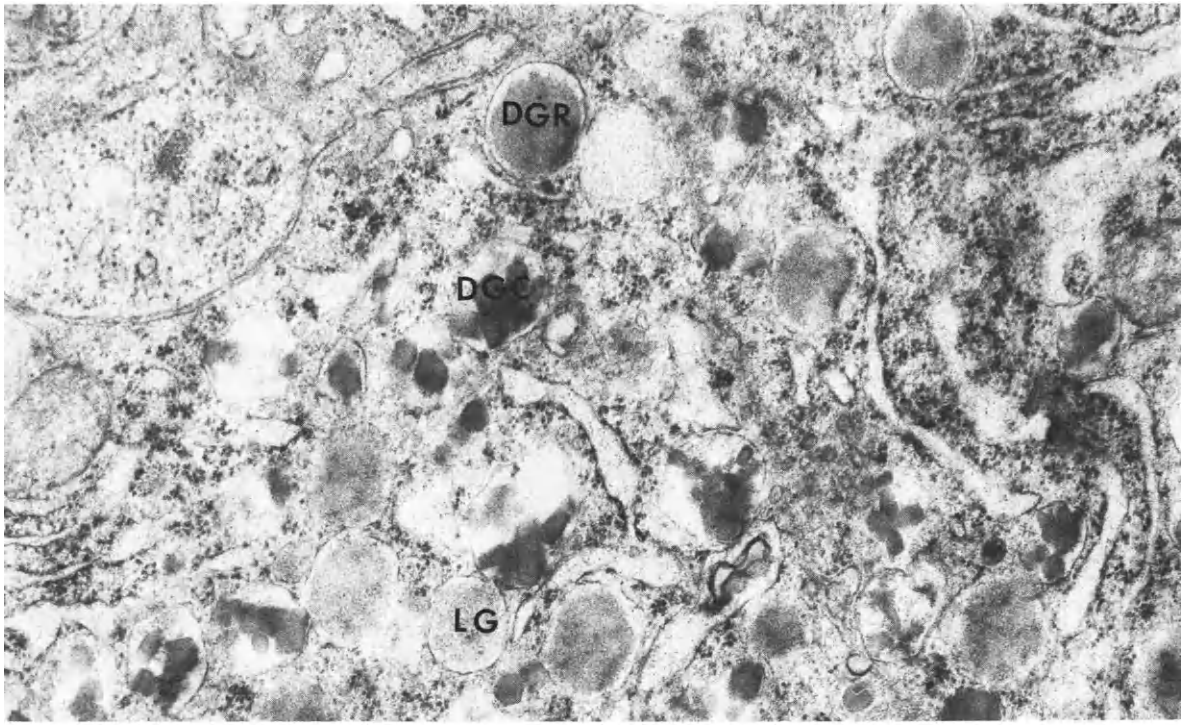
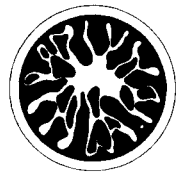
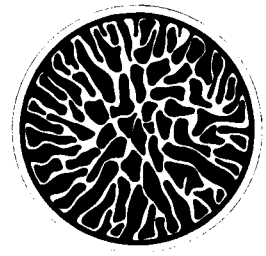
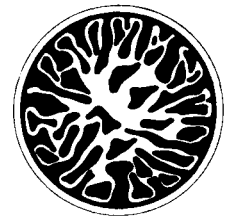


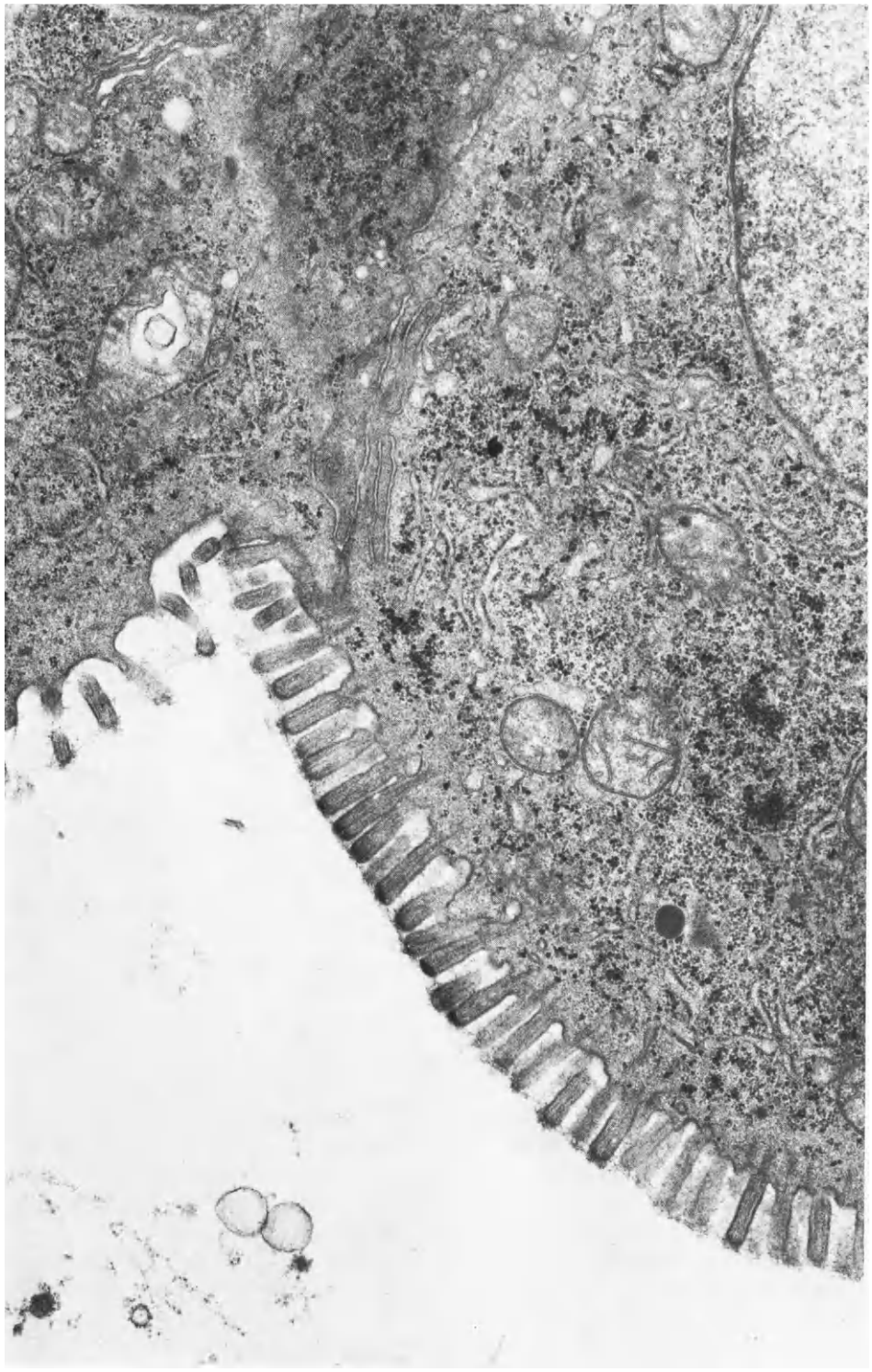

**Figure 2** Part of a B cell in a human fetus at 20 weeks of gestation. Note tl ;ranules with several electron-dense crystalline inclusions (DGC), dark granules with one large round electron-dense core (DGR) surrounded by a white halo, and light or pale granules (LG) with a more electron-lucent amorphous content (X 28,500).

Table 1 Schematic Representation of the Development of the Fetal Rat Duodenum, Jejunum, and Ileum

| | Gestational age (days) | | |
|---|---|---|---|
| | 17.5 | 19.5 | 21.5 |
| Duodenum |  |  |  |
| Jejunum |  |  |  |
| Ileum |  |  |  |

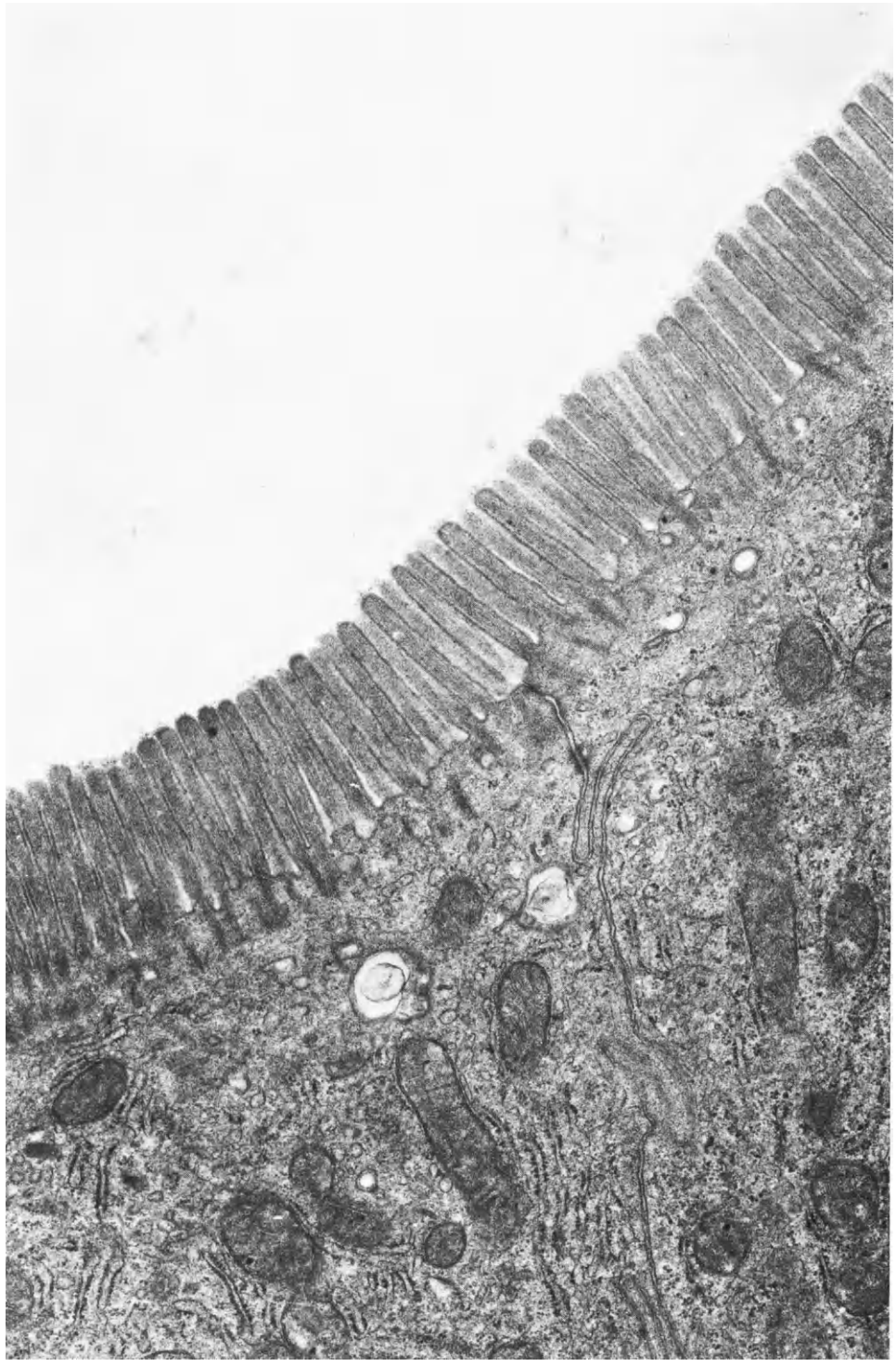
*Source*: B. Reusens, C. Remacle, and J. J. Hoet, unpublished results, 1981.

The assimilation of fructose by the gastrointestinal tract of the lamb increases as its function matures with increasing gestational age. Fructose absorption through the small intestine increases from 10% in 1 hr at 100 days to 40% in 1 hr at 107 days; in the colon, it goes from 10 to 47% at the same ages. Water absorption increases from 120 ml/day at 100 days to 1000 ml/day at 110 days (Wright and Nixon, 1961). The absorption of amino acids becomes apparent only after the twentieth day of fetal life in the rat (Donnelly, 1971). The absorption of hemoglobulins by the gut at birth is very specific for each species. The rabbit loses this characteristic by 24 days of fetal life, ungulates at 2 days, and murines at 20 days of extrauterine life. Immunoglobulins may reach the stomach via the amniotic fluid, but it is not known at what time during fetal life this absorption is initiated (Brambell et al., 1951). These observations support the specific role of the absorptive function of the gastrointestinal tract, which together with the maturation of the endocrine system of the fetal pancreas may prepare the B cell for its specific secretory function at the time of birth. The maturation of this complex system of the enteroinsulin axis may be the limiting factor to the very early development of hyperinsulinism, which may be observed during the latter stage of fetal life in the rat (Hoet and Reusens, 1976). However, an insulinogenic placental factor has been postulated to produce the hyperinsulinism which may be inducable only after maturation of the complex system (Sodoyez-Goffaux et al., 1981).



(A)

Figure 3 Maturation of the epithelial cells in the rat duodenum in relation to fetal age, with increase in density and length of the microvilli. (A) Fetal age 19.5 days. (Reusens et al., 1980).



(B)

**Figure 3** Rat duodenum. (B) Fetal age 21.5 days. (Reusens et al., 1980.)

In the human fetus, the histological maturation of the digestive system is precocious and has already occurred by the third month. Nevertheless the development of the epithelial and the endocrine cells of the system is progressive throughout fetal life (Moxey and Trier, 1978). The gastrointestinal hormones motolin, gastric inhibitory polypeptide, pancreatic polypeptide, pancreatic glucagon, neurotensin, and enteroglucagon are present in fetal blood in the third trimester and they may be elevated in the case of stress. Gastrin and secretin are also present, but do not increase in these conditions (Lucas et al., 1979). The maturation of the absorptive and endocrine function of the gastrointestinal tract enhances the insulin secretion effect of glucose when glucose reaches the B cell via the "helper system" present in the gastrointestinal tract more than when it is carried through the bloodstream. This is a well-known characteristic of insulin secretion of adult life, which may already be operational in the latter part of fetal life.

## The Endocrine Pancreas of the Newborn

### Structural Features

The normal neonatal human endocrine pancreas is similar to that of the fetus in late pregnancy, with predominantly mantle islets (Robb, 1961; Van Assche, 1970). Adult types of islets (cell types intermingled) are not common before the fourth year of life (Ferner and Stockenius, 1951). The different cell types are the same as those present during fetal life, but their proportion and location within the cell will vary according to age (Rahier et al., 1981). The volume of islet cells is positively correlated with birth weight in normal infants and remains constant throughout 3 months after birth (Hultquist, 1971). Furthermore, there is a positive correlation between the percentage of B cells in the islets and the insulin content in the microdissected islets (Van Assche et al., 1969; de Gasparo et al., 1969b).

### Functional Features

At birth, the fetus must rapidly adapt from a relatively secure homeostasis to an independent existence. Toward the end of gestation, preparations are made for this transition. There is a rapid accumulation of hepatic glycogen and an increase in the activity of the enzymes required for its mobilization. At birth, the blood sugar of the baby reflects that of the mother, but the level then falls as a consequence of the sudden severence of the maternal supply of glucose. Subsequently, the blood sugar level rises, as glucose is provided by the fetal liver, and is maintained at a steady level (Cornblath and Reisner, 1965). In the infant born to a mother with normal glucose tolerance, the basal levels of insulin are low compared to adult levels and do not increase when the fetal blood sugar is raised through glucose administration to the mother during parturition (Thomas et al., 1967). They are also comparatively stable over the first few days of extrauterine life (Joassin et al., 1967).

In the immediate postnatal period the neonate is relatively unresponsive to glucose challenge; however, after 2 hr of age the unfed newborn responds to intravenous glucose challenge with a biphasic insulin response (Isles et al., 1968). The response to oral glucose challenge is characterized by a gradual rise in plasma insulin concentration, which remains elevated for a considerable time (Pildes et al., 1969). The prolonged elevation of insulin persists even after the blood glucose has declined. The newborn infant rapidly develops the ability to release insulin in response to a glucose stimulus. The secretory responsiveness of the B cell to different nutrients

develops, however, in successive steps in relation to postnatal development (Grill et al., 1981). In addition, the changes in insulin secretion are related to age. The glucose-responsive pool of insulin is actually smaller in islets from 12-month-old rats as compared with 2-month-old one (Gold et al., 1981).

Plasma glucagon levels are similar in the maternal peripheral blood and the umbilical cord blood in normal term deliveries (Bloom and Johnston, 1972; Milner et al., 1973). At birth there is no glucagon response to elevated blood glucose; however, the glucagon secretory mechanisms are functional at birth, as hypoxia can raise the plasma glucagon level (Johnston and Bloom, 1973). Glucose and insulin administered together are effective in lowering plasma glucagon levels (Luyckx et al., 1972). The rise may be related to splanchnic nerve stimulation, adrenal secretions, or a change in gastro-intestinal function.

## The Infant of the Diabetic Mother

### Structural Features

There is marked hyperplasia and hypertrophy of the pancreatic islets in infants born to mothers with reduced glucose tolerance (Cardell, 1953; Van Assche and Gepts, 1971). These changes have been attributed to high maternal blood sugars and an increased glucose content of the amniotic fluid as early as 1920, before the hormonal significance of the islets was understood (Dubreuil and Anderodias, 1920). Similar histological features have been described in infants born to mothers whose diabetes only became overt in later life (Van Beeck, 1939; Woolf and Jackson, 1957). The islets in the pancreas of the infant of the diabetic mother are extremely vascular and numerous minute islets composed of only a few cells are observed (Van Assche et al., 1969; Hultquist, 1971). Growth of the islets may result from proliferation of islet cells, the continued production of endocrine cells from the exocrine matrix, or a combination of both processes. It seems likely that the majority of the new endocrine cells are derived from the exocrine matrix, as little cell division is observed in the endocrine cell population (Wessels, 1964; Pictet and Rutter, 1972). Under conditions of islet hyperplasia in the pancreatic tissue of adult experimental animals, the peri-insular acinar cells show greater mitotic activity than those situated further from the islet or endocrine cells (Duff and Starr, 1944; Hughes, 1947; Hellerstrom et al., 1962; Kramer and Tan, 1968).

The islet hypertrophy in the newborn of diabetic women is related mainly to an increase in the number of B cells in the PP-poor zone; the number of B cells is not increased in the PP-rich zone (Milner et al., 1981). In 30% of cases, the pancreas of these infants showed infiltration of eosinophils in and around the islets. This infiltration was also found in the offspring of mothers that were not treated with insulin during pregnancy. These changes were evident at the earliest age studied, 19 weeks of gestation (Cardell, 1953; Driscoll et al., 1960; Van Assche et al., 1970).

Hultquist (1971) has also been able to demonstrate that the volume of islet tissue is correlated with maternal glucose levels within 6 hr of delivery. Maternal and fetal blood glucose levels also appear to influence the size of the B-cell nucleus. These features were not related to birth weight in premature infants of less than 32 weeks gestational age. The association between maternal blood sugar, birth weight, and the volume of islet tissue was less apparent when complications such as maternal ketonemia or toxemia were reported. This was also true when retinopathy was diagnosed (Hultquist, 1971).

## Functional Features

Infants born to diabetic mothers may be either oversized, small for dates, or of normal birth weight. The concept that prolonged exposure of the fetus of the diabetic pregnancy to hyperglycemia stimulates the B cells of the fetal islets to produce excessive amounts of insulin was first proposed by Pedersen and his colleagues (1954). How to integrate the functional behavior of the different endocrine cells in the overall clinical picture of the infant of the diabetic mother is not clear at present. The effects of hyperinsulinism on the fetus become apparent in the last trimester of pregnancy, but exactly when is not certain. Sosenko et al. (1979) have suggested that this occurs at 34 weeks of gestation in humans.

In the fetal rat, functional hyperinsulinism is not evident at 19.5 days and only becomes manifest at 21.5 days in pups born from rats with moderately severe diabetes (Kervran et al., 1978). This hyperinsulinism resulting from a change in the cell number, structure, and function of the total islet must be secondary to the induction of glucose sensitivity in the fetal pancreas. This state will be induced after the exposure of the fetus to an excess of nutrients from the mother (Freinkel, 1980), some of which may reach the fetal gastrointestinal tract, where the enteropancreatic axis will have progressively matured (Hoet and Reusens, 1976; Reusens et al., 1980). In the latter part of fetal life, the insulin content of microdissected islets is increased in proportion to the numbers of B cells in infants of diabetic mothers (de Gasparo et al., 1969b; Van Assche et al., 1969). The increased percentage of islets is also related to the birth weight (Naeye, 1965; Heding et al., 1980). This demonstrates that the intracellular stores of insulin are maintained by biosynthesis in the presence of a greatly increased hormone output. This is further demonstrated by the fact that the C-peptide and the proinsulin levels are higher in infants of diabetic mothers if they have no insulin antibodies (Heding et al., 1980). The former levels are also correlated with birth weight (Sosenko et al., 1979). The hypercellularity of the islets might be induced by the influx of high concentrations of amino acids, shown by de Gasparo et al. (1978) in cultures of B cells in vitro. A specific effect of amino acids could therefore be responsible for the high cellularity of the islets, while the glucose would, rather, be the agent for the secretion stimulation process.

The increased insulin and proinsulin secretion results in lipogenesis, protein anabolism, and in an increase in cell numbers in many vital organs (Naeye, 1965). An insulin-induced increase in lipogenesis is, at least in part, responsible for the characteristic obese appearance of the overweight infant of the uncontrolled diabetic mother. This result of altered maternal metabolism might also affect other fetal tissues and functions, which might be less apparent but which may have long-lasting biological consequences. The high birth weight, corrected for gestational age, in infants of insulin-treated gestational diabetics is correlated not only with fasting maternal plasma glucose, but also with plasma triglyceride and plasma alanine, serine, valine, isoleucine, and glycine (Freinkel and Metzger, 1979). It indicates that a complex interaction exists in the maternofetal unit, besides the glucose exchanges, which influences the making of the vital organs and functions of the fetus.

The administration of insulin to fetal rats (Picon, 1967), lambs (Liggins, 1974), and monkeys (Susa et al., 1979) highlights the role of the hyperinsulinism as a stimulus to the overall increase in body weight as well as in individual organs, clearly seen in the liver, the heart, and probably the spleen. In contrast, no effect was seen on the lung,

kidney and brain. Gruenwald (1974) has reported a decrease of the brain weight relative to the gestational age or body weight among infants of diabetic mothers. Nevertheless, the functions or the maturation of the organs cannot be assessed by the observed or expected weight. The lung maturation of infants of diabetic mothers is retarded, especially in overweight infants (Naeye, 1979). In the monkey fetus treated with insulin, the glycogen composition of the liver is correlated with the weight increase. It is apparent that not all organs have the same sensitivity to nutrients.

The difference in sensitivity of the organs may be related to the presence or absence of insulin receptors. The latter are present early and increase in the liver during fetal life, whereas they disappear with advancing gestation in the fetal lung. Monocytes of the infant of the diabetic mother do not show any down-regulation, which should be induced by high insulin levels, as usually observed in the adult (Neufeld et al., 1978). The absence of a down-regulation might be a specific feature of the fetus and might allow a continuous effect of the high insulin levels. Evidence for the insulin effect has been obtained at the biochemical and cellular levels. Experimental studies in the rat have shown that insulin stimulated the uptake of glucose, amino acid incorporation, and the synthesis of protein by the fetal heart from day 16 of gestation onward (Clark, 1971a,b). It might be that insulin has its metabolic effect through the insulin receptors, whereas the growth-promoting effect occurs through the somatomedin receptors. Whether the differentiation for insulin, growth hormone, and growth-promoting factors occurs during fetal life is not known (King et al., 1980).

Glucagon metabolism is also altered in the baby of the diabetic mother. The rise in plasma glucagon seen at 2 hr of age in normal healthy babies does not occur in those born to diabetic mothers (Johnston and Bloom, 1975). In insulin-treated primate fetuses, glucagon also had a tendency to be reduced. The elevated insulin levels present in the infant of the diabetic mother may be responsible for the suppression of the observed glucagon secretion. The foregoing indicates that the fetus has its own specific endocrinological and metabolic adaptation to the nutritional environment imposed on it by the altered metabolism of the mother. The consequences of this prematurely imposed adaptation may endanger the structure and function of vital organs, with long-term effects on further development.

### The Intrauterine Growth-Retarded Fetus and Newborn of the Diabetic Mother

Limited information is available on the endocrine pancreas in the growth-retarded fetus of the diabetic mother with vascular and renal complications. It is not obvious if these fetuses are hyper- or hypoinsulinic; however, this is an important issue in view of the role of fetal insulin as a growth-promoting factor.

D'Agostino and Bahn (1963) showed that in these fetuses the total pancreatic mass was decreased owing to a reduction in exocrine tissue, but a limited hypertrophy of the B cells was still evident. Naeye (1965) observed a slight increase in the percentage of islets in hypotrophic infants; however, different organs were still underweight. A small heart was due to a subnormal number of myocardial fibers, and a small liver weight to fewer hepatic cells, with a reduced total nuclear mass. There was also a diminished number of cells in the fetal adrenal cortex, with reduced cytoplasmic mass per cell. Growth retardation in diabetics might be related to amniotic fluid infections, which are more frequent in diabetic pregnancies. Poor maternal nutrition during pregnancy appears to retard or prevent the development of antimicrobiological activity in

the amniotic fluid (Tafari et al., 1977). These infections also have a role in the increased frequency of psychomotor impairment found in children of diabetic mothers. They are a more likely cause of the reported neurological impairment reported by Naeye (1979) than ketotis.

In the rat severe ketotic diabetes induces fetal growth retardation, B-cell degranulation, and low insulin levels (Aerts and Van Assche, 1977; Kervran et al., 1978). When the insulin level per gram of body weight was expressed, a major reduction was observed in growth-retarded pups (B. Reusens and J. J. Hoet, unpublished observations). In addition, an important slowing down of the maturation of the fetal gastrointestinal tract was observed which correlated with the fetal weight. Furthermore, the stimulation of the islets in the postnatal period of pups born to severely ketotic animals which were hypotrophic was not apparent. It therefore appears that the islets of Langerhans and the secretion mechanism in the B cells do not have the same functional increase in hypotrophic animals as in hypertrophic ones. Finally, in three cases of growth-retarded fetuses of diabetic mothers a normal insulin level and not a high insulin level in the cord blood was noted (F. A. Van Assche, R. Bouillon, and L. Aerts, unpublished observations, 1981.)

### The Intrauterine Growth-Retarded Fetus and Newborn of the Nondiabetic Mother

A few cases of extreme intrauterine growth retardation associated with pancreatic agenesis have been reported. (Douron and Buyl-Screuwens, 1969; Sherwood et al., 1974). These infants were hyperglycemic and showed signs of catabolism. Furthermore, in newborn infants with neonatal diabetes (which are nearly always small for dates) evidence exists that they have delayed maturation of the B-cell function (Ferguson and Milner, 1970; Pagliara et al., 1973; Hill, 1974; Liggins, 1974). The delay in the development of the endocrine pancreas has been demonstrated by Van Assche et al. (1977a) and a lack of insulin receptors has been shown (Ballabriga and Martinez, 1979).

In animal experiments, insulin depletion or B-cell ablation reduces the growth potential of the fetus. Liggins (in Hill, 1974) produced intrauterine growth retardation by removing the pancreas in the fetal sheep. Intraperitoneal injections of alloxan in the fetal rabbit (Harding et al., 1975) or streptozotocin in the fetal rhesus monkey (Hill, 1976) led to intrauterine growth retardation in a large number of fetuses. De Prins and Van Assche (1982) have shown that in rat fetuses with experimental growth retardation (unilateral ligation of the uterine arteries at day 17) the glucose and insulin levels in the peripheral blood were lower than in controls from day 21 of gestation until birth (day 23). The percentage of endocrine tissue was not at variance in the fetuses with growth retardation compared to the controls, but the proportional amount of granulated B cells was specifically reduced at the day of birth in the growth-retarded newborn rats.

It can be concluded that when poor nutritional supply occurs in late pregnancy fetal hypoglycemia will result that will not have the permissive action which is conducive for the fetal B cells to synthesize and secrete insulin normally. A later consequence will be the reduction of fetal B-cell growth (Van Assche and De Prins, 1983).

Observations in the human and in animal experimentation indicate that the pathological characteristics of small-for-dates infants of diabetics are not specific for such infants. A subnormal amount of cytoplasm is found in the cells of many organs, as well as a reduced number of cells in the liver, spleen, pancreas (exocrine), kidney, and adrenals. The reactions of the islets of Langerhans are at variance, however, in the two clinical situations. In the growth-retarded infant of a nondiabetic mother the number of

B cells is reduced and the functional activity appears to also be impaired; in the growth-retarded infant of the diabetic mother the percentage of B cells is not increased in the same proportion as in the hypertrophic infant and therefore shows a ratio close to that of a normal-sized infant. However, the available data indicate that these cells have not acquired a normal functional maturation and are therefore probably deficient in insulin and its growth-promoting effect.

Recently it has been shown in the rat that maternal hypoglycemia inducing fetal hypoglycemia and fetal hypoinsulinemia finally results in intrauterine growth retardation (Gruppuso et al., 1981). In the human we have confirmed that there is a correlation between maternal hypoglycemia and intrauterine growth retardation (F. A. Van Assche and F. De Prins, unpublished observations). In the human, nutritional supplementation to the mother in intrauterine growth retardation may be helpful (Beischer et al., 1977); however, even after this, the infants are still below the twenty-fifth percentile at birth (Habicht et al., 1974; F. A. Van Assche and F. De Prins, unpublished observations). One can only assume that fetal growth retardation in diabetics and nondiabetics is multifactorial.

### The Determining Factors in the Adaptation of the Fetal B Cell

At birth, the plasma levels of growth hormone are elevated in the normal newborn, but low in infants of diabetic mothers. The data appear to indicate that the regulation of growth hormone secretion has been modified in infants of diabetic mothers (Westphal, 1967). Studies on the endocrine pancreas of the anencephalic infant have demonstrated the importance of an intact hypothalamic-hypophyseal axis in the ability to respond to a premature glucose challenge. Two types of anencephalics can be recognized: those with a functional hypothalamic-hypophyseal connection and those without one. The morphological and functional development of the endocrine pancreas is similar in anencephalic and normal infants if the mother has normal glucose tolerance. However, the hyperplasia and hypertrophy of the islets normally seen in the offspring of diabetic mothers are not present in the absence of an intact hypothalamic-hypophyseal axis (Van Assche, 1968; Van Assche et al., 1969; de Gasparo and Hoet, 1971). These observations indicate that although the hypothalamus and pituitary are unnecessary for the normal development of the endocrine pancreas, which has been confirmed in animal studies involving experimental decapitation (Van Assche, 1971; de Gasparo et al., 1974), they are essential for the increased multiplication of the fetal B cell under the abnormal conditions of diabetic pregnancy. This effect may be directly under hypothalamic-hypophyseal control or mediated via the adrenal or thyroid glands. However, isolated adrenal insufficiency does not prevent B-cell hyperplasia (Hoet et al., 1975). Growth hormone has been shown to sensitize the rat B cell to secretagogues (Martin and Gagliardino, 1967), and the low growth hormone levels in the anencephalic infant (Grunt and Reynolds, 1970; Grumbach and Kaplan, 1973) may be important in this respect (Salazar et al., 1969; Van Assche 1970, 1975; Hoet et al., 1975). A central influence on insulin output has also been shown to occur in the rabbit. Insulin output was, however, increased by decapitation and this increase was prevented by adrenocorticotropic hormone replacement (Jack and Milner, 1975).

Islets of Langerhans are also increased in size and number in the pancreas of erythroblastic infants born with $\alpha$-thalassaemia (Van Assche et al., 1970). Although the total amount of endocrine tissue, the insulin content of the pancreas and the

insulin levels in the cord blood are all increased, there is no change in the proportion of B-cells per islet (Van Assche et al., 1970). In infants of insulin-treated diabetic mothers who have developed antibodies against insulin, a direct correlation was found between the antibodies in the mother and those in the neonate. The total insulin levels were correlated with the insulin-binding antibodies of the infant. There was also a significant correlation between antibodies and C peptide in the infants, which is a consequence of an increased endogenous insulin secretion. The infants with antibodies had asymptomatic hypoglycemia more often, which may be related to insulin antibody complexes and a greater secretion stimulus effect. It indicates that the insulin antibodies in the newborn infant are not without consequences (Heding et al., 1980) and that insulin with low immunoreactivity should be used in treating diabetic mothers.

### The Clinical Consequences of Abnormal Pancreatic Development

The fetus responds to a glucose challenge in utero by increasing the proportion of B cells and, as a result, has an increased insulin secretory capacity and output. The adaptation to an abnormal intrauterine environment with an increased insulin secretion will inhibit the maturation of pulmonary lecithin, which is held responsible for the respiratory distress syndrome of these neonates (Smith et al., 1975). Furthermore, they have an increased risk of symptomatic hypoglycemia after birth and are more prone to obesity and diabetes mellitus in later life (Farquhar, 1969; Shah and Farquhar, 1975). If the human B cell is only capable of a finite number of cell divisions, as appears to be true in the rat (Logothetopoulos, 1972), an adverse intrauterine environment resulting in B-cell hyperplasia could jeopardize the regenerative potential in later life. A reduced capacity for cell division in the B-cell population in conjunction with normal B-cell development and function may be one of the many factors determining the increased incidence of diabetes mellitus in individuals born to diabetic mothers. The question arises as to whether the changes are reversible after birth. It is possible that the B cells which are overstimulated during intrauterine life sustain a permanent defect. Hultquist and Olding (1975) have shown that in infants of diabetic mothers there is increased fibrosis in the islets of Langerhans 2 weeks after birth. An answer to the problem of the long-term effect can be found by using an experimental model of diabetes and pregnancy in the rat. There are striking similarities in the morphological and functional features of the endocrine pancreas of the rat fetus in experimental mild diabetes and the endocrine pancreas of the human fetus of diabetic mothers (Aerts and Van Assche, 1977). In the follow-up of pups born to experimental diabetic rats it can be concluded that even mild experimental diabetes in the rat causes persistent changes in the endocrine pancreas of their offspring (second generation) that are not perceptible in basal conditions but which become apparent in situations stressing B-cell activity, such as an intravenous glucose load or pregnancy. This inadequacy of B-cell compensation in the offspring of the second generation may be interpreted as a possible cause of gestational diabetes, which is even manifest in the fetuses of the third generation (Aerts and Van Assche, 1979). Careful control of diabetic pregnancy is important, as infants born to diabetic mothers whose blood sugar levels have been carefully controlled with insulin throughout the pregnancy have less hyperplasia and hypertrophy of the pancreastic islets or none at all (Van Assche, 1970; Hultquist, 1971). Adequate maternal control also reduces clinical complications arising in the neonatal period (Persson, 1974, 1975).

It has also become apparent that dietary control alone is not entirely effective in suppressing the tendency of the fetuses of gestational diabetic mothers to be overweight (Borberg et al., 1980), whereas insulin therapy is. It seems likely, therefore, that the infants of diet-treated gestational diabetics will keep the usual features of an infant of a diabetic mother. The long-term consequences of the premature adaptation during fetal life to an abnormal metabolic environment may also not be prevented, and this raises the question as to whether the use of dietary control without insulin is entirely justified, even for mothers with mild gestational diabetes.

Pregnancy modifies maternal glucose homeostasis, and failure to respond to the challenging needs of pregnancy with an increased number of B cells and enhanced insulin secretion may have serious consequences for the mother. Gestational diabetics not treated with insulin may show further deterioration of endocrine function, with associated vascular complications, years later, that the insulin-treated mother does not develop (O'Sullivan et al., 1971). A subset of mothers who are obese and have a positive family history for diabetes clearly showed a reduction in the incidence of decompensated or overt diabetes in later life if treated with insulin during pregnancy. This is one more indication that the decision of diet and/or insulin treatment in gestational diabetics cannot be taken lightly.

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# 5

# Fetal Fat and Glucose Metabolism

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## INTRODUCTION

The task of reviewing fetal fat metabolism and carbohydrate metabolism together in this volume, where previously they had been considered separately, has inevitably led to a sharpening of terms of reference. The subject is considered with particular emphasis on the role of fat and carbohydrate as sources of energy for the fetus; thus fat is considered as a fuel and no attention is paid to structural fat or fat with a specialized function such as brain phospholipid or lung surfactant. Glucose was chosen rather than carbohydrates in general because it is the hexose most directly involved in meeting fetal fuel requirements. This chapter should be read in conjunction with Chapter 18, which deals with fetal energy metabolism.

In the preparation of this chapter there has been the inevitable problem of extrapolation across species. Evidence from human experimentation has been given prominence and animal studies have been cited selectively. The reader will note that many differences between species in the development of fat and glucose metabolism appear to be related more to the length of gestation than to the class of animals studied.

## FAT METABOLISM

Fat that will be used to provide energy, possibly in the fetus and certainly after birth, is stored as triglyceride in white and brown adipose tissue and liver. Triglyceride must be hydrolyzed to enable fat transport to take place and the important lipid components found in the circulation are triglyceride, fatty acid, glycerol, and ketone bodies.

Although the newborn of most mammalian species burn fat as the major energy source, the prenatal development of lipid stores varies widely between species (Hahn and Novak, 1975). Small rodents such as the rat and mouse are born with very little adipose tissue and have 1-2% body fat (Widdowson, 1950). The newborn rabbit contains 5% fat, half of which is in brown fat, a quarter in the liver and the rest in other body tissues including white fat (Hardman et al., 1970). The term human infant is quite different from laboratory animals, having between 12 and 16% body fat. Adipose tissue stores develop late in gestation, the concentration of fetal lipids before 32 weeks being low and constant (Roux et al., 1971). In the last 2 months of pregnancy subcutaneous fat increases exponentially from 20 to 350 g, and deep body fat from 10 to 80 g (Southgate and Hey, 1976) (Figure 1).

Fatty acid is quantitatively the most important component of circulating triglyceride, but ketone bodies may also be a significant source of energy, especially toward the end

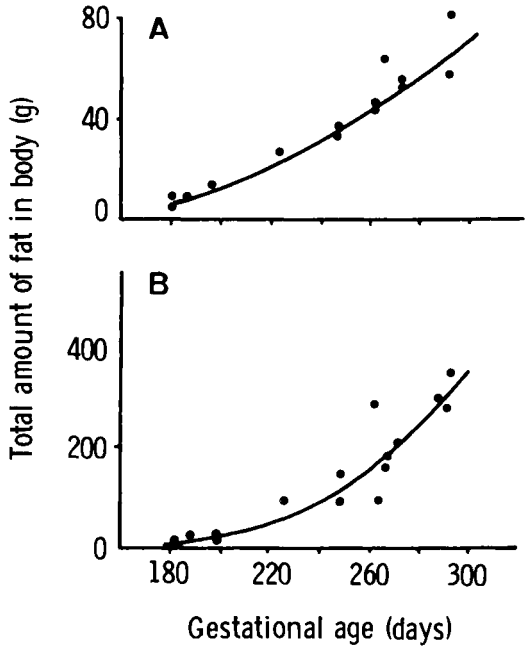


**Figure 1** Subcutaneous fat (A) and deep body fat (B) of human infants of normal weight for their gestational age. (From Southgate and Hey, 1976.)

of pregnancy and during labor, when ketosis is common. Maternofetal transport of fatty acid, glycerol, and ketone bodies has been demonstrated in a number of species, including man. Fatty acid transport has been demonstrated in the rat (Hummel et al., 1975), rabbit (Elphick et al., 1975), guinea pig (Bohmer and Havel, 1975), and monkey (Portman et al., 1969). Glycerol crosses the rat and rabbit placenta (Gilbert, 1977) and placental permeability to ketones has been demonstrated in man and the rat (see Robinson and Williamson, 1980).

Studies with perfused human placenta in vitro suggest that fatty acids of maternal origin could provide up to 20% of the fatty acid stored as triglyceride in the term fetus (Dancis et al., 1973), but it is not clear if there is any association between the placental capacity for fatty acid transfer and the amount of subcutaneous fat found in the newborn of other species. Maternal plasma free fatty acid levels of women not in labor undergoing elective cesarean section were higher than and positively correlated with those in the umbilical cord (Elphick et al., 1976). Women who received lipid infusions during labor had higher arterial concentrations of free fatty acids, glycerol, and hydroxybutyrate at delivery than control mothers. The umbilical artery and vein concentrations of the three metabolites were raised in the experimental group, as was the venous-arterial difference, indicating net transfer to the fetus (Elphick et al., 1978b; Rubaltelli et al., 1978).

### Lipogenesis

Fatty acids may be synthesized in three ways. The most important quantitatively is de novo synthesis from glucose, acetate and carbon dioxide in the cell cytosol under the control of acetyl-coenzyme (acetyl-CoA) carboxylase, biotin, and the fatty acid

CYTOSOLIC DE NOVO SYNTHESIS

$$\text{Acetyl-CoA} + CO_2 \xrightarrow[\text{biotin}]{\text{Acetyl-CoA carboxylase}} \text{Malonyl-CoA}$$

$$\text{Acetyl-CoA} + 7\ \text{Malonyl-CoA} \xrightarrow{\text{Fatty acid synthetase}} \text{Palmitoyl-CoA}$$

MITOCHONDRIAL FATTY ACID ELONGATION

$$\text{Acetyl-CoA} + \text{Acyl-CoA} \longrightarrow \text{Acyl-CoA (n + 2)}$$

MICROSOMAL FATTY ACID ELONGATION

$$\text{Malonyl-CoA} + \text{Acyl-CoA} \longrightarrow \text{Acyl-CoA} + CO_2\ (n + 2)$$

**Figure 2** Pathways of fatty acid synthesis.

synthetase complex (Figure 2) among which the carboxylase is rate limiting. Fatty acid elongation also takes place in mitochondria by the sequential addition of two carbon units in a process thought to be the reverse of $\beta$-oxidation. This pathway is of minor importance, except in the heart, where no alternative exists (Hulsman, 1962). In the microsomes malonyl-CoA replaces acetyl-CoA as the chain lengthener, but this mechanism is also thought to be quantitatively unimportant.

The development of lipogenesis has been studied in the rat (Taylor et al., 1967; Ballard and Hanson, 1967), where there appears to be an association between hepatic lipogenesis and lipid supply to the fetal and neonatal tissues. Fatty acid synthesis is active in hepatic cytosol in late fetal life, declines after birth when the diet becomes fat-rich milk, and then increases again at weaning when the dietary fat intake falls. Ketones of maternal origin may be incorporated into fetal lipids in a variety of tissues (Edmond, 1974; Seccombe et al., 1977). Intrauterine growth retardation can be induced in the rat by ligation of one uterine artery. Liver slices taken from such growth-retarded fetuses 3 hr after delivery show impaired gluconeogenesis and synthesis of phospholipids (Nitzan and Groffman, 1971). Fetal tissue from rabbit and guinea pig can synthesize fat in vitro and in vivo from acetate (Popjak, 1954) and from fatty acids (Illife et al., 1973; Jones, 1973). Fetal hepatic lipogenesis does not occur in the pig (Mersmann et al., 1973). It will be recalled that the pig is born with negligible fat stores, unlike the other species of long gestation quoted, suggesting that fetal hepatic lipogenesis may play an important part in the creation of the subcutaneous energy reservoir.

Triglyceride synthesis has been studied using tissues from "young" fetal rhesus monkeys (60-142 days gestation, term is 165 days), "term" fetuses (156 days), and

neonatal and adult animals. Hepatic phospholipid and triglyceride synthesis from palmitate was fastest in the "young" fetus. Glucose incorporation into triglyceride was greatest in the newborn, two-thirds the neonatal rate in the fetus and one-third that in the adult. All rates were expressed per unit wet weight of liver. When it is remembered that the fetal liver is both hemopoietic and hepatocytic in composition, the fetal hepatocytic capacity for lipid synthesis was probably underestimated.

Human fetal tissue of 12-20 weeks gestation is capable of incorporating glucose, fructose, acetate, citrate, and amino acids into lipids in vitro (Villee and Loring, 1961). Triglyceride synthesis from palmitate has been demonstrated in slices of liver, lung, and brain from abortions of 12-16 weeks gestation (Yoshioka and Roux, 1972).

In contrast to cytosolic lipogenesis, mitochondrial or microsomal fatty acid chain elongation becomes more active after birth, in parallel with increases in the numbers of these organelles. In organs such as brain and lung, in which fatty acid synthesis is important for the building of structural lipids or surfactant, respectively, de novo fatty acid synthesis can be demonstrated before or after birth during the period of maximum growth velocity of the organ.

## Lipolysis

Triglyceride must be hydrolyzed before it can pass into or out of cells. Intracellular lipolysis is controlled by triglyceride lipase, whereas the breakdown of triglyceride in the circulation is governed by lipoprotein lipase, otherwise known as clearing factor lipase. Triglyceride lipase is activated at the cell surface by hormones such as glucagon and catecholamines or by sympathetic stimulation. The signal is translated within the cell by cyclic adenosine 5′-monophosphate and a protein kinase in turn. Lipoprotein lipase is liberated from the endothelial cells of capillary walls spontaneously and in response to highly positively charged compounds of which the most important clinically is heparin. Dunlop and Court (1978) examined the lipolytic properties of cells isolated from human fetal subcutaneous tissue as an index of the cellular potential to be adipocyte precursors. Specimens from fetuses aged 10-22 weeks uniformly hydrolyzed exogenous triglyceride and were stimulated by noradrenalin. The demonstration of hormone-sensitive lipolysis was taken as evidence of the adipocytic nature of the cells. Heparin also increased fatty acid release from the cells, showing that lipoprotein lipase was present too. Lipoprotein lipase has been studied more systematically in the fetal and newborn rat (Cryer and Jones, 1978). Enzyme activity in lung, skeletal muscle, heart muscle, and brown fat emerged substantially in the first 24 hr of postnatal life. In fetuses delivered prematurely there was also an increase in lipase activity on the first day, showing that the change was linked more closely to the transition to extrauterine life than to gestational age.

Triglyceride is seen in the circulation postnatally as chylomicrons or very low density lipoprotein following the ingestion of fat-rich food, and there is little reason a priori to anticipate lipemic fetal plasma. Nonetheless, blood is collected from time to time, particularly from rabbit fetuses, which is frankly turbid, and studies in fetal guinea pigs have shown the liver to be the source of the circulating triglyceride (Bohmer et al., 1972), but the mechanism by which it reaches the circulation remains unexplained.

## Fat Oxidation

The breakdown products of triglyceride are glycerol and fatty acids. Fatty acids generate ketones. All three products are present in the fetal circulation. They may be of fetal or maternal origin. Quantitatively the most important are fatty acids, which are transported by albumin. Cellular uptake and cytosolic transport involve specific carrier proteins. The first step in fatty acid oxidation is esterification to form an acyl-CoA ester (Figure 3). The ester must pass the inner mitochondrial membrane to undergo β-oxidation. The membrane is impermeable to the ester and translocation is achieved by the formation of an acyl carnitine, for example, palmitoyl carnitine under the influence of palmitoyl carnitine transferase A situated on the outer surface of the inner mitochondrial membrane. Once inside, the reverse reaction occurs under the control of carnitine palmitoyl transferase B, yielding palmitoyl-CoA, which is subsequently β-oxidized.

Failure of a fetal cell to burn fatty acids could arise from a lack of free fatty acids, of carnitine, or of one of the enzymes described above. Fetal rat liver has a decreased capacity to oxidize fatty acids, which is probably mainly due to limited production of palmitoyl carnitine by carnitine palmitoyl transferase A (Bailey and Lockwood, 1973). After birth, fatty acid oxidation increases markedly and remains high until the time of weaning. Parallel changes take place in rat heart before and after birth for probably the same reasons (Warshaw and Terry, 1970). Carnitine may also be rate limiting. In the neonatal rat heart the carnitine concentration is one-quarter that in the adult and the addition of carnitine to heart homogenates increases the rate of fatty acid oxidation

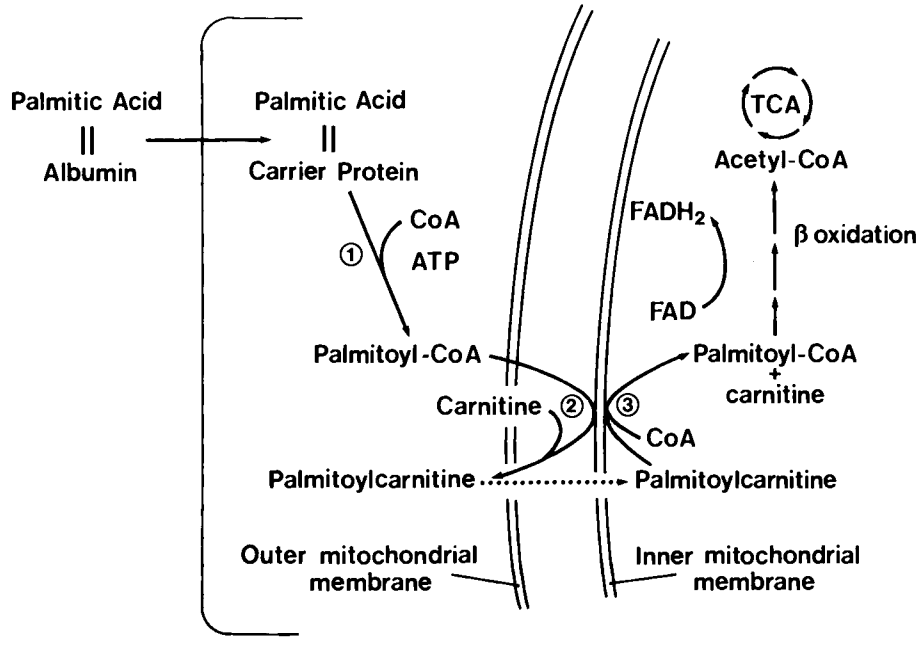


Figure 3 Pathway of fatty acid oxidation: (1) acyl-CoA synthetase, (2) carnitine palmitoyl transferase A, and (3) carnitine palmitoyl transferase B.

in vitro (McGary et al., 1975; Robles-Valdes et al., 1976). The primary source of carnitine for the rat pup is breast milk, in which the concentration is highest in the first 2-3 days of suckling. Hepatic carnitine levels increase dramatically in the early neonatal period in parallel with the increase of fatty acid oxidation. Carnitine in breast milk infant formulas or parenteral nutrients for human infants may have an important subsidiary role in the development of fatty acid oxidation.

The contribution of fatty acid oxidation to overall fetal energy production varies between species, from negligible to moderate. Fetal rhesus monkey tissues can oxidize fatty acids to a limited extent (Roux and Myers, 1974), as can human fetal brain, liver, placenta, and lung slices in vitro (Yoshioka and Roux, 1972). Brown adipose tissue from the 28-day rabbit fetus released $CO_2$ from palmitate in vitro and oxidation was stimulated by noradrenalin (Hudson and Hull, 1977). The rate of oxidation did not differ between fetal and early neonatal brown adipose tissue, illustrating a possible difference between this and other tissues.

Ketone bodies in the fetal circulation may arise from the partial oxidation of fetal fatty acid, but they may also be of maternal origin, since the placenta is freely permeable (Sabata et al., 1968). Fasting during pregnancy causes a rapid rise in maternal ketone levels in the rat (Herrera et al., 1969) and in man (Felig and Lynch, 1970) and maternofetal ketone body transfer during labor may be appreciable. During the latter part of pregnancy the capacity to oxidize hydroxybutyrate has been demonstrated in vitro in fetal liver, brain, and placenta in both the rat (Shambaugh et al., 1977) and human (Adam et al., 1975; Patel et al., 1975). On balance, ketones in the fetal circulation are used more for lipid synthesis than as a source of immediate energy (Seccombe et al., 1977).

## GLUCOSE METABOLISM

Fetal glucose utilization is large, being 5-8 mg/kg per minute, and is approximately two to three times that in the adult when compared on a body weight basis. All fetal tissues can burn glucose, most do preferentially, and some are obligatory glucose consumers, the most important being the brain, which is responsible for about one-third of the total glucose utilization (Adam et al., 1975; see Jones, 1979). Erythrocytes, renal cortex, and the adrenal medulla are also thought to be obligatory glucose consumers.

The observation that the fetal respiratory quotient approximates to unity led to the erroneous idea that glucose provided for all fetal energy needs, but more recent work has shown that other fuels, principally lactate and amino acids, are responsible for up to 50% of the energy requirement (see Battaglia and Meschia, 1978; and Chapter 18).

### Glucose Delivery and Production

The fetus receives most of its glucose directly from the mother by facilitated diffusion across the placenta, the rate of delivery being directly related to both maternal arterial glucose concentration and the maternofetal arterial glucose gradient. If the fetus is undisturbed and healthy, the fetal blood glucose concentration varies between 30 and 50% of that in the mother in the majority of species studied (see Bassett and Jones, 1976; Hay, 1979). Under nonfasting or fasting conditions in man fetal blood glucose

is about 90% of the mother's (Milner and Hales, 1965; Oakley et al., 1972), but it is doubtful if blood can be collected from a human fetus in unstressed conditions. Maternal starvation reduces the maternal blood glucose and thereby the rate of glucose delivery to the fetus (Boyd et al., 1973; Bassett and Madill, 1974; Simmons et al., 1974). The fall in maternal blood glucose concentration that occurs toward the end of human pregnancy was also thought to restrict glucose delivery to the fetus, but recent studies employing [1-$^{13}$C]glucose have shown that maternal systemic glucose production rates increased as the arterial concentration fell and were similar in normal and well-controlled gestational diabetic women (Kalhan et al., 1979). The increase in maternal glucose production offset the fall in arterial concentration and was sufficient to meet the glucose requirements of the fetus. Comparisons of maternal and fetal blood at delivery showed that the maternal and fetal glucose pools were in equilibrium in both normal and diabetic subjects. Short maternal fasts did not trigger fetal systemic glucose production.

Measurements of oxygen consumption and glucose delivery to the same fetus show that the maximum contribution made to fetal oxidative metabolism by placentally derived glucose is 50%, falling to 20% during maternal starvation. This immediately focuses attention on the ability of the fetus to synthesize glucose and the use by the fetus of nonglucose fuels as energy sources. Recent work has shown that after glucose and amino acids, lactate is the third most important fuel for the fetal lamb. Fetal lactate may originate from placental metabolism of glucose and reflect a contribution of maternal glucose metabolism to fetal fuels (Burd et al., 1975; Char and Creasy, 1976). The subject of fetal energy balance is considered more fully in Chapter 18.

Gluconeogenesis in the fetus could be an alternative source of glucose. In the adult glucose production takes place mainly in the liver and kidney. In species of short gestation, such as the rat and rabbit, gluconeogenic enzymes are absent or present only in low activity in fetal liver and glucose synthesis does not occur in appreciable amounts until after birth (see Bassett and Jones, 1976). By contrast, fetuses of longer gestation such as the guinea pig, pig, sheep, monkey, and human have hepatic gluconeogenic activity and glucose production has been demonstrated in vitro using human, guinea pig, or sheep liver. The contribution by these pathways in normal fetal life has not been defined; they may assume importance in the ability of a species to withstand premature delivery.

The extent by which a fetus can augment exogenous glucose delivery by glucogenesis has been recently reviewed (Sparks, 1979). Transplacental glucose delivery is close to obligatory glucose requirements under normal circumstances, suggesting that the need for endogenous glucose production is minimal. However, during maternal starvation, oxygen consumption and glucose turnover do not alter in the fetal lamb, despite a fall in umbilical glucose uptake (see Sparks, 1979). Similar observations have been made in the rat (Girard et al., 1977). Both suggest that fetuses of short or long gestation are able to supplement transplacentally acquired glucose by endogenous glucose production when necessary.

The fetus could produce glucose by glycogenolysis, which is considered in the next section, by glucogenesis (i.e., the production of new glucose from other carbohydrates), or gluconeogenesis (i.e., from any substrate excluding other carbohydrates). Other carbohydrates, particularly galactose and fructose, which feature in postnatal nutrition as components of lactose and sucrose, do not participate in fetal energy metabolism to any extent. Gluconeogenesis is governed by the availability of substrate and the activity of key enzymes.

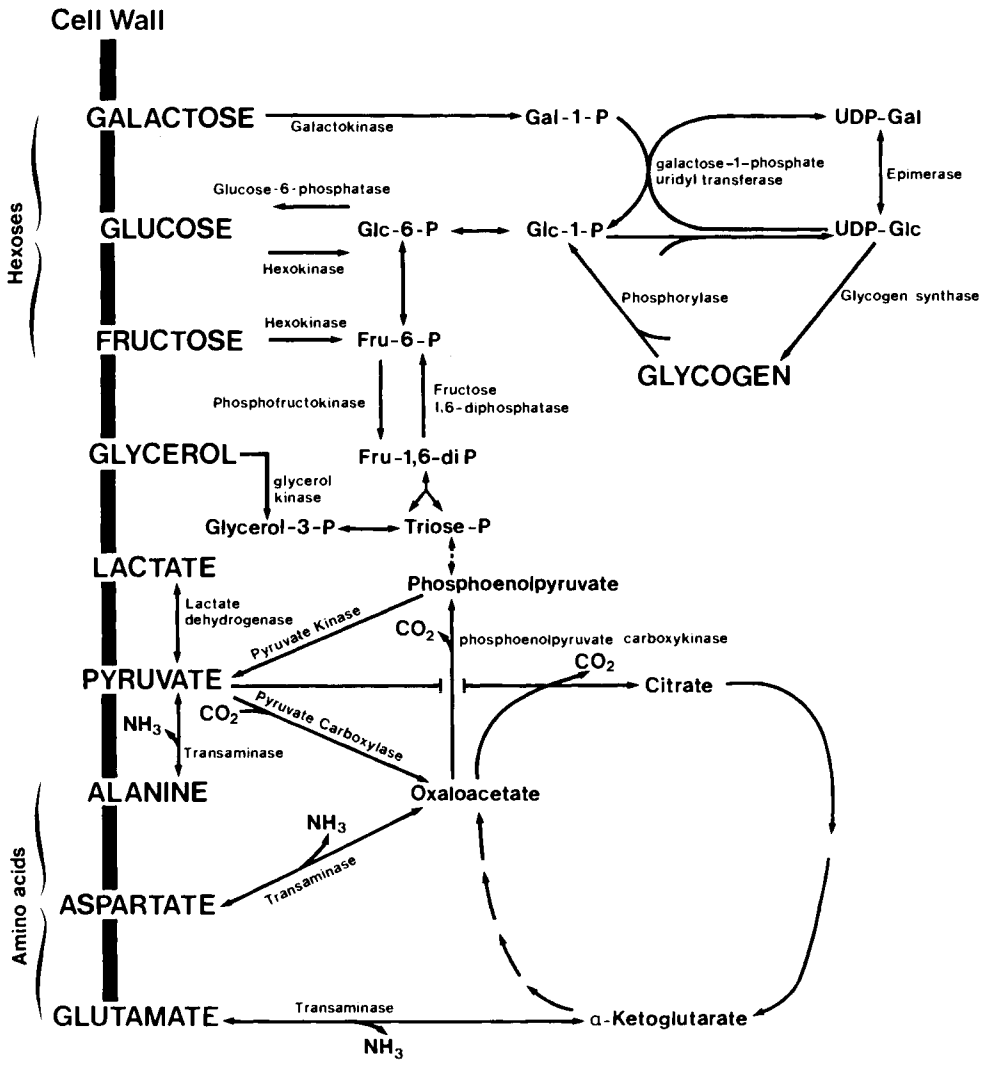


Figure 4 Interrelation between glucose and glucogenic metabolites in a fetal cell.

Glucogenesis from other sources such as lactate and amino acids involves reversal of glycolysis at the three-carbon level (Figure 4). The three most important enzymes controlling this are pyruvate kinase, pyruvate carboxylase, and phosphoenol pyruvate carboxykinase. Phosphoenol pyruvate carboxykinase exists in the cytosol and in mitochondria and absence of the cytosolic form has been linked with absence of hepatic gluconeogenesis in fetal rats (Philippidis and Ballard, 1969). The physiological roles of pyruvate kinase and pyruvate carboxylase are also likely to be important in fetal life, but their precise role has not been defined (see Sparks, 1979).

Glycerol is a potential gluconeogenic substrate which is available to the fetus in low concentrations. The key enzyme controlling glycerol metabolism to glucose or glycogen is glycerol kinase (Figure 4). Glycerol may be incorporated into glucose by the

fetal rat and rabbit (Bossi and Greenberg, 1972; Gilbert, 1977). Glycerol kinase activity increases in late gestation, with a further postnatal surge (Lin, 1977).

The ontogeny of key gluconeogenic enzymes differs markedly between species. The longer gestation fetuses such as the sheep, cow, and guinea pig show gluconeogenic capacity both in vitro (liver slices) or in utero. The midterm human fetal liver contains gluconeogenic enzymes, but in lower concentrations than those found in the adult (see Greengard, 1977). Gluconeogenesis from alanine has been demonstrated in the first-trimester human fetal liver grown in organ culture (Schwartz and Rall, 1975a).

### Glucose Storage

Glucose stored as glycogen is a major fuel reserve laid down by the fetus for use in the immediate neonatal period, but which also in some circumstances may be mobilized in utero. Glucose-6-phosphatase, the enzyme that controls the generation of glucose from glycogen, is not found in all tissues, with the result that liver glycogen is the main reservoir of stored glucose available for use anywhere in the body, whereas other fetal tissues (e.g., heart and skeletal muscle) have glycogen stores for local use.

Glycogen synthesis and breakdown are governed by glycogen synthase and phosphorylase, respectively. Each enzyme exists in an active form, depending on its state of phosphorylation. The active form of phosphorylase is phosphorylated, whereas that of synthase is dephosphorylated. In the adult the balance of activity for glycogen synthesis or breakdown depends largely on the phosphorylation state of the enzymes, but in the fetus the concentration of enzyme, especially that of synthase, is more important.

Enzymic balance favoring glycogen synthesis in the liver occurs in the presence of a high glucose concentrations. Galactose also stimulates hepatic glycogenesis, but this

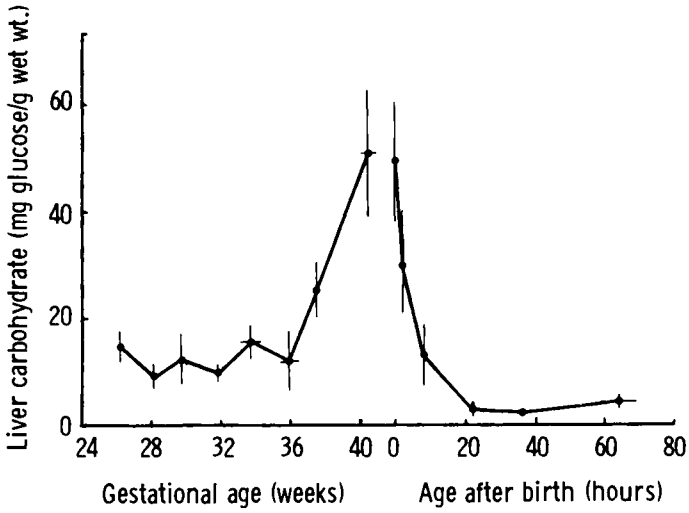


**Figure 5** Liver glycogen in man before and after birth. The left-hand side shows the mean (±SE) hepatic carbohydrate concentration in the fetus during the last trimester; the right-hand side shows the changes after birth in babies of more than 37 weeks of gestation. (From Shelley and Neligan, 1966.)

is not normally important until postnatal feeding with breast milk is established. If a pregnant rat is fed galactose in subtoxic doses, fetal hepatic glycogen stores are also increased (Sparks et al., 1976), indicating that a hexose which may be harmful in too high a dose can have a beneficial effect at a lower dose. Glycogenolysis is stimulated by hypoglycemia or by catecholamines or glucagon.

Toward term there is a rapid accumulation of hepatic glycogen (Figure 5). In the rat this proceeds at the rate of 20 mg/g of liver per day to reach a final concentration in excess of 100 mg/g. Similar changes take place at a slower rate in other species (Shelley et al., 1975). The accumulation is controlled by synthase and the formation of the synthase enzyme is under the control of adrenocortical glucocorticoids (see Jost and Picon, 1970).

Glycogen breakdown requires the two enzymes phosphorylase and glucose-6-phosphatase. Both are present in the fetus, but they do not become active until after birth when complete depletion of human liver glycogen can occur within 12 hr (Shelley and Neligan, 1966).

## ENDOCRINE CONTROL

Both substrate and enzymes play important roles in the ontogeny of fetal fat and glucose metabolism, but as development progresses hormones also participate and become controlling factors in the transition to postnatal life. Insulin, glucagon, and catecholamines are the hormones principally involved and this review will be restricted to them, although it is recognized that other hormones, for example, glucocorticoids and adrenocorticotrophin, are known to be involved in the development of enzymes controlling glycogen synthesis and may affect fetal lipid metabolism experimentally (see Hahn and Novak, 1975).

Comparison between species is fraught with difficulty unless the reader keeps clearly in mind a number of principles. He must consider the ontogeny of the endocrine cell, the response of the endocrine cell to stimuli of secretion, hormonal transport in the circulation, the development of hormonal receptors on the effector cell, and, finally, the development of the enzymatic and metabolic response in the effector cell to hormonal receptor activation. This has been recently reviewed for insulin and glucagon in some detail (Milner, 1979) and will be illustrated here by comparing the fetal hepatic response to the two hormones in man and the rat.

In the last 4 days of intrauterine life of the rat both plasma insulin and glucagon concentrations rise several fold, but with the preservation of an insulin-glucagon ratio of more than 10 (Girard et al., 1974). Immediately after birth there is a precipitous fall in plasma insulin and a steep rise in plasma glucagon, with a consequent drop in the insulin-glucagon ratio to unity which is maintained during fasting for 16 hr (Girard et al., 1973). When a maternal fast was imposed for the last 96 hr before term, the effect on the fetus was to lower blood glucose and gluconeogenic substrate and to raise blood ketone bodies (Girard et al., 1977). This was associated with a fall in fetal plasma insulin and a rise in glucagon anticipating the changes that occur normally in the immediate postnatal period. There was an increase in activity of key gluconeogenic enzymes in fetal liver of starved pregnancies, suggesting that the fetal metabolic response was both ketogenic and gluconeogenic, both actions being characteristic of glucagon (Schade et al., 1979).

Rat fetuses injected with exogenous insulin at term become hypoglycemic and form more hepatic glycogen (Manns and Brockman, 1969). Conversely, fetuses injected with

insulin antibody or glucagon become hyperglycemic and have decreased glycogen (Hunter, 1969; Manns and Brockman, 1969; Picon et al., 1970). These observations show indirectly that the fetal rat hepatocyte has receptors for insulin and glucagon, but that the concentration of receptors on the hepatocyte membrane differs with age and between the two hormones. Binding of [$^{125}$I] glucagon by 15-day fetal hepatic membranes was 1% of the adult level and 23% of that on day 21 (Blazquez et al., 1976). In contrast, insulin binding on day 15 was 11% of the adult level and 45% of that on day 21. Thus at equimolar concentrations of the two hormones the fetal rat hepatocyte is more sensitive to the action of insulin than glucagon. Both hormones become equipotent, that is, achieve their adult membrane binding characteristics by 30 days postnatally.

A comparison of the biochemical ontogeny of the rat and human hepatocyte reveals similarities, especially when enzymic activity is expressed per unit wet liver weight and as a fraction of that found in the adult (Greengard, 1977). Of particular interest is the observation that some enzymes which normally appear postnatally in the rat can be evoked by the administration of glucagon to the fetus. For example, exogenous glucagon stimulates the activity of phosphorylase (Philippidis and Ballard, 1970), phosphoenol pyruvate carboxykinase (Yeung and Oliver, 1968), glucose-6-phosphatase, and tyrosine amino transferase (Greengard and Dewey, 1967). Glucagon may play a part in the normal enzymic ontogeny of glycogenolysis and gluconeogenesis. Precocious induction of these pathways may result if hyperglucagonemia is the consequence of metabolic or drug-induced alteration of the fetal environment.

The onset of oral feeding with a fat-rich milk after birth results in lipogenesis despite a low insulin-glucagon ratio and a need for thermogenesis. This implies that the (pre-) adipocyte, like the hepatocyte, is more sensitive to insulin than to the stimuli of lipolysis.

Little is known about glucagon physiology in the human fetus. Immunoreactive glucagon is present in the pancreas from week 7 (Assan and Girard, 1975) and the D cells are the first in the islet to granulate (Like and Orci, 1972). Fragments of human fetal pancreas of 8-20 weeks gestational age incubated or perfused in vitro released glucagon consistently in response to epinephrine or arginine (Assan and Girard, 1975). Since it is not possible to collect blood from an unstressed human fetus, there is no information on the physiological concentration of glucagon in the fetal circulation. In the same way it is difficult to be confident that measurements of plasma insulin in cord blood samples at different stages of development reflect those of the unstressed fetus in utero, but the human B cell is capable of secreting insulin to a wide variety of stimuli, but not glucose, from 12 weeks gestation (see Milner et al., 1975, 1979). Sensitivity of the B cell to glucose as an insulin secretogogue probably develops at weeks 28-30 (Milner, 1981).

Pieces of human fetal liver from abortuses of 5-25 weeks have been grown in organ culture (Schwartz and Rall, 1975a). The incorporation of alanine into glucose and glycogen was stimulated by glucagon and theophylline. Unfortunately the two agents were not tested independently and it is impossible to deduce if the effect was mediated by glucagon or by theophylline. Insulin abolished the stimulation of gluconeogenesis by glucagon plus theophylline. Insulin increased tissue glycogen accumulation from fetuses of 7 or more weeks gestation and glucagon depleted hepatic glycogen stores from 6-week fetuses (Schwartz et al., 1975). When both hormones were present in pharmacological and presumably maximum concentrations, the glucagon effect overrode

the insulin effect. Further work led to the conclusion that the two hormones exerted their action via the D form of glycogen synthetase and did not influence phosphorylase activity (Schwartz and Rall, 1975b). In an extension of this work, human fetal livers were perfused in vitro with a synthetic recirculating medium. When the medium contained no glucose, there was a transient release of glucose from the liver which was suppressed by the addition of glucose to the medium. Hepatic glycogen was the predominant source of glucose and could account for all the glucose liberated. When glucagon was added to the perfusion medium, hepatic glucose production doubled, but the addition of insulin had no effect on hepatic glucose production or uptake (Adam et al., 1978). The authors comment, however, that the experimental model was inappropriate for detection of an insulin effect. These observations illustrate how the human liver is able to respond to insulin and glucagon in the second quarter of fetal life and may lead the reader to speculate on their actions on fetal hepatic carbohydrate and lipid metabolism in utero.

Two experiments of nature illustrate the role of insulin on human adipose development. The overweight infant of the diabetic mother (IDM) and the scrawny baby with transient diabetes mellitus provide a dramatic contrast. The IDM is overweight mainly due to excess lipid (Osler, 1960) which develops in the last 10-12 weeks of pregnancy owing to fetal hyperinsulinism. The fact that both normal and excess lipogenesis occur only in the last quarter of pregnancy could be due to the appearance of insulin receptors on preadipocytes at this time or it could be related to the onset of glucose sensitivity in the fetal B cell. The infant with transient neonatal diabetes provides more compelling evidence for a physiological role of insulin in adipocyte development. These babies lack subcutaneous fat at birth, which subsequently develops normally in response to therapy and following resolution of the illness (Gentz and Cornblath, 1969).

At birth the concentration of glucagon in the human infant is low and there is a rise by the age of 2 hr which is greater in small-for-dates infants and less in IDMs than in normal infants (Bloom and Johnston, 1972; Milner et al., 1973). The perinatal changes in glucagon and insulin are qualitatively similar in man and the rat, but quantitatively less dramatic in the human. They are appropriate to the change from lipo- and glycogenesis characteristic of late fetal life to a pattern of lipolysis and glycogenolysis which typifies early postnatal existence pending the establishment of oral feeding. Infants studied at the age of several weeks when they were gaining weight rapidly were still characterized by having high plasma glucagon and low plasma insulin concentrations, conditions characteristic in the adult of catabolism, not anabolism (Milner et al., 1981). This could indicate a balance of insulin and glucagon receptors in the human neonate different from that found in the adult.

Although glucagon is lipolytic and may play a controlling role in lipid metabolism (Schade et al., 1979), the primary control of adipocyte lipolysis after birth depends on catecholamines. Although considered here as a hormone, norepinephrine is released mainly as a sympathetic neurotransmitter and adrenomedullary catecholamine secretion is probably of secondary importance. Catecholamines accelerate lipolysis from fetal adipose tissue in vitro (Elphick et al., 1975) or when infused into the fetal circulation (Dawkins, 1964; Comline and Silver, 1972), but under normal circumstances endogenous fetal catecholamine release is likely to be negligible. The stress of labor for the fetus is accompanied by increased catechol secretion (Eliot et al., 1981) and in early postnatal life adipocyte lipolysis is activated by norepinephrine released from postganglionic sympathetic neurons terminating in both brown and white adipose tissue (Hull, 1974).

Sympathetic activity may also influence fat and carbohydrate metabolism indirectly by changing the secretion of insulin and glucagon from the islets of Langerhans, and experimental evidence suggests that cutting the umbilical cord may initiate this chain of events (Grajwer et al., 1977).

## CLINICAL ASPECTS

### Adaptation to Extrauterine Life

This book is concerned mainly with the fetus and in particular the human, but the moment of birth is a most artificial time to cease the narrative on fat and carbohydrate metabolism, as dramatic changes occur as a result of birth. The newborn infant, unlike the fetus, has been fair game for the clinical investigator and a clear account of the relevant metabolic changes can be given with little recourse to data from laboratory species.

In the normal term infant the plasma glycerol level rises sharply in the first minutes of life and free fatty acids (FFA) in the early hours, while glucose levels fall gently (see Cornblath and Schwartz, 1976). By the age of 12 hr FFA levels have stabilized at three to four times the umbilical cord concentration, whereas glucose has fallen by approximately one-third. The respiratory quotient of the infant falls over the same interval, from 1.0 to 0.7, signifying a transfer to fat as the principle source of energy. The typical IDM has an exaggerated fall in blood glucose and an attenuated rise of FFA because of hyperinsulinemia (see Pedersen, 1977). The preterm infant, especially the very low birthweight infant, lacks both hepatic carbohydrate and subcutaneous adipose stores, with the result that circulating fuel levels are low and may run out, leading to exhaustion of cardiac and respiratory muscle and cerebral damage. Small-for-dates infants are also characterized by a tendency to hypoglycemia, the etiology of which is complicated because these babies are a heterogeneous group, some of them being hyperinsulinemic (Le Dune, 1972).

Studies with adipose tissue samples collected by needle aspiration from newborn infants (see Hahn and Novak, 1975) have yielded results consonant with the measurements of circulating metabolites in the human infant and experimental data collected from other species in the neonatal period.

The supply of fuel to the neonatal brain is of paramount importance in determining the subsequent quality of life. Persson and his colleagues have studied this in man by making determinations of cerebral blood flow, cerebral arteriovenous differences of oxygen, and circulating metabolites in infants under general anesthesia immediately prior to elective surgery (Settergren et al., 1976). A net cerebral uptake was demonstrated for glucose, acetoacetate, and D-β-hydroxybutyrate and a net release of lactate and pyruvate. The calculated rate of cerebral blood flow and uptake of ketone bodies was greater in infants than had been previously reported in adults. The contribution of ketone bodies to cerebral metabolism assuming complete oxidation was approximately 13%. The effect of feeding and starvation was tested in similar studies using infant rats, where it was shown that the contribution to cerebral metabolism made by ketone bodies rose during starvation (Dahlquist and Persson, 1976). The implication is that the products of fat oxidation form a valuable fuel for the human neonatal brain, especially when nourishment is subnormal or absent.

## Nutrition Before and After Birth

Appreciation that fetal fat and glucose metabolism are influenced by the plane of maternal nutrition led investigators to consider the effect of different patterns of maternal feeding, starvation, and intravenous nutrient supplementation on fetal well-being.

The influence of maternal diet on white adipose tissue fatty acids is illustrated by the fact that infants born to Dutch mothers had small but significantly higher concentrations of palmitic, oleic, and linoleic acids in their fat stores than did infants of English mothers (Widdowson et al., 1975). This is probably the consequence of a greater consumption of "soft" margarine and other unsaturated fats by the pregnant Dutch women.

Women are often anorexic and may be nauseated during labor. This coupled with the stress of parturition leads to high circulating FFA levels and, in some cases, ketosis. Severe ketoacidosis in diabetic pregnancy is associated with a high perinatal mortality (Drury et al., 1977) and ketonuria in diabetic pregnancy has been causally related to mental impairment of the offspring (Churchill et al., 1969). Obstetric practice has been influenced by such findings so that it is common for ketonuria during labor to be treated by intravenous glucose infusion. Glucose has also been given to women in the first and second stage of labor for the benefit of their small-for-dates fetuses (Sabata et al., 1973). Maternal hyperglycemia was associated with fetal hyperglycemia and reduced FFA levels as reflected in the cord blood. A beneficial effect for the infant was claimed, but experiments with lambs indicate that this should be interpreted with caution. Glucose infused into chronically catheterized fetuses was associated with a modest rise in plasma lactate when the lamb was well oxygenated, but a rapid progressive rise in lactate and a fall in pH when the fetus was mildly hypoxic (Shelley et al., 1975). Human fetal hypoxia associated with maternal glucose infusion could have similar consequences.

The complexity of such an apparently simple procedure as giving a pregnant woman intravenous glucose is illustrated by the effect of maternal glucose infusion on placental fatty acid transfer in rabbits (Elphick et al., 1978a). Maternal and fetal circulating glucose and insulin levels rose and maternal plasma FFA levels fell. Despite this, there was little change in fetal plasma FFA levels. The authors concluded that the maternal glucose infusion or the high plasma insulin which resulted increased the flow of FFA to the fetus, probably from sources other than the maternal FFA. These results should be born in mind when changes in human diabetic pregnancy are being considered.

The adverse outcome of ketotic diabetic pregnancy is more likely to be due to the acidosis than to ketone bodies per se. When pregnant rabbits were starved for 48 hr at term, there was lipid mobilization by the mother, increased FFA transfer to the fetus, which was stored as tissue triglyceride, particularly in the liver and adipose tissue (Edson and Hull, 1977). Conditions which increase FFA and ketone body transfer across the human placenta, in the absence of acidosis, may have a beneficial rather than a deleterious effect on fetal outcome.

Women in labor have been given lipid infusions for short periods to study placental permeability, rather than as an attempt to augment fetal fuels. The evidence indicates that such treatment can increase fetal circulating FFA and triglyceride levels (Rubaltelli et al., 1978), but whether more prolonged treatment might have a beneficial or deleterious effect on the newborn infant awaits further study.

It is now normal practice to feed a newborn baby with milk, preferably human, from the early hours of life. This has come about with the realization that some infants, especially the small-for-dates and preterm ones, are at risk of fuel shortage and may

suffer cerebral and other damage in association with hypoglycemia (Smallpiece and Davies, 1964). The pattern of fuel delivery resulting from milk feeding is well illustrated by a study in which oxygen consumption, the respiratory quotient (RQ), and plasma metabolite levels were measured in IDM and small-for-dates babies from the first to the eleventh day of life (Gentz et al., 1976). The RQ fell to a nadir at 24-48 hr of age and then rose progressively. The highest hydroxybutyrate levels were found at the same time as the lowest RQ levels and provided confirmatory evidence of a high rate of fat oxidation. Feeding caused the RQ to rise to unity. Oxygen consumption rose to a maximum 60-90 min after a feed and was accompanied by an increase in rectal temperature. Oxygen consumption also rose with advancing postnatal age and increasing milk intake, reflecting the caloric cost of growth.

Not all babies can tolerate oral feeding from birth and some require parenteral supplementation or even total parenteral nutrition. Glucose infusions are used to supply water and calories and the glucose tolerance of low birth weight infants treated in this way has shown what infusion rates can be tolerated safely (Cowett et al., 1979). Glucose was infused via a peripheral vein at rates of 8, 11, or 14 mg/kg per minute for 3 hr and plasma glucose, insulin, and timed urine glucose and volume were measured. At each infusion rate a steady blood level was noted by the end of 1 hr. In the infants receiving 8 mg/kg of glucose there was no change in plasma glucose insulin or glycosuria. All the infants receiving 14 mg/kg of glucose became hyperglycemic and glycosuric. Those given 11 mg/kg of glucose could be divided into two groups: those that became hyperglycemic and glycosuric and who had no insulin response to glucose and those who maintained normal plasma glucose and had a rise in plasma insulin in response to the infusion. These results show how intravenous glucose alone can provide more than enough calories for the infant's basal requirements and that despite glycosuria at the higher infusion rates there was 99% glucose retention and no osmotic diuresis.

The search for parenteral calories has led to the use of intravenous fat emulsions in the newborn, usually as part of a parenteral feeding regimen. This provoked an interest in neonatal lipid tolerance which can be studied conveniently using Intralipid. Preterm infants who were of normal body weight cleared the emulsion faster than those who were small for dates. The small-for-dates preterm infants had impaired lipolysis and triglyceride accumulation which was improved by heparin (Olegard et al., 1975). This was confirmed by Andrew et al. (1976, 1978), who also demonstrated that triglyceride hydrolysis improved with advancing gestational age. They showed that plasma triglyceride, FFA, and ketone bodies were higher in small-for-dates babies at the end of an Intralipid infusion and fell more slowly thereafter, and suggested that this might be due not only to a deficiency in lipoprotein lipase but also to impaired $\beta$-oxidation of FFA to ketones.

### Hypoxia, Acidosis, and Cold

Hypoxia in the newborn is commonly associated with respiratory and metabolic acidosis, so that it may be difficult to disentangle hypoxia from acidosis in clinical problems. Cold exposure is also bad for babies because an increased metabolic rate is necessary to maintain a constant body temperature. If the cold stress is so severe as to cause hypothermia, there may be serious pathological sequelae (Mann and Elliott, 1957).

The rate of FFA mobilization in the newborn depends not only on the neuronal and hormonal stimulation and inhibition of lipolysis, but also on changes in the uptake, re-esterification, and oxidation of FFAs within adipose tissue. In the human infant early postnatal lipolysis occurs in a thermoneutral environment due to increased sympathetic activity, but cold exposure can exaggerate the rise in plasma glycerol and FFA occurring naturally (Pribylova and Rylander, 1972). Acidosis in the first 2 postnatal hours has little or no effect on plasma changes in glycerol and FFA (Persson and Tunell, 1971); however, animal experiments have shown that either acidosis or hypoxia can inhibit cold-induced lipolysis (Baum, 1967; Poyart and Nahas, 1966) and similar changes may occur in man if the stress were more prolonged. Hypoxia has a further adverse effect on neonatal fat and carbohydrate metabolism because of a reduced efficiency in fuel oxidation.

Cold exposure has no short-term predictable effect on blood glucose levels and a complex action on circulating concentrations of the hormones involved in regulating fat and glucose. Exposure to environmental temperatures of 22.5-28.0°C for 1 hr caused no significant change in mean plasma concentrations of insulin, glucagon, growth hormone, or thyrotropin in newborn term, preterm, or small-for-dates infants (Fekete et al., 1972). Similarly, when exchange transfusions were performed using warm or cold blood, there was no significant difference between the two groups of babies in plasma insulin, glucagon, or growth hormone levels, despite the cold transfusions being associated with a greater net retention of glucose and smaller net loss of FFA by the baby (Milner et al., 1972); however, animal studies indicate that it may be naive to draw conclusions from plasma concentration measurements. Cold exposure in newborn rabbits was associated with a significant rise in both plasma glucose and insulin, as well as the well-known elevation of FFA (Cser et al., 1977). The glucose rise may have been the consequence of increased glycogenolysis or decreased glucose utilization and the insulin rise secondary to the glucose rise. The full analysis of neonatal metabolite and hormonal responses to cold exposure will only become possible by the use of a model in which frequent repeated measurements can be made.

### Drugs

The effects of prenatal or postnatal drug exposure on fat and carbohydrate metabolism do not appear to have been studied systematically. Some drugs have profound clinical effects, as will be seen in the following examples.

Diabetic women may be treated with oral sulfonylureas during pregnancy (Stowers, 1979). In this case the fetus is exposed not only to the effects of a disturbed maternal metabolism, but also to the drug which crosses the placenta. Such an infant is born hyperinsulinemic and with therapeutic circulating levels of an insulinotropic drug that is degraded slowly because of the immaturity of the neonatal liver. These babies can suffer profound intractable hypoglycemia and treatment by exchange transfusion may be necessary to remove the drug (Kemball et al., 1970).

As described above, heparin stimulates lipolysis by activation of lipoprotein lipase. Heparin added in low doses to neonatal infusion fluids may have desirable metabolic effects, as well as being antithrombogenic. The effect of heparin on plasma metabolites is seen vividly when exchange transfusion is performed using blood preserved with heparin or acid citrate and dextrose (Cser and Milner, 1974).

A group of drugs with great clinical relevance to perinatal metabolism are the $\beta$-sympathomimetics and $\beta$-blockers. The $\beta$-mimetics are commonly used to suppress

uterine contractions in the management of preterm labor or fetal distress and may be administered for periods ranging from hours to weeks. β-Blockers are given to pregnant women less commonly, but patients with thyroid or cardiac disease may receive them throughout pregnancy. The placenta is permeable to both classes of drug (see Van Petten, 1975; Cottrill et al., 1977). Interest in fetal toxicity has focused mainly on embryopathic and the cardiovascular effects, but the control of lipolysis by the sympathetic nervous system means that any drug acting on it may be predicted to affect fat and carbohydrate metabolism.

Brettes et al. (1976) reported improved fetal growth of small-for-dates infants whose mothers were treated with the β-sympathomimetic ritodrine and claimed that this was due to increased uterine blood flow and improved fetal nutrition, an interpretation which has subsequently been queried (Lunell and Sarby, 1979). An alternative possibility was that ritodrine could induce maternal carbohydrate intolerance. This prompted Blouin et al. (1976) to study glucose tolerance in ritodrine-treated and control pregnancies. No difference was found between the groups in either mother or baby. Other work reported subsequently suggests that had lipid metabolism been studied, differences between ritodrine and control pregnancies might have been observed. Isoxuprine, another β-mimetic, caused a doubling of plasma FFA, no change in plasma glucose, but a significant rise in plasma insulin and the insulin-glucagon ratio when given to rabbit pups nursed in a thermoneutral environment. When the animals were kept in the cold, there was the anticipated rise in plasma FFA and this was increased further by isoxuprine treatment (Cser et al., 1977). It would be interesting to find out if the infants, who are usually preterm, born to women treated with β-mimetics have an exaggerated rise in plasma FFA postnatally and to consider whether the fetal growth-promoting effect claimed for ritodrine might be due to increased transplacental passage of lipid, as proposed for IDM by Szabo and Szabo (1974).

Propranolol is the most common β-blocker in clinical use today. Neonatal hypoglycemia lasting for 2-3 days features among the reported complications of propranolol therapy in pregnancy (see Habib and McCarthy, 1977), but it is difficult to demonstrate direct causality or a convincing pathophysiology. Propranolol given to rabbit pups as a single injection abolishes the rise in blood glucose, serum FFA, and oxygen consumption induced by cold (Dober et al., 1978). The pharmacological actions of transplacentally acquired propranolol in the human lasts for days (Cottrill et al., 1977) and these babies probably have abnormal fat and carbohydrate homeostasis with an impaired ability to respond to cold exposure.

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# 6

# Maternal, Fetal, and Neonatal Amino Acid and Protein Metabolism

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## INTRODUCTION

We have taken the position that the circulating free amino acid pool lies at the center of the anabolic and catabolic utilization of the protein precursors. Although the plasma free amino acid content represents a very small fraction of the total amino acids of the body, its quantitative and qualitative changes can be used, for example, to estimate the protein status and judge the type and severity of deviations of amino acid and protein economy. Besides the slow changes of the relatively well regulated composition of the circulating amino acid pool to nutritional and body compositional changes, rapid responses of the plasma amino acid profile can also be used as sensitive indicators of endocrine interactions and metabolic adjustments involved in different physiological and pathological conditions.

As clinicians, we prefer to consider the plasma free amino acid alterations and interrelations in the mother and fetus, as well as in the neonate immediately after birth. We should like to call attention to the potential significance of such quantitative and qualitative changes in relation to different stages of pregnancy, transplacental amino acid supply, fetal growth, biochemical maturity, and neonatal metabolic adaptation under normal and abnormal conditions. The present knowledge concerning these aspects stems largely from the amino acid analysis of body fluids, and it is this easily accessible experimental and clinical tool which continues to contribute to our understanding of the function of the circulating free amino acids.

In our consideration of protein metabolism we have tried to incorporate concepts which seem to us to be real advances, such as the protein synthesis rate in the fetus and newborn. The reader will notice some omissions and should appreciate that this review is not intended to cover all maternal, fetal, and neonatal aspects of amino acid and protein metabolism. Our overriding consideration has been to be clinical in orientation and practical in presentation. This review is, in fact, for the pediatrician, obstetrician, and medical student.

## THE MATERNAL CIRCULATING AMINO ACID POOL IN NORMAL PREGNANCY

### Changes in Total Plasma Amino Acids

Using the α-aminonitrogen level as a measure of the circulating amino acid pool, Bonsnes (1947) showed more than 30 years ago that the maternal amino acid concentration is lowered during pregnancy (average, 3.2 mg/100 ml), returning to normal levels (average, 4.1 mg/100 ml), on the first or second day after birth. This relatively early drop of the

Table 1 The Total Plasma Concentration (μM/liter) of 19-20 Amino Acids in Nonpregnant Women and Pregnant Women at Different Gestational Periods

| Nonpregnant | Period of gestation (weeks) 15-20 | 33-37 | 38-41 | 42-45 | References |
|---|---|---|---|---|---|
| 1827 | – | 1571 | 1373 | – | Lindblad and Baldesten, 1967; Lindblad and Zetterström, 1968 |
| 1861 | 1658 | 1526 | 1445 | 1613 | Young and Prenton, 1969 |
| – | 1522 | 1660 | 1603 | – | Cockburn et al., 1970, 1971 |

circulating amino acid pool during pregnancy is supported by recent research dealing with the maternal plasma aminogram at immature, premature, term, and post-term deliveries (Ghadimi and Pecora, 1964; Lindblad and Baldesten, 1967; Lindblad and Zetterström, 1968; Young and Prenton, 1969; Cockburn et al., 1970, 1971). From Table 1 it is seen that the sum of the amino acid concentrations is definitely lower in the first half of the second trimester and this value is either maintained or continues to drop during the later stages of pregnancy. It should be noted that in Young and Prenton's (1969) data the progressive decline in total plasma amino acids is followed by a rise after 42 weeks, which together with the decreased fetal-maternal ratio might indicate an impaired placental amino acid transfer in prolonged pregnancy.

### Changes in the Pattern of Plasma Amino Acids

The levels of the majority of amino acids in maternal plasma are lower than those of nonpregnant women. However, the observed degree of reduction varies greatly. In Young and Prenton's study (1969) 7 of 19 amino acids, namely, glycine, leucine, ornithine, lysine, arginine, serine, and α-amino-n-butyrate, were found to be significantly reduced. These findings are in agreement with those of Zinneman et al. (1967), whose values in the third trimester were compared with those 6-8 weeks after delivery.

It should be noted that a few amino acids maintained their level or showed a tendency to increase. An example of the latter is alanine, which was found to be elevated in each series of examinations. Although the increase of its concentration was statistically insignificant, it appears to be a constant characteristic of the maternal amino acid pattern in normal pregnancy.

## FETAL-MATERNAL AMINO ACID RATIO

### Total Plasma Amino Acids

Recent studies using column chromatography for the determination of total and individual amino acids fully confirmed the active amino acid transport suggested by the uphill gradient between the α-aminonitrogen content of maternal and fetal plasma (Christensen and Streicher, 1948; Clemetson and Churchman, 1954). Table 2 summarizes the mean fetal-maternal ratio of total amino acids calculated from the data reported during the last 20 years by different authors. It can be seen that at full-term

Table 2 Mean Fetal-Maternal Amino Acid Ratio Within Different Periods of Gestation (Calculated from Date)

| Range of gestational age at delivery | | | | |
|---|---|---|---|---|
| Immature (15-25 weeks) | Premature (33-38 weeks) | Mature (39-42 weeks) | Postmature (42 weeks) | References |
| – | – | 2.0 | – | Butterfeld and O'Brien, 1963 |
| 3.4 | 2.2 | 1.9 | – | Ghadimi and Pecora, 1964 |
| – | 2.1 | 1.9 | – | Lindblad and Baldesten, 1967; Lindblad and Zetterström, 1968 |
| 2.8 | 1.8 | 1.9 | 1.6 | Young and Prenton, 1969 |
| 2.3 | 1.8 | 1.7 | – | Cockburn et al., 1970, 1971 |
| – | – | 1.6 | – | Glendening et al., 1961 |
| – | – | 1.6 | – | Velazquez et al., 1976 |

vaginal deliveries the ratio was found to be 1.9 or 2.0 and 1.6 or 1.7 by four (Ghadimi and Pecora, 1964; Lindblad and Baldesten, 1967; Young and Prenton, 1969; Butterfield and O'Brien, 1963), and three (Cockburn et al., 1971; Glendening et al., 1961; Velazquez et al., 1976) groups of investigators, respectively. The mean ratios at immature and premature birth (Ghadimi and Pecora, 1975; Lindblad and Baldesten, 1967; Lindblad and Zetterström, 1968; Young and Prenton, 1969) support the conclusion drawn from earlier studies (Christensen and Streicher, 1948; Clemetson and Churchman, 1954) dealing with the transplacental gradient of α-aminonitrogen, that the fetal-maternal transplacental gradient is more pronounced in the earlier stages of pregnancy. This trend is particularly evident if ratios between 15 and 25 weeks are compared with those obtained at full-term vaginal deliveries. It is of interest and importance that Young and Prenton (1969) observed a further drop in the overall fetal-maternal ratio in prolonged pregnancy (42-45 weeks), pointing toward a possible progressive impairment of placental amino acid transfer as gestation proceeds beyond 42 weeks.

### Individual Plasma Amino Acids

In order to explore the changes of fetal-maternal ratios of individual amino acids during normal pregnancy, the observations of four groups of authors have been thoroughly analyzed and used to calculate average ratios as indices of active placental amino acid transfer.

The mean fetal-maternal ratios corresponding to three stages of pregnancy are given in Figure 1, from which the following conclusions may be drawn: (1) The individual variations of the ratios of the cord vein levels to the mother's venous level are large at deliveries in each period tested; (2) the transplacental gradients of most amino acids at 15-20 weeks exceed those observed in the later periods; (3) for a number of amino acids (alanine, valine, leucine, isoleucine, aspartic acid, glutamic acid, phenylalanine, proline, citrulline, ornithine, and methionine) there is a considerable, mostly

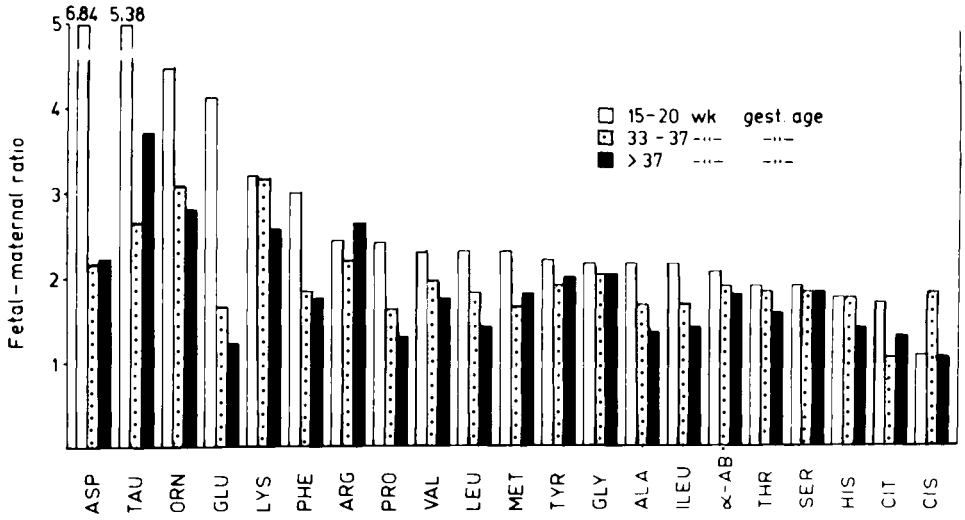


**Figure 1** The means of fetal-maternal ratios of amino acids within three periods of gestation calculated from data reported by four groups of authors (From Ghadimi and Pecora, 1964; Lindblad and Baldesten, 1967; Lindblad and Zetterström, 1968; Young and Prenton, 1969; Cockburn et al., 1970, 1971.)

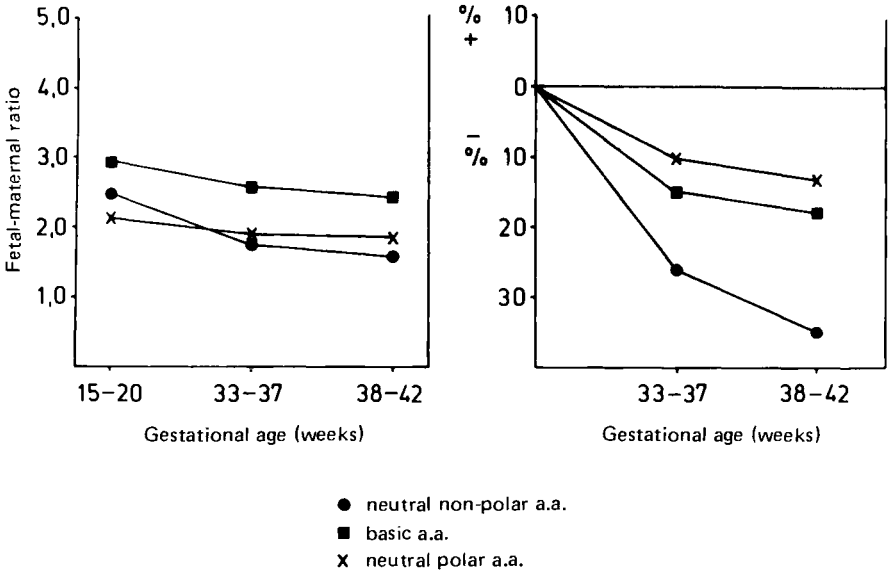


**Figure 2** (Left) Mean fetal-maternal ratios of the three transport groups of amino acids within three gestational age periods derived from the data reported by four groups of authors. (From Ghadimi and Pecora, 1964; Lindblad and Baldesten, 1967; Lindblad and Zetterström, 1968; Young and Prenton, 1969; Cockburn et al., 1970, 1971.) (Right) Mean percentage changes of the fetal-maternal ratios of the three transport groups of amino acids in relation to the mean values obtained at 15-20 weeks of gestation.

progressive decrease of the fetal-maternal ratios with gestational age; and (4) the ratios of a few amino acids, such as glycine, serine, cystine, tyrosine, and arginine, are maintained or fall only slightly with the progress of pregnancy.

### Different Transport Groups of Amino Acids

The data summarized in Figure 1 have also been grouped according to the side-chain chemistry by which membrane transport processes are able to distinguish one amino acid from another. The mean fetal-maternal ratios of three transport groups and their percentage changes with gestational age are depicted in Figure 2. The largest decline is shown by the group of neutral, nonpolar amino acids (leucine, isoleucine, valine, alanine, methionine, proline, phenylalanine). The rate of fall of the fetal-maternal gradient with gestational age is much lower in the neutral, polar and dibasic groups of amino acids.

## FETAL-MATERNAL RELATIONSHIP OF THE PLASMA FREE AMINO ACIDS IN TOXEMIA, PROLONGED PREGNANCY, RETARDED FETAL GROWTH, AND MATERNAL NUTRITION

### Total Plasma Amino Acids in Toxemia

Among the abnormal, complicated pregnancies, toxemia is the most important condition which may impair the placental transport of nutrients to the fetus. Its importance in relation to the fetal amino acid supply is underlined by fetal malnutrition frequently associated with preeclamptic toxemia. The decreased fetal-maternal α-aminonitrogen gradient reported by Clemetson and Churchman (1954) suggests that the placental amino acid supply is adversely affected by toxemia. From these data it follows that the fall in the fetal-maternal α-aminonitrogen ratio is brought about by a marked rise in the maternal level (Table 3). These early observations already suggested a decreased fetal amino acid uptake from the maternal blood due to severe toxemia.

Figure 3, based on the data of Cockburn et al. (1971), visualizes the total maternal vein and cord vein concentrations of 20 amino acids estimated by ion exchange chromatography in normal and toxemic pregnancies. In fact, both circulating amino acid pools increase in toxemia, but the elevation in the maternal pool (30%) is considerably greater than that of the fetal one (12%), resulting in a fall of the overall fetal-maternal ratio

Table 3 Maternal and Fetal α-Aminonitrogen Levels and Ratios

| Type of pregnancy | Number of cases | Maternal $\alpha$-$NH_2$-N[a] | Fetal $\alpha$-$NH_2$-N[a] | Fetal-maternal $\alpha$-$NH_2$-N ratio[a] |
|---|---|---|---|---|
| Nontoxemia | 11 | 2.96 ± 0.11 | 4.84 ± 0.14 | 1.65 ± 0.049 |
| Mildly toxemic | 9 | 3.23 ± 0.21 | 4.96 ± 0.24 | 1.56 ± 0.077 |
| Preeclamptic | 10 | 3.52 ± 0.22 | 4.87 ± 0.031 | 1.37 ± 0.076 |

[a]Values expressed as mg/100 ml ± SE.
*Source*: Clemetson and Churchman (1954).

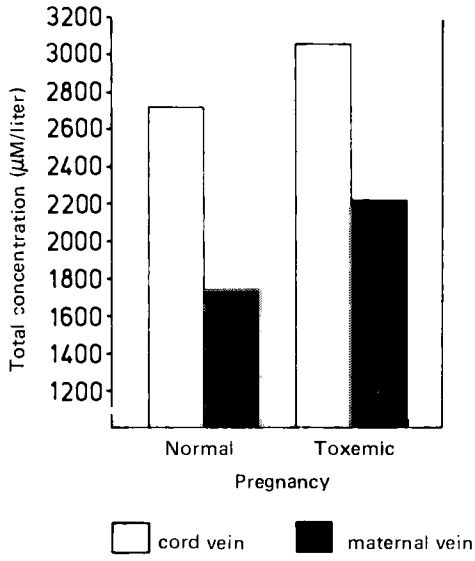


Figure 3 Total umbilical vein and maternal vein concentrations of 20 amino acids in normal and toxemic pregnancies. (From data reported by Cockburn et al., 1971.)

(1.60 versus 1.40). In contrast, Lindblad and Zetterström (1968) found no such differences in total amino acid concentrations and hence a decreased value in the overall fetal-maternal ratio.

### Three Transport Classes of Amino Acids in Toxemia

From Figure 4 it can be seen that Cockburn et al. (1971) found a maternal and fetal rise in the combined concentration of the neutral, nonpolar and neutral, polar amino acids. Since two neutral, polar amino acids were not determined by Lindblad and Zetterström (1968), only the concentration of the neutral, polar class is depicted, whose cord level shows a tendency to increase. The total concentration of the three branched-chain amino acids belonging to the neutral, nonpolar group has been separately calculated and demonstrated. Both groups of authors observed an increase of the concentration of leucine, isoleucine, and valine in maternal plasma, while the cord level remained unchanged. The same fetal-maternal relationship applies to the basic amino acids according to the observations of Cockburn et al. (1971). In Lindblad and Zetterström's study (1968), however, both the fetal and maternal levels of basic amino acids were maintained.

### Prolonged Pregnancy and Retarded Fetal Growth Associated with Nontoxemic Pregnancy

Besides toxemia, prolonged (post term) pregnancy is also frequently associated with placental insufficiency and intrauterine growth retardation. The question arises as to whether the alterations of the relationship between the fetal and maternal circulating free amino acids are comparable to those observed in toxemic mothers. This question can be answered by Young and Prenton's (1969) interesting study performed in women with normal and prolonged pregnancy giving birth to small-for-dates infants.

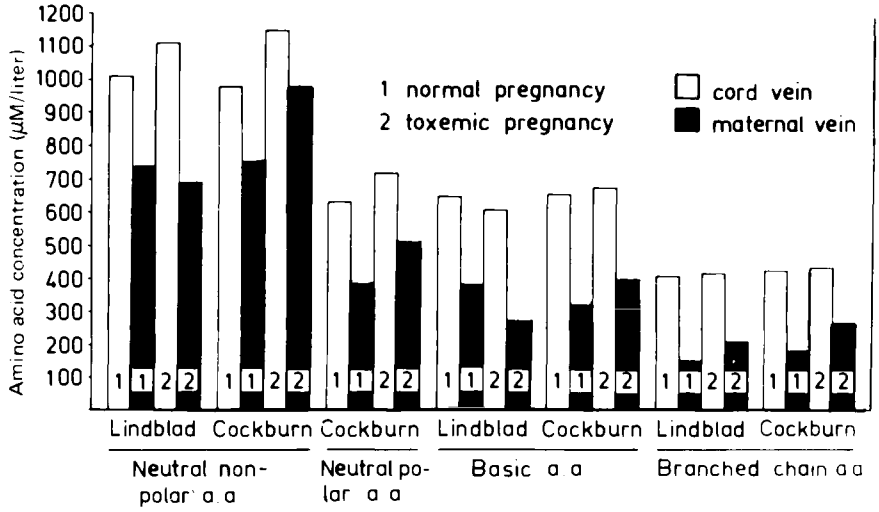


**Figure 4** The total maternal and cord vein plasma concentration of three transport groups of amino acids calculated from the data reported by Lindblad and Zetterström (1968) and Cockburn et al. (1971). The total concentrations of the branched-chain amino acids are also separately shown.

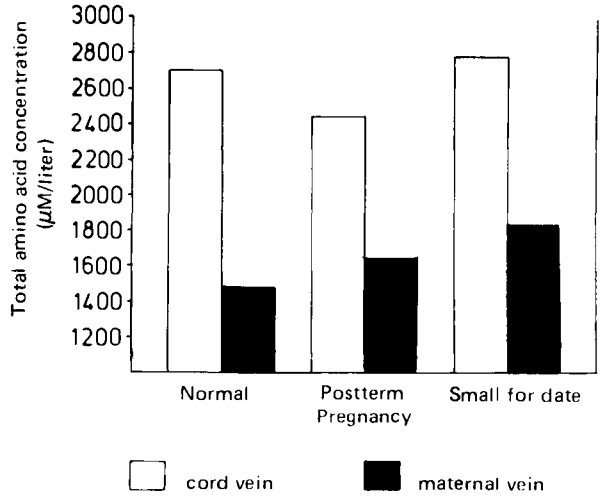


**Figure 5** Total cord vein and maternal vein concentrations of amino acid in normal, post term and small-for-dates pregnancies. (From Young and Prenton, 1969.)

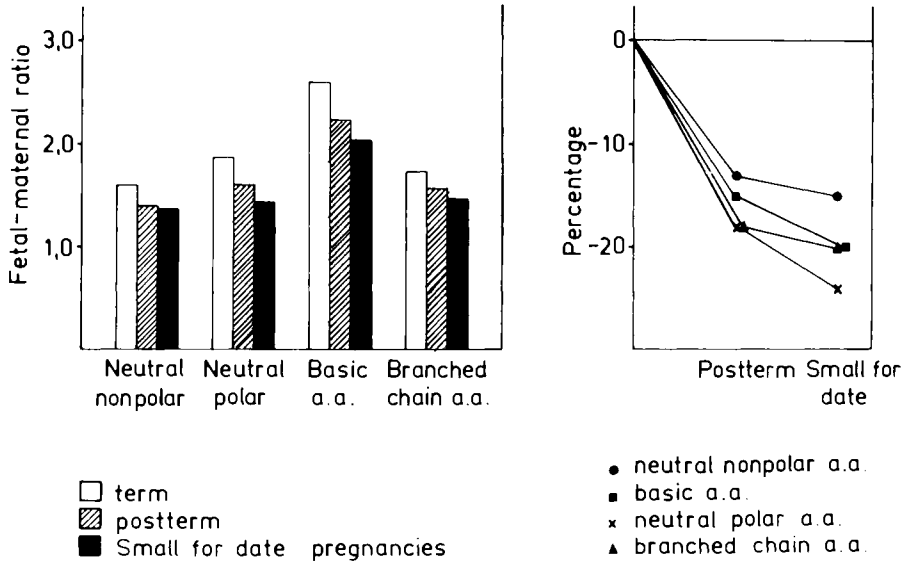


**Figure 6** (Left) Mean fetal-maternal ratios of the three transport groups of amino acids at normal term, post term and small-for-dates deliveries. The mean ratios of the branched-chain amino acids are also separately shown. (From Young and Prenton, 1969.) (Right) Percentage fall of the mean fetal-maternal ratios of the different groups of amino acids in post term and small-for-dates deliveries in relation to the normal.

Figure 5 demonstrates that the total concentration of 19 amino acids in the maternal vein of the post term and small-for-dates groups exceeds that obtained in normal pregnancy. This tendency is particularly evident in the small-for-dates groups.

Figure 6, constructed from Young and Prenton's data (1969), presents mean fetal-maternal ratios of the different amino acid classes and their relative drops in post term and small-for-dates pregnancies in relation to control values. The largest fall occurs in pregnancies associated with intrauterine growth retardation. Thus, as in severe toxemic pregnancy, the decrease in mean fetal-maternal ratios of the three transport groups of amino acid is mostly, if not exclusively, due to the increased maternal plasma amino acid content.

### Maternal Malnutrition

Among the factors impairing fetal growth and development, poor maternal nutrition should be considered as one of the most likely causes of fetal undernutrition. Since the maternal protein intake seems to be correlated with anthropometric measures (Kamran et al., 1975; Dieckman et al., 1951) and several other variables of the newborn (Metcoff et al., 1976), the question arises as to whether the plasma aminogram undergoes characteristic changes in pregnant mothers subjected to dietary deprivation, and if so, the extent to which the maternal amino acid profile influences the fetal plasma aminogram. This question appears all the more important, since in preeclamptic toxemia the plasma amino acid profile of the undernourished fetus does not appreciably differ from the normal.

Studying a low socioeconomic group of pregnant mothers in West Pakistan, Lindblad et al. (1969, 1970) found a significant increase in the maternal plasma levels of glycine and ornithine. Cord plasma was characterized by a general hyperaminoacidemia with significantly increased proline levels. The aminogram of the newborn during the first hours after birth showed increased levels of alanine, proline, glycine, and taurine, and there was a delay in the decline of the branched-chain amino acids. This amino acid pattern observed within a few hours of extrauterine life is similar to the plasma aminogram of kwashiorkor (Holt et al., 1963).

The amino acid patterns of underprivileged Ethiopian mothers during delivery and their newborn was also found to be consistent with that characteristic of postnatal protein energy malnutrition (Gebre-Medhin et al., 1978). In this study the glycine-valine ratio was used as an indicator of nutritional deficiency: It was significantly increased in both maternal and cord vein plasma. The higher value of the quotient was solely due to the increased glycine level, whereas in postnatal protein energy malnutrition a decreased valine level also contributed to the increased ratio. It is of interest that the fetal-maternal gradients of amino acids were generally depressed, suggesting a possible impairment of placental amino acid transport.

In contrast to these data obtained from mother's plasma and infant's cord blood at delivery, McClain et al. (1978) related the occurrence of fetal malnutrition with the plasma amino acid profile of mothers from a low-income group at 25 weeks of gestation. Mothers who subsequently gave birth to malnourished infants had significantly lower plasma levels of 10 of the 18 amino acids measured than mothers giving birth to well-nourished infants. The total amino acid concentration was almost 20% lower, but not all amino acids were reduced in the same proportions. Ornithine, arginine, and aspartic acid were over 30% lower, while isoleucine, valine, methionine, and cystine were at or above the normal concentrations observed in mothers with normally grown fetuses. It is notable that arginine, ornithine, and lysine, the three most markedly reduced amino acids, belong to the same transport group.

## SIGNIFICANCE AND SCOPE OF PLACENTAL TRANSFER OF AMINO ACIDS AND FETAL-MATERNAL AMINO ACID RELATIONSHIP

The fetal-maternal amino acid relationship is maintained by a transport system that (1) involves transfer against a gradient, (2) shows discrimination between the D and L isomers of the amino acids, (3) does not involve binding proteins in the fetal plasma in quantities sufficient to account for accumulation, (4) is competitively inhibited by similar amino acids common to a specific transport site and transport group, and (5) can be saturated by increasing concentrations of amino acids.

All of these characteristics suggest that an active transport process is involved in the placental transfer of amino acids. The mechanisms of this system have been extensively studied using a variety of experimental techniques and animals. An excellent review of this literature based on numerous studies of the author is found in Young (1976). More recent data have been published for the primate (Stegink et al., 1975, 1979) and the human by Dancis and his groups (Schneider et al., 1979).

The maternal circulating free amino acid pool represents a potential means to differentiate gestational periods in normal pregnancy (Schoengold and de Fiore, 1977). In view of the overall maternal amino acid pattern at different gestational ages, the pattern-recognizing technique appears to be a better indicator than the use of a control

chart based on the large individual variations of absolute amino acid concentrations. Since, however, the most striking changes occur during the first trimester of normal pregnancy (Schoengold and de Fiore, 1977), the predictive power of the maternal plasma amino acid pattern decreases with the progress of gestation. Therefore serial samples starting very early in pregnancy are needed, which makes the pattern-recognizing technique rather impractical.

The diagnostic value of the deviation of the maternal plasma aminogram from the normal, however, appears to be a more promising field to investigate. As a result of further efforts, besides the elevated levels of branched-chain amino acids, compounds belonging to other transport groups might also turn out to be useful in screening toxemic pregnancies. So far, we think, the best has not been made of the toxemic maternal amino acid profile.

In view of the observations concerning the small-for-dates syndrome, a more detailed study of the relationship between maternal plasma amino acids and the rate of fetal growth in the third trimester and beyond might also offer promise for detecting intra-uterine malnutrition. This is underlined by a recent report showing that the total plasma free amino acids measured during the third trimester are positively correlated with birth weight (Kamran et al., 1975). It is of interest that the maternal amino acid profile has been found to be correlated with the occurrence of fetal malnutrition as early as 25 weeks of gestation (McClain et al., 1978).

Nutrition and the nutritional status of the mother may also alter maternal plasma free amino acid homeostasis and hence the transplacental amino acid supply of the fetus. The amino acid imbalance during different stages of pregnancy, caused by either dietary deprivation or protein or amino acid supplementation, represents an enormous problem of fetal nutrition. In spite of the great interest in this field, we know little about the physiological and metabolic effects of maternal dietary amino acid pattern on the fetus. Nothing is known, for example, about the effects of the deficit or excess of individual or different groups of amino acids on the transplacental transfer and amino acid uptake of the human fetus. Studies on animals (Zamenhof et al., 1968, 1974; Rio et al., 1970; Portela et al., 1977) showing abnormalities of body and brain composition due to amino acid-imbalanced diet underline the importance and necessity of exploring the interrelationship between the dietary amino acid pattern and transplacental delivery of amino acids in mothers subjected to dietary deprivations, or the excess of one or more amino acids. Under such conditions the significance of placental active transport may switch over from an advantage to a disadvantage to the fetus. Therefore whenever oral amino acid or protein supplementation or parenteral infusion of amino acid mixtures is planned, one should always consider that the fetal-maternal gradient permits a potentially deleterious or toxic fetal hyperaminoacidemia. The damaged offspring of a hyperphenylalaninemic mother is a good reminder of such threats of active placental transport.

## FETAL UPTAKE OF AMINO ACIDS FROM THE UMBILICAL CIRCULATION

Studies based on the individual differences between concentrations of amino acids in the plasma of the umbilical cord vein and artery have provided information on the fetal uptake of amino acids from the umbilical circulation in both sheep (Lemons et al., 1976) and man (Pohlandt, 1978). The bulk of the fetal retention consists of neutral amino acids, 87% in the fetal lamb and 71% in the human fetus. The basic

amino acids constitute the second largest group, showing a net flux from the placenta into the umbilical circulation and contributing to the total fetal amino acid uptake. In Pohlandt's study (1978) performed on neonates delivered by cesarian section or spontaneously, alanine and lysine were the two amino acids whose net uptake constituted a sizable amount of the total amino acids retained. This observation corresponded with earlier results reported by Heinrich et al. (1974).

For the acidic amino acids there is either no net flux to the fetus or, on the contrary, a transfer from the fetus to the placenta. This particularly applies to glutamate, whose production appears to exceed the requirements of fetal protein synthesis. Lemons et al. (1976), dealing with the umbilical uptake of amino acids in chronic, unstressed fetal lamb preparation, suggested that glutamate might originate from deamination of glutamine, and its transfer from the fetus to placenta might represent a major pathway of nitrogen excretion in intrauterine life.

## AMINO ACIDS IN THE AMNIOTIC FLUID

Levy and Montag (1969) were the first to report the use of ion exchange chromatography for the quantitation of free amino acids in amniotic fluid at birth. Several subsequent reports have documented the concentrations of amino acids in amniotic fluid obtained at hysterotomy pregnancy terminations (Cockburn et al., 1970; Scott et al., 1972), by transabdominal amniocentesis (O'Neill et al., 1971; Dallaire et al., 1974), or at birth (Emery et al., 1973; Cockburn et al., 1973).

### Changes During Pregnancy

Several of the studies reported have recognized the general association between amino acid concentration and gestational age (Scott et al., 1972; O'Neill et al., 1971; Dallaire et al., 1974). Early in the pregnancy many amino acids are present in the amniotic fluid in relatively high concentrations, which decrease during the first half of pregnancy. This was clearly demonstrated in the human fetus of 48-140 days gestation by Scott et al. (1972). Changes were also striking for the branched-chain amino acids. Alanine had the highest average concentration during early gestation (Cockburn et al., 1970; Scott et al., 1972). Emery et al. (1973) reported values between the ninth and fourtieth week of gestation and showed that this trend of changes continues throughout pregnancy. They also found that the concentration of a few amino acids (cysteic acid, phosphoethanolamine, ethanolamine, and proline) remained more or less the same.

There is a general further reduction in the amniotic fluid concentration at term both in humans (Emery et al., 1973; Cockburn et al., 1973) and in the rhesus monkey (Kerr and Kennan, 1969). The pattern of changes in amniotic fluid amino acids throughout pregnancy can most clearly be seen in this later study made in the rhesus monkey: Most of the individual amino acids of the amniotic fluid showed a gradual and progressive decrease as pregnancy progressed.

### Amniotic Fluid Amino Acids in Relation to Maternal and Fetal Serum and Fetal Urine

In early pregnancy the concentrations of amino acids in fetal serum and amniotic fluid are generally higher than those in maternal serum and fetal urine (Cockburn et al.,

1970; A'Zary et al., 1973). The total concentration of amino acids was found to be 2410 μmol/liter in amniotic fluid, 5059 μmol/liter in fetal serum, 1908 μmol/liter in maternal serum, and 1437 μmol/liter in fetal urine. A positive correlation between amino acid levels in amniotic fluid with those in fetal serum and urine was reported, suggesting an exchange mechanism between amniotic fluid and fetal serum, with fetal urine acting as a diluent of amniotic fluid (Cockburn et al., 1970; A'Zary et al., 1973). In fact, among the various fluids (maternal venous plasma, umbilical arterial plasma, amniotic fluid, and fetal urine) the closest relationship existed between amniotic fluid and urine, suggesting that fetal urine makes a significant contribution to amniotic fluid.

Amniotic fluid amino acid concentrations at term are usually less than those in the umbilical or maternal venous plasma. Little correlation was found between amino acids in amniotic fluid and those in maternal vein plasma and umbilical arterial plasma (Cockburn et al., 1973). There were only 3 significant correlations between amino acids in amniotic fluid and fetal urine, as opposed to 10 in earlier pregnancy. It was concluded that factors other than fetal urine exercise a more important influence on the composition of amniotic fluid at term. However, Felig et al. (1972), studying the amino acid metabolism during starvation in human pregnancy, have found that changes in the maternal plasma paralleled those in amniotic fluid, indicating that levels of amino acids in amniotic fluid are profoundly influenced by maternal nutrition.

## Nutritional and Diagnostic Aspects of Amniotic Fluid Amino Acids

The observation that the amniotic fluid is swallowed by the fetus suggests that it may have a role in fetal nutrition. Theoretically there does not appear to be any need for enteral nutrition in the fetus in view of the normally abundant transplacental mechanism of providing the nutrients necessary for growth and development. But still, since the volume of amniotic fluid swallowed daily by the fetus in the later part of pregnancy is considerable, the amino acid content of this fluid may play a role in fetal nitrogen metabolism. In fact, it has been demonstrated (Pitkin, 1979) that the fetus ingests, absorbs, and utilizes proteins from the amniotic fluid, which raises the possibility of taking therapeutic advantage of this route of amino acid nutrition. A report (Plesse and Wilken, 1977) describing the intraamniotic infusion of amino acid mixtures in cases of suspected fetal growth retardation has provided some basis regarding such an approach; but the concept of "feeding the fetus" via the amniotic route is nothing more than an intriguing speculation at present and the general agreement is that any nutritive function of amniotic fluid must be very minor.

With the development of amniocentesis a number of inborn errors of metabolism, for example, the adrenogenital syndrome, have been diagnosed antenatally by direct chemical analysis of the amniotic fluid. The maternal circulation and placenta maintain fetal amino acid homeostasis even if the fetus is suffering from an inborn error of amino acid metabolism. The precursors of the affected metabolic pathway are readily metabolized and cleared by the mother. As a result, amniotic fluid concentrations remain normal as long as the mother is not affected with the disease. Accordingly, it is doubtful that prenatal quantitation of amniotic fluid amino acids will detect fetal aminoacidopathies. In fact, O'Neill et al. (1971) had found a normal amniotic fluid amino acid pattern in one fetus at term with maple syrup urine disease. This may not be true, however, for infants with renal tubular transport defects.

## BIOCHEMICAL MATURITY, AMINO ACID METABOLISM, AND ESSENTIALITY AND NONESSENTIALITY OF AMINO ACIDS

The statement that "we may give ourselves a false picture if we always select indispensable amino acids as though they were the more representative amino acids" (Harper, 1974) particularly applies to intrauterine and early postnatal life. Rapid growth, increased amino acid requirement, and enzyme immaturity are circumstances under which a nonessential amino acid can become essential. The premature infant is a good example of the consequences of the immaturity of a number of its enzyme systems. Not only does it require greater quantities of protein and essential amino acids to take care of the needs for growth, but it also needs certain amino acids that are not essential by the classic experimental criterion which led to the segregation of the amino acids into the two categories. As a result of biochemical and nutritional studies, it has become obvious that in addition to the eight amino acids considered essential for the adult, other amino acids are also essential for premature infants (Kenny and Kretchmer, 1959; Kretchmer, 1959; Gaull et al., 1972; Snyderman et al., 1959).

### Amino Acid Synthesis

The significance of the immature enzymatic mechanism of biosynthesis of an amino acid is best exemplified by the conversion of methionine to cystine via the transsulfuration pathway. Cystathionase is completely absent in the human fetal liver: Its appearance seems to be a postnatal phenomenon (Gaull et al., 1972). Hence the fetus is entirely dependent upon the mother for its supply of cystine, even postnatally cystine should be regarded as a dietary essential for a certain period.

The essentiality of tyrosine for premature infants has been partly based on the absence of phenylalanine hydroxylase (Kenny and Kretchmer, 1959), which in the adult mammalian liver converts phenylalanine to tyrosine, and partly on investigations of the amino acid requirement of the premature infant. However, a number of recent reports (Ryan and Orr, 1966; Friedman and Kaufman, 1971; Jakubovic, 1971; Räihä, 1973) have shown that phenylalanine hydroxylase activity is present in human liver from the eighth week of gestation. This finding makes the essentiality of tyrosine in the human neonate questionable, provided that there is adequate phenylalanine present to meet the demand for both amino acids.

Histidine can be readily synthesized by the normal adult human; in infants, however, withdrawal of histidine from the diet results in a decreased retention of nitrogen and a diminished weight gain (Snyderman et al., 1959). Thus the rate of synthesis is not sufficient to meet its demand for growth and could in this period of life be classed as essential.

### Amino Acid Oxidation

In a number of mammalian species studied under chronic steady-state conditions the glucose consumed by the fetus has been shown to be inadequate to meet its oxygen consumption (Battaglia, 1979). Another major source of carbon, for the fetal lamb at least, is provided by the umbilical uptake of lactate; glucose and lactate could account for approximately 75% of the oxygen consumption. Recently a series of

studies reviewed by Battaglia (See Chapter 18) confirmed the role of amino acids as metabolic fuels in the fetal lamb and demonstrated that amino acid catabolism (expressed as the urea production rate) is sufficient to account for approximately 25% of the oxygen consumption.

### Gluconeogenesis in the Fetus

The ability of the liver to synthetize glucose from pyruvate, lactate, and amino acids requires the presence of four key enzymes–pyruvate carboxylase, phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-diphosphatase, and glucose-6-phosphatase–to allow the reversal of glycolysis at thermodynamically irreversible steps. In most species these enzymes, except for PEPCK, have been found to be present in substantial amounts during fetal life (Yeung and Oliver, 1968; Ballard et al., 1969). Liver PEPCK is also absent in the human fetus, but rapidly increases after birth (Marsac et al., 1976). Since the fetus receives a constant infusion of glucose from the mother throughout gestation, fetal gluconeogenesis may not be necessary for glucose homeostasis. The effect of fetal hormones on the gluconeogenic enzymes are not known, but some of these can be modified in utero by various hormonal treatments. For example, hormones which increase cellular cyclic adenosine 5'-monophosphate (catecholamines, glucagon) were able to produce premature induction of PEPCK in fetal rat liver (Yeung and Oliver, 1968), whereas insulin prevents the postnatal development of PEPCK activity in the same species (Girard et al., 1973a).

Ballard et al. (1969) reported that there was substantial gluconeogenic activity from pyruvate in both fetal calf and fetal lamb livers in vitro. The fetal lamb has a high rate of urea production, and as much as 25% of the fetal oxygen consumption could be accounted for by the catabolism of amino acids, suggesting that gluconeogenesis is probably occurring in the fetal lamb (Battaglia, 1979). In fact, active gluconeogenesis from alanine determined with an intravenous infusion of [U-$^{14}$C]alanine and [6-$^{3}$H]glucose or [U-$^{14}$C]glucose was found in the fetal lamb (Prior and Christenson, 1977). This is contrary to what occurs in the nonruminant fetus, where the gluconeogenic process does not develop until after birth.

### Clinical Implications of Enzyme Immaturity Involved in Amino Acid Metabolism

Besides the consequences of an insufficient conversion of methionine to cystine, and of phenylalanine to tyrosine, the absence of degrading enzymes or their partial development can also be of serious consequence. If an amino acid is not metabolized, and enzymatic adaptation does not occur soon, its concentration may reach high levels in the plasma and tissues if the oral or parenteral intake exceeds the requirement. Therefore high-protein diets or a high parenteral amino acid load in a neonate may cause harmful distortion of the plasma and tissue aminogram.

Under such conditions a number of potential consequences should be considered. First, the imbalanced pattern of amino acid intake is usually associated with marked metabolic disturbance. Second, in view of the slow development of some enzymes involved in amino acid degradation, toxicity may occur easily with certain amino acids. Third, the early stages of brain development, such as in premature infants, appear particularly sensitive to the toxic effects of amino acid excess. And fourth, although the enzymes of the urea cycle are present in the human fetal liver at an early stage of development (Räihä and Suihkonen, 1968), the urea-producing capacity is limited in

the premature infant. Therefore a protein or amino acid excess can easily produce a considerable overload to the immature kidney, leading to marked metabolic acidosis, an elevated blood urea level, and hyperammonemia.

All these dangerous implications can be more or less disregarded if the protein or amino acid supply does not greatly exceed the requirements, even in rapidly growing, low birth weight infants. One should avoid marked overestimates and try to arrive at optimal needs for the different stages of biochemical development. The clinician should never forget that a careful correlative evaluation of the specific needs and biochemical immaturity of the developing organism is the most important aspect of the nutritional management of the low birth weight infant.

## PROTEIN SYNTHESIS IN THE FETUS AND NEWBORN

In fetal tissues, as in regenerating liver and malignant growth, both the extracellular and intracellular concentrations of free amino acids are higher than in the adult and indicate that a faster turnover rate of the tissue proteins accompanies their deposition.

There are two ways of investigating the rate of protein synthesis in the fetus: (1) by measuring the amino acid and protein accumulation in a known time of gestation by investigating the nitrogen and amino acid composition of the fetal organs and carcass and (2) by measuring the protein synthesis rate, utilizing the rate of uptake by the protein of a continuously infused labeled amino acid.

In the past the first method was used only in experimental animals, but recently Widdowson et al. (1979) collected very important information regarding the human fetus. The amino acid composition of the body and organs of fetuses of accurately known gestational age (79 days to term) were measured. The body and organs of each fetus were homogenized and the proteins hydrolyzed by hydrochloric acid. The values included the small amounts of free amino acids, together with the much larger amounts originally in the form of protein. It became clear that the contribution of each amino acid to the total amount of amino acids in the body did not change appreciably throughout the period of gestation. The total amount of amino acids and nitrogen incorporated into the fetal body during growth were calculated by measuring the amino acids and the total amount of nitrogen in the body at different gestational ages. As Figure 7 (Widdowson et al., 1979) shows, nitrogen does not begin to increase rapidly until about 160 days of gestation, and thereafter, as the fetus begins to gain weight faster, the deposition of nitrogen and protein increases too.

### The Well-Nourished Fetus

For obvious reasons, only animal data are available. The observations to be described were made on fetal lambs. Fetal lambs were chosen because they are large enough for repeated blood sampling and they approximate the size of the human fetus. The studies were made at 135 days of gestation on lambs weighing 2.41 kg (± 0.17 kg); indwelling catheters had been inserted into the carotid artery and jugular vein 1 or 2 days previously (Young et al., 1979).

[L-$^{14}$C] Lysine was given intravenously for 6 hr, and its rate of uptake by the protein in the steady state was estimated by determining the specific activity of the labeled lysine in this pool and relating it to that in the intracellular free amino acid pool. These values were obtained by killing the fetus, removing the organs quickly, and homogenizing aliquots in cold 10% trichloroacetic solution. The amino acid content

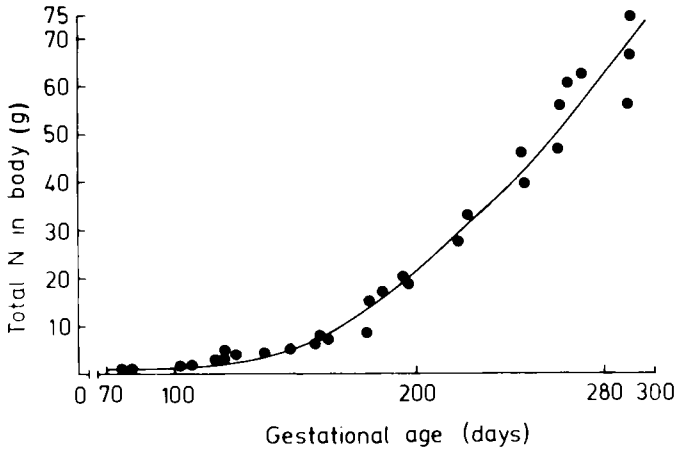


**Figure 7** The relationship between the total body nitrogen of the human fetus and gestational age. (From Widdowson et al., 1979.)

of the precipitated proteins (after hydrolysis with HCl) and supernatant were determined and the radioactivity associated with lysine was also measured. Fractional protein synthesis rates were calculated from the ratio of the specific activities in the protein-bound and intracellular pools. The results were expressed as the protein half-life in days (Table 4).

The mean values of about 1 day in the brain, liver, and heart were very short in comparison with the adult (Buttery et al., 1975), but were comparable with those in the newborn lamb (Soltész et al., 1973) with the exception of skeletal muscle.

The short half-life of the protein in fetal organs may be due either to the presence of a greater proportion of the rapidly turning over proteins or to some difference in fetal physiology which allows the whole spectrum of tissue proteins to be turning over more rapidly. The half-life of placental proteins was similar to that in the fetal liver.

Since the protein content of the tissues was not measured in this study, the protein deposition rate could not be measured and compared with the protein synthetic rate.

In conclusion, it can be stated that fetal amino acid and protein metabolism is in a dynamic state. The faster deposition rate of protein is accompanied by a quick incorporation of labeled precursors.

Since insulin is thought to be a key regulator of amino acid uptake and protein synthesis in adult tissues (Manchester, 1970), the question arises as to whether the same applies to hormonal regulation of fetal protein metabolism. Clark (1971) has shown that insulin increased the incorporation of [L-$^{14}$C]leucine into the protein of fetal rat heart in vitro; but in recent experiments in in utero catheterized fetal lambs using the method of continuous infusion of labeled lysine, Young et al. (1979) found that insulin apparently reduced the protein synthesis in each organ studied, including the fetal cardiac muscle.

### The Malnourished Fetus

Direct measurements of protein synthesis rate in fetal pathology such as intrauterine malnutrition have not been made, but Metcoff et al. (1973) tried to relate changes in

Table 4 Changes with Age in Mixed Protein Half-Life in Sheep

| | Fetus[a] (135 days) | Lamb[b] (3 days) | Adult[c] (1 year) |
|---|---|---|---|
| Brain | 1.62 ± 0.41 | – | – |
| Liver | 0.67 ± 0.15 | 0.7 ± 0.05 | 6.9 |
| Skeletal muscle | 7.81 ± 2.96 | 3.1 ± 0.19 | 40.7 |
| Cardiac muscle | 1.00 ± 0.24 | 2.3 ± 0.42 | 21.6 |
| Number | 6 | 6 | 4 |

[a]Young et al. (1979).

[b]Soltész et al. (1973).

[c]Buttery et al. (1975).

the protein synthesis of maternal leukocytes to the overall synthesis rate of the maternal organism. They have shown a deficient protein-synthetic capacity of the leukocytes, indicated by RNA polymerase activity in women having fetally malnourished babies. They speculated that if the leukocyte reflects metabolic processes in other organs, it would appear that there are metabolic defects in the protein synthesis of these mothers. It was also thought to be possible that the process which affected the metabolism of the maternal leukocyte also affects the cells of the fetus in a similar fashion.

In contrast to the maternal leukocyte, the protein synthesis in the placentas of low birth weight infants were increased, probably in compensation to their smaller size. Laga et al. (1972) studied the placentas of women of low socioeconomic status in Guatemala and also found that the in vitro capacity for protein synthesis rate was not impaired in spite of a lower weight when compared with the placentas of women in Boston.

### The Newborn

The method of continuous infusion of a labeled amino acid, modified from that of Waterlow and Stephen (1967) and similar to that used in the fetal studies, was used to measure protein synthesis rate in the newborn lamb (Soltész et al., 1973). $^{14}$C-Labeled leucine was given intravenously for 5 hr. Chemical analysis and calculation of protein synthetic rate were the same as in the fetus. Very high synthetic rates similar to the fetal values were found (Table 4).

For obvious reasons this method of using radioactive label cannot be employed for the measurement of the protein synthesis rate in the human baby. Instead, amino acids labeled with the stable, nonradioactive $^{14}$N were used in the human. Tracer levels of [$^{14}$N]glycine were given orally and the protein synthesis rate was measured and calculated after the analysis of urinary urea for $^{14}$N by mass spectrometry. In an earlier report (Nicholson, 1970) utilizing this method, rates of protein synthesis in premature infants were found to be comparable to those of adults. Only three infants were studied, however, and the adult data for comparison were taken from other studies. Recently Young et al. (1975) have measured the total human body protein synthesis at various ages from young premature infants to the elderly. They found that

the protein synthesis rate in young infants (1-46 days old) was more than five times higher than in young adults (17.4 versus 3.0 g/kg per day).

## POSTNATAL CHANGES IN THE CIRCULATING AMINO ACID POOL IN THE NEWBORN INFANT

The most striking feature of the postnatal changes of the plasma aminogram in the newborn is a significant drop in most amino acids (Lindblad, 1970). At 4 hr after birth the venous concentrations of branched-chain amino acids, lysine, and alanine were found to be significantly decreased and that of glycine significantly increased (Table 5). At 28 hr of age isoleucine, lysine, and alanine showed a further fall below the cord levels.

### Normally Grown Full-Term and Preterm Infants

Mestyán et al. (1969a), using the ratio of the plasma concentrations of nonessential glycine, serine, glutamine, and taurine to that of the essential leucine, isoleucine, valine, and methionine (Whitehead, 1964), also demonstrated that the plasma amino acid pattern of full-term infants changes rapidly and profoundly in early extrauterine life. In Figure 8 it can be seen that the ratio of the two groups of amino acids rises at a rapid rate, reaching a maximum 12-24 hr after birth. Thereafter the quotient falls so that on the third day its value is about 2.0, and is then maintained during the rest of the observation period. From Figure 8 it is apparent that the increase in the ratio after birth is due to the simultaneous but opposite change in the combined plasma levels of the two groups of amino acids. These observations are, in fact, essentially in agreement with those of Lindblad (1970) obtained by ion exchange chromatography, since the concentrations of the branched-chain amino acids included in the denominator of the amino acid quotient decreased and those of glycine included in the numerator increased.

**Table 5** Cord Vein Plasma Levels of Free Amino Acids (CB) Compared to Those of the Cubital Vein Plasma (CUB) at 4 hr of Age[a]

| Amino acid | Number | Mean | Standard deviation | Significance of difference |
|---|---|---|---|---|
| Valine | CB 10 | 224 | 25 | +++ |
| | CUB 12 | 139 | 20 | |
| Leucine | CB 10 | 118 | 32 | +++ |
| | CUB 12 | 67 | 13 | |
| Isoleucine | CB 10 | 62 | 12 | +++ |
| | CUB 12 | 36 | 5 | |
| Lysine | CB 10 | 318 | 32 | +++ |
| | CUB 5 | 212 | 33 | |
| Alanine | CB 10 | 441 | 75 | ++ |
| | CUB 9 | 311 | 65 | |
| Glycine | CB 10 | 239 | 35 | + |
| | CUB 9 | 293 | 48 | |

[a]Values expressed as μM/liter plasma.

*Source*: Lindblad and Baldesten (1967) and Lindblad (1970).

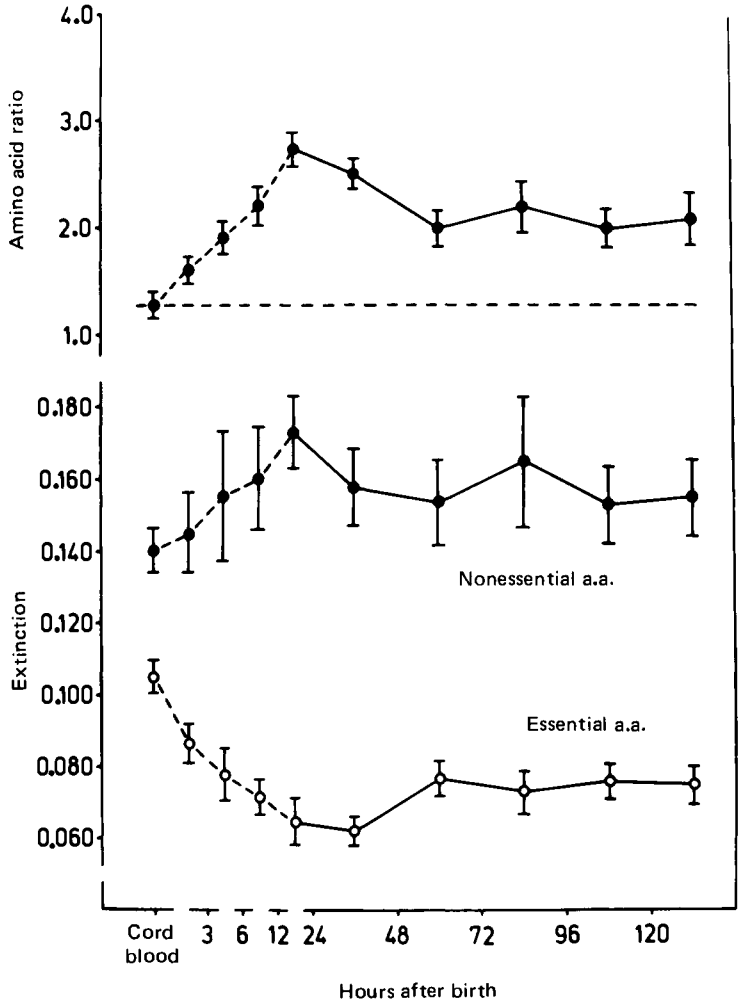


**Figure 8** The postnatal changes of the plasma amino acid ratio and the changes of the plasma concentrations of the nonessential (glycine, serine, glutamine, and taurine) and essential amino acids (leucine, isoleucine, valine, and methionine) indicated by the extinction values of the two groups of amino acids. (From Mestyán et al., 1969a).

**Table 6** Plasma Amino Acid Ratios Obtained in Preterm Infants

| | Time after birth | | | | | |
|---|---|---|---|---|---|---|
| | 3 hr | 3.6 hr | 6-12 hr | 12-18 hr | 18-24 hr | 9-10 days |
| Amino acid ratio | 1.6 | 2.2 | 2.3 | 2.6 | 2.6 | 2.7 |
| Standard deviation | ±0.38 | ± 0.41 | ± 0.52 | ± 0.41 | ± 0.45 | ± 0.62 |
| Number of premature infants | 8 | 15 | 16 | 11 | 12 | 20 |

*Source*: Mestyán et al. (1969a).

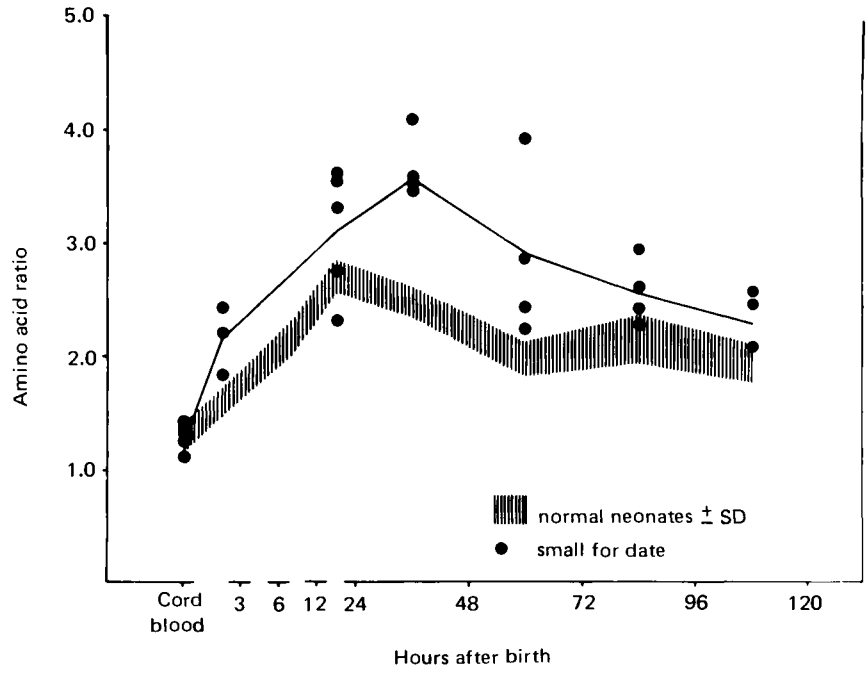


Figure 9 Postnatal changes in the plasma amino acid ratio (as in Figure 8) in five infants born to toxemic mothers. The solid line shows the average and the striped area represents the means ± SD observed in 50 normal full-term infants.

Table 6 shows the means of the amino acid ratio obtained in preterm infants at different time intervals within 24 hr of birth and on the ninth or tenth postnatal day (Mestyán et al., 1969a). It can be stated that premature birth is followed by a qualitatively and quantitatively similar response in the plasma concentration of the two groups of amino acids included in Whitehead's ratio test.

In newborn with a short gestational period, Lindblad (1970) found higher levels of phenylalanine, taurine, and tyrosine than in normals a few hours after birth.

### Growth-Retarded Full-Term and Preterm Infants

Lindblad (1970) reported that in neonates born to hypertensive (toxemic) mothers the plasma aminogram during the first hours following birth was characterized by a rapid fall of the branched-chain amino acids. If, however, toxemia was associated with the small-for-gestational age (SGA) syndrome, the branched-chain amino acids stayed high for some hours and then declined rapidly below the cord levels. It is of interest that during the same time interval alanine and proline concentrations showed a tendency toward increased levels. We shall return to this observation later.

In SGA full-term and preterm neonates born to mothers with uncomplicated or toxemic pregnancies, Mestyán et al. (1969b, 1971) observed a significantly larger postnatal rise of the plasma amino acid ratio than normally (Figure 9). The larger response was mostly due to the marked fall in the combined concentrations

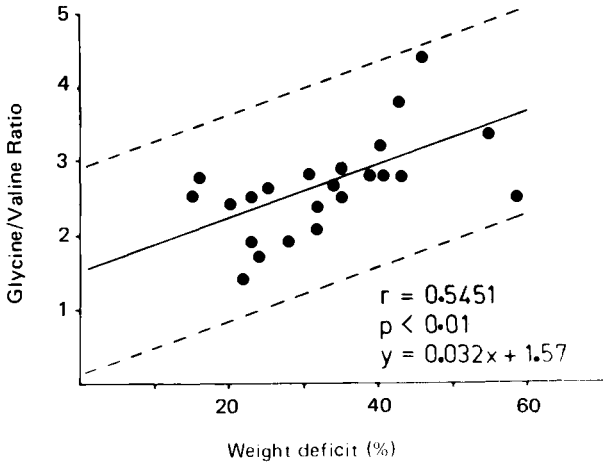


**Figure 10** Glycine-valine ratio in relation to the percentage of weight deficit in intrauterine malnourished newborn infants. (From J. Mestyán and G. Soltész, unpublished data, 1978.)

of leucine, valine, and methionine included in the denominator of the quotient Thus the postnatal changes of the plasma aminogram can be used as an indicator of fetal malnutrition.

Lindblad (1971) proposed the use of the glycine-valine ratio as a biochemical indicator of fetal malnutrition due to maternal nutritional deficiency. This ratio index, which is, in fact, a simplified form of the Whitehead quotient, characteristic of extrauterine protein energy malnutrition, was found to be significantly elevated in the maternal and cord plasma of underprivileged pregnant mothers.

Hibbard and Kenna (1975) did not observe an increased glycine-valine ratio in dysmature neonates born to normally nourished mothers. This also shows that one has to differentiate between intrauterine malnutrition due to maternal undernutrition and other causes as far as the characteristics of the maternal and cord plasma aminogram are concerned. However, postnatally, even in intrauterine growth retardation due to causes other than maternal malnutrition, an increased glycine-valine ratio may be observed. Figure 10 shows that the larger the percentage weight deficit of the newborn infant, the higher the postnatal value of the glycine-valine ratio. From this observation of J. Mestyán and G. Soltész (unpublished data, 1978), it follows that the predictive value of the postnatal glycine-valine ratio depends largely on the severity of the impairment of fetal growth.

### The Relationship Between Postnatal Changes of Plasma Amino Acids and Other Plasma Nutrients

It is well known that marked changes occur in carbohydrate and fat metabolism after birth. The concentration of blood glucose falls and that of free fatty acids rises, pointing toward the characteristic shift in the mobilization and utilization of metabolic fuels.

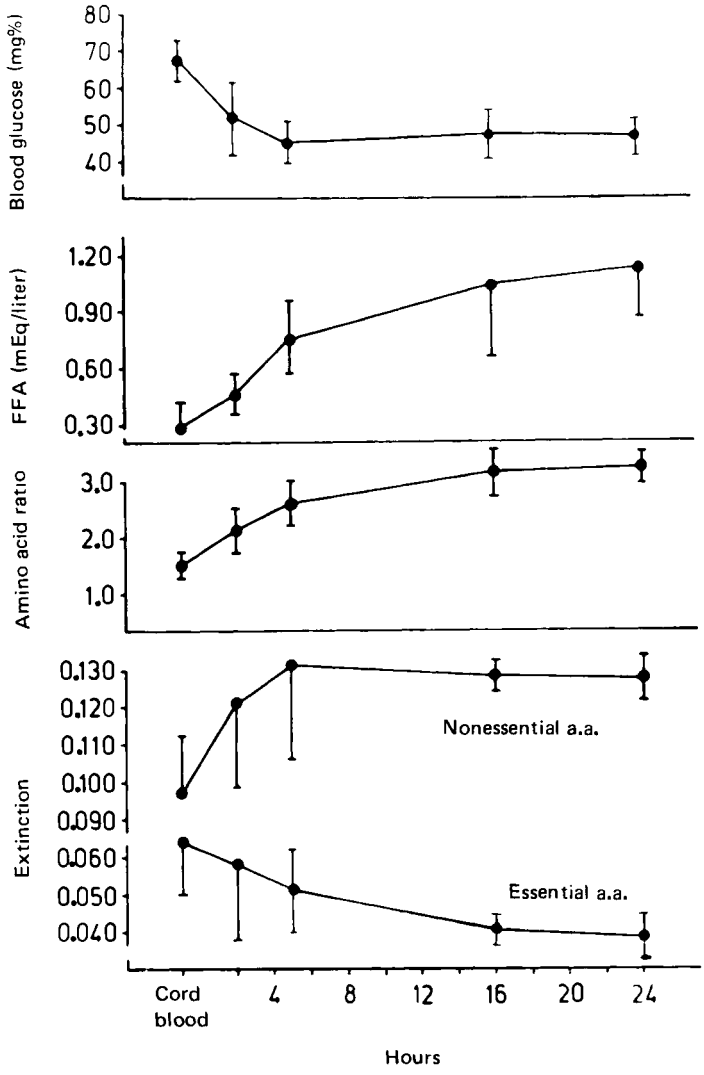


**Figure 11** The mean (± SD) postnatal response of blood glucose, plasma free fatty acids, and amino acid ratio in six normal full-term infants. (From Mestyán et al., 1969b.)

The observations of Mestyán et al. (1972) show that in parallel to these changes in normal full-term infants, the plasma amino acid ratio rises at a rapid rate, reaching its maximum between 12 and 24 hr (Figure 11). Qualitatively the same reactions characterize the metabolic adaptation of SGA neonates born after either uncomplicated or toxemic pregnancies.

### The Participation of Protein (Amino Acid) Oxidation in the Energy Expenditure of the Newborn

In a series of studies dealing with the metabolic pattern of low birth weight infants receiving various nutritive mixtures intravenously (Rubecz and Mestyán, 1973; Mestyán

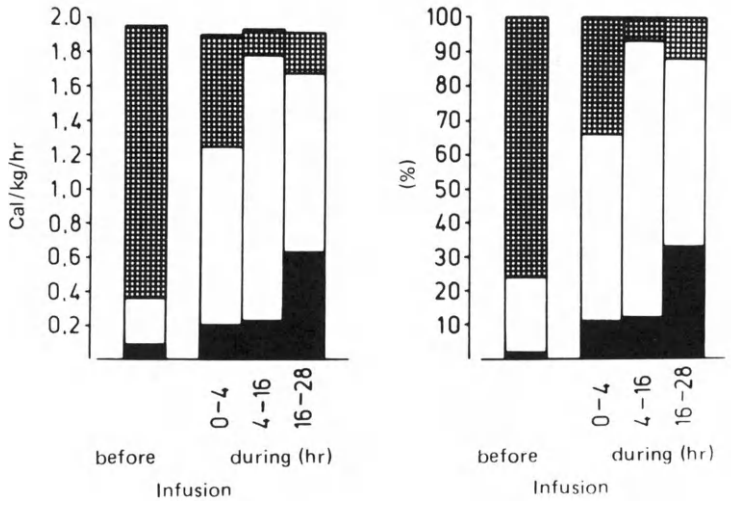


**Figure 12** The absolute and relative contribution of fat (▦), carbohydrate (□), and protein (amino acid) (■) oxidation to the total energy expenditure of a premature infant before and during intravenous infusion of an amino acid mixture. (From Mestyán and Rubecz, 1973.)

and Rubecz, 1973), it has been shown that the utilization of fatty acids in the early postnatal period generally accounts for more than 75% of the oxygen consumption in the thermoneutral environment. The relative contributions of fat, carbohydrate, and protein (amino acid) oxidation to the total energy expenditure are shown in Figure 12. Heat production in the unfed neonate at 18 hr after birth was already dominated by fat utilization. The administration of amino acid solution for parenteral feeding completely modified the metabolic pattern: The participation of carbohydrate and amino acid oxidation increased, while fat metabolism became the smallest energy component.

## PLASMA AMINO ACIDS IN DIFFERENT NEONATAL CONDITIONS

### Neonatal Hypoglycemia

From animal studies (Yeung and Oliver, 1967; Ballard and Hanson, 1968; Ballard, 1971b; Tildon et al., 1971; Girard et al., 1973b) it is known that the activity of enzymes involved in gluconeogenesis appears at birth and increases rapidly postnatally. Several reports dealing with the mechanism of hypoglycemia in intrauterine malnourished neonates suggest that, in addition to diminished stores of hepatic glycogen (Shelley, 1961; Shelley and Neligan, 1966), reduced endogenous production of glucose by gluconeogenesis may also contribute to the development of hypoglycemia. This suggestion is strongly supported by the reduced glycemic response to arginine (Dacou-Voutetakis et al., 1972) and alanine (Mestyán et al., 1974), the decreased disappearance rate of alanine (Mestyán et al., 1974), and the elevated plasma levels of gluconeogenic amino acids in SGA infants (Haymond et al., 1974).

Mestyán et al. (1975) found correlations between the extent of postnatal plasma amino acid accumulation, blood glucose concentration, and the severity of intrauterine

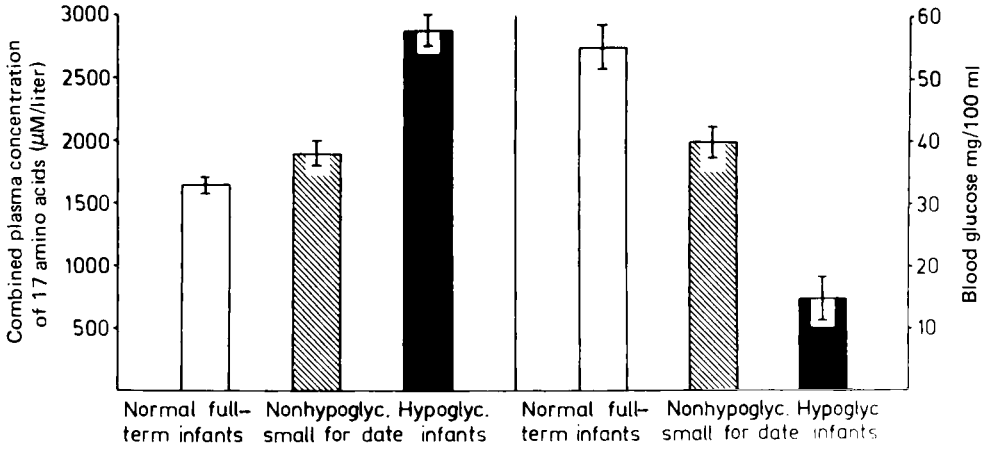


Figure 13 Mean total plasma concentration of 17 amino acids and mean blood glucose concentration in three groups of newborn infants. (From Mestyán et al., 1975.)

malnutrition. The relationship was most striking in hypoglycemic SGA infants, in whom the very low glucose values were associated with very high plasma concentrations of amino acids (Figure 13). This hyperaminoacidemia, which was mainly due to the increase in concentrations of gluconeogenic alanine, glycine, proline, and valine, was possibly the result of a large outflow of amino acids from peripheral pools and a decreased extraction from the plasma by the splanchnic organs, in particular the liver. The significant inverse correlation between the accumulation of amino acids and the blood glucose level observed by Mestyán et al. (1975) strongly suggests a decreased hepatic gluconeogenic capacity probably due to a delay in the maturation process and functions involved in endogenous glucose production. Recent results obtained in experimental intrauterine growth retardation in rats show a significantly reduced activity of PEPCK, which is a key enzyme of hepatic gluconeogenesis (Pollak et al., 1979). In view of the observations in hypoglycemic intrauterine malnourished infants outlined above, it is of interest that Roux and Jahcan (1974) and Manniello et al. (1977) reported significantly higher total plasma amino acid levels at birth in intrauterine growth-retarded rats as compared to controls of normal body size, with particular increases in gluconeogenic amino acids (alanine, glycine, proline, and valine).

### Maternal Diabetes

Hyperinsulinism seems to have a primary etiological role in the hypoglycemia of infants of diabetic mothers (Pildes, 1973). However, the aminogram observed by Soltész et al. (1978) in full-term and preterm neonates did not show any characteristics of hyperinsulinism, such as a marked decline in the concentration of branched-chain amino acids, tyrosine, and phenylalanine. Instead of hypoaminoacidemia, the total concentration of amino acids and the level of a few individual amino acids (glycine, alanine, taurine, and valine) were significantly elevated in full-term babies, but some of these infants suffered from asphysia, which was in fact responsible for the hyperaminoacidemia and hyperalaninemia. However, no significant difference was found in the total plasma concentration of amino acids between premature control infants and premature infants

Table 7 Mean Total Plasma Amino Acid, Blood Glucose, and Blood Lactate Concentrations in Asphyxiated and Nonasphyxiated Preterm Infants

| | Mean (±SE) total plasma amino acid concentration (μM) | Mean (±SE) total blood lactate concentration (mM) | Mean (±SE) blood glucose concentration[a] (mg/100 ml) |
|---|---|---|---|
| Nonasphyxiated preterm infants ($N = 13$) | 2081 ± 91 | 1.95 ± 0.09 | 52.8 ± 5.7 |
| Asphyxiated preterm infants ($N = 16$) | 2713 ± 82 | 3.38 ± 0.29 | 72.4 ± 12.6 |
| $P$ value | $P < 0.001$ | $P < 0.001$ | – |

[a]Nine infants received glucose infusion prior to admission.

*Source*: Schultz et al. (1977).

of diabetic mothers whose Apgar scores were normal. Thus this study did not reveal a typical postnatal plasma amino acid pattern associated with maternal diabetes.

### Plasma Amino Acids and Perinatal Asphyxia

According to the observations of Schultz et al. (1977), perinatal asphyxia may also cause hyperaminoacidemia in the newborn infant (Table 7). The changes in the plasma amino acid pattern were similar to those observed in hypoglycemic neonates (Table 8). Among the 17 amino acids determined, the increase in alanine concentration was particularly marked and accounted for a large portion of the increment of the total plasma amino acid content.

In view of the close correlation between the pyruvate and alanine levels found under different conditions (Felig and Wahren, 1970; Marliss et al., 1972) and studies performed on isolated perfused rat liver (Hems et al., 1966; Phillipidis and Ballard, 1969; Ballard, 1971a), it appears reasonable to assume that the increased availability of pyruvate may result not only in increased lactate production, but also in an increased conversion of pyruvate to alanine catalyzed by alanine aminotransferase. Failure of amino acid removal due to impaired hepatic gluconeogenesis can be an additional mechanism contributing to amino acid accumulation in the plasma.

### Plasma Amino Acids and Cold Stress (Hypothermia)

Schultz et al. (1979) reported a significant elevation of alanine concentrations in the plasma of hypothermic preterm infants. Molnár et al. (1979) observed a more general hyperaminoacidemia in newborn rabbits exposed to a heat-losing environment, but it was alanine which showed the largest contribution to the marked increase in total plasma amino acids.

Hypothermia induces lactate acidosis, which, like in asphyxia (Schultz et al., 1977), is probably responsible for the amino acid accumulation in the plasma. The increased lactate production caused by cold stress represents an increased availability of pyruvate (Felig and Wahren, 1970), which may result in an increased conversion of pyruvate to

Table 8 Concentrations of 17 Amino Acids in Nonasphyxiated and Asphyxiated Newborn Infants

| Amino acid | Nonasphyxiated preterm infants[a] | Asphyxiated preterm infants[a] | |
|---|---|---|---|
| Taurine | 252 ± 13 | 315 ± 18 | ++ |
| Aspartate | 46 ± 5 | 40 ± 3 | |
| Glutamate | 48 ± 3 | 68 ± 7 | + |
| Citrulline | 25 ± 2 | 26 ± 2 | |
| Proline | 175 ± 14 | 237 ± 17 | ++ |
| Glycine | 285 ± 24 | 308 ± 21 | |
| Alanine | 267 ± 19 | 409 ± 20 | +++ |
| Cystine | 122 ± 24 | 140 ± 13 | |
| Valine | 156 ± 8 | 210 ± 13 | ++ |
| Methionine | 22 ± 3 | 31 ± 3 | + |
| Isoleucine | 47 ± 4 | 56 ± 6 | |
| Leucine | 98 ± 8 | 122 ± 12 | |
| Tyrosine | 141 ± 17 | 117 ± 8 | |
| Phenylalanine | 107 ± 9 | 128 ± 10 | |
| Lysine | 179 ± 21 | 358 ± 27 | ++ |
| Histidine | 52 ± 10 | 74 ± 8 | |
| Arginine | 57 ± 8 | 76 ± 6 | |

[a]Values expressed as μM/liter plasma, ± SE.

[b]+, $P < 0.05$; ++, $P < 0.01$; +++, $P < 0.001$.

*Source:* Schultz et al. (1977).

alanine catalyzed by alanine aminotransferase. In addition to the increased release of amino acids, a failure of hepatic amino acid removal may also contribute to hyperaminoacidemia and hyperalaninemia.

### The Significance of Plasma Amino Acids in the Metabolic Adaptation of the Newborn Infant

Both the measurement of the individual amino acids (Lindblad, 1970) and the amino acid ratio (glycine, serine, glutamine, and taurine to leucine, isoleucine, valine, and methionine (Mestyán et al., 1969b) revealed a regular and definite trend in the postnatal changes of the circulating amino acid pool. The resulting plasma amino acid pattern in normally grown infants, as it has been pointed out by Lindblad (1970), resembles that found in caloric insufficiency; the branched-chain amino acids decline, while glycine levels increase. In fact, the simultaneous and inverse changes of these amino acids are reflected by the increase in the plasma amino acid ratio, which is a useful indicator of the postnatal redistribution of amino acids.

The combined concentrations of branched-chain amino acids in SGA infants born to toxemic or nontoxemic mothers fell to levels significantly lower than normal, causing a more pronounced postnatal rise in the plasma amino acid ratio. Similarly increased ratios have been found in extrauterine protein calorie malnutrition. This similarity can be added to other common features of intrauterine and extrauterine malnutrition.

Lindblad and Zetterström (1968) and Young and Prenton (1969) have shown that the plasma aminogram in the venous umbilical circulation of SGA infants does not differ significantly from that of normally grown neonates. Mestyán et al. (1969b, 1971) also concluded that intrauterine malnutrition with possibly impaired placental amino acid transfer does not manifest itself as an altered fetal aminogram in terms of the plasma amino acid ratio.

In view of the decreased fetal-maternal ratios of the branched-chain amino acids due to increased maternal levels in pregnancies associated with intrauterine growth retardation (Lindblad and Zetterström, 1968; Young and Prenton, 1969), it is surprising, but very interesting, that the fetal levels are maintained. Thus the fetus seems to be able to regulate the pattern of its circulating amino acid pool in spite of the diminished rate of growth and possibly impaired transplacental amino acid supply. However, after cutting the umbilical cord, a marked postnatal fall in the branched-chain amino acids takes place, indicating a deficient pool of these amino acids.

It is this response of this group of amino acids which is mostly responsible for the high postnatal amino acid ratio proposed by Mestyán et al. (1971) as a diagnostic aid of intrauterine malnutrition and as an indirect index of a possibly impaired placental amino acid transport. This way of looking at the well-being and nutritional status of the fetus and newborn also applies to the postnatal response of other nutrients. The marked fall in blood glucose, for example, is a well-known and clinically easily applicable biochemical feature of intrauterine malnutrition.

Hormonal and metabolic responses to neonatal fasting play, in all probability, an important role in the characteristic alterations of the plasma amino acid pattern. The closely related changes of blood glucose, plasma free fatty acids, and amino acids (Mestyán et al., 1972) in the metabolic adaptation of the newborn point toward interrelated alterations of the metabolism of the three main nutrients. Since amino acids are major substrates for gluconeogenesis, it is logical to assume that during fasting in the early neonatal period, when carbohydrate supply is inadequate, increased amounts of amino acids are catabolized as chief sources for replacing glucose. The increased mobilization and oxidation of fatty acids might be an additional powerful stimulus for enhanced gluconeogenesis, with the consequent transcellular shift of amino acids.

The mobilization and utilization of amino acids as a source of energy, particularly as precursors of glucose, are now recognized as a key processes in the metabolic transition from intrauterine to extrauterine life. These processes initiated by progressively increasing gluconeogenesis are important in preventing hypoglycemia and in restoring the transient fall in blood glucose after birth. If there is a delay in the development of the homeostatic mechanisms of gluconeogenesis, the resulting hypoglycemia may be accompanied by a marked accumulation of amino acids and distortion of the plasma aminogram. These changes of the circulating pool of severely malnourished neonates appear to be characteristic features of inactive gluconeogenesis, suggesting a largely limited hepatic uptake of mobilized amino acids.

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# 7

# Diabetes Mellitus and the Fetus

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## INTRODUCTION

Diabetes is a disease that produces a disturbance of homeostasis. This has profound effects on intrauterine development and causes complications during pregnancy and the neonatal period.

Intensive research has led to an improved understanding of the pathology of the disease and its effect on the developing fetus and has pioneered the "team approach" in perinatal medicine. The aims of the team include correction of maternal hyperglycemia, avoidance of congenital malformations, detection of intrauterine fetal distress during pregnancy and labor, and delivery of a normal mature infant without neonatal complications. Thus it is appropriate that this chapter be written by a physician, an obstetrician, and a pediatrician, all with a special interest in diabetes complicating pregnancy.

## HISTORICAL REVIEW

In the preinsulin era both maternal and fetal mortality in diabetic pregnancy approached 50% (Williams, 1909). Following the introduction of insulin therapy in 1922, there was a major decline in the maternal mortality rate to 5% or less (Lawrence and Oakley, 1942). The main therapeutic aim during these two decades was to prevent ketoacidotic coma in the mother, and although this early example of "fetal medicine" clearly altered the environment of the developing fetus, it had a very limited impact on perinatal mortality. During this period the ratio of stillbirths to neonatal deaths was 2 to 1, with most losses occurring in utero between 36 and 40 weeks. Preeclampsia and polyhydramnios were frequent and the infants were usually oversized, immature, and prone to neonatal complications, especially hypoglycemia. The incidence of major congenital malformation observed was also higher in these infants than in those delivered by nondiabetic women.

Limited understanding of the pathophysiology of these pregancies led to empirical forms of therapy. Early delivery, frequently by cesarean section, was adopted to avoid late pregnancy intrauterine deaths, which were thought to be due to "postmaturity" (Peel, 1972). The subsequent increase in prematurity, together with functional immaturity characteristic of the infant of the diabetic, resulted in a major increase in neonatal mortality (Peel, 1972), of which more than 50% could be attributed to hyaline membrane disease (Driscoll et al., 1960).

In America, Smith and Smith (1937) introduced the concept of a "hormonal imbalance" in diabetic pregnancy and subsequently White and Hunt (1943) instituted a policy of routine stilboestrol and progesterone therapy, which was maintained in Boston until 1975 (Kitzmiller et al., 1978) despite the negative results of the Medical Research Council trial of hormone therapy conducted in Britain in the early 1950s (Medical Research Council, 1955).

The importance of careful medical supervision and "rigid control" of diabetes in pregnancy was, however, recognized shortly after the introduction of insulin therapy (Peckham, 1931; Skipper, 1933). These observations were subsequently confirmed by Lawrence and Oakley (1942), who subdivided their patients according to whether supervision was complete, partial, or nonexistent and demonstrated perinatal mortalities of 23, 50, and 70%, respectively, in the three groups. The authors also observed a significantly greater mean birth weight in unsupervised pregnancies (4.59 kg) than in those where supervision was regarded as complete (3.15 kg).

The concept that the better the control of the diabetes the better the outcome for the fetus and, in particular, that perinatal results which approximate those observed in nondiabetic pregnant women can be achieved by tight control has only recently been widely recognized. Furthermore, although optimal results can be achieved in this area of fetal medicine when interested physicians, obstetricians, and pediatricians care for diabetic patients, the outcome is much less satisfactory when these pregnancies are not managed by specialized teams (Persson, 1978).

While the improved control of blood glucose levels that has been achieved during the last decade has undoubtedly been a major factor in the improved perinatal outcome of diabetic pregnancy (Karlsson and Kjellmer, 1972), this period has also witnessed a revolution in techniques for fetal surveillance that has led to major changes in obstetric management. Notable among these are antepartum and intrapartum continuous fetal heart rate monitoring and fetal blood sampling in labor. In addition, the amniotic fluid lecithin-sphingomyelin ratio for assessing fetal pulmonary maturity has helped to prevent those neonatal deaths which resulted from hyaline membrane disease following planned early delivery (Drury et al., 1977).

Major changes have also occurred during this time in our understanding of the neonatal pathophysiology of the infant of the diabetic mother and in the nature and provision of neonatal intensive care facilities, both of which have contributed to the greatly reduced perinatal mortality rates of 2-5% reported in recent years (Essex and Pyke, 1979; Drury et al., 1977; Gabbe et al., 1977b).

In this chapter we have attempted to review recent research into the pathophysiology of diabetic pregnancy and to demonstrate the impact that these research findings have had on the evolution of the modern clinical management of this condition.

## EFFECT OF MATERNAL DIABETES ON THE FETUS

### Overview

Maternal diabetes has a profound influence on the growth, body composition, and endocrine pancreatic function of the fetus. The plethoric, cushingoid appearance and excessive size that characterizes infants of diabetic mothers was first recorded by Bennewitz in 1826. Enlargement of the islets of Langerhans and of the beta cells in the pancreas was described a century later (Dubreuil and Anderodias, 1920). Subsequent studies have demonstrated a relationship between these morphological changes

Table 1 Neonatal Complications

| |
|---|
| Congenital anomalies |
| Birth trauma |
| Asphyxia |
| Respiratory distress |
| Cardiomegaly |
| Polycythemia |
| Renal vein thrombosis |
| Hyperbilirubinemia |
| Hypocalcemia |
| Hypoglycemia |
| Feeding problems |

in the pancreas and an increased amount of extractable insulin in the pancreatic islets (Steinke and Driscoll, 1965). Diabetic pregnancy is also associated with a higher incidence of fetal and neonatal complications than occurs in normal pregnancy (Table 1).

According to the classical Pedersen hypothesis, episodic maternal hyperglycemia leads to increased anabolism and enhanced fetal growth, especially of the adipose tissue, liver, and heart. The Pedersen hypothesis has had a great impact on both experimental and clinical research and has recently been expanded to include the stimulatory effects on the fetus of other elevated substrates in the mother, in particular amino acids, on insulin production (Freinkel and Metzger, 1979) and on the growth of the beta cells (Milner, 1979). Perhaps more important, this theory has stimulated clinicians to improve the quality of diabetic control during pregnancy and it is generally believed that this has markedly improved the outlook for intact survival and health of the offspring.

## Fetal and Neonatal Insulin Secretion

In nondiabetic mothers, differentiated beta cells, which are able to synthesize and store insulin, appear in the fetus at 10-11 weeks of gestation (Like and Orci, 1972). Insulin, solely derived from the fetal pancreas, is present in the fetal circulation from the twelfth week and in amniotic fluid from around the seventeenth week of gestation. Insulin has long been assigned a role as a modulator of fetal growth because of its anabolic properties and the association between macrosomia and beta-cell hyperplasia in infants of diabetic mothers. However, because many insulin-treated diabetic patients develop insulin-binding antibodies that are transported across the placenta to the fetus, and which interfere with the radioimmunoassay of insulin, it has only recently become possible to demonstrate hyperinsulinism in these infants. This has been achieved by radioimmunoassay of C-peptide, which is secreted in equimolar concentrations with insulin, but which does not cross-react with insulin antibodies. Significantly higher levels of C-peptide have been reported in the cord blood of the infants of diabetic women as compared to normal controls, confirming hypersecretion of insulin at the time of birth (Block et al., 1974; Ogata et al., 1980; Sosenko et al., 1979). Although infants of diabetic mothers, as a group, have C-peptide levels in cord blood that are two to three times higher than those of control infants at comparable blood glucose levels, they show great individual variations (Heding et al., 1980). This might be attributed partly to the influence of the stress of parturition on insulin secretion around birth and partly to

variations in the maternal blood glucose levels during the hours preceding delivery, which makes it unlikely that cord levels of C-peptide accurately reflect basal insulin secretion in utero. The presence of insulin-binding antibodies derived from mothers raises additional problems. Firstly, they may interfere with the transport and action of insulin (as indicated by a positive correlation between these antibodies and C-peptide), and secondly, they may lead to an increase in insulin secretion by the fetus (Heding et al., 1980). This latter effect may result from the amount of insulin required to maintain free insulin at a constant concentration in the presence of binding antibodies.

The stage of fetal development at which hypersecretion of insulin starts is not known, but significantly raised levels of both insulin and C peptide have been demonstrated in amniotic fluid during the last trimester of diabetic pregnancies (Ogata et al., 1980; Tchobroutsky et al., 1980; Weiss et al., 1978); and in a recent study the average C-peptide concentration in amniotic fluid 2-3 weeks prior to delivery was found to be approximately six times higher in diabetic than in nondiabetic pregnancies (Persson et al., 1982).

Although it is evident that the fetus is capable of secreting insulin early in pregnancy, there is little information about the sensitivity of different tissues to insulin during development, and there have been few studies on the development of insulin receptors in human fetal tissues. Cell membranes of placental tissue both in early gestation and at term are markedly enriched with insulin receptors (Posner, 1974). These receptors are mainly located on the brush border of the syncytiotrophoblast and are thus in direct contact with maternal blood in the intervillous space (Whitsett and Lessard, 1978). Their physiological role is unclear, but it has been suggested that they may be involved in the regulation of placental growth and the degradation of maternal insulin. Insulin binding to nonclassic targets such as red blood cells and monocytes from cord blood in healthy infants is greater than in corresponding adult cells, owing to increases in receptor concentrations and receptor affinity (Herzberg et al., 1980; Neufeld et al., 1978; Thorsson and Hintz, 1977). How these binding data relate to the biological effects of insulin in fetal tissues is unknown. So called "down-regulation" of insulin receptors by insulin as seen in obese adults is not present in the infants of chemical diabetic mothers, who, on the contrary, have a higher receptor concentration in monocytes than normal newborns (Neufeld et al., 1978).

### Size at Birth

Infants of diabetic mothers form a very heterogeneous group and the two variables which appear to have a pronounced effect on both fetal nutritional status and postnatal fuel homeostasis are the degree of maternal diabetic angiopathy and the metabolic control which is achieved during pregnancy. One extreme is represented by the plethoric and macrosomic infant with increased amounts of total body protein, glycogen, and fat, often born to mothers with diabetes of short duration (White's classes A and B) and whose diabetic control during pregnancy is unsatisfactory. The other extreme is represented by the intrauterine growth-retarded baby delivered by mothers with severe diabetic microangiopathy (White's classes F and R) with impaired placental function. In this latter situation energy stores of fat and glycogen are diminished and cell size and numbers are reduced in many organs.

The acceleration in fetal growth is clinically not manifest until after the twenty-eighth week. Although these fetuses may be both heavier and larger than average for

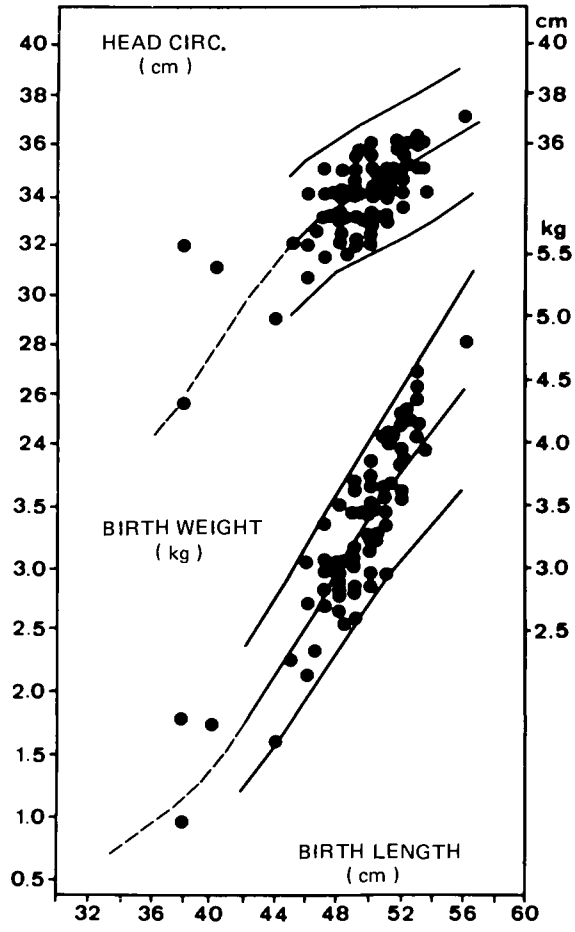


Figure 1 Head circumference, body weight, and birth length in 73 infants of insulin-diabetic women (White's classes B, C, and D) in relation to reference values shown as the mean and the tenth and ninetieth percentiles.

their period of gestation, their growth is usually asymmetrical, affecting birth weight relatively more than birth length. This asymmetry tends to become obvious when the birth weight exceeds 3.5 kg, as illustrated in Figure 1, which shows the relation between body weight and length at birth. Available data suggest that strict regulation of maternal blood glucose is accompanied by a reduction in infant birth weight, a more normal appearance at birth, and a reduction in both perinatal mortality and morbidity. Despite this, no significant relationship has been found between the degree of metabolic control achieved in the mother and the size of the infant at birth (Karlsson and Kjellmer, 1972; Persson, 1974). This lack of correlation may be attributed to the rather crude and superficial way of expressing the degree of diabetic control, which in these studies was calculated as an average blood glucose level from intermittent daily glucose determinations during the last trimester alone. A significant positive correlation has, however, been found between the mean diurnal plasma glucose during the last trimester of pregnancy and both infant birth weight and the

cord plasma insulin at birth in a small group of normal and untreated chemical-diabetic women (Gillmer, 1978a). A direct relationship between maternal hemoglobin $A_{1C}$ determined during the last trimester of diabetic pregnancy and the infant birth weight has been reported by some workers (Widness et al., 1978), but not others (Fadel et al., 1981). Studies on the body composition of infants of diabetic mothers clearly suggest that the major part of the overweight is due to an excessive accumulation of adipose tissue. As lipogenesis occurs mainly during the last 8 weeks of gestation and as adipose tissue is one of the classic target organs of insulin action, it is not altogether surprising that a positive correlation has been demonstrated between both the skinfold thickness and adipose cell diameter of the newborn infant and maternal blood glucose values during the third trimester of pregnancy (Whitelaw, 1977; Persson et al., 1979). This finding suggests that the mass of fetal adipose tissue increases as the glucose concentrations of the mother rises and is consistent with the hypothesis that fetal hyperglycemia and hyperinsulinism enhance triglyceride synthesis in adipose tissue. This concept has, however, been questioned by Szabo and Szabo (1974), who suggested that glucose may not be the major precursor of fetal triglyceride and fatty acids, but only of the alpha-glycerophosphate necessary for triglyceride formation, and that the higher free fatty acid (FFA) concentration in pregnant diabetics would result in an increased transfer of FFAs to the fetus. This view is supported by observations in various animal species (Van Duyne et al., 1962; Portman et al., 1969; Hull, 1975) of placental transfer of FFAs from the mother to the fetus in vitro (Szabo et al., 1969; Dancis et al., 1973) and in vivo (Sabata et al., 1968; Sheath et al., 1972; Elphick et al., 1976). Additional evidence that this transfer occurs in man may be derived from the observation that there is a mean umbilical vein-artery FFA concentration difference of about 0.06 mmol/liter, which is positively correlated with the maternal FFA concentration at delivery (Sabata et al., 1968; Sheath et al., 1972; Elphick et al., 1976). The Szabos also supported their hypothesis by showing that the fasting maternal FFA concentration is positively correlated with birth weight (Szabo et al., 1975). This observation was not, however, been confirmed in two recent studies (Gillmer et al., 1977b; Treharne et al., 1977). In the first of these studies similar mean percentile birth weights were recorded in a group of untreated chemical-diabetic patients and a group of insulin-dependent diabetic patients, despite marked differences in mean diurnal plasma glucose and FFA concentrations of both glucose and FFA, whereas the insulin-dependent diabetic patients had elevated glucose concentrations but subnormal plasma FFA concentrations. It is therefore possible that fetal size may be influenced more by the total amount of available substrate crossing the placenta, rather than by the maternal concentrations of glucose or FFA alone. In both normal and chemical-diabetic pregnancies infant birth weight and cord plasma insulin concentrations are positively correlated (Shima et al., 1966; Thomas et al., 1967) and more recently a significant correlation has been demonstrated between birth weight and the binding of insulin by monocytes in normal infants (Neufeld et al., 1978). In insulin-dependent diabetic women the occurrence of macrosomia (i.e., birth weight above the ninetieth percentile for gestational age) and neonatal hypoglycemia (i.e., serum glucose below 30mg/100ml) was associated with elevated cord C-peptide concentrations (Sosenko et al., 1979). It is, however, noteworthy that in a group of insulin-dependent diabetic women a positive correlation between infant birth weight and cord plasma C-peptide was only found among those who had no insulin-binding antibodies (Heding et al., 1980).

Many of the observations discussed above are consistent with the Pedersen theory of maternal hyperglycemia-fetal hyperinsulinism, but do not prove a causal relationship. Of particular interest in this context are infants with nesidioblastosis who have an increased beta-cell mass together with an uncontrolled hypersecretion of insulin. They are usually macrosomic at birth and sometimes have a physical appearance which is similar to that of infants of diabetic mothers. Assuming that fetal hyperinsulinism in this condition augments fetal growth, then this occurs without any alteration in the substrate levels in maternal blood. This is in keeping with recent observations on the effect of experimentally induced chronic fetal hyperinsulinism in the rhesus monkey without maternal diabetes (Susa et al., 1979). The insulin-treated fetuses had higher body weights than the controls and showed enlargement of certain organs, in particular the liver, heart, spleen, and placenta, whereas the crown heel length, brain, and kidney size were unaffected. These insulin-induced changes in the rhesus monkey show a striking resemblance to those observed in the classic macrosomic infant of the diabetic mother.

## Metabolic Changes Following Birth

### Carbohydrate Metabolism

One of the characteristic findings in the offspring of diabetic mothers is hypoglycemia in the neonatal period. It is well recognized that the blood glucose concentration declines significantly during the first hours after birth in both normal infants and infants of diabetic mothers. This initial decline of blood glucose usually occurs at a faster rate in infants of diabetic mothers and of chemical-diabetic mothers than in normal infants. Of particular clinical importance is the observation that glucose infusion to diabetic mothers leads to a marked elevation of glucose in the fetal circulation associated with an increased incidence of neonatal hypoglycemia in the immediate postnatal period (Adam, 1971; Light et al., 1972). On the other hand, if the maternal blood glucose is maintained within the normal physiological range during the hours preceding birth, it seems possible to prevent neonatal hypoglycemia (Adam, 1971).

In untreated chemical-diabetic and normal control women a variety of indices, derived from maternal diurnal glucose profiles and oral glucose tolerance tests, have shown a highly significant negative correlation with the absolute plasma glucose concentration of the infant 2 hr after delivery and a positive correlation with the rate of glucose utilization during the first 2 hr after birth (Gillmer et al., 1975b). These data are consistent with the Pedersen hypothesis.

The endogenous glucose production rate has been determined 3 hr after birth in infants of insulin-treated diabetic mothers and controls by means of a [1- $^{13}$C] glucose tracer dilution technique (Kalhan et al., 1977). Due to recycling of tracer, this technique underestimates glucose production. Simultaneous measurements using $^{13}$C- and $^{2}$H-labeled glucose showed that carbon recycling contributed to between 3 and 20% of the endogenous glucose production rate in healthy newborn infants (Kalhan et al., 1980). In the normal newborn the systemic glucose production rate determined by [1-$^{13}$C] glucose tracer ranged between 3.8 and 4.9 mg/kg per minute, whereas in infants of diabetic mothers the production rate was only 60% of the normal value. This lower glucose production rate in infants of diabetic mothers could be attributed to either an insulin effect and/or a deficient stimulation of glycogenolysis in the liver by pancreatic

glucagon or sympathetic neural noradrenalin. The results of some investigations suggest that the spontaneous glucagon secretion in the immediate neonatal period is deficient in infants of diabetic mothers (Bloom and Johnston, 1972; Williams et al., 1979), and that glucagon secretion following intravenous alanine administration is reduced (Williams et al., 1979). In a study, which unfortunately did not include controls, infants of chemical diabetic and insulin-dependent mothers showed a modest but continuous rise of plasma glucagon during the first 6 hr after birth and before the first feeding (Kuhl et al., 1980). No significant difference in the glucagon response during the first 2 hr after delivery was observed by Gillmer (1978b) in infants of normal and untreated chemical diabetic women. The concentration of catecholamines in urine following hypoglycemia in infants of diabetic mothers has also been considered to be inappropriately low (Stern et al., 1968). It seems clear, however, that infants of diabetic mothers are able to respond with an increase in sympathoadrenal activity during stress, as indicated by the significantly higher plasma noradrenalin concentrations in umbilical arterial and venous blood at birth in comparison to control infants (Young et al., 1979). Whether the counter-regulatory responses of glucagon and catecholamines to hypoglycemia in the immediate neonatal period are inappropriate or not in infants of diabetic mothers, however, remains unclear.

Remarkable, but as yet unexplained, are the occasional episodes of profound hypoglycemia, without clinical signs and symptoms, which may occur during the first hours after birth in infants of diabetic mothers and even normal neonates. It is possible that other energy sources such as amino acids and ketone bodies (acetoacetate and 3-hydroxybutyrate) are available to the central nervous system. Although nothing is known about the role of amino acids as a source of energy for the newborn human brain, several studies have shown that ketone bodies can replace glucose as substrate for the central nervous system. At the age of 3-4 hr the concentrations of 3-hydroxybutyrate are, however, below those at which measurable cerebral arteriovenous differences have been found in infants and children (Persson et al., 1973a; Settergren et al., 1980). The significantly lower systemic glucose production rate in infants of diabetic mothers may indicate that part of their energy requirement is covered by oxidation of glucose derived from other sources, for example, elevated stores of glycogen present in various tissues, such as the medulla and spinal cord (Persson et al., 1978). This is supported by the available evidence which suggests that asymptomatic neonatal hypoglycemia in infants of diabetic mothers does not result in intellectual impairment later in life (Persson et al., 1978).

Further evidence supporting the concept of functional postnatal hyperinsulinism in infants of diabetic mothers is their increased ability to dispose of a glucose load (Persson, 1975). The observation that values for the glucose disappearance rate are inversely correlated with both basal glucose and FFA concentrations could be compatible with functional hyperinsulinism of varying degrees. This view is supported by the findings of Phelps et al. (1978). These authors measured basal values for C-peptide and their response to intravenous glucose 2-4 hr after birth in infants of diabetic mothers and controls. The 10-min C-peptide increment following glucose administration was significantly correlated with the rate constant for glucose disappearance (kt).

### Lipid Metabolism

Normal newborn infants show a distinct rise in arterial plasma glycerol within minutes after birth, which is followed by a progressive rise in plasma FFAs, suggesting a rapid

onset of lipolysis followed by a somewhat delayed lipid mobilization (Persson and Tunell, 1971). This postnatal rise in plasma FFA occurs at a slower rate in infants of diabetic mothers (Persson et al., 1973a). The mean value of glycerol concentration is, however, no different from that seen in control infants. In the absence of turnover data for FFAs in infants of diabetics, it could be speculated that this lower plasma FFA level reflects a decreased outflow of fatty acids from adipose tissue and/or an increased removal rate of fatty acids from blood. One possible explanation for a decreased outflow of FFAs from adipose tissue could be a more rapid re-esterification of liberated fatty acids with alpha-glycerophosphate, derived either from glucose entering the cell or from glycogen stores present within the cell. A state of functional hyperinsulinism could lead to an accelerated entry of glucose into adipose tissue cells and other tissues and thus explain the low plasma glucose concentrations. This interpretation is, however, difficult to reconcile with the strong antilipolytic effect of insulin, which according to in vitro data occurs at a significantly lower concentration of insulin than that needed to affect the rate of uptake of glucose by adipose tissue. The significantly higher glycogen content of subcutaneous adipose tissue in newborn infants of diabetic mothers as compared to controls would, however, support the hypothesis of re-esterification of FFA with alpha-glycerophosphate derived from glycogen within adipose tissue (Novak et al., 1972). Another possible explanation is enhanced oxidation of fatty acids within adipose tissue.

The characteristic findings of hypoglycemia, suppressed plasma FFA concentrations, and increased disappearance rates of intravenously administrated glucose in infants of diabetic mothers during the first postnatal hours are usually transient. Plasma concentrations of glucose, FFAs, and 3-hydroxybutyrate as well as kt values usually revert to normal within the first few days after delivery.

### Energy Metabolism

The changes occurring in energy metabolism as measured by the oxygen uptake ($VO_2$) and respiratory quotient (RQ) have been studied extensively in normal infants, but surprisingly rarely in infants of diabetic mothers. In the normal full-term infant of non-diabetic mothers $VO_2$ increases progressively with time and there is a concomitant increase in body temperature with falling RQ values during the first days after birth. The gradual decrease of RQ from around 1.0 at birth to 0.7 at 1-2 days of age has been interpreted as reflecting the change from carbohydrate to fat oxidation; however, RQ values around 1.0 which have been determined immediately after birth cannot be used as a measure of the tissue metabolic respiratory quotient (Persson and Tunell, 1971). Measurement of the oxygen uptake and carbon dioxide production during the first 2 hr after birth together with simultaneous changes in blood gases have shown that oxygen uptake during the first 8 min after birth is approximately 10-11 ml/kg per minute (Persson and Tunell, 1971; Tunell et al., 1976). Thereafter oxygen uptake gradually decreases to 5-6 ml/kg per minute. The respiratory exchange ratio, that is, the relation between carbon dioxide elimination and oxygen uptake, was consistently greater than 1.0 during the first 20 min after birth, reflecting the elimination of accumulated carbon dioxide. It was suggested that 2 hr after birth would be the earliest age at which RQ measurements could give reliable information about the energy balance of the infant (Persson and Tunell, 1971). Measurements of oxygen uptake, RQ, and plasma concentrations of glucose, FFA, and 3-hydroxybutyrate were performed in infants of diabetic mothers and infants of chemical diabetic mothers before the first feed and during an

early feeding regimen (Gentz et al., 1976). Before the first feed and between 2 and 16 hr after birth the values for oxygen consumption rates and respiratory quotient fell within the normal range. When feeding was started, oxygen uptake increased significantly with increasing age and milk intake. Respiratory quotient values decreased during the first 24-48 hr, but rose thereafter to the highest values at 7-11 days, when the milk intake was adequate to cover energy requirements and body weight was increasing. The plasma concentrations of glucose, FFA, and 3-hydroxybutyrate were all within the normal range. The highest concentrations of 3-hydroxybutyrate were found 1-2 days after birth, when the highest RQ values were recorded. The RQ values were also inversely correlated with the plasma levels of 3-hydroxybutyrate, supporting the interpretation that the initial fall of the RQ reflects an increase in oxidation of fat. These results also suggest that within 2 hr of birth the energy requirement in infants of diabetics was provided by oxidation of a mixture of substrates. Lower values of oxygen consumption with normal RQ values have, however, been recorded by other investigators in the infants of diabetic mothers during the first 36 hr after birth (Courpotin et al., 1975), indicating a decreased availability of substrates for tissue oxidative metabolism.

### Perinatal Mortality

Many centers have reported a major improvement in perinatal outcome. Strict comparative evaluation of published reports on both perinatal mortality and morbidity in diabetic pregnancies must, however, be made with caution. This is because of differences in socioeconomic factors, the time at which the studies were conducted, the criteria for the selection of patients, and the definition of disease entities. The continuous improvement in the prognosis for the offspring is illustrated by the national figures from Sweden for the period 1973-1978, shown in Figure 2. Total perinatal mortality decreased from 73 per 1000 in 1973 to 39 per 1000 in 1978. It has been suggested that this improvement stems from improved metabolic control of the pregnant mother. When trying to evaluate the influence of treatment on the outcome of diabetic pregnancy, it is, however, important to be aware that the perinatal mortality rate in

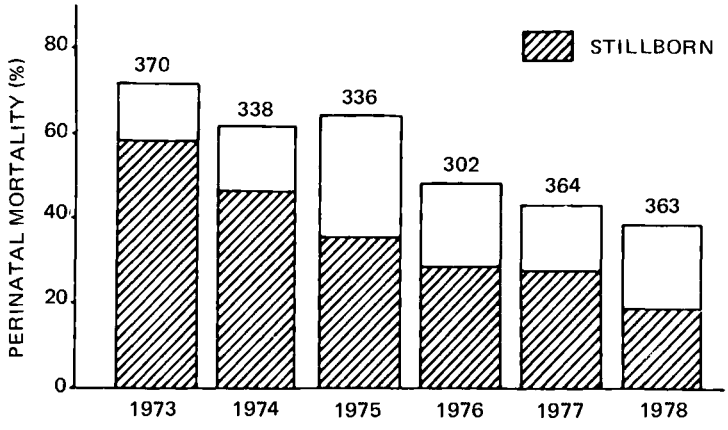


Figure 2 Perinatal loss per 1000 births in 2073 diabetic pregnancies in Sweden between 1973 and 1978.

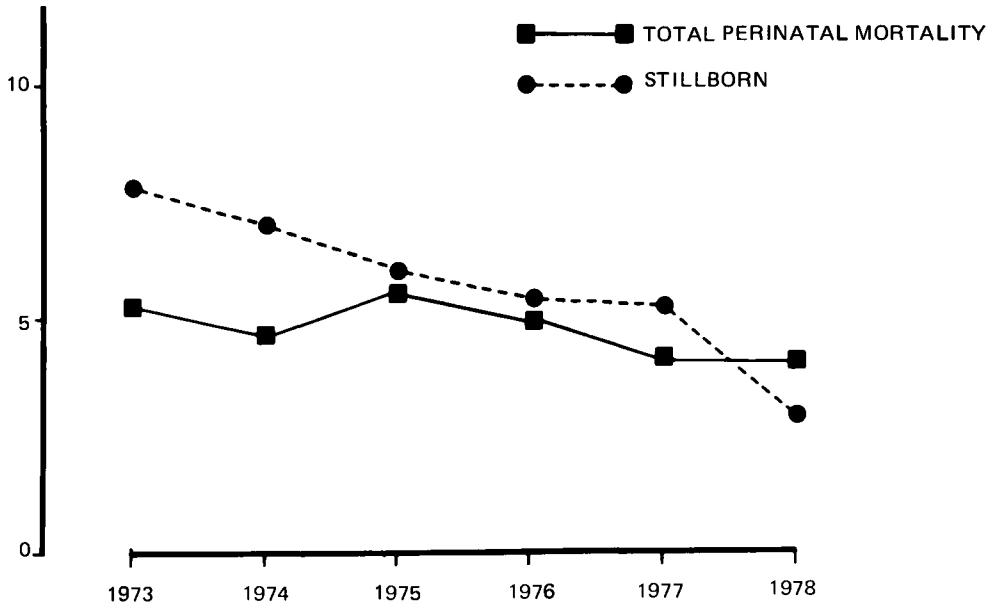


Figure 3 Ratio of the perinatal mortality rate (diabetic to nondiabetic pregnancies) in Sweden between 1973 and 1978.

nondiabetic pregnancies also declined between 1973 and 1978. This is probably due in part to socioeconomic changes, but it also results from improved medical, obstetric, and pediatric care. As illustrated in Figure 3, the total perinatal mortality in diabetic pregnancies was four to five times that of the nondiabetic population, without any appreciable change during the study period. In contrast, the ratio between the stillbirth rate in diabetic and nondiabetic pregnancies declined significantly, from around eight in 1973 to around four in 1978, probably as a result of a more intensive supervision of maternal diabetes during pregnancy and labor.

### Congenital Malformations

It has long been recognized that congenital malformations occur more frequently in the offspring of diabetic women than in those of normal women. White and Hunt (1943) reported an incidence of 1 in 6 (16.7%) for the infant of the diabetic mother, as compared with 1 in 55 (1.8%) for the infant of the nondiabetic mother.

Recently several series have been published, all of which confirm a significant excess of malformations in these babies, but with an overall incidence ranging from 4.4 (Jervell et al., 1980) to 11.9% (Schneider et al., 1980).

Most large studies have, however, reported an incidence of about 6-8% (Drury et al., 1977; Malins, 1979; Pedersen, 1979). The reasons for variations between studies have been highlighted by Malins (1979), but the most important are probably the differing definitions applied in diagnosing congenital malformations and the varying severity of the maternal diabetes in different series. Furthermore, few series indicate the extent to which termination of pregnancy has been performed, and with the increasing use of screening programs for the detection of neural tube defects (Wald et al., 1979) this variable will assume increasing importance in all published analyses.

**Table 2** White's Classification as Applied in the Copenhagen Series

| White's classes | Age of onset (years) | | Duration (years) | Retinopathy (benign) |
|---|---|---|---|---|
| A | Chemical diabetes diagnosed during pregnancy and treated with diet ± drugs | | | |
| B | ⩾ 20 | and | < 10 | Absent |
| C | 10-19 | or | 10-19 | Absent |
| D | < 10 | | ⩾ 20 | Present |
| F | Nephropathy and/or proliferative retinopathy | | | |

*Source*: Molsted-Pedersen (1980).

**Table 3** Frequency of Congenital Malformation in Relation to White's Classification—Copenhagen Series 1926-1978

| White's classes | Total number of infants | Infants with congenital malformations | | |
|---|---|---|---|---|
| | | Number | Percentage | |
| A | 415 | 17 | 4.1 | |
| B | 486 | 29 | 5.9 | 5.1 |
| C | 443 | 23 | 5.2 | |
| D | 586 | 67 | 11.4 | 12.5 |
| F | 111 | 20 | 18.0 | |
| Total | 2041 | 1156 | 7.6 | |

*Source*: Molsted-Pedersen (1980).

In an attempt to relate the incidence of congenital malformations to the duration and severity of diabetes, Molsted-Pedersen (1980) divided the 2041 patients managed in Copenhagen between 1926 and 1978 into groups, using a modification of the White classification (Table 2). The overall incidence of congenital malformations in these diabetic patients was 7.6%, compared with 2.6% in a control group of 8789 nondiabetic mothers. The breakdown according to White's classes is shown in Table 3. It is apparent that the incidence of congenital malformations was similar in White's classes B and C and double that of the controls, while that in class A patients was nearly 60% higher than that of the control patients. The largest numbers of congenital malformations occurred in classes D and F, with a four- and sevenfold increase, respectively, compared to controls.

Malins (1979), in his analysis of the results in Birmingham, England (Table 4), found no increase over controls in Class A and similar rates (8.2-10.7%) in classes B to F. He did, however, observe a significantly greater incidence of malformations in women who developed diabetes under 20 years of age (11.2%) compared to those with disease of later onset (6.1%). There was, however, no significant difference in the incidence of congenital malformations in women with diabetes of less than 10 years duration (7.4%) and those with diabetes for more than 10 years (10.4%).

Table 4 Incidence of Congenital Malformations in the Birmingham Series

| White's class | A | B | C | D and F |
|---|---|---|---|---|
| Total number of women | 116 | 281 | 173 | 131 |
| Congenital malformations (N, %) | 2 (1.7) | 23 (8.2) | 18 (10.4) | 14 (10.7) |
| Fatal malformations (N, %) | 0 (0) | 9 (3.2) | 9 (5.2) | 8 (6.1) |

*Source*: Malins (1979).

The nature of the fetal malformations observed in association with diabetes mellitus is varied, but in most series cardiovascular and central nervous system defects predominate (Kučera, 1971; Pedersen, 1977a; Jervell et al., 1980). Skeletal malformations, especially those involving the lower limbs, are also very common (Malins, 1979). These range from syndactyly and polydactyly to sacral agenesis. This latter deformity, the so-called "caudal regression" syndrome, although not confined to the infants of diabetics, occurs 250 times more frequently in the offspring of diabetics than in those of nondiabetic women (Kučera, 1971). Considerable interest in this observation has arisen because of the earlier finding that insulin injected into the yolk sac of chick embryos within the first 3 days of incubation causes either partial or total suppression of tail structures (Duraiswami, 1952; Zwilling, 1952).

The cause or causes of congenital malformations in infants of diabetic mothers are unknown. The possible role of genetic, metabolic, and teratogenic factors have been reviewed by Gabbe (1977). In an attempt to investigate the cause of fetal malformations associated with diabetes, Deuchar (1979a,b) performed both in vivo and in vitro studies on rats with diabetes induced immediately after conception using either streptozotocin or alloxan. She demonstrated significantly more congenital malformations, including sacral defects, in untreated diabetic rats than in controls, and also that these defects could be abolished by controlling the maternal diabetes with insulin. Because insulin has been shown to interfere with ossification (Smithberg and Runner, 1963) and may promote proliferation of cartilage rather than bone (Reynolds 1972), Deuchar (1979a) suggested that fetal hyperinsulinemia may be a factor in causing this rare congenital anomaly.

Excessive fetal insulin secretion in late pregnancy may account for abnormal skeletal ossification, but it cannot account for the majority of congenital anomalies observed in the infants of diabetic mothers. The reason for this is that while the critical period for teratogenesis in the human is between the third and sixth week of gestational age (Mills et al., 1979) beta-cell differentiation is not observed before 10-11 weeks (see above). Furthermore, as insulin does not cross the placenta (Adam et al., 1969), exogenous insulin administered to the mother cannot be a direct cause of fetal abnormalities.

Maternal insulin therapy may, however, exert an indirect effect on the fetus by altering the metabolic environment in which it develops. The evidence for this is, at present, only indirect, but Pedersen and Molsted-Pedersen (1979) have reported lower congenital malformation rates in diabetic patients, especially of White classes D and F who had been attending a diabetic clinic in Copenhagen before pregnancy (8.5%),

compared to those from provincial Danish cities and country areas (19.4%). A few of this latter group had attended a diabetic clinic before pregnancy and were thought to have less satisfactory metabolic control at the time of conception. Evidence to support this hypothesis has recently been presented by Miller et al. (1981), who found significantly higher hemaglobin $A_{1C}$ concentrations during the first trimester of pregnancy in a group of diabetic women delivering infants with major congenital anomalies compared to those with normal infants.

Fuhrmann et al. (1983) recently reported that significantly fewer congenital malformations occurred in a group of diabetic women in whom good control was attained before pregnancy compared to a group in whom control was not achieved until 14 weeks of gestation.

Central nervous system defects occur twice as frequently in the fetus of the diabetic as in that of the nondiabetic (Kůcera, 1971) and some authors have recommended routine measurements of amniotic fluid alpha-fetoprotein in all diabetic pregnancies to enable open neural tube defects to be detected (Molsted-Pedersen, 1980). Amniocentesis is, however, a procedure which is not without risk (Medical Research Council, 1978) and although maternal serum alpha-fetoprotein levels have been reported to be generally lower in diabetic women (Wald et al., 1979), this latter technique would appear to be preferable as a routine screening procedure.

Although there has been a major reduction in the perinatal mortality rate associated with diabetic pregnancy during the last 20 years, the incidence of fatal congenital malformations has not changed significantly during this time and these deaths now account for half or more of the perinatal mortality in most series (Gamsu, 1978). On current evidence, it therefore seems logical to encourage diabetic patients to seek advice about their treatment before attempting to become pregnant.

## MEDICAL MANAGEMENT

### Types of Diabetes

Until recently the varying clinical patterns recognized as diabetes were regarded as representing quantitative rather than qualitative differences in the degree of insulin deficiency. It is now believed that insulin-dependent diabetes is precipitated by environmental factors, such as virus infections, in individuals with an HLA-related genetic predisposition to the disease (Nerup et al., 1974), whereas the twin studies of Pyke (1977) suggest that non-insulin-dependent diabetes is associated with a different form of inherited islet cell defect, independent of the HLA system and with a high degree of penetrance.

There is no clear cut-off point between normality and mild non-insulin-dependent diabetes, and recognition of mildly impaired carbohydrate tolerance is only important if clinical intervention can be shown to be beneficial. Outside pregnancy there is little evidence that this is the case. The latest recommended definitions for the diagnosis of diabetes distinguish, on oral glucose tolerance test (GTT) criteria, between "diabetes mellitus" and "impaired glucose tolerance," on the grounds that subjects with the latter condition rarely develop complications, seldom show deterioration in their condition, and sometimes revert to normal (World Health Organization, 1980). The usual dietary advice given for mild carbohydrate intolerance is, in any case, identical to the guidelines for good nutrition in the community at large.

Within pregnancy, a different situation prevails; in pregnant women with normal and mildly impaired carbohydrate tolerance, maternal measurements, including the mean diurnal glucose concentrations and various indices that can be derived from an oral GTT, are well correlated with neonatal glucose concentrations 2 hr after birth (Gillmer et al., 1975a,b). On the assumption that maternal diabetes causes fetal hyperinsulinism, and that this is the cause of neonatal glucose changes, these observations form a rational basis for a dividing line between normal and abnormal—namely, that degree of glucose intolerance below which clinically significant fetal hyperinsulinism is rarely, if ever, seen. After 50 g of oral glucose, 1- and 2-hr plasma glucose values of 165 and 120 mg/100 ml, respectively, define this line (Beard, 1976).

### Screening for Diabetes in Pregnancy

It is frequently observed that gestational diabetic women have poor previous obstetric histories. Abell et al. (1976) carried out oral GTTs on 11,551 unselected women booking for antenatal care and found a previous perinatal mortality of 13.8% in those with abnormal carbohydrate tolerance, with no difference between gestational and previously diagnosed forms of the disease; other studies have given similar results (O'Sullivan, 1975a). Unfortunately, none of the studies carried out to evaluate screening for carbohydrate intolerance in early pregnancy have based the criteria for diabetes on indices derived from the neonate. The American Diabetes Association Workshop Conference on Gestational Diabetes (1980) recommended that the criteria established by O'Sullivan and Mahan (1964) should be retained; these are based on a 100-g oral GTT, a diagnosis of diabetes being made if plasma glucose values meet or exceed two of the following levels: fasting, 105 mg/100 ml; 1 hr, 190 mg/100 ml; 2 hr, 165 mg/100 ml; and 3 hr, 145 mg/100 ml (determined by the Somogyi-Nelson method).

O'Sullivan carried out oral GTTs on an unselected pregnant population and based the validation of his diagnostic criteria firstly on the identification of high-risk women (O'Sullivan, 1975a) and secondly on late follow-up findings which indicated a 16-year cumulative incidence of diabetes of 60% (O'Sullivan, 1975b). These studies showed only small differences between tests carried out at different stages of pregnancy, though more detailed metabolic studies have shown lower fasting and higher postprandial blood glucose values in late pregnancy (Lind et al., 1973; Gillmer and Persson, 1979) (Figure 4).

It is clearly not possible to carry out full oral GTTs on total antenatal populations, and much attention has been given both to simpler screening tests and to clinical pointers from which to identify high-risk populations for more detailed testing. O'Sullivan and co-workers described a screening program in which a single blood glucose estimation was made 1 hr after a 50-g oral glucose load in 752 pregnant women at their first antenatal visit, without dietary preparation (O'Sullivan et al., 1973). A total of 109 (14%) had a value in excess of 130 mg/100 ml and a 100-gram oral GTT was performed on these patients, of whom 19 (2.5%) were diagnosed as gestational diabetics on the above criteria. This study showed that a single blood glucose measurement at a timed interval following oral glucose was more effective in identifying gestational diabetics than the usual clinical criteria of a previous large baby, a family history of diabetes, a poor obstetric history, and obesity. Nevertheless, age seemed to be an important factor, abnormal oral GTTs being much rarer in women under the age of 25 (O'Sullivan et al., 1973). These results have recently been confirmed in a larger

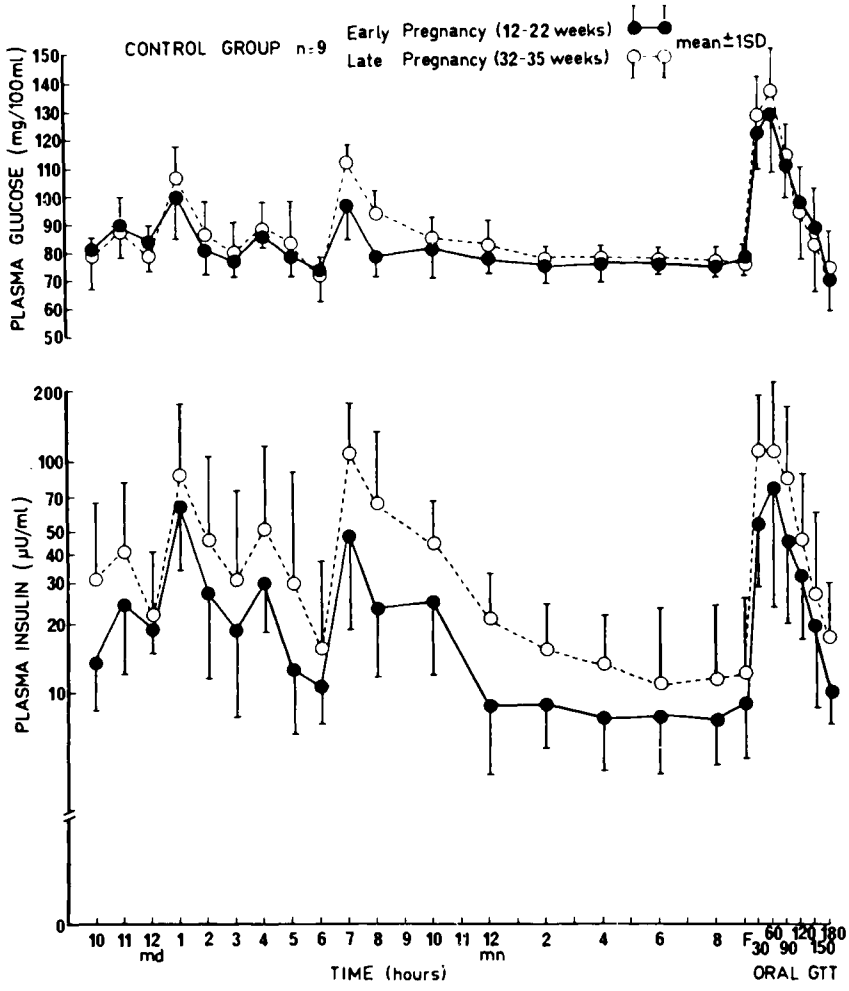


Figure 4 Plasma glucose and insulin concentrations during a diurnal profile and oral glucose tolerance test (GTT) in nine normal women studied in the second and third trimesters of pregnancy (md, midday; mn, midnight). (Based on Gillmer et al., 1975a.)

study by Beard et al. (1980), who used a 50-g oral GTT. They diagnosed chemical diabetes (based on an area in excess of 775 under the 3-hr GTT curve) in 1.3-1.9% of their obstetric population in successive years.

A good case can be made for a screening system based on fasting blood glucose values, as although these show considerable within-patient variation in normal subjects (O'Sullivan and Mahan, 1966), this does not seem to be the case in mild diabetes (Holman and Turner, 1979). In practice, however, a more convenient procedure is to estimate the blood glucose at a known interval after an ordinary meal. Lind and McDougall (1981) have described a screening program based on this concept. They made "random" blood glucose estimations at 28 weeks and assessed the result in relation to plasma glucose measurements previously made at half-hourly intervals after normal meals in 186 healthy women at the same time in pregnancy. Of the 763 women tested, 10 had a plasma glucose in excess of the ninety-ninth percentile limit

of postprandial glucose values and had an oral GTT. Three of these women were diagnosed as having chemical diabetes. This screening system may therefore be less sensitive but more specific than that advocated by O'Sullivan et al. (1973). The apparent efficacy of any screening procedure will, however, depend inevitably on the criteria used for interpreting the test carried out on screen-positive subjects, although in pregnancy the outcome of *that* pregnancy is likely to be a more relevant end point.

A major problem in any screening program is timing and it is fortunate that congenital malformations are less common in gestational than established diabetes (Molsted-Pedersen, 1980), because screening prior to the critical stages of organogenesis does not seem possible. Early screening provides maximum opportunity for action, whereas late screening achieves the highest pickup rate. As management during the third trimester seems most critical to fetal well-being, there is a good case for a simple screening procedure at 28 weeks.

Despite the poor previous obstetric histories of gestational diabetics, there is some evidence to suggest that with improved obstetric care the need to identify mild carbohydrate intolerance in pregnancy is less pressing. Hadden (1980) found that mothers whose glucose tolerance lay from one to three standard deviations above the mean had only slightly more pregnancy complications than those with a normal oral GTT, and no special management was needed in these cases. Mestman (1980) also found that pregnant women with an abnormal oral GTT but a normal fasting blood glucose (8.8% of his study population) had a perinatal mortality that was as low as that in the general population. A total of 25% of the infants of gestational diabetics did, however, experience some morbidity. The high late incidence of true diabetes in women with gestational carbohydrate intolerance (Mestman, 1980; O'Sullivan and Mahan, 1980) has not been confirmed by Hadden (1980), who found a very poor correlation between index oral GTTs and tests carried out 10 years later.

It is possible that use of glycosylated hemoglobin ($HbA_{1C}$) may contribute to the identification of women at risk from gestational diabetes in early pregnancy. This estimation, however, reflects mean blood glucose up to 3 months previously and will therefore probably not respond to deteriorating carbohydrate tolerance until after the critical period of organogenesis. In any case, the value of $HbA_{1C}$ in distinguishing subjects with mild carbohydrate intolerance has not yet been validated by comparison with conventional glucose tolerance testing procedures. This measurement may well, however, be of use in monitoring diabetic control as pregnancy progresses.

In conclusion, a simple 28-week single-sample screen for carbohydrate intolerance in pregnancy will usually alert the obstetrician and pediatrician to cases in which diabetes-related problems are likely to arise, and may help to identify women likely to develop diabetes in later life.

## Classification of Diabetes in Pregnancy

Gestational diabetes is currently defined as glucose intolerance recognized during pregnancy (American Diabetes Association Workshop Conference on Gestational Diabetes 1980); knowledge of whether the condition antedated the pregnancy is rarely available, though observed regression to normality during the puerperium provides indirect evidence. Several attempts have been made to subdivide more severe forms of diabetes according to fetal prognosis; the first of these was that of White (1949); her original classification has been redefined many times and one version is shown in Table 2. The White classification attempts to take into account factors that are likely to influence

Table 5 Prognostically Bad Signs during Pregnancy (PBSP) and White Classifications Combined—Copenhagen Series 1959-1972[a]

| | PBSP | | | |
|---|---|---|---|---|
| | Present | | Absent | |
| White class | N | PNM | N | PNM |
| A | 35 | 143 | 132 | 30 |
| B | 47 | 191 | 132 | 38 |
| C | 68 | 294 | 155 | 90 |
| D | 119 | 252 | 171 | 105 |
| F | 48 | 375 | 13 | 308 |

[a]N, Number of cases; PNM, perinatal mortality rate per 1000 deliveries.
*Source*: Modified from Pedersen et al. (1974).

the outcome of pregnancy; these include age, the duration of diabetes, and the presence of microvascular complications. However, other factors, especially diabetic control, are also important. Pedersen and Molsted-Pedersen (1965) identified certain prognostically bad signs during pregnancy; these included clinical pyelonephritis, precoma, severe acidosis, preeclampsia, and neglect of the diabetes. The results of these authors, shown in Table 5, confirmed a rising perinatal mortality throughout the White classes, but also showed that for each class the presence of prognostically bad signs during pregnancy significantly increased the chances of perinatal death (Pedersen et al., 1974). Metabolic control, however, appears to exert an independent effect on pregnancy outcome and several studies have shown that strict blood glucose control during pregnancy improves the outcome, regardless of age, the duration of diabetes, or the presence of complications (Karlsson and Kjellmer, 1972; Bibergeil et al., 1975).

In reporting clinical studies, use of White's classes and prognostically bad signs during pregnancy aids comparative data interpretation, and for this reason it should be encouraged. In centers with a high standard of medical, obstetric, and pediatric care, perinatal mortality is now so low that identification of high-risk subjects is vital if significant clinical advances are to be made.

## Treatment of Diabetes in Pregnancy

### Diet

Eating patterns influence the prevalence of diabetes, and their modification profoundly affects the course of the disease. Thus even without significant weight change, reduction of carbohydrate intake lowers blood glucose (Wall et al., 1973). There is also evidence that the degree of refinement of the carbohydrate consumed affects blood glucose excursions (Simpson et al., 1980). Current dietary recommendations for diabetics include both restriction of energy to the requirement for ideal body weight recommendations maintenance and the provision of 45-50% of this energy from unrefined carbohydrate sources.

The situation is more complicated in pregnancy and although optimal pregnancy outcome has long been equated with "good" maternal nutrition (Baird et al., 1954), concern has been expressed that high-energy diets predispose to excessive maternal

weight gain (Emerson et al., 1972) and obstetric complications such as preeclampsia. The effects of a carbohydrate-restricted diet similar to that prescribed for diabetes have been described in a study of a population in which such a diet was given with the aim of preventing preeclampsia (Kerr-Grieve et al., 1979). These authors observed a significant reduction in maternal weight gain and birth weight in comparison to a control population. Both caloria deprivation and carbohydrate restriction per se could have contributed to these findings.

The extent to which moderate caloric restriction can be undertaken in obese pregnant subjects has been investigated by Borberg et al. (1980). They compared metabolic parameters in mothers and offspring from thin, normal, and obese women with an unrestricted food intake, and obese women in whom daily energy intake was restricted to 1800-2000 calories, with 150-180 g of carbohydrate. Weight gain was less in the dieted obese women than in any of the other groups, but there was no clear effect of dieting on birth weight in this small group of subjects, though obese women, whether dieted or not, had fatter babies than the normal or thin women. In this study, carried out on nondiabetic mothers, the dieted obese subjects showed a marked reduction both in basal insulin concentrations and insulin response to a glucose challenge, demonstrating that changes in food intake can moderate the metabolic effects of pregnancy. It is difficult to extrapolate these results to diabetic subjects, but the observation that chemical diabetes may arise during pregnancy through failure to increase insulin secretion in response to the diabetogenic stress of pregnancy (Gillmer and Persson, 1979) suggests that calorie restriction may be the appropriate initial management of mild diabetes in pregnancy, as it is in the nonpregnant state.

One potential cause for concern over the use of calorie restriction during pregnancy is the possible deleterious effect of elevated ketone levels described by Berendes (1975). None of the studies quoted above support the view that moderate caloric restriction can be harmful through this mechanism, and in the study of Borberg et al. (1980) fasting 3-hydroxybutyrate concentrations were highest in the thin women, while values following oral glucose did not differ significantly between any of the four groups studied.

### Oral Hyperglycemic Agents

Sulfonylurea formulations have never been shown to have harmful or teratogenic effects on the fetus (Malins et al., 1964); nevertheless, there is a general feeling that insulin is a preferable method for treating the pregnant diabetic because of the possibility of premature stimulation of fetal beta-cell activity. Chlorpropamide crosses the placenta and, when used in a dose of 200 mg/day to treat chemical diabetes during pregnancy, has been shown to cause higher cord insulin concentrations and lower plasma glucose concentrations after birth in the infants of treated diabetic patients than in those of untreated diabetic controls (Stowers and Sutherland, 1975). Despite the long half-life of this drug (approximately 40 hr), a dose of 100 mg daily used for at least 6 weeks before delivery did not cause hyperinsulinism or hypoglycemia in the newborn, although there was a more marked insulin response to an intravenous GTT and a suggestion of an increased rate of glucose disposal in these infants compared to untreated controls (Sutherland et al., 1973). The scanty literature on the use of biguanides in pregnancy has been reviewed by Coetzee and Jackson (1979), who reported the use of metformin 1500 mg daily in 60 pregnant chemical diabetics: 54% of established and 29% of gestational diabetics were classified as metformin failures and changed to other forms of treatment. As these failures were rejected from the follow-up group, it is perhaps not

surprising that a small fall of 19 mg/100 ml in the mean blood glucose was observed in the 33 patients continuing the treatment until term. The value of this form of therapy remains unproven.

### Insulin

*Non-Insulin-Dependent Diabetes* Most of the identified inconsistencies relating to the outcome and management of gestational diabetes stem from the fact that the term is defined on the basis of the timing of diagnosis, rather than from the severity of the disease. While some studies (Mestman, 1980; Hadden, 1980) concluded that medical intervention is generally unnecessary in gestational diabetes, provided that there is good antenatal supervision, O'Sullivan et al. (1974) found that treatment of the gestational diabetic with diet and insulin can reduce fetal loss. Unfortunately it is impossible to conclude from O'Sullivan's otherwise excellent study whether the addition of insulin to the dietary treatment conferred any benefit. However, Oppermann and Camarini-Davalos (1980) showed a clear effect of insulin in reducing the incidence of macrosomia in a group of 243 gestational diabetic women, 90 of whom received insulin. There is, moreover, good evidence that mild maternal glucose intolerance can induce marked disturbances of fetal metabolism (Ogata et al., 1980).

It is perhaps surprising that little effort has been made to identify a subset of gestational diabetics in whom special benefit might be expected from aggressive medical management. Only Gabbe (1980) has attempted to do this, identifying a high-risk group in whom there was a previous intrauterine death, an elevated fasting glucose, or toxemia of pregnancy.

The management practice of Gyves et al. (1980) seems to have much to recommend it. These workers achieved a 1.1% perinatal loss rate in 183 gestational diabetic pregnancies using simple dietary measures, supplemented by insulin if the 2-hr postprandial blood glucose was above 120 mg/100 ml, despite admission to hospital to ensure dietary compliance. Ambulatory management was encouraged and careful obstetric care given, without any special policy of early intervention.

Unfortunately little has been written about the type of insulin regime that should be used in the mildly diabetic pregnant woman. Gyves et al. (1980) used daily insulin doses ranging from 10 to 228 units. O'Sullivan et al. (1966) also provided some information about total insulin dosage, but not about the type of insulin used or the number of patients receiving single or multiple daily injections. At first sight the use of a single daily injection of highly purified crystalline insulin zinc suspension might seem attractive; this has been advocated for use in nonpregnant diabetics by Holman and Turner (1977), on the basis of demonstrated benefits from chronic insulin infusion in non-insulin-dependent diabetics (Turner et al., 1976). In pregnancy, however, there is not only the risk of nocturnal hypoglycemia in insulin-treated patients (Gillmer et al. 1975a) (see Figure 5), but also an increased insulin demand in the non-insulin-dependent diabetics as pregnancy proceeds, greater than that seen in insulin-dependent diabetics (Rigg et al., 1980). It may, therefore, be more prudent to use a shorter-acting formulation such as isophane insulin, or a soluble-isophane mixture, given twice a day. Roversi et al. (1980) have used three or more daily injections of highly purified soluble insulin in 280 gestational diabetic women with excellent results, but there is as yet no evidence that such a regime confers significant benefit in comparison with simpler programs. Although highly purified insulin of minimum antigenicity should always be used, even these preparations may cause formation of insulin antibodies during pregnancy (Mylvaganam et al., 1980).

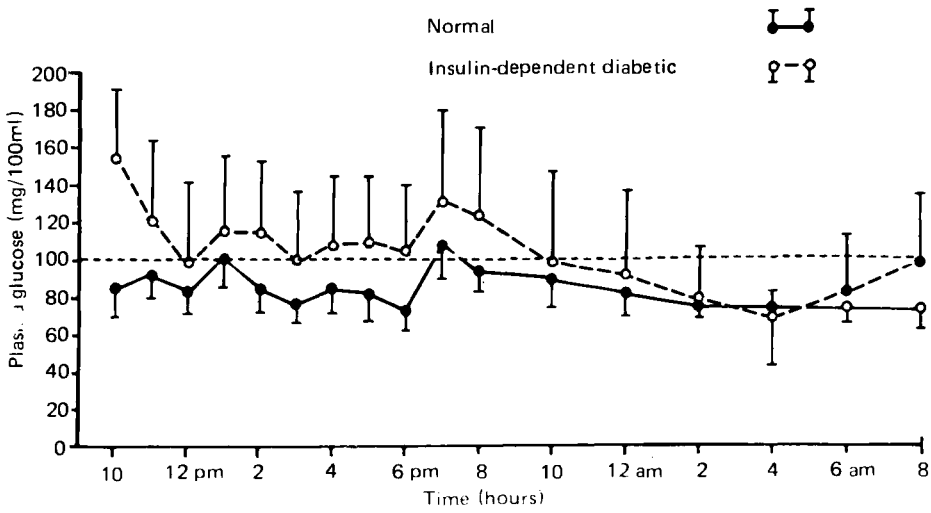


**Figure 5** Plasma glucose concentrations during a diurnal profile in 24 normal women and 13 insulin-dependent diabetics in the third trimester of pregnancy. (Based on Gillmer et al., 1975a.)

*Insulin-Dependent Diabetes* The importance of "excellent" rather than "good" blood glucose control throughout pregnancy has become increasingly apparent in the past two decades and is supported by both physiological observations and clinical studies (Gillmer and Persson 1979, Ogata et al., 1980). Attaining excellent diabetic control depends first and foremost on a high level of patient motivation and, secondly, upon expert medical advice throughout pregnancy. The use of home glucose monitoring increases the patient's involvement in treatment, but the value of this in actually improving diabetic control has been questioned (Stubbs et al., 1980; Worth et al., 1980). Nevertheless, many physicians recommend home glucose monitoring for their pregnant insulin-dependent diabetics.

In addition to the factors mentioned above, metabolic control of the insulin-dependent diabetic is significantly influenced by the presence of endogenous insulin secretion, as evidenced by circulating C-peptide. Asplin et al. (1979) have shown that retention of glucose-responsive beta-cell function significantly improved metabolic autoregulation in a group of nonpregnant insulin-dependent diabetics studied with detailed diurnal metabolic profiles. Similar findings have been noted in pregnant women (Stangenberg et al., 1982).

The satisfactory clinical results reported in most studies of pregnant insulin-dependent diabetics have been achieved using twice-daily insulin injections. Most physicians recommend a combination of soluble, together with an extended action formulation such as isophane or an insulin zinc suspension. The duration of action of the highly purified porcine formulations such as Insulatard (Nordisk) and Monotard (Novo) appears to be shorter than with older preparations, either as a result of species differences or reduced antibody formation. As a result, only small additions of soluble insulin are usually required nowadays and the main priority is to avoid undesirable hyperglycemia prior to the next injection, rather than before lunch and at bedtime, as was previously the case with less pure beef insulin preparations. The regime of background crystalline insulin zinc suspension supplemented by preprandial soluble insulin, advocated by Phillips et al. (1979), may prove to be as satisfactory as any others.

The demonstration that $HbA_{1C}$ provides an index of mean plasma glucose during periods of up to 3 months (Gonen et al., 1977) has led some to use this measurement for evaluation of diabetic control in pregnancy. As previously indicated, the retrospective nature of the measurement is a disadvantage, but it does appear to be a useful tool for checking patient compliance as pregnancy proceeds (Leslie et al., 1978).

While the good results reported in current studies may induce a complacent attitude toward present management techniques, there is evidence that the difficulty of administering insulin in a way that approximates its normal physiological release into the portal system leads to significant departures of metabolite levels from the normal, even in diabetics thought to be well controlled on the basis of plasma glucose concentrations (Alberti et al., 1975). One attempt to resolve this problem has been the development of insulin administration by continuous subcutaneous infusion (Pickup et al., 1978; Tamborlane et al., 1979). This technique has been used in pregnancy by Potter et al. (1980), who employed the method during the third trimester without technical difficulties and described some improvement in glucose concentrations during diurnal profile studies carried out in patients so treated. However, the increasing use of improved but more elaborate methods of diabetic management in pregnancy is inhibited by the large numbers of patients required to demonstrate the clinical superiority of these new techniques over current practice.

## OBSTETRIC MANAGEMENT

### Fetal Surveillance

#### Ultrasound

There is frequently a need for planned preterm delivery in diabetic pregnancy and accurate knowledge of the gestational age of the fetus is therefore extremely important. This can be achieved by measurement of either the fetal crown-rump length in the first trimester of pregnancy (Robinson, 1973) or the biparietal diameter (BPD) of the head in the second trimester (Campbell, 1969). The former measurement is the more accurate and in nondiabetic pregnancy predicts gestational age to within 4 or 5 days. Pedersen and Molsted-Pedersen (1979) have, however, cast some doubt on the reliability of crown-rump length measurements in the fetus of the diabetic. They found that the estimated gestational age of the fetuses of a group of 31 diabetic women who were certain of the date of their last menstrual period was on the average nearly 1 week less than expected, and in 10 cases the deficit exceeded 9 days. The authors also noted that only two of this latter group had been attending a diabetic clinic before pregnancy and have suggested that the apparent early growth retardation of these fetuses may be a consequence of poor maternal diabetic control. They have recently also observed an association between early growth retardation and major fetal congenital malformations (Pedersen and Molsted-Pedersen, 1981).

Anencephaly, which occurs three times more frequently in diabetic than in nondiabetic pregnant women (Kučera, 1971), can be detected early in pregnancy by ultrasound scanning. Renal agenesis, which is six times more common in diabetic pregnancy, may also be diagnosed in this way.

Serial BPD measurements have now been in widespread use mainly to detect fetal growth retardation for just over a decade. However, despite the fact that most articles on the outcome of diabetic pregnancy published in the last few years state that these

measurements have been made, few studies of serial measurement of biparietal growth in the fetus of the diabetic appear to have been published and current data is conflicting. Murata and Martin (1973) demonstrated that growth of the biparietal diameter of the diabetic fetus was within the normal range up to 37 weeks, with no difference according to the severity of the diabetes as judged by the White classes. Thereafter, however, the fetus of the diabetic mother differed from the normal, in that it did not display any slowing of growth. Szalay et al. (1975), on the other hand, found similar values in diabetic and normal pregnancies until term, but the number of patients studied after 37 weeks was small. More recently Aantaa and Forss (1980) reported that the BPD of the fetus of the diabetic was smaller than normal between 20 and 32 weeks of pregnancy, but were unable to demonstrate any difference from the normal between 36 and 40 weeks.

In general, BPD measurements do not appear to be of value for the prediction of fetal weight (Sabbagha, 1977) and fetal chest area has been found to be a better predictor of the large-for-dates infant (Wladimiroff et al., 1978). Multiple measurements of the fetus have been shown to enable prediction of the birth weight to within ±200 g with 95% confidence (McCallum and Brinkley, 1979), but are not widely used.

### Antenatal Fetal Heart Rate Monitoring

Both contraction stress tests (CST), in which an intravenous oxytocin infusion is used to provoke uterine activity, and resting or nonstressed tests (NSTs) have been widely used in diabetic pregnancy to assess fetal well-being. The former test has been used mainly in the United States, and the latter in Europe.

In the well-controlled diabetic it has been shown that the CST reliably predicts survival up to 1 week after it has been performed and as false-negative results are rare, pregnancy can be allowed to continue safely by use of this test until fetal maturity is assured (Gabbe et al., 1977b). In this study of 242 women with insulin-dependent diabetes (White classes B to R) the incidence of late decelerations in labor and low Apgar scores at birth were shown to be significantly greater in pregnancies where the CST was abnormal. Curet and Olson (1980) in a study of 362 high-risk pregnancies showed that the perinatal mortality in patients with a positive CST was six times higher than that in patients with a negative test. Of the 12 diabetic patients in this study, 4 had a positive CST, compared with 10% in that of Gabbe and co-workers. Three reports of an intrauterine death within 7 days of a negative CST have been published (Baskett and Sandy, 1975; Parer and Alfonso, 1977; McCranie and Niebye, 1977). These cases, which should not detract from the value of this investigation in high-risk pregnancy, however, serve to emphasize current ignorance of the cause of most of the fetal deaths that occur in diabetic women.

The nonstressed test has the advantage over the CST in that no intravenous infusion is required and it can therefore be repeated as frequently as desired. However, if the fetal heart rate record is commenced when the fetus is inactive and the fetal heart tracing appears nonreactive, the test may falsely be considered positive. As periods of fetal inactivity may persist for up to 40 min, this investigation can be extremely time-consuming. In addition, as maternal hypoglycemia has been shown to be associated with reduced baseline variability (Gillmer and Persson, 1979), this possibility must also be considered when an abnormal tracing is obtained. Current practice in most units is to perform the NST two or three times weekly or more frequently if any abnormality is detected (Gillmer and Beard, 1975). If necessary, the test may be repeated several times in 1 day. Others have, however, suggested that if the NST is abnormal, a CST should be performed (Haukkamaa et al., 1980).

## Hormonal Methods of Assessing Fetal Well-Being

*Estrogens* Measurement of urinary estriol excretion over 24 hr was the first test to be widely applied for fetal monitoring in diabetic pregnancy. The values obtained were generally found to be lower than the mean in nondiabetic women, though usually within the normal range (Gillmer and Beard, 1975). Several investigators have found urinary estriol levels to be useful in the management of diabetic pregnancy. Easterling and Talbert (1970) defined three patterns of estriol excretion. The first in a group of six patients was characterized by a progressive rise in the 24-hr urine estriol excretion in the latter weeks of pregnancy and was associated with a normal outcome in all cases. The second consisted of a rise followed by an abrupt fall of more than 20% within 24-48 hr of delivery. One intrauterine death occurred in the eight patients in this group. The third group of seven patients had consistently low levels of urinary estriol. All the infants in this group were small for dates and two intrauterine and one neonatal death were observed.

Rivlin et al. (1970) reported a study of 186 diabetic patients of whom 49 were insulin dependent. No clinical use was made of the 24-hr urinary estriol results obtained in these patients. A total of 16 perinatal deaths occurred and the authors concluded that 9 of these could have been avoided if the estriol values observed had been used in clinical management.

Schwarz et al. (1969) found that when pregnancy was allowed to continue when the urinary estriol levels remained in the normal range, only 8 of 113 gestational diabetics required preterm delivery and only 4 of 20 insulin-dependent diabetics had to be delivered before 38 weeks. The overall perinatal loss in this series was 2.6 and 0.9% in the patients with gestational diabetes. Persson et al. (1970), in a similar study, demonstrated that only 12 of 51 insulin diabetics needed to be delivered before the thirty-eighth week of pregnancy. The perinatal mortality in this series was 4%. Goebelsmann et al. (1973) demonstrated that variations in urinary estriol excretion may be greater in diabetic patients and that this could be corrected by calculation of an estriol-creatinine ratio. This group emphasized the need for daily estimations with results available on the same day. They observed that a 35% fall in estriol values from the mean of the three highest consecutive values or levels that remained two standard deviations below the mean were associated with fetal compromise.

Estrogens were first measured in the blood of pregnant diabetic women more than 40 years ago, when Smith and Smith (1937) reported reduced levels in diabetics with toxemia. Roy and Kerr (1964) measured blood estrogens (estriol, estradiol, and estrone) in nine diabetics with uncomplicated pregnancies and found these to be in the normal range, though with a slightly reduced mean value. Masson and Sutherland (1973) also reported plasma estriol values lower than the normal range.

More recently attention has turned to measurement of unconjugated estriol (Distler et al., 1978). These investigators performed a blind prospective study in 62 women. They measured unconjugated and total plasma estriol and also 24-hr urinary estriol-creatinine ratios daily for 1-8 weeks prior to delivery. The mean values of both total and unconjugated estriol were higher than those of normal women and also displayed greater variability. From analyses of the day-to-day variation (based on the percentage rise or fall from the highest mean of three consecutive preceding values), they concluded that unconjugated plasma estriol estimation was the best test available for predicting fetal jeopardy in diabetic pregnancy. De Hertogh (1979), on the other hand, compared plasma unconjugated estriol and estradiol. He found that while

unconjugated plasma estriol displayed similar increases in normal and diabetic pregnancy, unconjugated estradiol increased more in pregnant diabetic patients than in normal women. He also concluded that unconjugated plasma estriol was the most suitable estrogen to measure for assessment of fetal well-being during diabetic pregnancy.

The largest study of daily urinary estriol determinations published to date in insulin-dependent diabetics is that of Gabbe et al. (1977b). Of the 238 women of White classes B to R they studied, 43 displayed abnormal values or a significant fall in estriol concentrations and there were 2 stillbirths in this group. The remaining 195 women all had a normal daily urinary estriol excretion and only 1 had a stillbirth. There was no change in the total plasma, estriol concentration before fetal death, but the serum unconjugated estriol value had fallen by 40%.

Several recent series describe the value of daily urinary estriol estimations for feto-placental monitoring in pregnant diabetics (Kitzmiller et al., 1978; Martin et al., 1979). In others urinary estriol measurements were only made two (Jervell et al., 1979; Haukkamaa et al., 1980) or three (Schneider et al., 1980) times a week. In contrast, Drury et al. (1977) concluded that urinary estrogen measurement would not have avoided any of the deaths in their series and had only a small part to play in the management of diabetic pregnancy.

In summary, the debate concerning the value of estrogen assays in the management of diabetic pregnancy continues. On current evidence it appears, firstly, that urinary estriol measurements (or preferably the estriol-creatinine ratio) should be made at least twice a week and ideally every day. Secondly, plasma estrogen measurements may be preferable to those made on urine, although contrary to original expectations, these assays also show considerable diurnal varability (Hull et al., 1978). Finally, of the plasma estrogens that can be measured on a routine basis, unconjugated plasma estriol probably has the greatest potential value in clinical application.

*Human Placental Lactogen* Human placental lactogen (HPL) is a polypeptide hormone, which displays close structural and biological similarity to human growth hormone (Li et al., 1971). Although less potent than human growth hormone, it appears to contribute to increased free fatty acid mobilization and enhanced insulin response to glucose of late pregnancy (Yen, 1973).

Plasma HPL concentrations are weakly correlated with placental mass (Spellacy et al., 1971a) and have generally been found to be higher in diabetic than in nondiabetic pregnant women (Gillmer and Beard, 1975; Garoff, 1976; Gillmer et al., 1977a).

Measurement of serial HPL concentrations has been shown to be of value in predicting placental insufficiency (Saxena et al., 1969), but has not been widely used in diabetic pregnancy. Spellacy et al. (1971b) reported 5 stillbirths in 33 patients with diabetes mellitus, 4 of the 5 deaths being associated with normal HPL levels. The exception was a patient with hypertension complicating her diabetes. Most workers are in agreement that HPL concentratons are reduced in the diabetic with placental insufficiency, although both Sexena et al. (1969) and Ursell et al. (1973) stressed that a significantly reduced level for a diabetic may fall in the normal range of nondiabetic patients. A further problem is the very marked spontaneous and unexplained fluctuation in plasma concentrations which have been observed both in normal (Pavlou et al., 1972) and diabetic pregnancies (Gillmer et al., 1977a) during diurnal studies. These findings emphasize the need to collect blood samples on successive days if a clinically significant interpretation of plasma HPL concentration is to be achieved.

Persson et al. (1973b) compared the value of serial HPL and urinary estriol measurements in 45 diabetic pregnancies. They found that all women with normal HPL values

had normal estrogen excretion. Human placental lactogen levels and estriol values were both low in two of the three perinatal deaths in this series, but whereas HPL levels were normal in the third patient, estriol excretion was reduced. These authors concluded that urinary estriol measurements offered the better hormonal alternative for assessment of fetoplacental well-being in diabetic pregnancy. Saxena et al. (1969) also reported three stillbirths in diabetic patients with normal HPL levels.

Plasma HPL determinations do not appear to have been used in most recently published series of diabetic pregnancies (Drury et al., 1977; Kitzmiller et al., 1978; Gabbe et al., 1977b). The series reported by Jervell et al. (1979) from Oslo is an exception, but these workers did not give any details of the HPL results obtained.

Several authors (Selenkow et al., 1971; Ursell et al., 1973; Garoff, 1976) have reported a greater increase in insulin requirements in patients with higher plasma HPL concentrations, but Soler et al. (1975) and Spellacy and Cohn (1973) did not find any association between HPL levels and insulin requirements.

### Preterm Labor

Spontaneous preterm labor is more common in diabetic women than in the nondiabetic population. Molsted-Pedersen (1979) (in Copenhagen) reported an incidence of 15% in class A patients and 32% in classes B to F, compared to an overall 5% incidence for nondiabetics in Northern Europe (Anderson, 1978).

The cause of this pregnancy complication is unknown, but in view of the predisposition of the infant of the diabetic to the problems associated with immaturity, preterm labor has even more serious implications for the infant of the diabetic than for that of the nondiabetic.

Conventional therapy using beta-sympathomimetic agents to arrest preterm labor is, however, not without hazard in diabetic women. These drugs bind beta-2-adrenergic receptors in uterine muscle, effecting uterine relaxation. In addition to their cardiovascular side effects of tachycardia and hypotension, they also cause profound metabolic changes. These include: (1) glycogenolysis with consequent hyperglycemia and an increase in blood lactate concentration; (2) lipolysis with increased blood levels of free fatty acids, glycerol, and 3-hydroxybutyrate (Fredholm et al., 1978); and (3) a significant fall in plasma potassium concentrations (Thomas et al., 1977). Borberg et al. (1978) reported diabetic ketoacidosis in a diet-treated class A diabetic receiving intravenous ritrodine (Figure 6). Large amounts of intravenous insulin were required to achieve normoglycemia. Considerable attention to the regulation of blood glucose concentrations is therefore necessary when using these drugs in women with impaired carbohydrate tolerance and especially in insulin-dependent diabetic women. Simultaneous intravenous insulin administration in high doses is mandatory if ketoacidosis is to be avoided. Barnett et al. (1980), describing their experience in 10 diabetic patients in preterm labor, advocated starting the intravenous insulin infusion at 16 units/hr and advised simultaneous administration of 100-120 mmol of potassium every 24 hr to prevent hypokalemia.

Glucocorticoid drugs have been used for some years to promote lung maturation when preterm delivery is anticipated (Liggins and Howie, 1972). These drugs have many actions which are metabolically antagonistic to those of insulin (Grodsky, 1975) and in insulin-dependent diabetics produce a profound disturbance of control, necessitating intravenous insulin infusion (Borberg et al., 1978). The combination of

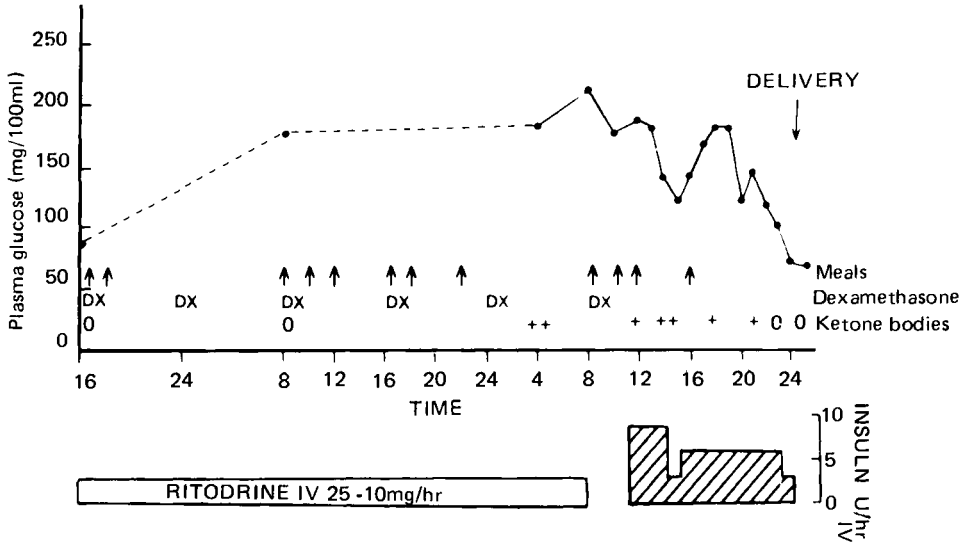


**Figure 6** Plasma glucose concentration, urine ketones, diet, and drug therapy in a chemical-diabetic patient in preterm labor. (From Borberg et al., 1978.)

glucocorticoids with adrenergic drugs results in a marked enhancement of the hyperglycemic effect of the latter (Kauppila et al., 1978) and this therapy should only be initiated in centers specializing in the treatment of pregnant diabetic women (Molsted-Pedersen, 1979).

### Timing and Mode of Delivery

Shortly after the introduction of insulin, it was recognized that the fetus of the diabetic was at increased risk of unexplained antepartum death, especially during the last 4 weeks of pregnancy, and also of intrapartum death, frequently as a consequence of trauma during the delivery of a macrosomic infant. These observations resulted in a widespread policy of planned delivery by cesarean section as soon as the baby was considered large enough to be viable (Titus, 1937). In most units delivery was performed between 36 and 38 weeks in uncomplicated pregnancies, or even earlier if there were obstetric or diabetic complications of pregnancy. This policy, although not accepted by all (Shir, 1939), resulted in a significant increase in deaths from hyaline membrane disease of the large but immature neonate (Gellis and Hsia, 1959).

The obstetric dilemma of timing delivery to avoid both intrauterine and neonatal death has been greatly simplified during the last decade with the introduction of improved methods for assessing fetal well-being and pulmonary maturity. The most important of these has been the introduction in the early 1970s of the amniotic fluid lecithin-sphingomyelin (L/S) ratio measurement for assessment of pulmonary surfactant activity (Gluck et al., 1971). Gluck and Kulovich (1973) were the first to report delayed maturation of the L/S ratio in classes A, B, and C diabetic pregnancy. Shortly afterward Whitfield et al. (1973) reported falling or static L/S ratios in serial amniotic fluid samples of some diabetic women. Subsequently Farrell (1976) collated 100

reports on the L/S ratio from the world literature and found a 7-18% incidence of respiratory distress syndrome in diabetic pregnancies, even when the L/S ratio was within limits normally indicative of fetal pulmonary maturity. Lowensohn and Gabbe (1979), who had found no difference between L/S ratios in diabetic and nondiabetic pregnancy (Gabbe et al., 1977a), concluded that methodological differences were the probable cause for discordant results in different laboratories.

An improved method for separation of pulmonary phospholipids using two-dimensional rather than one-dimensional thin-layer chromatography has been reported by Gluck's team (Hallman et al., 1976). This technique has resulted in the concept of a "surfactant phospholipid profile" (Kulovich et al., 1979) and has led to the recognition that measurement of phosphatidylglycerol has an important role in assessing fetal pulmonary maturity. This phospholipid is found in measurable quantities only in lung tissue. It is secreted into the alveoli with lecithin and appears to be a catalyst and stabilizer of lecithin activity in surfactant (Hallman et al., 1976). Recent studies (Cunningham et al., 1978; Kulovich and Gluck, 1979) have suggested that phosphatidylglycerol provides a better prediction of the likelihood of respiratory distress syndrome than the L/S ratio in pregnancies complicated by diabetes, especially if there is asphyxial stress during labor. Cunningham (1981) has cautioned that delivery should be delayed (if possible) when phosphatidylglycerol is absent from amniotic fluid or only present in low concentrations and the L/S ratio is less than 3. In addition, Hallman and Teramo (1979) have demonstrated an association between low phosphatidylglycerol levels and neonatal hypoglycemia, an observation which is compatible with the concept that delayed lung maturation results from fetal hyperinsulinemia (Stubbs and Stubbs, 1978).

Although surfactant production is delayed in the fetus of the diabetic, most studies suggest that pulmonary maturity is achieved by 38 weeks (Robert et al., 1976; Cunningham, 1981), especially if careful metabolic control is maintained during pregnancy (Curet et al., 1979). Most authors at present favor delivery of the infant of the diabetic at this gestation and recommend that the amniotic fluid L/S ratio or phospholipid profile be measured beforehand (Drury et al., 1977; Brudenell, 1978; Gabbe and Quilligan, 1981). With meticulous control in uncomplicated pregnancies it may, however, be reasonable to allow pregnancy to continue to 39 weeks or even term (Persson et al., 1975), especially in the gestational (class A) diabetic. Indeed, Gyves et al., (1977) delivered 46% of their insulin-requiring diabetics between 38 and 41 weeks gestation with no increase in perinatal mortality or morbidity.

Whereas most diabetic women were, until recently, delivered by cesarean section, many authors have reported successful vaginal delivery in approximately half their diabetic patients (Gabbe et al., 1977b; Brudenell, 1978). Even with apparently good diabetic control, macrosomic infants occur more frequently in diabetic than in nondiabetic pregnancies (Persson, 1978), and it is generally accepted that patients with a malpresentation or any suspicion of cephalopelvic disproportion should be delivered by elective cesarean section. Although spontaneous labor more commonly occurs when diabetic pregnancy is prolonged beyond 38 weeks, the larger size of the fetus and increased risk of shoulder dystocia in these circumstances must be taken into consideration when planning the mode of delivery (Kitzmiller et al., 1978).

Labor should ideally be induced only when the cervix is favorable. If delivery is indicated when the cervix is unfavorable, vaginal prostaglandins may be employed to "ripen" the cervix, but continuous fetal heart rate monitoring must be performed because of the risk of fetal asphyxia during this treatment. Continuous fetal heart rate

monitoring is also mandatory (Quinn and Murphy, 1981). Using this technique, Gabbe and co-workers (1977b) observed late decelerations in 16% of their diabetic patients, but this was no different from the incidence in nondiabetic women (20%). Kitzmiller and co-workers (1978) and Brudenell (1978), on the other hand, reported a 25% incidence of fetal distress in labor as defined by the present of late decelerations and a fetal scalp blood pH of less than 7.25. Brudenell (1978) also found that fetal distress tended to be more common in primigravidas (33%) than multigravidas (19%).

### Management During Labor

Hyperglycemia during labor predisposes to neonatal hypoglycemia (Light et al., 1972), and in the hypoxic sheep fetus has been shown to be associated with a greater rise in plasma lactate and decline in pH than occur in the normoglycemic fetus (Shelley et al., 1975). It would therefore appear to be important to maintain maternal blood glucose concentrations in the normal range during labor.

Although the so-called "artificial pancreas" has been recommended for this purpose (Nattrass et al., 1978; Santiago et al., 1978), equally good results can be achieved, at a fraction of the cost, using an intravenous insulin infusion (usually 1-2 units/hr, administered by a syringe pump) and a separate intravenous glucose infusion designed to provide approximately 10 g/hr (West and Lowy, 1977; Watkins, 1978). Blood glucose concentrations should be measured hourly at the bedside using test strips and a reflectance meter and should be maintained between 70 and 100 mg/100 ml. Brudenell (1978) found that this technique was associated with only a 13% incidence of fetal distress, compared to 33% in patients in whom subcutaneous insulin injections were used. This observation is in keeping with Shelley's findings in the sheep (see above). Epidural analgesia is generally preferred, because blood glucose concentrations tend to be more stable when the patient is free of pain.

### Management After Delivery

Endogenous insulin production in the normal and chemical-diabetic woman returns to prepregnant levels almost immediately after delivery (Lind and Harris, 1976). This dramatic increase in insulin sensitivity after delivery also occurs in insulin-dependent diabetic patients and the intravenous dose of insulin must be reduced by about 60-70% at this time to avoid hypoglycemia. After a normal delivery subcutaneous insulin injections at the prepregnancy dose can be resumed with the next meal. Following delivery by cesarean section the intravenous insulin and glucose infusions should be maintained until normal feeding is resumed. Gestational diabetic patients usually require no treatment after delivery, but their fasting plasma glucose concentration should be measured before they leave the hospital and a glucose tolerance test should be performed 3 months after delivery.

### Lactation in the Diabetic

Early experience suggested that failure of lactation was a common problem in the diabetic mother (White, 1937). Brudenell and Beard (1973), however, ascribed this to the increased incidence of cesarean section, prolonged separation of the mother and baby, and suckling difficulties in the premature neonate. Although no information is available on milk composition in the diabetic, many diabetic women successfully breast-feed their infants without any adverse effect on diabetic control or neonatal growth,

provided that appropriate dietary advice is given and adjustment of the insulin dose is made (Lawrence, 1980).

## NEONATAL MORBIDITY

Neonatal morbidity occurs in more than 50% of infants of insulin-dependent mothers and in approximately 25% of infants of chemical-diabetic mothers (Mestman, 1980). Some of the problems encountered in the clinical management of the newborn baby are shown in Table 1.

### Respiratory Distress

The respiratory distress syndrome was until recently the leading cause of neonatal mortality and morbidity in infants of diabetic mothers. In a retrospective study of 805 infants of diabetic mothers born between 1958 and 1968, the syndrome occurred in 23.4%, as compared to 1.3% in the nondiabetic control group (Robert et al., 1976). When controlled for other factors such as gestational age and route of delivery, it was found that infants of diabetic mothers had almost six times as much of a chance of suffering respiratory distress syndrome than infants of nondiabetic mothers. It has been proposed that fetal hyperinsulinism might interfere with the pulmonary phospholipid metabolism, leading to surfactant deficiency (Stubbs and Stubbs, 1978). This hypothesis is supported by experimental evidence, which suggests that insulin may antagonize the normal effects of cortisol on the enzymes responsible for surfactant synthesis in the lung (Smith et al., 1975). Clinical observation also suggests that the incidence of respiratory distress syndrome is much less common in infants of mothers in whom strict diabetic control has been achieved throughout pregnancy. In fact, this dramatic fall in respiratory distress due to surfactant deficiency represents the most remarkable change in morbidity that has occurred over the last decade. A more frequent pulmonary complication nowadays is transient tachypnea of the newborn. A rapid respiratory rate and cyanosis characterize this condition, but these usually disappear within 24-36 hr. This can be distinguished from respiratory distress syndrome by the less severe symptoms, the benign course, and the chest x-ray appearances. Recent studies suggest that transient tachypnea in infants of diabetic mothers may be due to lack of phosphatidylglycerol (Hallman and Teramo, 1979).

### Polycythemia

Another cause for respiratory problems is polycythemia. Enhanced extramedullary hematopoiesis and polycythemia are characteristic findings in infants of diabetic mothers. An increased rate of hematopoiesis might be caused by fetal hypoxia due to placental insufficiency and/or to elevation of maternal hemoglobin $A_{1C}$, which (because of its increased oxygen affinity) might lead to oxygen deficiency in the fetus. Recently Widness et al. (1980) demonstrated markedly elevated erythropoietin levels determined by radioimmunoassay in the cord blood of infants of diabetic mothers. These authors also reported similarly elevated erythropoietin concentrations in the fetal rhesus monkey that had been subjected to hyperinsulinemia during the latter part of gestation. The insulin-treated fetuses also had evidence of increased extramedullary erythropoiesis. These findings suggest that fetal hyperinsulinemia may stimulate erythropoietin production in utero, leading to increased erythropoiesis, but

whether this is a primary effect of insulin or secondary to chronic fetal hypoxia is unknown. Polycythemia may have serious consequences for the baby, since the blood viscosity increases exponentially when the hematocrit exceeds 60%. It may be necessary to treat the hyperviscosity syndrome, which is an entity characterized by cardiorespiratory distress and neurological symptoms, by phlebotomy and/or modified exchange transfusion.

### Cardiomegaly

A high incidence of cardiomegaly with or without symptoms is a common finding in infants of diabetic mothers. Recent studies using echocardiography have demonstrated hypertrophy of the ventricular walls and, in particular, of the interventricular septum in infants of diabetic mothers (Hirschfeld et al., 1979). Using this noninvasive technique, it is also possible to evaluate cardiac performance so as to detect circulatory abnormalities. Echocardiographic studies suggest that there is a very wide spectrum of circulatory disturbances in infants of diabetic mothers, including cardiomegaly with or without impaired circulatory function (Gutgesell et al., 1976; Hirschfeld, Fanaroff and Merkatz, 1979). The cause of cardiomegaly is unclear, but as poor metabolic control during pregnancy seems to be associated with a marked increase in the thickness of the interventricular septum (Hirschfeld et al., 1979), it may in part be due to fetal hyperinsulinism.

### Neonatal Small Left Colon Syndrome

This rare syndrome, which is of unknown etiology, produces typical signs and symptoms of lower intestinal obstruction similar to those of Hirschsprung's disease in the newborn, for example, failure to pass meconium, abdominal distention, and bile-stained vomitus. Contrast radiography shows a narrowing of the sigmoid and descending colon, with proximal dilatation of the large bowel. The clinical course is usually benign and of short duration, but colonic perforation has been reported. The syndrome seems to be more common in infants of diabetic mothers (Davis et al., 1974), and it has been suggested that it could be caused by increased glucagon secretion due to neonatal hypoglycemia, with a resultant inhibition of jejunal and sigmoid activity (Philippart et al., 1975). This delayed functional maturation of the gastrointestinal tract may help to explain the relatively high incidence of feeding problems of around 30% seen in infants of diabetic mothers (Persson et al., 1979). A relative increase of less mature ganglion cells of the left colon has been described in this condition (Davis et al., 1974), but others have not found this abnormality (Philippart et al., 1975).

## LONG-TERM PROGNOSIS

There is limited information on the long-term prognosis of the babies of diabetics. Follow-up studies have mainly dealt with two important aspects: the risk of appearance of diabetes in later life and psychosomatic development.

### Risk of Appearance of Diabetes

The majority of women with known diabetes during pregnancy have type 1 insulin-dependent juvenile diabetes, which is characterized by an increased association with

HLA *DRw3* and *DRw4*. Children of diabetic adults may therefore, as a group, show a greater genetic susceptibility to develop diabetes than those of nondiabetic parents. The incidence of juvenile diabetes among the children of diabetic mothers varies between 0.5-1.0% in different series (Pedersen, 1977b). In a recent study based on data from 464 children of diabetic mothers, the prevalence of insulin-dependent diabetes was calculated, using a method of age correction, to be 1.5% at the age of 25 years (Kobberling and Bruggeboes, 1980).

### Psychosomatic Development

Several authors have described a poor long-term prognosis in terms of neurological abnormalities and impairment of intellectual development. Churchill et al. (1969) reported that diabetic mothers who had had acetonuria during pregnancy had offspring with IQ scores lower than those of controls. This association was independent of factors such as the severity of the diabetes and insulin reactions during pregnancy and the duration of pregnancy. A drawback of this study was that neither acetonuria nor blood glucose was recorded systematicaly during pregnancy. Naeye (1979) in a recent reexamination of the same material concluded that these findings could be explained by amniotic fluid infection and were not due to hyperketonemia during pregnancy.

In a large Danish series which included 740 children born during the period 1946-1966 (between 1.5 and 26 years old at follow-up), an unusually large group of children (36%) had evidence of cerebral dysfunction or related conditions (Yssing, 1975). The incidence of cerebral palsy and epilepsy was three to five times higher than normal, whereas the incidence of mental retardation was no different from normal. The occurrence of major cerebral handicaps seemed to be related to high or low maternal age in pregnancy, maternal White classes D and F, low gestational age, low birth weight of the infant, and the presence of acute complications during pregnancy. Similar findings have been recorded by others (Bibergeil et al., 1975); however, follow-up results of children born since 1964 suggests that the long-term prognosis has improved. In a careful study which included examinations of the children at 1, 3, and 5 years of age, Stehbens et al. (1977) showed a delay of intellectual development in 6 out of 47 children at 5 years. In two other recent studies major handicaps were found in 4 out of 51 children at an average age of 7 (Cummins and Norrish, 1980) and in none of 49 children aged 5 at follow-up (Persson et al., 1978). Furthermore, both of these studies showed a normal distribution of IQ scores and no evidence of abnormally low IQ values.

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# 8

# Fetal Breathing

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## INTRODUCTION

It is commonly held that the "first breath" is taken at the time of birth. However, it is now apparent that the first breath of air has been preceded by a long experience of intrauterine breathing activity, albeit with a fluid-filled respiratory tract and a consequently small tidal volume. Thus although many major changes within the respiratory system take place at birth, most notably the filling of the lungs with air and the commencement of gas transfer, birth may be seen as part of a continuing developmental process. With many recent studies highlighting the relatively inefficient mechanics of breathing in the newborn, the importance of adequate prenatal respiratory experience is becoming recognized.

The notion that fetuses make breathing movements has been considered for several centuries, at least since the time of Leonardo da Vinci. During the nineteenth century, much was written on the subject, particularly in German obstetric publications. The first permanent recordings of suspected fetal respiratory movements were made by Ahlfeld (1905) using a pressure sensor applied to the maternal abdomen. Many subsequent accounts of rhythmical, respiratory-like movements of the fetus during late pregnancy were published during the early part of the twentieth century. These early obstetric observations apparently prompted an interest in the subject by experimentalists such as Barcroft and Barron (1937) and Snyder and Rosenfeld (1937), among others. The majority of these early studies were performed on fetuses either totally or partially removed from their anesthetized or restrained mothers, but still attached by the umbilical cord. Many of these studies showed that respiratory movements could be induced in the otherwise apneic fetus, but that they rarely occurred spontaneously. For instance, Barcroft (1946) demonstrated that a single inspiratory effort or series of them could be elicited in the sheep fetus by tactile, thermal, or chemical stimuli.

Although many studies showed that fetuses could be induced to make respiratory-like movements, their absence in the unstimulated exteriorized fetus was widely interpreted as an indication of their absence in the healthy fetus in utero. It was not until the refinement of recording techniques in chronic animal studies that the existence of spontaneous breathing movements in the healthy fetus gained acceptance (Merlet et al., 1970; Dawes et al., 1970). These early studies in sheep were followed shortly by the demonstration of rhythmical respiratory-like movements of the chest and abdomen in human fetuses using newly developed, noninvasive ultrasonic techniques (Boddy and Robinson, 1971). Following these pioneering studies, there has been a rekindling of interest in prenatal respiratory activity, by the obstetrician fired by the

need for a simple, noninvasive test of fetal well-being, and by the respiratory physiologist with a desire for a greater understanding of the early development of the respiratory system and the changes it undergoes at birth.

## TECHNIQUES

### Animal Studies

The earliest studies on animals involved the direct observation of fetuses removed totally or partially from the uterus with the umbilical circulation intact. This approach has almost entirely been replaced by the measurement of physiological events using electronic sensing equipment implanted into fetuses in utero. Notwithstanding the great care taken in their preparation and in the maintenance of physiological blood gas status, acute unanesthetized exteriorized fetuses rarely make spontaneous breathing movements (e.g., see Moss and Scarpelli, 1979). Possible reasons for the failure of the exteriorized fetus to behave as it would in utero have been discussed by Comline and Silver (1974).

The most widely used method of detection of fetal respiratory activity in chronic animal studies involves the measurement of pressure changes within the respiratory tract. A fluid-filled catheter is inserted into the cervical trachea and positioned such that its tip lies within the thorax (Dawes et al., 1972). The influence of pressure deflections of nonfetal origin may be eliminated by subtracting the pressure recorded from the amniotic sac from the intrathoracic pressure signal. A more sensitive method of pressure detection, less prone to extrinsic influence, is the implantation in the trachea of a miniature pressure transducer located at the tip of a flexible catheter (e.g., Gaeltec, United Kingdom). Whichever system is used, it must have a low "noise" level and high sensitivity, because in the sheep fetus, for example, the majority of respiratory pressure fluctuations are smaller than 5 mmHg.

Electromyography, the detection of electrical activity of contracting muscles, has been used to analyze the involvement of individual muscle groups in fetal breathing. Pairs of small stainless steel electrodes are sewn into the muscle and connected to differential ac amplifiers and appropriate filters. Details of this technique and the equipment required have recently been given by Harding and Poore (1982). The recorded signals of 50-100 $\mu$V are usually integrated and displayed on a polygraph. In this way, the contributions of the diaphragm (Maloney et al., 1975a), intercostal muscles, and the muscles of the larynx (Harding et al., 1977, 1980) can be defined. Electromyography permits the reliable detection of even very small inspiratory efforts, whereas these may cause pressure deflections within the noise level of most pressure-recording systems. On the other hand, nonrespiratory muscle activity may occasionally be falsely classified as respiratory in electromyogram recordings.

That respiratory movements are the result of activity in the phrenic nerve has been confirmed by Bahoric and Chernick (1975). Similarly, Wyszogradski et al., (1975) used the phrenic neurogram to demonstrate that gasping in exteriorized, anesthetized fetal sheep was attributable to phrenic discharges. It is unlikely, however, that recording phrenic nerve activity offers any advantage over recording the electromyogram of the diaphragm, except perhaps during early gestation, when motor end plates are not fully differentiated (Dickson, 1939).

Real-time ultrasound has been used to observe fetal breathing movements in sheep for periods of less than 1 hr (Wittmann et al., 1981). This technique offers the advantage,

when combined with physiological recordings, of correlating fetal body movements with breathing movements.

### Human Studies

Techniques for the observation of fetal breathing movements in human subjects must be noninvasive and noninjurious to the developing fetus. It is widely considered that ultrasonic devices satisfy both of these criteria. Ultrasonic techniques used to monitor fetal breathing have recently been reviewed by Harding and Poore (1982) and only a brief description will be given here.

The initial ultrasound observations of fetal breathing employed an A-scan technique (Boddy and Robinson, 1971). This type of equipment provides a continuous indication of the distance from the probe of surfaces which reflect sound waves. Hence echoes could be obtained from the surface of the fetal chest or abdomen showing rhythmical fluctuations in their distance from the surface of the maternal abdomen. By their rhythmical nature and location and correlation with maternal abdominal movements, they were taken to represent fetal breathing movements (Boddy and Mantell, 1972). This technique has now been largely replaced by real-time B-scan devices which provide a continuous image of the fetus. With A-scan devices, uncertainty exists as to whether a surface movement is respiratory, due to transducer movement, or due to nonrespiratory fetal movement. This problem has largely been overcome with real-time systems, where the outline of the fetus (longitudinal or transverse sections) may be viewed. Respiratory movements and gross body movements are readily distinguishable (Patrick et al., 1978a). The spatial resolution of modern real-time systems is on the order of 0.5 mm and even very shallow respiratory movements can be detected. The signals are viewed on a video screen and may be stored on magnetic tape or film for later analysis. Automated measurements of chest and abdominal wall movements have been performed using a phase-locked tracking system (Bocking et al., 1982).

Another ultrasonic technique using relatively inexpensive equipment takes advantage of Doppler frequency shifts in the fetal circulation associated with inspiratory efforts (Boyce et al., 1976). Using a simple 2-MHz probe, Doppler shifts may be detected from two locations, the fetal hepatic circulation and the umbilical vein (Mantell, 1980; Goodman and Mantell, 1980). The frequency of the audible Doppler signal originating from the umbilical vein is modulated downward during each inspiratory effort while the frequency of the signal from the abdominal veins is increased.

## DESCRIPTION OF FETAL BREATHING MOVEMENTS

### The Form of the Movements

#### Animal Studies

Recordings of intrathoracic pressure in fetal sheep in utero show both isolated and rhythmical respiratory movements (Dawes et al., 1972). Rhythmical movements are typically shallow (1-3 mmHg) and rapid (1-4 per second), although the frequency is highly variable. During the last month of gestation these "rapid, irregular" breathing movements occur in episodes during which rapid eye movements (REM) are present and the electrocorticogram is devoid of large-amplitude slow waves. The volume of fluid which moves along the trachea during the inspiratory efforts is usually less than 1 ml (Dawes et al., 1972; Maloney et al., 1975a).

The second type of inspiratory effort occurs less commonly and less predictably. It occurs at low frequency (1-4 times per minute) and is longer lasting and has been described by Dawes et al. (1972) as a "gasp." It is now felt that this term is inappropriate owing to dissimilarities between this type of inspiratory effort and asphyxial gasping (Harding, 1980). This type of isolated, deep inspiratory effort apparently has no counterpart in human fetal breathing and may be analogous to the inspiratory effort associated with regurgitation in the ruminating animal. Evidence in support of this notion is based largely on the involvement of laryngeal muscles and is set out below.

Fetal breathing movements are accompanied by electrical activity in the muscle of the diaphragm (Maloney et al., 1975a) and in the phrenic nerve (Wyszogrodski et al., 1975; Bahoric and Chernick, 1975). In confirmation of the belief that fetal breathing is a result of central neural activity, unitary discharges have been recorded in the medulla of fetal sheep in phase with phrenic nerve activity and deflections in tracheal pressure (Bystrzycka et al., 1975).

The intercostal muscles play a minor part in rhythmical fetal breathing movements during the last month of gestation in the sheep, although they may be active during isolated, deep inspiratory efforts in the sheep (Harding et al., 1980). This finding concurs with the observed depression of intercostal activity in the newborn lamb (Henderson-Smart and Read, 1978) and cat (Duron, 1969) during REM sleep. The depression of intercostal activity is thought to be due to tonic presynaptic inhibition of 1A afferents by descending reticular pathways (Pompeiano, 1966). Prior to the differentiation of recognizable behavioral states in fetal sheep (110-120 days), respiratory intercostal activity may be recorded (Dawes et al., 1980a), suggesting that descending inhibitory pathways may not yet have developed. The presence of inspiratory and expiratory intercostal nerve activity in exteriorized fetal sheep at term (Bystrzycka et al., 1975) may be attributable to the conditions under which the experiments were performed.

The inertia of the contents of the fetal respiratory tract coupled with the high degree of compliance of the chest wall and absence of intercostal activity undoubtedly contribute to the paradoxical movements of the chest wall and abdomen (Poore and Walker, 1980). This rocking motion may also be observed in newborn humans and lambs, particularly during REM sleep (Henderson-Smart and Read, 1978). There is, however, no respiratory activity in the major abdominal muscles (rectus abdominus) during normal fetal breathing movements (R. Harding, unpublished observations).

The intrinsic muscles of the fetal sheep larynx are active in relation to fetal breathing movements from as early as 90 days of gestation. The abductor muscles (posterior cricoarytenoid) discharge in phase with the diaphragm during rhythmical breathing movements, but are inactive during isolated, deep inspiratory efforts (Harding et al., 1980) (Figure 1). In the air-breathing situation, vigorous contraction of the diaphragm in the absence of simultaneous active abduction of the arytenoid cartilages would lead to passive closure of the glottis. This sequence of events occurs immediately before regurgitation in the ruminant and assists cranial projection of the bolus of digesta. It is not known whether regurgitation occurs in the fetus during deep inspiratory efforts; however, these "breaths" resemble regurgitative attempts in the ruminant, not only in the absence of laryngeal abduction, but also in their relation to the slow-wave electrocortical state (Harding et al., 1980). These deep inspiratory efforts should not be confused with asphyxial gasping (Towell and Salvador, 1974), during which the activity of both the laryngeal abductor muscles and the diaphragm is particularly intense (R. Harding, unpublished observations).

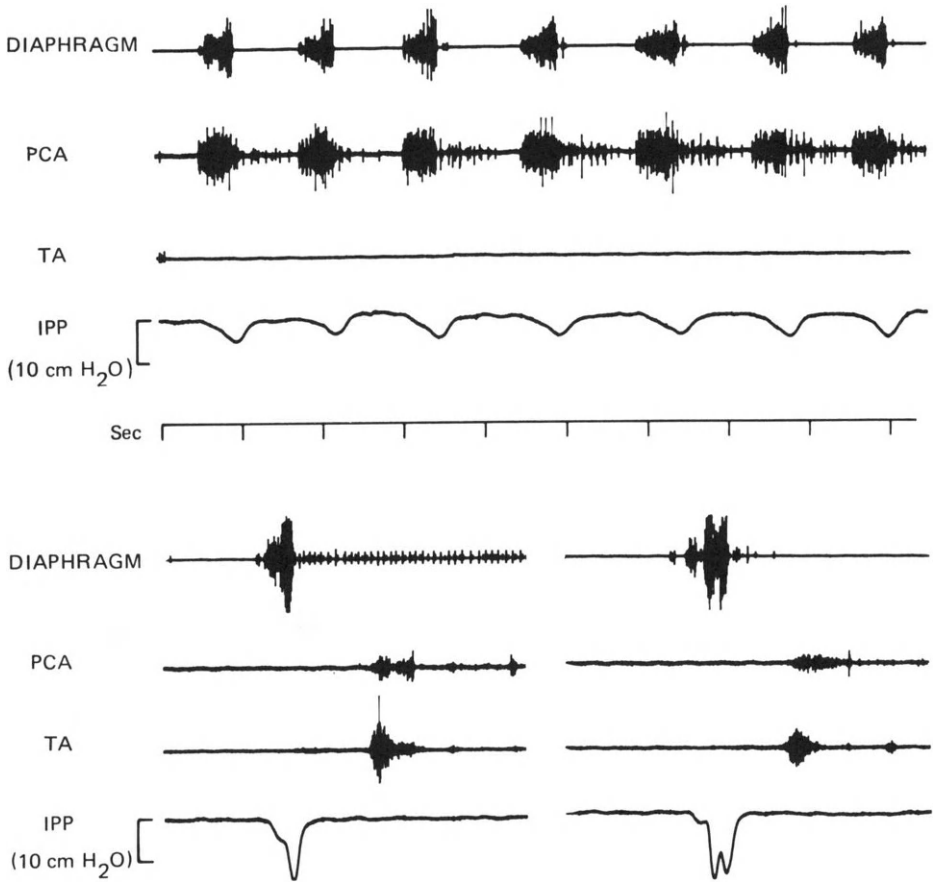


**Figure 1** Two types of inspiratory effort in the fetal sheep. The traces are electromyograms of the diaphragm, posterior cricoarytenoid (PCA), and thyroarytenoid (TA) muscles and the intrapleural pressure (IPP). The upper recording shows a series of rhythmical breathing movements during the REM, low-voltage electrocortical state. Activity in the PCA occurs during each inspiratory effort. The lower recording shows two examples of isolated, deep inspiratory efforts during the non-REM, high-voltage electrocortical state. Typically the diaphragm contracts in two or more phases and is not accompanied by activity in the PCA. The adductor muscles discharge afterward. (From Harding, 1980.)

The activity of the adductor muscles of the larynx is also related to breathing movements in the sheep fetus. Recordings from thyroarytenoid, one of the major adductors in the sheep, show a cessation of activity during each burst of diaphragmatic electromyogram and a low-level tonic discharge during apnea (Harding et al., 1980). In this they resemble the "expiratory" units recorded in the medulla by Bystrzycka et al. (1975). Although the adductor muscles are usually tonically active during apnea, the glottic aperture is not firmly sealed and fluid may be passed through it.

The reciprocity between the activities of laryngeal adductor muscles and the diaphragm is also seen during fetal swallowing. With each swallow the arytenoid cartilages are briefly and strongly adducted while the activity of the diaphragm is inhibited

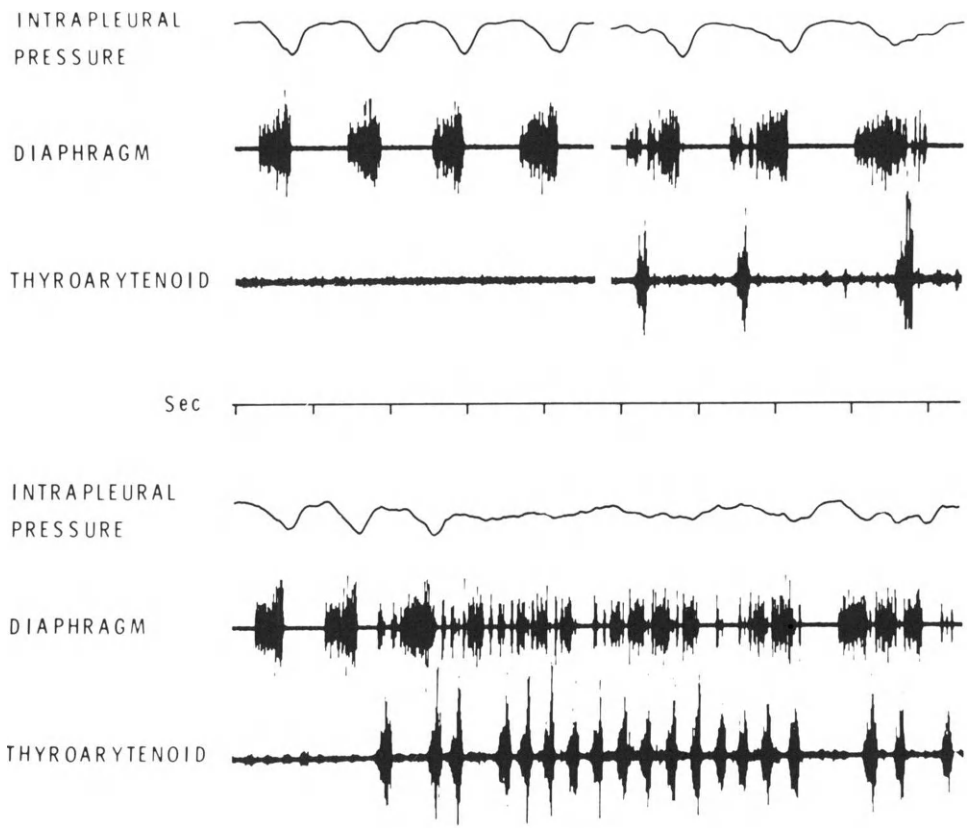


**Figure 2** The influence of swallowing on inspiratory activity in the fetal sheep. Swallows are indicated by brief, large-amplitude bursts of electromyogram activity in the thyroarytenoid. The upper left recording shows a series of inspiratory efforts in the absence of swallowing. The upper right shows the brief inhibitory effect that single swallowing movements have on the diaphragm. The lower recording shows the disrupting effect of a series of rapid swallows (3 Hz) on inspiritory activity.

(Harding et al., 1978a). During bouts of swallowing at up to 3 Hz the diaphragm becomes repeatedly inhibited, giving rise to the appearance of a series of staccato inspiratory efforts (Figure 2). This phenomenon has also been seen in the lamb being suckled (Harding and Titchen, 1981). The high degree of coordination between opposing muscle groups appears to be organized at the level of the brainstem and is independent of afferent traffic from the larynx.

### Human Studies

The paradoxical motions of the fetal chest and abdomen during fetal breathing were described first by Mantell (1976) using a pair of A-scan ultrasound transducers. He noted rhythmical movements of the anterior chest wall of 2-3 mm and out-of-phase movements of the anterior abdominal wall of 6-8 mm. This observation was subsequently confirmed by Patrick et al. (1978a) using real-time B-scan sonography. These authors reported wide variations in the extent of the movements of the body surfaces.

However, even very small breaths may be recognized by the characteristic reciprocal movements of the anterior walls of the chest and abdomen. The extent of the movements of the chest and abdomen may not be an accurate representation of the vigor of diaphragmatic contraction. Poore and Walker (1980) found only a poor correlation between intrathoracic pressure and movement of the fetal chest in sheep. The reciprocal movements of the chest and abdomen may be interpreted as being a result of contraction of the diaphragm which forces the abdominal wall outward and retracts the chest wall. The largest excursion occurs at the level of the umbilicus, which can be used as a convenient landmark in order to make measurements at a consistent site (Gennser and Marsal, 1979).

Sudden, deep inward and outward movements of the chest wall have been reported in several studies employing ultrasound (e.g., Lewis and Trudinger, 1977; Patrick et al., 1978a), and have been referred to as hiccups. Typically they occur each 3-6 sec and in episodes lasting 6-16 min. Their cause is unknown and they do not occur in the sheep fetus. However, some similarity exists between the fetal hiccup and the isolated, deep inspiratory effort in the sheep. Postnatally, the hiccup is a sudden, deep inspiratory effort, the glottis being drawn closed, resulting in the characteristic sound. This maneuver resembles the inspiratory effort of regurgitation in the ruminant. In the neonate hiccups are usually associated with feeding and reflux. Thus the hiccup, like the isolated, deep inspiratory effort of the sheep fetus, may be a consequence of gastric or esophageal stimulation.

## Patterns of Fetal Breathing

### Animal Studies

Fetal breathing movements are characteristically episodic in both sheep and humans, the only species studied over long periods in utero. The movements tend to occur in bouts separated by periods of respiratory inactivity. Perhaps one of the most exciting and provocative findings in the history of research into fetal breathing is that in the sheep the episodes of breathing movements occur only during bouts of rapid eye movements in the presence of a low-voltage, high-frequency electrocorticogram; apnea, on the other hand, coincides with an absence of eye movements and with slow-wave, high-voltage electrocortical activity (Dawes et al., 1972). These two behavioral states occupy approximately equal amounts of time in the sheep fetus after 115-125 days. The incidence of both fetal breathing and the fetal low-voltage electrocortical state (REM) shows an approximately parallel circadian variation in the sheep (Boddy et al., 1973). The peaks occurred late in the evening and the troughs early in the morning; however, the physiological changes underlying this variation have not yet been identified.

The relationship between behavioral states in utero and postnatally requires careful consideration. There seems to be little doubt that the fetal condition in which there are few ocular movements and slow waves of high amplitude are present in the electrocorticogram is analogous to the state of quiet (non-REM) sleep in the postnatal animal (Ruckebusch, 1972). However, it is becoming increasingly apparent that a significant proportion of time spent in low-voltage, high-frequency electrocorticogram must be regarded as arousal or wakefulness, particularly late in gestation. It has been estimated by Ruckebusch et al. (1977) that periods of arousal occupy about 10% of time during the last 10 days of gestation in the sheep fetus. Their criteria for arousal

was based on the absence of rapid eye movements and increased fetal movement with changes in heart rate. These conclusions have, however, been contested, and more recently the monosynaptic evoked response of the sciatic nerve has been used in an attempt to more clearly distinguish between the state of arousal and REM sleep (Ioffe et al., 1980). However, the variability of the evoked response is large, preventing its use as a criterion of state. A similar approach was adopted by Rigatto et al., (1982) in fetal sheep. They found that monosynaptic and polysynaptic spinal reflexes were greater during the slow-wave electrocortical state than during the REM state, the converse of the relationship observed by Ioffe et al. (1980).

Postnatally, rapid swallowing (feeding) is one activity which, in the lamb, consistently takes place during wakefulness. Thus the presence of rapid bouts of fetal swallowing may be a reliable index to the existence of the state of arousal in utero (Harding, 1980). Support for this concept is that phasic activity in the nuchal muscles, increased heart rate variability, and augmented breathing movements usually accompany spontaneous swallowing bouts.

Developmental changes in the pattern of spontaneous breathing movements were noted by Dawes et al. (1972) in fetal sheep both when delivered into a saline bath and in utero. From about midterm the size and frequency of the movements during bouts increased, but there was reported to be no obvious change with age in their incidence or periodicity. The latter point has been recently examined in detail by Bowes et al. (1981a) using computer analysis of repeated 2-hr recordings made between 100 days and term in the sheep fetus. It was found that breathing movements were almost continuous at the younger age and that periods of apnea of increasing length evolved, such that by 130-140 days alternating episodes of breathing and apnea take place. These authors were, however, unable to relate the changing pattern of breathing to development of sleep states. The studies of Bernhard et al. (1959) and Ruckebusch (1972) have shown that slow-wave electrocortical activity develops late in gestation (115-125 days) and it is likely that it is the emergence of this state which accounts for the development of increasing amounts of fetal apnea observed by Bowes et al. 1981a.

### Human Studies

It is now generally recognized that real-time scanning affords, at present, the most reliable measure of fetal breathing movements in human subjects. Some doubt has been expressed concerning the validity of measurements made using A-scan methods (Farman et al., 1975; Thaler et al., 1980); nevertheless, many of the baseline observations made using A-scan methods yielded information that has been subsequently confirmed by real-time scanning. For example, Boddy and Robinson (1971) found that during late pregnancy fetal breathing movements occurred in bouts for 55-90% of the recording time at a frequency of 30-70 per minute. Prolonged recording with real-time equipment has shown that at 34-35 weeks of gestation, fetal breathing movements were present for, on the average, 32% of the time at a mean frequency of 49 breaths per minute (Patrick et al., 1978a). Other recent studies have shown a widely varying range of the frequency and incidence of breathing movements, and it has become recognized that many factors of fetal and maternal origin exert an influence.

Continuous recordings over 24 hr at 34-35 weeks of gestation have shown that the incidence of fetal breathing increases following maternal food intake and during the early hours of the morning (Patrick et al., 1978b). Each increase in the incidence

of fetal breathing was preceded by a rise in the maternal plasma glucose concentration. In the sheep there is some evidence that fetal breathing movements may be depressed by hypoglycemia (Boddy and Dawes, 1975) and it has been demonstrated that intravenous administration of glucose to women during normal pregnancy increases the incidence of fetal breathing movements (Boddy and Dawes, 1975; Hohler et al., 1977). The stimulatory effect of maternal glucose load on fetal breathing has not, however, been confirmed by Ritchie (1979).

The reason for the nocturnal increase in the incidence of fetal breathing movements is not understood. It is not related to maternal glucose levels and may be associated with a circadian rhythm such as has been reported in the sheep (Boddy et al., 1973). Some recent evidence by Patrick et al. (1981) indicates that the nocturnal facilitation of fetal breathing may be related to elevated levels of *maternal* glucocorticoids. In the sheep fetus, however, there is no correlation between the incidence of fetal breathing and *fetal* glucocorticoids (Boddy et al., 1974a).

The 24-hr pattern of human fetal breathing has recently been subjected to mathematical analysis (Campbell, 1980; Campbell et al., 1980). Box-Jenkins modeling techniques and analysis of frequency spectrum distribution were used to analyze data obtained between 30 and 39 weeks of gestation. This analysis confirmed that the incidence of fetal breathing was nonrandom and that significant repeat patterns occurred at intervals of 100-500 min.

As yet few attempts have been made in human subjects to examine the relationship between the fetal rest-activity cycle (Sterman, 1972) and fetal breathing, even though real-time scanners have been used to record both fetal body movements and breathing (Patrick et al., 1978a; Ritchie, 1979). This sort of data will be required to link episodes of fetal breathing to antenatal behavioral states as has been accomplished in the sheep. This may be a difficult task owing to the difficulty of recording fetal breathing movements when the fetus is moving using real-time ultrasound scanning. However, it seems a distinct possibility that fetal breathing is linked to the 40- and 80-min cycles of gross fetal activity which presumably reflect fetal states of sleep or arousal (Sterman, 1972; Timor-Tritsh et al., 1978; Granat et al., 1979).

A recent attempt has been made to assess fetal behavioral states from heart rate variability and body movements (Timor-Tritsch et al., 1980). Fetal breathing movements, detected by an abdominal strain gauge, were less regular during the "active" state than during the "quiet" state. Because the authors chose only a 1-min epoch for state definition, it is doubtful if the states thus identified can be equated with either the prenatal rest-activity cycle or the postnatal states of REM sleep, quiet sleep, and wakefulness. This type of investigation highlights the need for careful evaluation of the behavioral state, especially where electrocortical recordings cannot be made.

In this regard it is often tempting to compare the relationship between fetal behavioral states and respiratory activities in the most widely studied animal species, the sheep, with that in the human fetus. It must be remembered, however, that central nervous system development in the sheep is precocious, with two electrocortical activity states being fully differentiated at 125 days of gestation (0.8-0.9 of term). In comparison, these states and their electrocortical correlates are not established until after birth in the term human infant (Ellingson, 1972).

Although fetal breathing movements have been detected as early as 11 weeks (Boddy and Dawes, 1975), the bulk of quantitative data relates to the last 10 weeks of pregnancy. Where breathing patterns have been examined earlier than 30 weeks, they have

been found to differ significantly from those obtained nearer term. In the study of Fox et al., (1979) there was a significant increase in the incidence of fetal breathing with increasing gestational age. For example, between 24 and 29 weeks breathing movements were present, on the average, for only 12% of the time, whereas by 30-34 weeks this had increased to 33%. A further increase to 51% was noted at 35-39 weeks. This finding has not been supported by the findings of two other groups. Between 28 and 40 weeks, Trudinger and Knight (1980) observed no difference in the incidence of fetal breathing. A similar conclusion was reached in a comparison of 24-hr recordings made at 30-31 and 38-39 weeks in which the incidence was 31% in each case (Patrick et al., 1980). The brevity of the observation period—for example, 30 min in the studies of Fox et al. (1979) and Trudinger and Knight (1980)—may contribute to the discrepencies between these groups, given that the percentage of time spent breathing during any 1 hr can range from 0-86% (Patrick et al., 1980).

The frequency of breathing movements during episodes is reported to become progressively reduced and more regular between 28 weeks and term (Trudinger and Knight, 1980). The change in the breath-to-breath interval is small, increasing from a mean value of 1 sec at 30 weeks to 1.3 sec at 40 weeks. These values are in good agreement with the data of Patrick et al. (1980).

## PHYSIOLOGICAL INFLUENCES ON FETAL BREATHING MOVEMENTS

Most of the available evidence relating to the physiological regulation of fetal breathing has been derived from animal experimentation, principally in sheep. Where it has been technically and ethically feasible to compare the responses of human fetuses with those of animals there has been general agreement. One of the most common challenges met by the fetus is asphyxia, and considerable attention has been paid to the influence of blood gas levels and pH on fetal breathing. Such studies have yielded information not only on the recognition of abnormal fetal respiratory patterns, but on the prenatal development of respiratory control mechanisms. Although a considerable amount of experimental work in this area was performed prior to the development of chronic animal preparations, it will not be included in this review, owing to doubts over the physiological status of the exteriorized fetus.

### Oxygen Levels

#### Hypoxia

In sheep, maternal administration of gas mixtures low in oxygen sufficient to reduce fetal $paO_2$ from 20-24 to 10-12 mmHg while leaving $paCO_2$ and pH largely unchanged caused a reduction in the incidence of fetal breathing movements over a 1-hr period (Boddy et al., 1974b; Maloney et al., 1975b). This finding confirmed earlier observations in fetal rabbits of Snyder and Rosenfeld (1937). The inhibition of breathing movements in fetal sheep was paralleled by a reduced amount of time spent in the REM state and an increase in the proportion of time spent in the non-REM, slow-wave electrocortical condition. There is also a reduction in the incidence of limb movements (Natale et al., 1981). The fetal response to hypoxia may thus be interpreted as one appropriate for the conservation of oxygen. The effects of experimental hypoxia in the human fetus are uncertain, but Ritchie (1980) concluded that the likely response is a reduced incidence of fetal breathing, as in the sheep.

Brief, partial occlusions of the umbilical cord produce essentially hypoxic conditions in the sheep fetus (Towell and Lysak, 1978) and abolish rhythmical breathing movements (Tchobroutsky et al., 1979). Similarly, brief occlusion of a uterine artery causes moderate hypoxia, which has been shown to abolish fetal breathing movements and trigger a switch of sleep state from REM to non-REM (Harding et al., 1981).

The fetal response to hypoxia is the reverse of that expected postnatally in the presence of functional chemoreceptors in the carotid bodies. On the evidence of electrophysiological studies in exteriorized fetal sheep, the carotid bodies are apparently not functional until the time of birth (Biscoe et al., 1969). In the lamb there is some evidence that the maturation of a rapid carotid body-mediated ventilatory response takes place gradually during the first few days of postnatal life (Belenky et al., 1977). The mechanisms responsible for the activation of the carotid bodies are still unknown; some of the possibilities have been considered by Purves (1981b) and Jansen et al. (1981).

### Hyperoxia

In fetal sheep, $paO_2$ may be raised by some 5 mmHg by allowing the ewe to breathe 50% $O_2$ in $N_2$. This maneuver maintained for 1 hr had no significant effect on the vigor or incidence of fetal breathing movements in the nonlaboring animal (Boddy et al., 1976). A similar finding in the human has recently been made by Ritchie and Lakhani (1980a). These findings suggest that availability of oxygen at the normal $pO_2$ of 20-25 mmHg does not limit the ability of the healthy fetus to make breathing movements. The reduced incidence of breathing movements in labor may, however, be attributable, at least in part, to a reduced oxygen supply (see p. 270). In human pregnancies in which fetal $paO_2$ is likely to be diminished, for example, in conditions of severe preeclampsia or fetal growth retardation, hyperoxia led to a significant increase in the incidence of fetal breathing (Ritchie and Lakhani, 1980b).

### Natural Variations in Fetal $paO_2$

By comparing the incidence of ovine fetal breathing with fetal $paO_2$ in the same sheep at different times and between sheep, Dawes et al. (1972) concluded that spontaneous fetal breathing was not influenced by natural variations in oxygen tension. However, Towell (1974) concluded from observations made in fetal sheep and goats that spontaneous mild fluctuations of fetal blood gases exist and may influence the incidence of fetal breathing. Mild hypoxemia was frequently present during episodes of fetal breathing. Contradictory observations have been made by Bissonette et al. (1980) and the resolution of these relationships will probably be dependent upon the continuous measurement of fetal blood gases.

Further evidence for the existence and causes of spontaneous fluctuations of fetal $paO_2$ have been obtained by the use of miniature implantable oxygen electrodes (Parker et al., 1971). Some of the variation was attributable to low-level, nonlabor uterine contractions producing intrauterine pressure changes of less than 5 mmHg (Jansen et al., 1979). Although the contractions produced only small changes in fetal $paO_2$ (2-5 mmHg), they were often coincident with a cessation or diminution of fetal respiratory movements and a change of sleep state from an REM to a non-REM condition (Nathanielsz et al., 1980).

### Asphyxia

Asphyxia caused by prolonged occlusion of the umbilical cord leads to the onset of fetal gasping following the abolition of rhythmical breathing movements (Towell and Salvador, 1974; Tchobroutsky et al., 1979). Asphyxial gasps are distinguishable from isolated, deep inspiratory efforts by their considerably longer inspiratory time (Manning et al., 1979a) and, in the sheep fetus, by the differential involvement of laryngeal abductor muscles (Harding et al., 1980).

A distinction must be drawn between gasping and normal fetal breathing movements. In many earlier experimental studies in exteriorized animals the appearance of gasping was often interpreted as representing the onset of respiratory activity by the fetus, particularly when anesthesia was used. The physiological stimulus to gasping is quite different to that for rhythmical fetal breathing movements, although the final motor pathway is common to both. Gasping is present after bilateral section of the vagus and carotid nerves (Dawes et al., 1972) and is therefore probably triggered by a central sensor. Jansen and Chernick (1974) have suggested that the primary stimulus to asphyxial gasping is severe hypoxemia and is independent of other peripheral chemoreceptors or of those on the surface of the medulla. Although severe hypoxemia, such as may be present at the time of birth, may lead to cessation of rhythmical inspiratory activity, the onset of gasping may provide a mechanism for rapid and effective aeration of the lungs. With the improvement of blood gas status brought about by gasping and the initiation of carotid body function, regular and continuous respiratory activity may begin. However, as Purves (1981a) suggested, the analysis of the physiological mechanisms involved in the onset of continuous respiration at birth has barely begun.

## Carbon Dioxide Levels

### Hypercapnia

A hypercapnic stimulus to the sheep fetus brought about by maternal inhalation of 4-6% $CO_2$ results in an increase in the incidence of fetal breathing (Boddy et al., 1974b): 1 hr of hypercapnia sufficient to raise fetal $paCO_2$ from 43 to 57 mmHg caused an approximate doubling of the incidence of fetal breathing and an increase in its vigor. The amount of time occupied by the REM state increased in proportion to the increase in the incidence of breathing movements. In the human (Ritchie and Lakhani, 1980a) 20 min of maternal inhalation of 5% $CO_2$ led to an increase in the mean incidence of fetal breathing from 16 to 47%. The increase was not attributable to the concomitant rise in maternal and fetal $pO_2$, because inhalation of 50% $O_2$ failed to mimic the effects of 5% $CO_2$.

Hypercapnia alone is not sufficient to initiate respiratory activity in fetal lambs in utero during the non-REM, slow-wave electrocortical state. Although raised levels of $CO_2$ have been found to initiate rhythmical respiratory activity in acute ovine fetal preparations (Moss and Scarpelli, 1979) and to increase the vigor of diaphragmatic discharges in chronic preparations (Bowes et al., 1981b), it is not clear from either of these studies whether $CO_2$ alone is capable of altering fetal behavioral states or whether the respiratory movements were initiated during the REM state. This doubt supports the view that in studies of this nature firm evidence of the behavioral state is of importance.

### Hypocapnia

Spontaneous fetal hypocapnia ($paCO_2$, 30-32 mmHg) associated with maternal panting led to a low incidence of fetal breathing in sheep (Boddy et al., 1974b). In two recent studies in late human pregnancy, maternal hyperventilation resulted in a reduced incidence of fetal breathing (Van Weering et al., 1979; Marsal et al., 1979). From studies such as those cited above, it may be concluded that central chemoreceptors are active in the fetus, although there is very little direct evidence to support this contention (Jansen, 1977).

### Natural Variations in $paCO_2$

Repeated sampling of fetal arterial blood in sheep and goats revealed that $pCO_2$ normally fluctuates and that it is directly related to the amplitude, but not the incidence, of fetal breathing movements (Towell, 1974). In another study of spontaneous changes in fetal blood gases (Bissonette et al., 1980) hypercapnia was normally associated with hypoxia. The presence of hypercapnia reduced the incidence of fetal apnea associated with hypoxia and reduced the level of $paO_2$ at which apnea developed.

## Acidemia

Infusions of HCl or $NH_4Cl$ into the circulation of fetal sheep causes prolonged stimulation of breathing movements in the absence of a change in $paCO_2$ (Molteni et al., 1980; Hohimer and Bissonette, 1981). The response has a latency of more than 4 hr, possibly attributable to the time required for the elevated hydrogen ion concentration to reach central chemoreceptors. These findings indicate that fetal respiratory activity is stimulated not only by respiratory acidosis, but also by metabolic acidosis, both of which are potent respiratory stimulants postnatally.

## The Role of Pulmonary Afferent Traffic

It is generally considered that vagal afferent traffic from the lungs plays a minor part in the regulation of fetal breathing. Bilateral section of the vagi in the neck does not substantially alter the pattern of breathing or its relation to behavioral states (Dawes et al., 1972; Condorelli and Scarpelli, 1976). This suggests that at resting lung volume there is neither an overall excitatory nor an inhibitory vagal input to the central respiratory pattern generator. That vagal inputs may influence fetal respiratory activity has been suggested by the work of Maloney et al. (1975b), who showed that during late gestation lung inflation (25 ml) led to a reduced frequency of bursts of diaphragmatic activity.

Pulmonary slowly adapting receptors, perhaps the most abundant vagal receptors from the lungs and lower airways, have a very regular tonic activity in the apneic fetus (Ponte and Purves, 1973). Even at subatmospheric intratracheal pressures these units had a substantial level of activity. The observed increase in activity with each inspiratory effort is likely to be small owing to the slight degree of pulmonary expansion which occurs on inspiration.

Thus it appears that while pulmonary stretch receptors are active in the mature sheep fetus, they play a small role in the regulation of the respiratory pattern. In particular, fetal apnea is not due to the inhibition of central respiratory activity by

vagal afferent traffic from the lungs. Immediately after birth, however, the vagal respiratory reflexes are particularly potent (Bodegard et al., 1969).

### Laryngeal Inputs

Stimulation of the laryngeal mucosa with water or milk in newborn lambs causes an abrupt and sometimes prolonged termination of breathing (Johnson et al., 1973). This reflex, which protects the lower airway from entry of foreign liquids or solids, appears to override the normal chemoreceptive drive to respiration. Laryngeal receptors with both short- and long-latency responses to liquid stimulation have been found in the neonates of sheep, cats, and monkeys (Harding et al., 1978b). Receptors with similar properties may be detected in the superior laryngeal nerve of fetal sheep (R. Harding, unpublished observations). They were insensitive to the presence of tracheal or amniotic fluid, unless it flowed rapidly over the laryngeal mucosa. Passage of distilled water through the fetal sheep larynx during episodes of breathing movements causes abrupt apnea, adduction of the larynx, and vigorous swallowing (Harding et al., 1977). It therefore seems unlikely that activity in laryngeal mucosal receptors contributes to the onset of apnea in the fetus.

### The Role of Somatic Afferent Traffic

Several recent reports have described the effects of generalized somatic afferent stimulation on fetal breathing movements. The purpose of these studies has been to determine the nature of possible mechanisms responsible for the initiation of continuous breathing at birth. Stimulation of the central end of a transected sciatic nerve in partially exteriorized fetal sheep frequently initiated a prolonged series of breathing movements (Condorelli and Scarpelli, 1975). The fetuses were anesthetized and had recently undergone surgical interference, both conditions which depress fetal respiratory activity (Dawes et al., 1972). Hence the results of such studies must be interpreted with caution. Using chronic in utero fetal sheep preparations, Chapman et al. (1977) found that the respiratory response to repetitive sciatic nerve stimulation was variable and ranged from an excitation to an inhibition. In a more recent study, the respiratory responses to a variety of somatic stimuli were shown to be dependent on the existing behavioral state of the fetus in utero (Ioffe et al., 1980). A strong linkage was demonstrated between somatic stimulation and breathing movements, such that each individual stimulus delivered during the REM state elicited a short-latency fall in intratracheal pressure. Because diaphragmatic electrical activity was not also recorded, it is by no means certain that the negative swings in tracheal pressure were due to respiratory muscle activity.

### The Influence of Higher Centers of the Brain

The observation that prolonged periods of apnea develop between 110 and 120 days in the sheep fetus (Bowes et al., 1981a) at the time when the electroencephalogram is developing episodes of large-amplitude slow waves suggests that higher centers of the central nervous system may play a role in the suppression of respiratory activity. Support for this concept is given by the observation that the incidence of breathing movements is increased and may even become continuous following destruction of the fetal pituitary gland (Robinson et al., 1980). Because the surgical technique employed in this series of ablation experiments may have resulted in damage to the preoptic areas

of the hypothalamus and the supraoptic nuclei, it is not possible to precisely define the locus of relevance to the respiratory effect.

Further support for the notion of descending inhibition of fetal respiratory activity during quiet sleep has been provided by the recent work of Dawes et al. (1980b), who showed that midbrain transection in some fetal lambs resulted in almost continuous breathing movements, even during slow-wave electrocortical activity. Other fetuses showed exaggerated inspiratory efforts instead of complete apnea during slow-wave sleep. The different breathing patterns may result from slight differences in the level of the transections. The results of these studies taken with those of Bowes et al. (1981a) on the development of patterns of fetal breathing suggests that the prolonged periods of apnea which develop in association with electrocortical slow waves may be due to active inhibition of the medullary respiratory centers from a site located above the midcollicular level.

After birth, the respiratory inhibition of quiet sleep is apparently overcome by high levels of peripheral and central afferent excitatory inputs. The identification of descending inhibitory influences associated with cortical slow waves is not only of interest to fetal physiologists, but may be of importance in the understanding of central apnea of the neonate and the sudden infant death syndrome.

### Temperature

Raising the temperature of the saline bath in which fetal sheep lay caused the onset of rapid inspiratory movements resembling panting; these persisted for periods of 5-10 min (J. S. Robinson cited by Dawes, 1973). In more recent studies on fetuses in utero between 120 and 135 days, maternal heating sufficient to raise fetal temperature by as little as 1°C caused prolonged bouts of high-frequency breathing movements (R. Harding and E. R. Poore, unpublished observations). The maximum frequency of electromyogram bursts in the diaphragm often reached 6 per second. The breathing movements retained their relation to the REM state, but this state usually occupied an increased proportion of time during the heating period. The "panting" often continued for 1-3 hr after the cessation of heating and after the maternal temperature had recovered. These observations indicate that thermal sensitivity is present in the fetus during late gestation.

Cooling of the skin of exteriorized fetal sheep has been found to induce slow, regular breathing movements, quite distinct from those which occur spontaneously in utero (Barcroft and Barron, 1937; Dawes, 1968; Harned and Ferreiro, 1973). However, Bystrzycka et al. (1975) considered the response to be inconsistent and less effective than cooling the snout. The response is likely to depend on the behavioral state of the fetus. Injections of chilled saline into the amniotic sac of fetal sheep can, on occasions, elicit a state resembling arousal (Harding, 1980).

### Circulating Levels of Adrenocorticotropic Hormone

A striking inverse relationship in fetal sheep between circulating adrenocorticotropic hormone (ACTH) concentrations and the incidence of breathing movements has been described by Boddy et al. (1974a). Fetal ACTH levels are known to be elevated during hypoxemia, fetal hemorrhage, hypoglycemia, periods of elevated catecholamine levels in blood, fetal infection, and during labor and immediately after surgery. In each of these states fetal respiratory activity is diminished or absent, and it is quite probable that this effect may be attributable to raised levels of ACTH (Nathanielsz et al., 1977).

### Glucose Levels

In the sheep, maternal hypoglycemia due to lack of feed intake is associated with a reduction in the incidence and strength of fetal breathing movements (Boddy and Dawes, 1975). Below a fetal blood glucose level of 8 mg/100 ml no breathing movements were present. In the sheep fetus, as in the human, glucose is the major metabolic substrate. Fetal breathing in the human is likewise influenced by circulating levels of glucose. In women who had fasted overnight the incidence of fetal breathing increased soon after receiving oral glucose (Boddy and Dawes, 1975). The stimulatory effect of the maternal glucose load has been observed in several studies in humans using real-time ultrasound scanners and Doppler ultrasound and it has become a means of standardizing, in part, the conditions under which observations are made (Hohler et al., 1977; Lewis et al., 1978; Natale et al., 1978; Goodman, 1980). The relationship between maternal glucose concentrations and fetal breathing has recently been reviewed by Natale (1980); it is suggested that the effect of glucose may be mediated by increased $CO_2$ production. The incidence of gross fetal body movements is not influenced by elevated maternal glucose levels, and it appears that the stimulatory effect is specific to breathing movements (Bocking et al., 1982).

## UTERINE MOTILITY AND FETAL BREATHING

### Labor

Early observations in human subjects using an A-scan ultrasound technique showed that the incidence of breathing movements fell from normal values of 55-90% with the approach of labor (Boddy and Dawes, 1975). Later studies, however, using real-time ultrasound systems have revealed a wide variation in fetal breathing patterns during labor. In a series of 25 patients in labor, the majority of whom received 5% dextrose intravenously, fetal breathing was found to be absent in about half, while the others showed brief episodes of fetal breathing of less than 30 sec (Wittman et al., 1979). A significant correlation was observed between the reduction in the incidence of fetal breathing and the presence of an abnormal fetal heart rate pattern. In another investigation of 22 patients in labor, Boylan and Lewis (1980) found a reduction in the mean incidence of fetal breathing from 36 to 1%; there was also a reduction in trunk movements. The authors concluded that diminished or absent fetal breathing during labor is not an indication of fetal compromise. A similar conclusion was reached by Richardson et al. (1979) from a study of 20 term fetuses during the first stage of electively induced labor. The incidence of breathing movements declined from a control value of 25.6 to 8.3% during the latent phase of labor, and then to 0.8% during active labor.

The mechanisms underlying the decline in the incidence of fetal breathing during labor are not fully understood. There are several possible explanations, including the effects of raised levels of ACTH or prostaglandins (see p. 272). Maternal blood glucose levels, known to be positively correlated with the incidence of fetal breathing movements, fall during labor, but administration of glucose during labor has no appreciable effect (Boylan and Lewis, 1980).

Fetal hypoxemia may also contribute to the decline of fetal breathing during labor. Uterine blood flow is diminished during contraction of the myometrium in humans (Borell et al., 1965) sheep, and dogs (Assali et al., 1958). The use of continuously recording oxygen electrodes has shown that fetal $paO_2$ falls greatly during each labor contraction in humans (Fall et al., 1979) and sheep (Jansen et al., 1979). Support for the role of hypoxemia in diminished fetal respiratory activity during labor is given

by the observation that administration of 50% $O_2$ in $N_2$ doubles the incidence of breathing movements in laboring sheep (Boddy et al., 1974b).

### Nonlabor Contractions

Uterine contractions which long precede labor were first described in sheep by Hindson and Ward (1973). They are typically of low intensity (<5 mmHg) and last for 5-10 min, recurring at intervals of 15-60 min. In sheep they may be detected as early as 0.5 of term (Harding et al., 1982) and they are probably analogous to Braxton-Hicks contractions in women. They are associated in late ovine pregnancy with mild hypoxemia (Jansen et al., 1979) and inhibition of fetal breathing (Nathanielsz et al., 1980). Uterine contractions occurring during episodes of rhythmical fetal breathing were frequently related to depression of the breathing movements or to their cessation accompanied by a switch from the REM state to the non-REM state with slow-wave electrocortical activity (Figure 3). The inhibitory effect of nonlabor uterine contractions on fetal breathing has recently been confirmed in the human fetus (36-42 weeks) by Wilkinson and Robinson (1982).

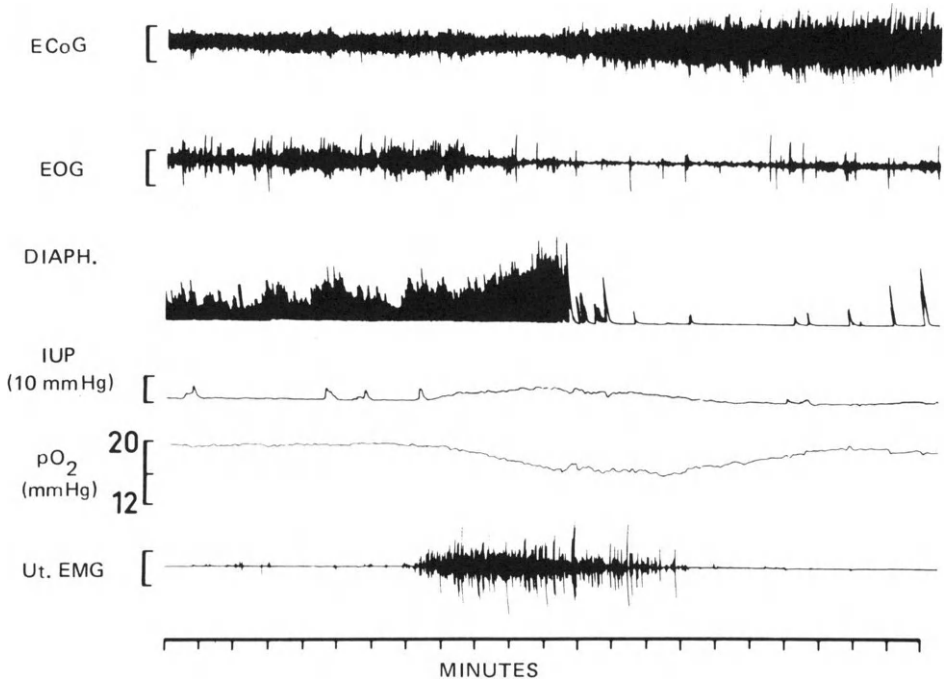


**Figure 3** The relationship between nonlabor contractions of the uterus and sleep states and inspiratory activity in a sheep fetus at 130 days of gestation. The traces are the electrocorticogram (ECoG 100 $\mu$V) electrooculogram (EOG, 200 $\mu$V), integrated electromyogram (EMG) of the diaphragm, intrauterine pressure (IUP), fetal carotid $pO_2$, and uterine EMG. Uterine muscle activity is associated with a rise in IUP, a fall in fetal $pO_2$, a cessation of ocular activity and breathing movements, and the appearance of high-voltage slow waves in the ECoG. (From Harding et al., 1981.)

A series of responses similar to those associated with nonlabor uterine contractions may be elicited in fetal sheep by the occlusion of a uterine artery for 5 min (Harding et al., 1981). This maneuver produces a fall in fetal $paO_2$ of 5-8 mm in the absence of significant hypercapnia or acidemia, suggesting that the fetal response may be due to acute hypoxemia. However, the stimulus to the fetus during uterine contractions is more complex than simple hypoxemia and involves physical distortion of the fetus. Using pairs of ultrasound transducers attached to opposite sides of the fetal chest in sheep, Poore et al. (1980) observed that the transverse and dorsoventral diameters of the fetal chest were altered by 1-1.5 cm during nonlabor contractions. Thus it is possible that excitation of thoracic proprioceptors (Remmers, 1973) may also play a role in the depression of fetal respiratory activity.

## THE ACTIONS OF PHARMACOLOGICAL AGENTS ON FETAL BREATHING

There has been considerable recent interest in the pharmacology of fetal breathing, partly because the majority of drugs administered during pregnancy cross the placenta into the fetal circulation and because a study of their effects may yield new information on the control of fetal respiratory activity. Maternally administered drugs may affect the fetus either directly, following entry into the fetal circulation, or indirectly by affecting blood flow through the placenta.

### Anesthetics, Analgesics, and Narcotics

Anesthetic agents administered to the maternal circulation depress or abolish fetal breathing in sheep, even in doses which cause only maternal sedation. In most cases the inhibition is long lasting. Small doses (15 mg/kg) of chloralose or pentobarbitone caused fetal apnea (Dawes et al., 1972). Even doses as low as 4 mg/kg of pentobarbitone, which had little effect in the ewe, resulted in termination of fetal breathing and the appearance of slow-wave electrocortical activity (Boddy et al., 1976). The inhibition of fetal breathing by barbiturates has been confirmed by Maloney et al. (1975a) and Condorelli and Scarpelli (1976).

In the studies of Boddy et al. (1976) the effects of pentobarbitone on fetal breathing could not be attributed to altered fetal blood gas levels or pH. These authors also tested the effects on fetal breathing of the narcotic analgesic agent meperidine (pethidine) (100-200 mg) administered to ewes. These relatively large doses had no consistent influence, although the stimulatory effect of hypercapnia was abolished. Meperidine crosses the placenta within 10-15 min of maternal administration and may establish higher circulating concentrations in the fetus than in the mother (Boddy, 1977).

The effects of meperidine have been tested in human subjects by two groups of workers. Administration of 1 mg/kg of meperidine to a small group of women before the onset of labor was related to a 50% decrease in the incidence of breathing movements (Gennser et al., 1976). In a double-blind trial in six patients of 34-35 weeks gestation, Lewis and Boylan (1979) were unable to demonstrate any effect of a smaller (5 mg) dose of meperidine.

The effects of maternal administration of diazepam have been tested in several studies which yielded conflicting findings. Boddy (1977) found that 20 mg of diazepam administered intramuscularly significantly reduced the incidence of fetal breathing. One-tenth of this dose, given intravenously in a double-blind trial, was without effect (Lewis and Oliver, cited by Lewis and Boylan, 1979). This finding

concurred with that of Gennser et al. (1976). When administered acutely to the circulation of the fetal or maternal sheep (0.18-0.22 mg/kg), diazepam diminished the amount of fetal breathing for 29-70 min; neither fetal nor maternal blood gases were altered at 10 and 60 min after the infusion (Piercy et al., 1977). However, the immediate effects of the infusion on fetal blood gases were not assessed. In contrast, chronic maternal administration of diazepam in the sheep resulted in an overall increase in fetal breathing activity (Worthington et al., 1978). The authors suggested that the increase may result from a "rebound" effect following periods of inhibition associated with successive administrations of the drug. This hypothesis could have been tested by comparing the effects of intermittent administration with those of a continuous slow infusion.

### Alcohol

Maternal ingestion of ethanol has also been implicated in the genesis of fetal apnea. Low levels of alcohol consumption had no effect on fetal oxygenation or pH in term human fetuses, but they produced a diminished incidence of fetal breathing (Fox et al., 1978). No evidence of fetal sleep states was obtained, but it is likely that the neural mechanisms involved in this response are similar to those mediating the effects of low levels of anesthetic agents. It is likely that fetal sleep states are affected, with abolition of the REM state.

### Cigarette Smoking

Maternal smoking has been linked with cessation of respiratory movements in the human fetus (Gennser and Marsal, 1974). The mechanisms underlying the effect of cigarette smoke on fetal breathing have been investigated by Manning and his colleagues. Direct injection of nicotine in the fetal sheep circulation has no effect on its breathing activity, whereas its maternal administration had effects similar to smoking (Manning and Feyerabend, 1976). In a subsequent study (Manning et al., 1978) it was shown that the injection of nicotine (0.14-0.25 mg/kg) into the maternal circulation caused a prompt fall in both fetal $pO_2$ and the incidence of fetal breathing movements. However, these dose levels are higher than would be obtained after smoking (Thaler et al., 1980). The effects of nicotine were blocked by pretreatment of the ewe with the $\alpha$-blocking agent phentolamine. In contrast, nicotine given directly to the fetus stimulated fetal breathing in a dose-related manner. This latter observation is in keeping with the stimulatory effects of other $\alpha$-adrenergic agonists (Boddy and Dawes, 1975). Nicotine was found to cross the placenta rapidly following maternal administration, fetal levels matching maternal levels within 5 min. It was concluded that the inhibitory effects of both maternal administration of nicotine and maternal smoking on fetal breathing are mediated by a fetal hypoxemia resulting from reduced uterine blood flow. In this case, uterine blood flow is diminished as a consequence of the sympathomimetic action of nicotine.

The effects of cigarette smoking on fetal breathing has recently been reassessed using real-time ultrasound in place of the A-scan technique employed in earlier studies (Thaler et al., 1980). The incidence of fetal breathing movement was unchanged after smoking two cigarettes, although there was a reduction in the number of maternally detected fetal body movements. The authors argued that the previously observed reduction in the incidence of fetal breathing may have resulted from erroneous classification of body movements as breathing movements. It can be argued, however,

that the reduced incidence of fetal body movements reported by Thaler et al. (1980) may have been attributable to altered maternal perception of such movements (Leader and Baillie, 1979).

### Naloxone and Opioids

Naloxone, when administered to previously apneic fetal sheep, initiated regular breathing movements for up to 30 min (Moss and Scarpelli, 1979). This opiate antagonist was administered intra-arterially at a high dose level (3 mg/kg) in partially exteriorized fetuses. A stimulatory effect of naloxone (0.8-4.0 mg/kg) on breathing in developing opossums was attributable to behavioral arousal (Farber and Maltby, 1980). As well as initiating inspiratory activity, naloxone enhanced the fetal respiratory response to maternal inhalation of $CO_2$ such that the threshold for the induction of fetal breathing was reduced and the sensitivity to $CO_2$ increased (Moss and Scarpelli, 1979). The authors anticipated the result because their previous work in adult animals had shown that $\beta$-endorphin depressed the respiratory response to $CO_2$ (Moss and Friedman, 1978). When naloxone (1 mg) was administered intra-arterially to chronically monitored fetal lambs in which fetal breathing movements were present 40-50% of the time, the respiratory responses were variable (R. Harding and E. R. Poore, unpublished observations). In no instance was a prolonged train of inspiratory efforts initiated by naloxone, nor was there any change in behavioral state. The most consistent finding was the appearance of several deep inspiratory efforts at intervals of 1-5 min in the absence of an obvious change of sleep state. This observation suggests that endogenous opioids are not involved in the genesis of slow-wave sleep and the accompanying absence of rhythmical respiratory activity in fetal sheep.

The difference between the respiratory effects of naloxone in fetal sheep which were in utero and in those which were partially exteriorized may be indicative of raised levels of endorphins in the latter. It is likely that the surgical intervention involved in fetal exteriorization would lead to an elevation of endorphin release (Wardlaw et al., 1979).

### Prostaglandins and Inhibitors of Their Synthesis

Recent evidence obtained in fetal sheep suggests that prostaglandins may play a role in the control of fetal breathing movements. It has been recognized for some time that type E prostaglandins, particularly prostaglandin $E_2$, produced apneic epidoses in newborn human infants under treatment for congenital heart disease (Olley et al., 1976). Infusions of inhibitors of prostaglandin synthesis (sodium mechlofenamate or indomethacin) into fetal arterial or venous circulation caused a marked increase in the incidence and depth of fetal breathing movements measured as tracheal pressure fluctuations (Kitterman et al., 1979). In two fetuses in which the electrocorticogram was recorded, it was found that fetal breathing occurred during high-voltage, slow-wave cortical activity, as well as during the low-voltage state. The respiratory response was not attributable to changes in blood gases or pH.

In a subsequent series of studies Kitterman et al. (1983) showed that intravascular infusions of prostaglandins $E_2$ and $F_2\alpha$ and analogs of cyclic endoperoxidases inhibited fetal breathing movements. Prostaglandin $E_2$ caused the most pronounced and consistent reduction of breathing movements, from a control value of 39.7 to 1.4%. These findings are consistent with the hypothesis that endogenous prostaglandin E, which is known

to rise in concentration in fetal blood 2-3 days before parturition (Challis et al., 1976), may be involved in the decrease in fetal breathing at this time (Boddy and Dawes, 1975).

In addition, prostaglandins may be responsible for the inhibition of fetal breathing following amniocentesis (Manning et al., 1977) and artificial rupture of the amniotic membranes prior to the onset of labor (Boylan and Lewis, 1980). It is likely that both of these procedures lead to an increase in circulating levels of prostaglandins (Mitchell et al., 1977).

### Serotonin

A recent attempt to modify sleep patterns in fetal sheep, using serotonin, has also yielded findings which may be of relevance to the regulation of fetal breathing (Quilligan et al., 1981). Infusions of a serotonin precursor (5-hydroxytryptophan, 120 mg) into fetal sheep led to the onset of slow-wave electrocortical activity which persisted for some 2 hr. This finding may have been expected, given the evidence that brainstem serotoninergic neurons may be involved in the ontogeny of cortical slow waves (Jouvet, 1967). As with inhibitors of prostaglandin synthesis, fetal breathing frequently occurred during slow-wave as well as REM sleep. The fetal $paCO_2$ rose and pH fell, possibly contributing to the more vigorous and rapid breathing movements during the infusion. It is possible that the dislocation of the relationship between central state and breathing movements is due to an effect of serotonin on centers other than those involved in the ontogeny of slow-wave cortical activity.

## FETAL BREATHING AND FETAL HEALTH

A major thrust in research into fetal breathing has been aimed at its possible use as an antenatal diagnostic tool. To this end, a large number of studies in experimental animals, predominantly sheep, and, with the advent of real-time imaging, in human pregnancy have been carried out in an attempt to identify normal and abnormal patterns of fetal breathing. In this regard, the clinician is at some disadvantage compared to the animal experimentalist, owing to the brief period of recording, usually less than 1 hr, which is at his disposal. This disadvantage has, to some extent, been reduced by the stimulatory effects of maternal glucose administration and by the development of tests designed to assess the fetal respiratory and cardiac response to acceptable challenges. It is now becoming recognized that a short observation period of fetal breathing alone is not an accurate guide to fetal health, but may become so when used in conjunction with other indices of fetal central nervous system activity.

### Animal Studies

Asphyxial insults may occur spontaneously in experimental animals or they may be invoked by such maneuvers as compression of the umbilical cord or the uterine arteries. Towell and Salvador (1974) studied the respiratory and blood gas responses to cord compression in chronic fetal goats. The asphyxia induced in these animals caused vigorous gasping for, on the average, 6.3 min, followed by a cessation of inspiratory activity. Mild asphyxia with persistent metabolic acidemia was brought about in fetal lambs by removing 14-20% of the maternal blood volume (Toubas et al., 1977). Fetal heart rate fell and arterial pressure rose. During the period of fetal

bradycardia there was no fetal breathing, although in the 24 hr after the experiment, fetal breathing was apparently normal. A similarly close relationship between fetal heart rate and breathing movements has been demonstrated by Harding et al. (1981) during 5 min of fetal hypoxemia induced by occlusion of a uterine artery. In the majority of experiments, heart rate fell by more than 10% and breathing movements ceased 1-2 min after the occlusion began. Fetal $pO_2$ fell to a mean value of 15 mmHg and there were insignificant changes in $pCO_2$ and pH. Further studies on the fetal respiratory response to acute asphyxia caused by clamping the umbilical cord for 10 min have been performed by Tchobroutsky et al. (1979). This maneuver resulted in fetal apnea with "moderate" hypercapnia, and gasping with "severe" hypercapnia. The variety of respiratory responses to acute asphyxia led to the conclusion that fetal heart rate was a more reliable index of acute asphyxia in the fetus.

Observations on the effects of chronic asphyxial and other long-term insults have largely been made in fetuses dying in utero from a variety of causes. Drawing on observations made in a large number of fetal lambs, Patrick et al. (1976) concluded that three respiratory patterns warned of imminent fetal demise. Normal patterns of breathing were absent for at least 24 hr before intrauterine death. The patterns with ominous implications were (1) continuous breathing at a rate of approximately 20 breaths per minute, (2) apnea with occasional gasping, and (3) apnea with occasional brief bursts of breathing and gasping. These patterns were not fixed and could change from one to another or revert to a normal pattern for short periods. A subsequent study from the Nuffield Institute group (Chapman et al., 1978) considered breathing patterns in additional cases of fetal lambs dying in utero. The results of these two studies have established that in fetal sheep, prolonged apnea is abnormal and suggestive of deteriorating fetal condition, and that continuous breathing may be indicative of imminent fetal death. Abnormal intermittent breathing may also indicate fetal ill health and may be associated with epileptiform seizures. It is emphasized by these authors that the presence of these breathing patterns does not necessarily indicate imminent demise and that recovery is possible.

Observations on respiratory movements in chronically monitored fetal monkeys dying in utero have been made by Manning et al. (1979a). In the uncompromised condition, judged by blood gas values, pH, and heart rate, the fetuses showed four patterns of breathing movements incorporating a mixture of rapid shallow breaths and isolated large-amplitude breaths. In this limited study, in which the fetuses died during labor, breathing movements ceased with the onset of labor and the development of a profound acidemia (mean pH 7.06). Death was preceded by a series of gasps which were distinguished from deep inspiratory efforts of the uncompromised fetus by their longer duration.

### Observations of the Human Fetus

The pioneering studies of Boddy and his colleagues using the largely superseded A-scan technique led to hopes that fetal breathing movements would prove to be a reliable indicator of fetal health (Boddy and Robinson, 1971; Boddy et al., 1973). In these early studies it was found that intrauterine fetal death was preceded by an absence of normal breathing movements and by the development of large-amplitude gasping movements. These studies showed that departure from a normal respiratory pattern may suggest a poor outcome of pregnancy. In particular, prolonged apnea and apnea with gasping were considered to be the most sinister signs (Boddy and Dawes, 1975).

Since these early studies were performed and with the widespread use of real-time ultrasound imaging a large number of publications have appeared which indicate that recognition of the high-risk fetus may be more difficult than was first realized. The principal problem is that the range of incidence of fetal breathing which may occur during a short observation period in both normal and abnormal pregnancies is so wide that prediction of the outcome for a particular fetus is hazardous.

A study of breathing movements in fetuses considered to be at high risk was performed by Trudinger et al. (1979b). Fetal compromise was indicated by a reduced amount of time spent breathing and a decreased variability in the breath-to-breath interval. When compared to biochemical tests of placental function, the predictive value of the patterns of fetal breathing movement was considered to be of greater value.

A study of 27 patients with fetuses considered to be at risk was conducted by Calvert and Richards (1979). Real-time assessment of fetal breathing was made over only 15-30 min, and the results considered along with fetal heart rate and a count of body movements. The findings confirmed those of earlier studies, showing a reduced incidence of breathing movements in fetuses at risk. There is some evidence that patterns of fetal breathing may be of value in the recognition of intrauterine growth retardation (Trudinger et al., 1979a). Fetuses which were considered to be growth retarded showed one of two patterns: (1) a reduced incidence of breathing in shorter episodes and with longer periods of apnea and (2) a significant reduction in the coefficient of variation for the breath-to-breath interval. The latter type of pattern may be analogous to the "picket fence" pattern of breathing observed in compromised fetal sheep (Patrick et al., 1976). The reduction in the variation of the breath-to-breath interval may be attributable to fetal hypercapnia (Ritchie and Lakhani, 1980a).

Over the last 5 years Manning and his colleagues have pursued the study of fetal breathing movements in the assessment of fetal condition. In earlier studies, a wide range of incidence of fetal breathing was found in abnormal pregnancies (Manning, 1977). Prolonged apnea or a substantially reduced incidence was indicative of a poor outcome. Recently, this group has improved the reliability of fetal breathing as an indicator of fetal well-being by combining it with heart rate monitoring (nonstress test). In a series of 398 observations made in 223 women it was concluded that the evaluation of fetal health is improved when more than one biophysical variable is monitored (Manning et al., 1979b). This approach has been further refined with the observation of five biophysical variables (Manning et al., 1981). As well as evaluating breathing movements, fetal body movements, fetal tone and heart rate, and qualitative amniotic fluid volume were simultaneously assessed in 1184 high-risk patients and related to the 5-min Apgar score. Combining the results of individual tests resulted in an improvement of the false-positive and false-negative frequency. The most accurate identification of the compromised fetus was obtained when the results of all five variables were taken into account.

## THE ROLE OF FETAL BREATHING IN THE DEVELOPMENT OF THE RESPIRATORY SYSTEM

Recently an increasing amount of attention is being directed toward an understanding of the "purpose" of fetal breathing and the role that it may play in the preparation of the fetus for extrauterine survival. In the absence of any firm evidence it has often been considered that prenatal use of the respiratory muscles is necessary or beneficial

for their efficient activity after birth. For example, in 1902 Ballantyne likened fetal breathing movements to thoracic gymnastics in preparation for the great extrauterine function of atmospheric respiration; however, recent studies have shown that the development of the lungs is more likely to be influenced by fetal breathing.

Denervation of the diaphragm in fetal sheep by bilateral section of the phrenic nerves has been found to result in pulmonary hypoplasia (Alcorn et al., 1980; Fewell et al., 1981). These observations lend support to the earlier findings of Wigglesworth et al. (1977), who noted impaired lung growth following destruction of the upper cervical spinal cord in fetal rabbits. In these experiments, however, it is not clear if the effect on lung growth is a consequence of the abolition of fetal breathing movements or due to the effects of diaphragmatic atrophy and the possible reduction in thoracic volume.

In a more refined series of experiments, Wigglesworth and Desai (1979) performed spinal transections at two levels in fetal rabbits. The higher section ($C_1$-$C_3$) left the phrenic nucleus and its outflow to the diaphragm intact, but interrupted descending drive from respiratory upper motor neurons; the lower section ($C_7$-$C_8$) preserved this pathway and presumably did not interfere with fetal breathing movements. High cord section, which would abolish fetal breathing movements, resulted in a significantly greater reduction in lung weight and DNA content than low cord section. The weight of the diaphragm was not affected. The conclusion was drawn that organized fetal breathing movements are essential for normal lung development. Similar observations were made in a series of experiments performed in fetal sheep by Liggins et al. (1981a). In addition to the effects of high cord section on lung growth, it was observed that lung maturation, based on pressure-volume relationships, was retarded, although the content of saturated phosphatidylcholine and phospholipids in the lung tissue was not affected. In a histological study of the lungs from phrenectomized fetal sheep, Alcorn et al. (1980) noted a structure consistent with immaturity, with the presence of both alveolar type I and type II cells lining the immature air spaces. These histological observations are thus in accordance with the physiological and biochemical findings of Liggins et al. (1981a).

In a further attempt to confirm the influence of fetal breathing movements on lung development, Liggins and his co-workers devised a means of blunting the effects of inspiratory muscle activity on pressure fluctuations within the chest (Liggins et al., 1981b). This was accomplished by replacing part of the chest wall with a silicone rubber membrane. The pressure deflections produced by fetal inspiratory efforts were smaller than those in intact fetuses. The weight and distensibility of the lungs were significantly reduced by the thoracotomy. These observations lend weight to the belief that rhythmical pressure fluctuations within the fetal chest are important to the normal development of the lung. Further experimentation may show whether the same conclusion can be applied to the development of the respiratory muscles themselves.

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# 9

# Physiological Control of the Fetal Cardiovascular System

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## INTRODUCTION

Cardiovascular function has particular significance during fetal life because delivery of oxygen and many substrates to developing fetal organs is accomplished by high blood flow rates which counteract low arterial concentrations. This chapter reviews the performance and the physiological control of the fetal heart and circulation. Growth and maturation of the cardiovascular system continues throughout development in utero and after birth, and although the progression of events in the development of the fetal circulation is similar in different species, the relative maturation at any stage of development varies considerably. Therefore gestational variations and important species differences are presented where data are available. Most of the present concepts of fetal cardiovascular regulation are based on physiological studies of chronically instrumented sheep studied in the last third of gestation, and there is a paucity of information relating to early fetal development. The relative advantages and disadvantages of chronic fetal lamb preparations have been discussed by Rudolph and Heymann (1974) and Boddy (1976). The use of the chronically catheterized fetal sheep has led to improvements in our knowledge of fetal condition and its higher level of cardiovascular performance and reflex responsiveness, and has unmasked a fetal behavioral cycle. These have brought new investigative challenges. Outstanding among these is the extraordinary pumping performance of the fetal heart, which contrasts with the structural immaturity of the myocardium. Neural regulatory mechanisms such as the unique pattern of autonomic nervous system activation during stress, the exact role of baroreceptors and chemoreceptors during fetal life, and the control of specific organ flows are poorly understood. Finally, the influence of fetal sleep and wakefulness on cardiovascular regulation promises to be an exciting and important area of investigation. The examination of what is presently known about these challenging areas of fetal cardiovascular control is a primary aim of this chapter.

## CARDIAC FUNCTION

### Development of Myocardial Structure and Function

A remarkable feature of the fetal cardiovascular system is the high level of pumping performance of the immature fetal heart. This can be illustrated by comparing average values of ventricular outputs measured in fetal, newborn, and adult life. Before birth, both the left and right ventricles contribute to fetal systemic flow in parallel, and the

Table 1 Hemodynamics in sheep

| Developmental stage | Cardiac output (ml/min per kg) | Heart rate (beats/min) | Ventricular output (ml/min per kg) | | Stroke volume (ml/kg) | |
|---|---|---|---|---|---|---|
| | | | Left | Right | Left | Right |
| Fetus (at term) | 450 | 150 | 150 | 300 | 1 | 2 |
| Newborn | 400 | 200 | 400 | 400 | 2 | 2 |
| Adult | 100 | 100 | 100 | 100 | 1 | 1 |

*Source*: Data from Heymann et al. (1973), Klopfenstein and Rudolph (1978), Peeters et al. (1979), and Berman and Musselman (1979).

sum of the two outputs (combined ventricular output) is commonly used to represent cardiac output. This convention is useful when total fetal cardiac output and its regional distribution in the body is under consideration (see p. 299); however, it is more appropriate to consider each of the fetal ventricles separately when comparing the myocardial performance of the fetus to that of the newborn and the adult, in which the ventricular outputs are arranged in series and equal in magnitude. At this time the most comprehensive data available are those from chronically instrumented sheep studied after recovery from surgery (Table 1).

In the mature fetus, combined ventricular output is about 450 ml/min per kilogram of fetal body weight. Working in parallel, the right ventricle ejects two-thirds of this (300 ml/min per kilogram), and the left ventricle ejects one-third (150 ml/min per kilogram). These outputs significantly exceed the resting cardiac output of about 100 ml/min per kilogram of body weight in the adult. Calculation of ventricular stroke volumes using a representative fetal heart rate of 150 beats/min at term yields values of 2 ml/kg for the fetal right ventricle and 1 ml/kg for the fetal left ventricle. These may be compared to 1 ml/kg for each of the adult ventricles working in series at 100 beats/min (Table 1). Thus an elevated heart rate together with an elevated stroke volume (of the right ventricle) explain the impressive pumping performance of the fetal heart in comparison with the adult heart. Recent examinations of cardiac performance in newborn lambs have revealed the extraordinary functional capacity of the heart at birth. In the first few days of life cardiac output and myocardial contractility strikingly exceed the adult values (Klopfenstein and Rudolph, 1978; Berman and Musselman, 1979). The stroke volume of the newborn is 2 ml/kg, equal to the fetal right ventricular stroke volume and twice the adult stroke volume. Because the newborn's heart rate is twice the adult level, cardiac output in the newborn is four times greater (Table 1).

The extraordinary level of performance of the perinatal heart in vivo poses many challenging questions in relation to the structural and functional mechanisms which underlie it. These are made more intriguing by studies of isolated cardiac tissue which have shown the physiological, biochemical, and ultrastructural immaturity of the myocardium at birth. The intrinsic contractile capability of mature fetal and newborn myocardium has been compared with adult tissue using isolated strips of papillary muscle or moderator bands studied in vitro (Friedman, 1972; Davies et al., 1975). These studies noted a higher resting tension and poorer contractile ability of perinatal tissue, indicated by less active tension developed under isometric conditions, and a

lesser extent and velocity of shortening when studied under isotonic conditions. These deficits could be due to a difference in the relative amount of contractile protein per unit mass of tissue, the nature and organization of the contractile protein in the sarcomere, or the structural and functional elements of the excitation-contraction coupling process itself. At this time, experimental evidence exists to support each of these explanations.

Biochemical studies have noted deficits in sarcoplasmic reticulum from fetal heart muscle, which accumulates calcium at a comparatively slow rate relative to adult hearts of the same species (Nayler and Fassold, 1977). From a functional standpoint, this could contribute to the lower active tension and higher resting tension of fetal myocardium, because sarcoplasmic reticulum modulates the tension developed during contraction and the resting tension by regulating the intracellular uptake and release of calcium.

Using an electron microscope, the components of the myocardium may be quantified as contractile (myofilaments) and noncontractile (nucleus, mitochondria, glycogen, and membranes). In many animal species the ratio of contractile to noncontractile tissue is significantly less in the mature fetus and newborn than in the adult (Friedman, 1972; Sheridan et al., 1979; Olivetti et al., 1980). When the relative mechanical performance of fetal and adult tissue is corrected numerically using the ratio of contractile to noncontractile tissue, the fetal and adult data tend to converge (Friedman, 1972), and with advancing age after birth improvement in tension development is parallelled by growth in the relative proportion of contractile proteins in the myocyte (Sheridan et al., 1979). These studies support the idea that the force-generating properties of the fetal sarcomere and the adult sarcomere are similar, and that the limited mechanical performance of fetal myocardium reflects a deficit in the total contractile tissue mass. However, in addition, other ultrastructural features of the fetal myocardium suggest "disorder" of the contractile apparatus. In contrast to the long, orderly rows of sarcomeres seen in adult tissue, myofibrils in developing myocardium are irregular in their orientation and the characteristic banded appearance of the mature myofibril is not evident early in gestation and is not complete at birth (Sheldon et al., 1976; Sheridan et al., 1979).

More study is required to understand the apparent paradox posed by the ultrastructural appearance of the myocardium on the one hand, and the impressive performance of the intact fetal heart on the other. Possibly, classic concepts of muscle contraction based on the banded appearance of adult tissue may not be appropriate for developing myocardium.

### Circulatory Factors Influencing Cardiac Output

The well-known graphical analysis of cardiovascular function initiated by Guyton (1955) specifies the interaction of cardiac and circulatory factors in determining cardiac output in the adult circulation. Recently this form of analysis has been applied to the fetal circulation (Gilbert, 1980), offering a conceptual basis for understanding the performance of the fetal cardiovascular system. In the basal state the fetus maintains a high venous return, and therefore cardiac output, via a high level of mean systemic pressure (driving pressure for venous return), which is twice the level recorded in the adult circulation. A slightly lower resistance to venous return contributes as well. Mean systemic pressure is elevated if vascular compliance is low and blood volume is increased; both these conditions pertain in the fetus.

This model can also help in understanding the limited capacity of the fetal heart to increase its output above the high basal level (Rudolph and Heymann, 1974). Increments in fetal blood volume produced by infusion of blood cause sharp elevations in right atrial pressure, but do not cause cardiac output to increase according to the Frank-Starling relationship (Gilbert, 1980). Decrements in fetal blood volume, on the other hand, cause both right atrial pressure and cardiac output to decline. These data support the suggestion that the fetal heart is operating near the upper limit of the Frank-Starling curve. They might also explain why the Frank-Starling mechanism, which operates in the primitive chick embryo heart (Faber et al., 1974), is not consistently found in the lamb fetus, leading to debate over its functional significance during fetal life (Kirkpatrick et al., 1976).

Following increments in fetal blood volume, mean systemic pressure is higher and resistance to venous return is lower (Gilbert, 1980). These circulatory conditions favor an increase in venous return (and cardiac output), but no significant increase results. Thus this analysis identifies cardiac factors, rather than vascular factors, as the cause of the limited capacity to increase total flow in the fetal circulation.

### Right Ventricle-Left Ventricle Interactions

The series arrangement of the right and left ventricles in adult life demands that inequalities of ventricular output cannot exist, beyond a few beats. Because the two ventricles of the fetus operate in parallel, there is a potential for long-term changes in the output of each one and in the balance of the two. This could be important, because the oxygen content of the blood ejected by each ventricle and its arterial distribution are different (see p. 299).

Electrical stimulation of the atria emphasizes how delicate the balance is between fetal ventricular outputs (Pitlick et al., 1976). With left or right atrial pacing, total cardiac output is not altered. However, changes in the individual outputs occur; for both left and right ventricles, pacing of the ipsilateral atrium results in a lower output than pacing of the contralateral atrium. This effect of atrial pacing is explained by small changes (<3 Torr) in the pressure difference across the foramen ovale. Left atrial stimulation, for example, increases left atrial pressure, resulting in augmented right ventricular filling and right ventricular output. Natural variations in atrial pressure might have functional significance in relation to the division of venous return between ventricles, because large unexplained variations are found in normal fetuses (Edelstone and Rudolph, 1979).

In adult life, cardiac geometry is such that the ventricles do not function independently, but interact throughout the cardiac cycle. The mechanisms of ventricular interference are best studied by breaking the normal series arrangement, allowing the output of each to be studied independently (Elzinga et al., 1980). These experimental studies of adult hearts have a circulatory similarity with the normal fetal pattern of parallel ventricular function. In diastole, the stiffness of each ventricle increases with the filling of the other side. Functionally this results in a smaller end-diastolic volume and a reduced ventricular output, in accord with the Frank-Starling mechanism. During systole, contraction of the left side of the heart enhances right ventricular ejection. Left ventricular behavior is more independent, probably because of the large pressure difference between adult ventricles.

Pressure interactions in perinatal hearts have been examined in passive nonbeating hearts of piglets (Versprille et al., 1978). At birth, mutual interference can be

demonstrated; increasing end-diastolic pressure of each ventricle causes a proportional increase in end-diastolic pressure of the other in the ratio of 0.6. This interference is age dependent; as the right ventricular geometry assumes the adult form during postnatal growth, the influence of the left ventricle on the right ventricle diminishes. These mechanisms might provide a further mode of interaction between the left and right ventricles in fetal life, in addition to changing pressure differences across the foramen ovale.

## RECEPTOR MECHANISMS

### Baroreceptor Control of the Circulation

In adult life, arterial baroreceptors are the major sensing elements of the cardiovascular regulatory system. During development there is considerable variation among species in the age at which baroreceptor reflex responses are functional, and the exact role of baroreceptors during fetal life has not been established. At birth, species differences correspond roughly to the general maturity of the animal. For example, cardiac slowing in response to elevation in arterial pressure is less well developed in the maternally dependent newborn puppy than in the more active and independent newborn lamb (Vatner and Manders, 1979). Nevertheless, the majority of species, including man, exhibit a depressed sensitivity of baroreceptor reflexes at birth and a progressive postnatal maturation to adult levels (Gootman et al., 1979; Vatner and Manders, 1979; Dawes et al., 1980).

During fetal life, functional baroreceptors have been demonstrated by measurements of phasic electrical activity in baroreceptor afferent nerves. This activity is synchronous with the arterial pulse (Ponte and Purves, 1973). At 0.6 gestation in the fetal lamb, elevations and small reductions of arterial pressure cause the heart rate to change in the opposite direction (Macdonald et al., 1980), signifying that a functional baroreflex control of the heart rate exists at this early state of development. However, the factors which govern fetal baroreceptor reflexes are poorly understood, and it is not certain that baroreceptor control of heart rate operates in the normal fetus in utero. When arterial pressure is raised in the fetal lamb, the degree of cardiac slowing is widely variable from trial to trial in the same animal on the same day (Maloney et al., 1977) and frequently heart rate does not change unless the arterial pressure is raised significantly above the normal range. This variability is not explained by changes in the sleep state of the fetus, the presence or absence of breathing movements or limb movements, or changes of blood gases (Dawes et al., 1980). With advancing gestation baroreflex control of the heart rate matures, as evidenced by an increasing proportion of positive responses to brief elevations of arterial pressure, and by an increasing sensitivity of these responses quantified by relating the degree of heart rate slowing to the extent of blood pressure elevation (Shinebourne et al., 1972). Other studies have not been able to show gestational changes of sensitivity, and this question is unresolved (Maloney et al., 1977; Dawes et al., 1980).

Mechanoreceptors in the atria, the ventricles, and the pulmonary artery are potential sites of cardiovascular reflexes which have not been systematically investigated in the fetus. Atrial or ventricular receptors causing reflex bradycardia may be implicated in the fetal response to lowered arterial pressure (Oberg, 1976). When fetal blood pressure is reduced by hemorrhage or by venous occlusion, tachycardia

only occurs when the pressure change is small (Wood et al., 1979; Macdonald et al., 1980). With larger pressure falls, the transient increase in heart rate is reversed and bradycardia occurs. Following atropine administration the bradycardia is abolished, and the classical inverse relationship between heart rate and the blood pressure of the adult circulation is unmasked. These studies show that in fetal hypotension, sympathetic acceleration of the heart is counteracted by increased vagal tone, and they emphasize that failure to demonstrate an adult form of response in the fetus cannot be interpreted to mean that receptor function is absent.

Baroreceptor activation can be expressed as changes in venous and arterial tone and cardiac contractility, in addition to changes in heart rate. The sensitivity of the baroreceptor-heart rate reflex does not necessarily reflect the capacity of the arterial baroreceptors to control blood pressure. For example, in adult life, dissociation between the control of heart rate and the control of blood pressure occurs in exercise and hypertensive states (Ludbrook et al., 1980). Whether fetal baroreflexes play an important role in regulating fetal blood pressure can be assessed by examining blood pressure variability after arterial baroreceptor denervation (Yardley et al., 1979). Surgical denervation of the carotid sinus, carotid artery, and aortic arch baroreceptor regions in fetal lambs results in effective denervation of the arterial baroreceptors and strikingly increases the natural variability of arterial pressure. Coefficients of variation of mean arterial pressure measured over 24-hr periods (standard deviation of the mean arterial pressure over the mean value of the mean arterial pressure) are two times greater in barodenervated fetuses as compared to intact fetuses. This response to denervation in the lamb fetus is surprisingly similar to the effect of barodenervation in active unanesthetized adult animals (Cowley et al., 1973) and supports the suggestion that arterial baroreceptors have a natural role in regulating fetal arterial pressure.

### Chemoreceptor Activity

Aortic chemoreceptors have been shown to be active in the mature sheep fetus by recordings of afferent electrical activity in the vagus nerve (Ponte and Purves, 1973). These receptors respond to asphyxia and chemical stimulation in a way which is similar to adult receptors, and they are presumed to be active in fetal life. Dawes and his associates (Dawes et al., 1969) have identified a primary role for these receptors in cardiovascular regulation in anesthetized fetal lambs. Because the aortic chemoreflex is activated by small falls of arterial oxygen tension from above-normal levels, it has been proposed as the first line of defense against arterial hypoxemia in fetal lambs. Fetal carotid body chemoreceptors, in contrast, are virtually inactive in anesthetized fetal lambs and only marginally responsive to physiological and pharmacological stimuli (Biscoe et al., 1969; Dawes et al., 1969).

These studies in anesthetized lambs support the long-held view that aortic chemoreceptors are active and play an important role in fetal cardiovascular regulation, whereas carotid chemoreceptors are inactive. Historically, the absence of fetal breathing movements in the anesthetized fetus and the failure to stimulate fetal respiratory activity by hypoxemia and carotid body stimulation has supported this view (Purves, 1981). However, recent studies of cardiovascular regulation and fetal breathing movements in unanesthetized fetal lambs in utero have provided new information which questions the idea of carotid body inactivity. Fetal breathing is stimulated by hypoxemia when suprapontine structures, which are presumed to inhibit fetal respiration, are absent (Maloney et al., 1980). The unanesthetized fetus responds to

moderate hypoxemia with bradycardia, and not with tachycardia as seen in studies of anesthetized animals (Walker et al., 1979). This response is due to vagal activation and should be distinguished from the bradycardia which results from profound hypoxemia of less than 10 Torr, which is not prevented by parasympathetic blockade (Lewis et al., 1980). Because fetal arterial pressure usually increases, the bradycardia has regularly been explained as a baroreflex response. However, recent observations support the idea that hypoxemic bradycardia is not due to baroreceptor stimulation. Firstly, arterial pressure does not increase in all hypoxemic fetuses, and the extent by which heart rate slows in different animals is the same whether or not arterial pressure rises (Walker et al., 1979). Secondly, brief episodes of fetal hypoxemia produced by occluding the uterine artery in sheep do not cause arterial pressure to increase, but the heart rate slows (Parer et al., 1980). Finally, pretreatment with alpha-adrenergic blocking agents prevents the hypoxemia-induced hypertension without affecting the fall in heart rate (Lewis et al., 1980). Chemoreceptor stimulation rather than baroreceptor stimulation might be the primary cause of the increased vagal activity and bradycardia which accompanies fetal hypoxemia. If so, what is the source of afferent activity? In adult animals the primary effect of carotid body stimulation is to slow the heart via activation of the medullary cardioinhibitory center and vagal outflow (De Burgh Daly, 1972). Aortic chemoreceptor stimulation, by contrast, accelerates the heart (Sleight, 1974). Thus carotid body sensitivity in the fetus in utero deserves durther examination.

## AUTONOMIC NERVOUS SYSTEM

### Natural Development of Autonomic Control of the Heart

Autonomic nervous control of the heart begins during fetal life in many animal species and in man. Ontogenetic development of the autonomic innervation of the heart has been studied in detail in the rapidly developing chick embryo (Pappano, 1977), and this provides a basis for understanding the sequence of development in the mammalian fetus. During ontogenesis the postsynaptic components of the neuroeffector process (the transmitter receptor and the target organ effector mechanism) appear before the presynaptic component (the efferent autonomic nerve). Thus responsiveness of the heart to neurotransmitters develops long before effective innervation is found. It should also be noted that the potential for autonomic control is marked by the development of effective neuroeffector transmission, and not by the anatomic appearance of neurons within the heart, which occurs much earlier in ontogeny. These principles are illustrated by the chronological sequence of parasympathetic innervation of the chick embryo heart. This begins with the appearance of the receptor for acetylcholine and the inactivating enzyme acetylcholinesterase (day 2); growth of cholinergic neurons within the sinoatrial node (day 6); the first detectable release of acetylcholine (day 10); and functional vagal neurotransmission and the appearance of the acetylcholine synthetic enzyme, choline acetyltransferase (day 12). (Hatching of the chick occurs on day 21.)

The sympathetic cardiac innervation in the chick embryo follows the same developmental sequence as the parasympathetic innervation (Pappano, 1977). Receptor responsitivity is established simultaneously, but the appearance of sympathetic nerves and the onset of effective neurotransmission are delayed in the sympathetic system in comparison with the parasympathetic system.

Available data show that a similar chronological sequence of autonomic development probably occurs in man and other mammals, although there are very marked relative differences between species (Pappano, 1977). Electrical stimulation of isolated human fetal atria can release acetylcholine and depress contractility from 13 weeks of gestation, but as the parasympathetic ganglia are functionally immature, it is unlikely that vagal transmission occurs at this age (Walker, 1975). Stimulation of the cervical vagus nerve produced a small inhibition of the fetal lamb heart as early as 0.4 gestation, the earliest age studied (Dawes, 1968). Effective parasympathetic neurotransmission is developed during fetal life in the guinea pig and the rabbit (Vlk and Vincenzi, 1977), but in the rat this is not achieved until after birth (Vlk, 1979; Marvin et al., 1980). Whether developmental changes occur in receptor sensitivity to acetylcholine after the onset of effective neurotransmission is of interest because of the apparent gestational changes in "vagal tone" in intact animals and in man (Schifferli and Caldeyro-Barcia, 1973; Walker et al., 1978).

Because cholinergic neurotransmission develops before adrenergic innervation, a potential for autonomic nervous imbalance exists during development in mammals (Pappano, 1977; Vlk and Vincenzi, 1977). However, as cholinergic and beta-adrenergic receptors probably appear at about the same time in ontogenesis, other sources of catecholamines could protect the developing fetus from unopposed vagal activity. In the fetal lamb beta-adrenergic agonists cause acceleration of the heart from at least as early as 0.4 gestation (Nuwayhid et al., 1975), prior to the growth of myocardial sympathetic innervation, which begins at approximately 0.6 gestation (Lebowitz et al., 1972).

Concentrations of norepinephrine can be used as an index of the magnitude of sympathetic innervation in developing myocardium because the heart stores of norepinephrine are located predominantly within sympathetic nerves (Friedman, 1972). Assessed in this way, myocardial innervation is incomplete at birth in many laboratory species because concentrations in the fetus are much less than in the adult. Significant growth of cardiac sympathetic innervation continues after birth in many animal species and it would be unwise to assume that the human fetal heart is fully innervated at birth. Comparative studies show that adrenergic innervation is better developed in newborn animals that are relatively more independent at birth (Pappano, 1977). The effectiveness of the immature sympathetic nervous system at birth may be enhanced by supersensitivity of the target organ due to suppressed uptake of the neurotransmitter and by high circulating catecholamine levels (Friedman, 1972; Geis et al., 1975; Tynan et al., 1977).

Histochemical, biochemical, and pharmacological studies of developing cardiac tissue indicate when the potential for autonomic control has developed. However, study of the fetus in utero is required to understand the natural development of autonomic control. The gestational development of basal autonomic tone is readily understood using the concept of the *intrinsic heart rate,* the natural frequency of discharge of the pacemaker cells in the sinoatrial node. The progressive natural reduction of fetal heart rate throughout gestation can be ascribed to two significantly different processes (Figure 1). In early gestation the natural decline in heart rate reflects a reduction in the intrinsic rate (phase 1). Isolated fetal atria show an age-dependent decrease in their rate of beating prior to 15 weeks of gestation (Walker, 1974), and as the isolated fetal heart does not respond to ganglion-stimulating drugs at this time, it is unlikely that the fetal heart is subject to tonic vagal inhibition (Walker, 1975). A second developmental

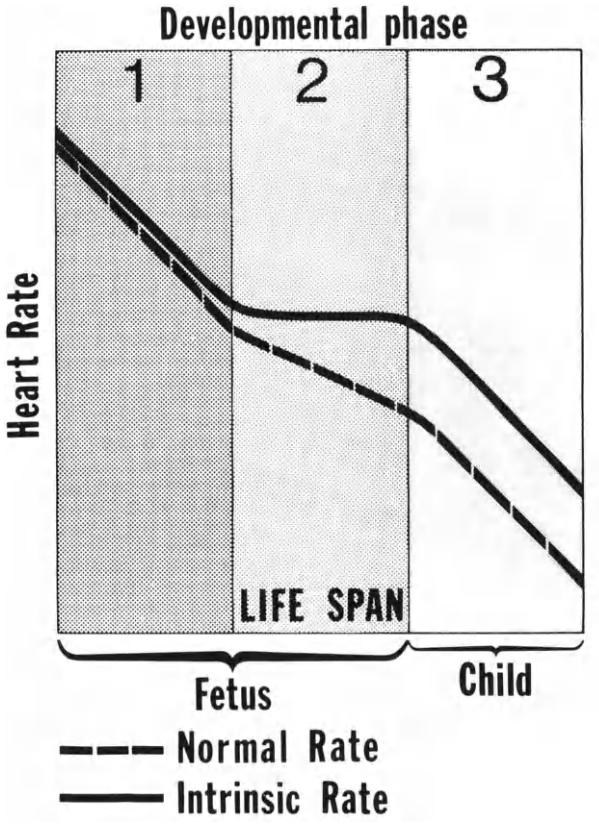


**Figure 1** Phases in the development of heart rate during fetal and postnatal life. The normal heart rate is the actual rate of beating when the heart is subject to autonomic nervous influences. The intrinsic heart rate represents the natural firing rate of pacemaker cells isolated from the autonomic nervous system.

period is characterized by growth of nervous influence on the heart. In this period (Figure 1, phase 2) the reduction of the normal heart rate can be ascribed to growth in vagal inhibition which predominates over a smaller tonic sympathetic stimulation (Vapaavouri et al., 1973; Walker et al., 1978). Intrinsic heart rate measured after administration of both atropine and propranolol is constant during developmental phase 2. In the human fetus this phase is from 15 weeks to term (Schifferli and Caldeyro-Barcia, 1973); in the fetal lamb, it is from 0.7 gestation to term (Walker et al., 1978).

Nonneurogenic changes become important in determining the general pattern of heart rate development once more after birth. The intrinsic heart rate falls from birth throughout early childhood in man (Cumming and Mir, 1970) and in neonatal life in lambs (Woods et al., 1977), with predominant vagal inhibition lowering the heart rate below the intrinsic rate. Detailed longitudinal studies have identified progressive slowing after a peak rate at approximately 1 month of age (Harper et al., 1976). Transient increases of newborn heart rate above fetal values which occur in the first hour after birth in the human fetus (Bustos et al., 1975) and during the first few days of life in lambs (Walker et al., 1978; Woods et al., 1977) are not shown in Figure 1.

### Vascular Responses

The functional vascular neuroeffector system is comprised of the postganglionic neuron, the synapse, and the effector smooth muscle cell. Study of isolated vessels in vitro offers the advantage of tracing the development of these components in detail from early gestation. Data from fetal lambs show that the development of functional postganglionic adrenergic innervation of vascular smooth muscle begins late in gestation and continues well after birth, and it is preceded by the development of vascular mechanisms involved in the response to vasoconstrictor agents.

Development of mechanisms associated with vascular adrenergic neurotransmission has been studied in detail using isolated carotid artery of fetal lambs between 0.4 gestation and term (Su et al., 1977a). Neuronal uptake (uptake I) and extraneuronal uptake (uptake II) of catecholamines were developed in the youngest fetuses and the enzyme systems for metabolic degradation of catecholamines, monoamine oxidase, and catechol-O-methyltransferase, were equally present in younger and older fetuses. Contractile responses to the sympathetic neurotransmitter noradrenaline developed later in gestation. Finally, contractile responses to selective electrical stimulation of the postganglionic sympathetic neuron were found. Therefore in the carotid artery, as in the heart, the mechanisms for adrenergic transmitter inactivation, transmitter action, and neuronal release of transmitter develop in sequence.

Contractile force developed by isolated fetal lamb ear artery in vitro is poorly developed prior to 0.6 gestation and then increases with age to reach a gestational maximum at approximately 0.9 gestation (Wyse et al., 1977). Significant development continues after birth, as the maximum responses to noradrenaline in the late-gestation fetus and newborn are only 20% of the adult vessel response. Responses to electrical stimulation also increase after birth, although lagging behind the development of the response to noradrenaline. Developmental increases in vessel responsiveness could be due to increasing sensitivity of the alpha-receptor, to maturation of the intrinsic contractile process, or to the structure of the vessel wall. As the alpha-receptor mechanism is complete prior to 0.6 gestation, development of the contractile process itself is the most probable cause of increasing responsitivity (Van Petten et al., 1978). Regional variations of blood vessel responsiveness exist which caution against the generalization of these concepts to all fetal vessels (Su et al., 1977b). Responses to noradrenaline increase rapidly late in gestation in the more peripheral, more muscular arteries such as the renal and carotid arteries, whereas the large elastic intrathoracic arteries, aorta, ductus arteriosus, and pulmonary artery maintain constant, and smaller, responses from 0.4 gestation to term.

In vivo studies of the blood pressure responses to injections of alpha-adrenergic agents have produced conflicting results. For example, it has been suggested that the fetal lamb is less responsive to catecholamines than the adult (Assali et al., 1978; Van Petten et al., 1978), but this has been disputed (Jones and Ritchie, 1978). Responsiveness in the whole circulation precedes responsiveness of the individual vessels which have been studied so far, possibly reflecting the diversity in regional vessels, and the duration of the pressor response is prolonged in the immature fetus despite the maturity of the catecholamine uptake and inactivation mechanisms indicated by in vitro studies (Harris and Van Petten, 1979). The maximum blood pressure increase achieved with noradrenaline injections increases with age in the fetal lamb from 0.5

gestation, indicating increasing responsitivity (Assali et al., 1978). The dosage required for half-maximal response remains constant, indicating that maturation of the post-synaptic elements of the neuroeffector mechanism contribute to the increasing responses, rather than increasing sensitivity of the alpha-receptor itself.

A measure of the *natural* control of the vasculature in vivo can be obtained by blockade of the prevailing level of autonomic tone with alpha-adrenergic blocking agents. Responses to the blockade of one element of the autonomic control system are difficult to interpret exactly because they must reflect in part the corrective response or lack of response in the other elements. Alpha-adrenergic blockade in chronic fetal lamb preparations results in a small decrease in the blood pressure of young fetuses studied between 0.45 and 0.7 gestation, but in a much greater fall in the last third of gestation (Rudolph and Heymann, 1974; Assali et al., 1978). These studies suggest that the fetal circulation is under sympathetic control from early gestation and that this increases as pregnancy approaches term, in parallel with the development of the peripheral neuroeffector mechanism.

### Circulating Catecholamines

The possibility that circulating catecholamines serve a regulatory function in the fetal cardiovascular system prior to the development of mature sympathetic innervation is supported by the early gestational development of adrenergic receptors and the supersensitivity of the fetal myocardium to catecholamines (Friedman, 1972). Unfortunately most knowledge of the origins and actions of circulating catecholamines in the fetus pertains to the last third of gestation in the fetal lamb (Jones, 1980), when sympathetic innervation of the heart and circulation is developing rapidly.

Plasma catecholamine concentrations in various animal fetuses increase over the last quarter of gestation, with adrenaline concentration showing the sharpest rise, reflecting the increasing adrenaline-noradrenaline concentration ratio in the fetal adrenal gland (Comline and Silver, 1966; Jones 1980). Anesthesia, gentle handling, and restraint stress of experimental animals causes large increases of catecholamines, especially of adrenaline (Buhler et al., 1978). In the unanesthetized lamb fetus basal values are similar to those found in adult animals. From 0.7 gestation there is a gradual rise which becomes rapid 2-3 days prior to delivery. Umbilical arterial catecholamine levels exceed umbilical venous levels in the basal state and during hypoxemia, reflecting the major role of the placenta in the clearance of fetal plasma catecholamines (Jones, 1980).

The cardiovascular actions of circulating catecholamines have been studied many times in the fetus, but they have not been properly defined. It is clear that noradrenalin, adrenaline, and dopamine cause vasoconstriction and can cause elevations of fetal blood pressure when injected intravenously. However, reflex bradycardia sometimes modifies the magnitude of the pressor response, particularly to adrenaline (Harris and Van Petten, 1979). Slow infusions of catecholamines, in contrast to injections, produce different cardiovascular actions. These studies are relevant to conditions such as hypoxia (Jones and Robinson, 1975), labor (Lagercrantz and Bistoletti, 1977), and maternal hemorrhage (Artal, 1980), in which high fetal levels of catecholamines are maintained. Infusion of either adrenaline or noradrenalin to produce plasma concentrations comparable to those seen in hypoxia cause an increase in blood pressure and variable changes in heart rate (Jones and Ritchie, 1978).

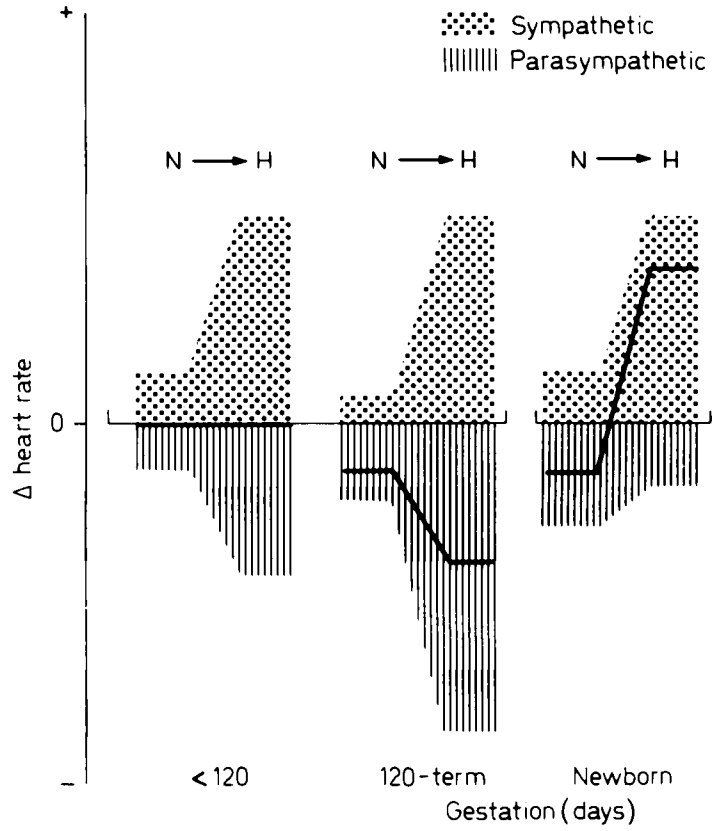


**Figure 2** Diagramatic representation of autonomic nervous influences on the heart rate of normoxemic (N) and hypoxemic (H) fetal and newborn lambs. The height and depth of shaded areas represent the antagonistic sympathetic and parasympathetic chronotropic influences. The zero line represents the intrinsic rate of the heart when isolated from autonomic nervous influence. The heavy line represents the actual heart rate in relation to the intrinsic rate. Prior to 120 days of gestation, increased sympathetic stimulation during hypoxemia is matched by an increasing parasympathetic inhibition; the heart rate is not changed. Later in gestation, sympathetic influence increases as before, but a larger increment in parasympathetic inhibition is reflected in a net bradycardia. In the newborn, augmentation of sympathetic influence plus a small contribution from parasympathetic withdrawal results in pronounced tachycardia. (From Walker et al., 1979.)

### Autonomic Activation During Stress

Cardiovascular responses of the fetal lamb to hypoxemia vary with the age of the lamb (Walker et al., 1979). Between 0.6 and 0.8 gestation heart rate and blood pressure are not significantly changed during hypoxemia, but in older fetuses bradycardia and hypertension occur. The increasing pressor response in the older fetuses parallels the increasing reactivity of the vascular neuroeffector mechanism (Assali et al., 1978; Van Petten et al., 1978). Plasma catecholamines are elevated to similar levels in younger as in older fetuses, although there are substantial variations in the responses of individual fetuses which could obscure a gestational trend (Jones and Robinson, 1975).

Absence of fetal heart rate changes during hypoxemia does not indicate failure of autonomic nervous system activation. Selective pharmacological blockade of the opposing vagal and sympathetic divisions of the autonomic nervous system "unmasks" considerable activation during hypoxemia (Walker et al., 1979). Prior to 0.8 gestation increased sympathetic stimulation during hypoxemia is matched by an increased vagal inhibition and heart rate is not changed, but later in gestation a larger increment in vagal inhibition causes bradycardia (Figure 2).

Opposing, augmented activity of the sympathetic and parasympathetic influences on the heart is also found in acute fetal hemorrhage (Macdonald et al., 1980) and in the chronically growth-retarded and hypoxemic fetal lamb (Llanos et al., 1980), in which plasma catecholamines are elevated (Jones, 1980). This pattern is not seen after birth, and the possible benefits for the fetus have not been elucidated.

## DISTRIBUTION OF CARDIAC OUTPUT

### Arterial Distribution

In the fetal circulation the right and left ventricles contribute to arterial blood flow in parallel, and many organs and the placenta receive blood flow from both ventricles. As a consequence it is convenient, when considering the delivery of oxygen and nutrients, to consider cardiac output in the fetus as the sum of the two ventricular outputs, the combined ventricular output. The measurements which best indicate values in the normal fetus are those available from the study of chronically catheterized fetal lambs after recovery from surgery. Data obtained for mature fetuses from 0.8 gestation to term from different laboratories are very similar (Cohn et al., 1974; Longo et al., 1978; Peeters et al., 1979). Combined ventricular output averages 464 ml/min per kilogram of body weight. Average percentages of combined ventricular output distributed to the various organs are the following: placenta, 40%; lungs, 9%; gastrointestinal tract, 5%; brain, 4%; myocardium, 3%; kidneys, 3%; spleen, 1%; liver, 0.3%; and adrenal glands, 0.1%. The balance of the flow (35%) is distributed to the fetal "carcass," principally the bones, skin, and skeletal muscle.

Measurements of cardiac output and its arterial distribution in relation to gestational age have been performed in acute studies of unanesthetized fetal lambs between 0.4 gestation and term (Rudolph and Heymann, 1970). The combined ventricular output of mature fetuses obtained in these circumstances (approximately 550 ml/min per kilogram) is higher than the chronically catheterized fetus, but the percentage distribution of blood flow is similar. With advancing gestation, cardiac output increases in proportion to fetal weight; that is, cardiac output per kilogram of body weight is unchanged from 0.4 gestation to term. The proportion of cardiac output distributed to the placenta (umbilical blood flow) decreases from 50% in the youngest fetuses to 40% just before term, reflecting a redistribution of cardiac output beteen the body and the placenta. Increasing flows to the lungs, gut, and brain can explain the proportional decrease in umbilical flow. No significant changes in the percentage of cardiac output or flow in relation to organ weight occurred in the kidney, heart, or skin and muscular tissues.

The distribution of cardiac output measured in studies of primates (Behrman et al., 1970; Paton et al., 1973) and previable man (Rudolph et al., 1971) cannot be compared easily with the lamb studies because of the general anesthesia used and the experimental difficulty arising from the progressive deterioration of the preparations. However, the

proportional distribution of cardiac output to fetal organs is similar to that seen in the lamb, with the exception of the brain. Brain flow represents approximately 15% of the cardiac output in the primate and human fetus, compared with 4% in the lamb. This is not wholly explained by the relatively greater development of the brain in the primate and human, as the brain blood flow per unit weight of tissue is highest in the sheep (Paton et al., 1973).

### Venous Return

The pattern of blood flow in the great thoracic veins and heart has been detailed in the fetal lamb in utero using the radioactive microsphere technique (Rudolph and Heymann, 1974). Inferior vena caval return represented 69% of the combined ventricular output, and the superior vena caval return 21%; the flow from the lungs, 7%, and heart, 3%, together made up the remaining 10%. Superior vena caval flow is returned to the right ventricle; only minimal amounts normally cross the foramen ovale to the left ventricle, although this may increase in fetal asphyxia (Dawes, 1968). In contrast, inferior vena caval flow is divided between the right and left ventricles; approximatley 40% of inferior vena caval flow passes through the foramen ovale into the left atrium. Blood flow shunted across the foramen ovale is influenced by changes in pulmonary vascular resistance; as pulmonary blood flow increases, the proportion of inferior vena caval return entering the left atrium falls (Rudolph, 1977). Superior vena caval return represents a greater proportion of venous return in the monkey and baboon fetus than in the lamb fetus (Paton et al., 1973), whereas in the previable human fetus the proportions are similar to values obtained in the sheep fetus (Rudolph et al., 1971).

Recently it has been appreciated that blood in the thoracic inferior vena cava is not homogeneous in its composition and ultimate arterial distribution (Edelstone and Rudolph, 1979). One-third of the thoracic vena caval flow is derived from umbilical venous flow crossing the ductus venosus, and two-thirds from abdominal inferior vena caval flow. These bloodstreams remain unmixed in the thoracic inferior cava and in the fetal lamb obvious streams of well-oxygenated blood derived from the umbilical venous circulation and poorly oxygenated blood from the lower body are visible through the thin-walled vena cava. Streaming of umbilical venous blood in the thoracic inferior vena cava and across the foramen ovale results in preferential distribution of oxygenated blood to the heart and brain during normal oxygenation and fetal hypoxemia (Reuss and Rudolph, 1980). Preferential streaming of ductus venosus blood has also been noted in the fetal monkey (Paton et al., 1973) and is extremely variable in magnitude, possibly resulting in wide variations in the oxygen content of blood supplying the heart and brain (Edelstone and Rudolph, 1979).

### Redistribution During Hypoxemia

Hypoxemia is believed to be the most likely acute stress faced by the fetus and it has been well studied. In the mature fetus, the general cardiovascular changes associated with mild hypoxemia (decrease in descending aortic blood $pO_2$ from the normal of 21 Torr to 12 Torr) are increased arterial pressure, bradycardia, no change in cardiac output, and no change in umbilical blood flow. With severe hypoxemia, particularly in association with metabolic acidemia, fetal cardiac output falls but umbilical flow remains constant (Cohn et al., 1974). Redistribution of cardiac output from the body of the fetus (principally muscles, skin, and skeleton) to the placenta maintains umbilical flow constant in these circumstances.

Peeters et al. (1979) have examined blood flow to fetal organs in detail over a range of fetal arterial oxygen content of 6-1 mM (36-10 Torr). Arterial oxygen content is different in fetal arterial vessels (Peeters, 1978), being greater in the ascending aorta than in the descending aorta, where it is greater than in the right ventricle. Consequently, these authors related specific organ flows to oxygen content in the supplying artery and to oxygen delivery, the product of blood flow and oxygen content. Blood flows to neural tissues (cerebrum, cerebellum, and brainstem), the myocardium, and adrenal glands increase progressively in inverse relation to arterial oxygen content, and oxygen delivery to these tissues remains constant. Blood flow to the lungs decreases progressively with hypoxemia, whereas blood flow to the kidneys, digestive tract, and carcass (bone, skin, and skeletal muscle) decrease abruptly in severe hypoxemia.

Because umbilical venous return is the source of oxygen in fetal life, the distribution of this flow has important consequences for oxygen delivery in normoxemic and hypoxemic states (Reuss and Rudolph, 1980). Factors affecting umbilical venous distribution are complex and extreme variations are found which are unexplained (Edelstone and Rudolph, 1979). However, during acute hypoxemia, the percentage of umbilical venous blood delivered to the myocardium and the brain of the fetal lamb increases. This mechanism together with the redistribution of cardiac output represent circulatory changes which protect these organs from decreased oxygen supply, despite a reduction in the total oxygen available from umbilical venous blood.

## REGIONAL CIRCULATIONS

### Coronary Circulation

The fetal coronary circulation illustrates the important role of high blood flow rates in the fetus, which counteract low arterial concentrations of oxygen in the normal state and during diminished oxygen availability.

In the adult heart about 75% of the oxygen contained in arterial blood is normally extracted during a single transit and changes in coronary flow are the primary mechanisms by which oxygen demand and supply are balanced. Because oxygen levels are significantly lower in fetuses than in adults, control of the fetal coronary circulation is of particular interest. Despite lower oxygen concentrations in ascending aortic blood, myocardial oxygen delivery (blood flow times arterial oxygen concentration) in the fetal lamb exceeds that in the adult sheep (Peeters, 1978), and myocardial oxygen consumption (blood flow times arteriovenous oxygen difference) is similar in the fetal lamb and in the adult sheep (Fisher et al., 1980). This is accomplished by significantly greater myocardial blood flow in the fetus, which is approximately twice the adult flow per 100 g of myocardium (Fisher et al., 1980).

Myocardial blood flow is linearly related to the reciprocal of arterial oxygen concentration, permitting the fetus to maintain a constant oxygen delivery and oxygen consumption during normal oxygenation and hypoxemia (Peeters, 1978; Rudolph et al., 1981). When fetal oxygen tension is reduced from the normal level of about 21 Torr to 12 Torr, coronary flow is increased two- to threefold (Cohn et al., 1974).

Coronary blood flow can increase four- to fivefold ("coronary reserve") in the adult heart (Klocke and Ellis, 1980), so that the basal level of fetal flow, though greater than in the adult, is within these limits. Fetal coronary reserve and factors regulating the coronary circulation before birth are largely unexplored. These may be particularly important during development, because the high rates of myocardial

flow and substrate metabolism primarily support the energy demands during contraction, rather than the growth requirements of the heart (Fisher et al., 1980).

Estimates of myocardial flow during development have yielded values which vary considerably from animal to animal and no trends between 0.4 gestation to term have been identified in lambs (Rudolph and Heymann, 1970). Because myocardial flow is linearly related to the oxygen content of fetal arterial blood, small natural variations in oxygen levels could account for blood flow differences.

### Pulmonary Circulation and Ductus Arteriosus

In the fetus most blood ejected by the right ventricle bypasses the lungs via the ductus arteriosus. Pulmonary blood flow under normal conditions of oxygenation averages 60-160 ml/minute per 100 g of lung tissue, representing about 9% of the combined ventricular output in mature fetal lambs (Cohn et al., 1974; Longo et al., 1978; Peeters et al., 1979). Large variations within and between studies could represent differences in fetal oxygenation, since small differences in pulmonary arterial oxygen content are accompanied by large differences in pulmonary blood flow (Peeters, 1978). Gestational changes of pulmonary blood flow have been measured in an acute study of unanesthetized fetal lambs between 0.4 gestation and term (Rudolph and Heymann, 1970). Early in fetal development (between 0.4 and 0.7 gestation) pulmonary blood flow increases in proportion to fetal body weight; in this period approximately 4% of the combined ventricular output is directed to the lungs. After 0.7 gestation there is a progressive increase to 8-10% of the combined ventricular output to the lungs at term. This period of development in fetal lambs sees dramatically accelerated growth of the pulmonary vascular bed, reflected in an increasing number of muscular resistance vessels per milliliter of lung tissue (Levin et al., 1976) and proliferation of the alveolar capillary network (Maloney et al., 1980).

The factors which maintain a "low" pulmonary flow during fetal life and cause its dramatic increase at the onset of air breathing have been the subject of great interest and investigation, notably by Dawes and his co-workers in a classic series of studies (reviewed by Dawes, 1968). These experiments performed in anesthetized lambs identified the predominent role of oxygen in regulating pulmonary vascular resistance before and after birth.

Fetal pulmonary vascular resistance increases and flow decreases progressively as oxygen tension is lowered to approximately 10 Torr (Cohn et al., 1974; Peeters et al., 1979). This pattern of response to oxygen is unique in the fetal circulation; kidneys, gut, skin, muscle, and bones increase their resistance to flow only at low oxygen concentrations (Peeters, 1978). Pulmonary blood flow on the average is reduced by about half, but in individual animals flow almost ceases (Lewis et al., 1976). Diversion of pulmonary flow to vital organs does not benefit the fetus to any important extent, as the proportion of cardiac output directed to the lungs is low. Increase in fetal arterial oxygen concentration above normal levels causes a dramatic fall in fetal pulmonary vascular resistance and redistribution of fetal cardiac output, represented by increased pulmonary flow and decreased flow through the ductus arteriosus and foramen ovale (Peeters, 1978).

Despite intensive investigation, the specific mechanisms by which oxygen exerts its pulmonary vascular actions in fetal life are unknown. Increasing reactivity of the fetal pulmonary vascular bed to hypoxemia with advancing gestation is not associated

with changes in the muscularity of pulmonary resistance vessels (Rudolph, 1977). Reflex pulmonary vasoconstriction acting via thoracic sympathetic nerves can be demonstrated in mature fetal lambs under normoxic conditions (Dawes, 1968). However, in chronically catheterized fetal lambs in utero there is little resting autonomic tone, as autonomic blockade (alpha-adrenergic, beta-adrenergic, and muscarinic) has no effect on pulmonary blood flow measured continuously with electromagnetic flow meters (Lewis et al., 1976). Furthermore, autonomic blockade does not alter the pulmonary vasoconstriction which accompanies fetal hypoxemia (Lewis et al., 1976). Prostaglandins of the F series which are present in tracheal fluid are vasoconstrictors of fetal goat lungs, but inhibition of prostaglandin synthesis does not alter fetal pulmonary vascular resistance (Cassin, 1980). Increases of pulmonary artery pressure and pulmonary blood flow following inhibition of prostaglandin synthesis in intact fetal lambs in utero are secondary to constriction of the ductus arteriosus (Levin, 1980).

The caliber of the ductus arteriosus has very important consequences for the distribution of fetal cardiac output and the magnitude of pulmonary blood flow. Early measurements in exteriorized fetal lambs with an open thorax indicated that the mean pulmonary artery pressure exceeded pressure in the aorta by 2-3 Torr, consistent with some constriction of the ductus arteriosus. However, no difference can be measured in fetal lambs in utero after recovery from surgery (Rudolph, 1977). Mechanisms which maintain patency of the ductus arteriosus have been extensively studied and recently reviewed by Coceani and Olley (1980) and Clyman (1980). Patency of the ductus arteriosus in the human fetus and in the lamb, rat, and rabbit is an active process effected by a prostaglandin, but in other animals the mechanism may be different (Clyman, 1980). Studies in lamb fetuses suggest that prostaglandin $E_2$ is the primary prostaglandin maintaining ductal patency, because it is the most potent ductus arteriosus-relaxing agent known; its action may be complemented by prostaglandin $I_2$, which is the major prostaglandin released by the ductus arteriosus (Coceani and Olley, 1980). Because local production of prostaglandins may not be adequate at fetal oxygen levels, circulating prostaglandin $E_2$ may be important.

Inhibition of prostaglandin synthesis by compounds such as indomethacin constrict the fetal ductus in vitro and in vivo. This effect is more marked in immature lambs, and in mature lambs pharmacological constriction of the ductus arteriosus is almost equal to the normal oxygen-induced constriction (Coceani and Olley, 1980). Sensitivity to constriction by oxygen, which is important for postnatal closure of the ductus arteriosus, is greater after 0.7 gestation in fetal lambs than in immature lambs (Clyman, 1980). Little evidence is available to assess the circulatory affects of prostaglandin synthesis inhibitors in human pregnancy, but some clinical data are available consistent with constriction of the human fetal ductus arteriosus following therapeutic or inadvertent exposure to indomethacin or salicylates (Levin, 1980).

### Cerebral Circulation

The cerebral circulation in the fetus is much like the coronary circulation in regard to absolute flow and response to hypoxemia. In the fetal lamb, brain blood flow measured with radioactive microspheres increases from 0.4 gestation to term, from approximately 30 ml/min per 100 g of tissue in the youngest fetuses to approximately 130 ml/min per 100 g in the term fetus (Rudolph and Heymann, 1970). Study of the cerebral circulation with flow transducers on arterial vessels is not practical, because

cerebral blood flow represents only 40% of the total cephalic flow in the fetal lamb (Makowski et al., 1972).

In normoxic conditions there are major regional differences in the intracranial flow distribution of the fetal lamb brain (Peeters, 1978). The flows to the cerebellum and brainstem are consistently higher than cerebral blood flow in normoxia; during acute hypoxemia intracranial flow is redistributed from the cerebrum and cerebellum in favor of the brainstem. In the primate fetus a similar pattern of intracranial flow distribution has been found, favoring the phylogenetically older brain regions (Paton et al., 1973). Measurements have not been made with regard to the functional neural activity of the regional brain structures or of the whole brain. In adult life, cerebral blood flow increases during rapid eye movement sleep and phasic increases in cerebral blood flow are superimposed on the tonically increased flow (Greenberg, 1980).

Mechanisms of fetal cerebral vascular control have not been extensively studied. Responses to hypoxemia have received the most attention because of the low arterial oxygen content during fetal life and the vulnerability of the fetus to oxygen deprivation. Oxygen consumption and glucose utilization of fetal lambs in utero are comparable to adult values, despite the lower oxygen and glucose concentrations in fetal arterial blood (Makowski et al., 1972). High fetal cerebral utilization of oxygen and glucose, like that of the myocardium, is supported by high levels of cerebral blood flow. During hypoxemia, brain blood flow increases as oxygen availability decreases, in proportion to the reciprocal of ascending aorta oxygen content (Sheldon et al., 1979). Increasing total flow combined with an intracranial redistribution of flow provide constant oxygen delivery to the brainstem, while oxygen delivery to the cerebral and the cerebellar regions declines slightly (Peeters, 1978). Decreasing fetal arterial oxygen tension from the normal value of 21 Torr to 12 Torr during hypoxemia doubles blood flow to the whole brain; superimposed acidemia does not change this response (Cohn et al., 1974).

Responsiveness to oxygen of the adult cerebral circulation is minimal in the physiological range of oxygen tensions, but below tensions of 50 Torr cerebral blood flow increases sharply (Greenberg, 1980). Thus over the same range of oxygen tension, fetal and adult cerebral circulations respond in the same pattern. The profound vasodilating effects of increased carbon dioxide tension in the adult cerebral vasculature are well demonstrated at physiological levels of arterial oxygen tension, where oxygen exerts no effects. In the fetus extreme sensitivity of the cerebral circulation to oxygen exists over the physiological range of oxygen tensions, and it is difficult to separate the respective influences of carbon dioxide and oxygen in the fetal cerebral circulation (Jones et al., 1978). Autoregulation of the cerebral circulation in the adult ensures that the cerebral blood flow remains fairly constant over a wide range of arterial pressures from 60 to 150 mmHg (Greenberg, 1980). This mechanism has been demonstrated in the anesthetized fetal lamb down to an arterial pressure of 40 mmHg (Purves and James, 1969), but it is not known whether this is important in normal fetal life in utero.

### Umbilical Circulation

Constancy of umbilical flow in the face of radical changes of cardiac output and its distribution to other fetal organs is a remarkable feature of the fetal circulation. Umbilical flow in the chronically instrumented fetal lamb averages approximately 200 ml/min per kilogram of body weight. This represents approximatley 40% of the

combined ventricular output in the mature fetus (Cohn et al., 1974; Longo et al., 1978; Peeters et al., 1979). Umbilical flow is remarkably constant under a variety of stresses in a given animal, but it can vary considerably between animals. Despite variations of umbilical flow in fetal sheep ranging between 154 and 444 ml/min per kilogram of body weight, in different animals oxygen uptake does not vary significantly (Clapp, 1978). This is explained by a reciprocal relationship between umbilical flow and the arteriovenous oxygen concentration across the placental circulation; at low flow rates the arteriovenous oxygen difference is found to be greater, and oxygen uptake (umbilical flow times arteriovenous oxygen difference) remains constant. This suggests that oxygen consumption is well regulated in fetal life and that umbilical flow is matched to the oxygen requirements of the fetus.

By contrast umbilical flow is remarkably constant and unresponsive to a variety of *acute* stresses, including fetal hypoxemia (Rudolph et al., 1981). In response to maternal hypoxemia, umbilical flow remains constant in the sheep fetus (Parer, 1978), whereas fetal oxygen consumption is reduced in proportion to the degree of hypoxemia. Constancy of umbilical flow during hypoxemia is maintained by redistribution of the combined ventricular output at the expense of fetal body organ flow, principally the skin and the musculoskeletal system (Cohn et al., 1974; Longo et al., 1978; Peeters et al., 1979). Fetal combined ventricular output usually remains constant; but even in severe hypoxemia and hypoxemia plus acidemia, when it falls, umbilical flow remains constant (Cohen et al., 1974). Fetal acidemia produced by changes of fetal arterial $pCO_2$ does not alter umbilical flow, except at extremely high or low values beyond the physiological range (Walker et al., 1976).

Despite considerable interest and investigation, factors which regulate the umbilical circulation are poorly understood. Because umbilical arteries are not innervated beyond the proximal 1-2 cm of umbilical cord, the umbilical circulation has long been considered to be a passive circulation in which perfusion pressure (fetal arterial pressure) is the primary determinant of blood flow (Dawes, 1968). Insensitivity or low sensitivity to respiratory gases and a variety of pharmacological agents supports this view (Berman et al., 1978). However, it should be emphasized that passivity of the umbilical circulation cannot explain the remarkable constancy of umbilical flow in many circumstances of increased arterial pressure, such as during hypoxemia (Cohn et al., 1974) and following infusion of vasoconstrictor drugs such as noradrenaline (Oakes et al., 1980). In these circumstances, increased umbilical vascular resistance is perfectly matched to increasing arterial pressure, so that umbilical flow remains constant. Whether this represents a fortuitous balance of umbilical vascular resistance in relation to vascular resistance in other fetal organs or a controlled response on the part of the fetus necessitates explanation.

Whether or not circulating vasoactive agents normally regulate the fetal umbilical circulation is uncertain because of the discrepant data available from studies of the fetus in utero and of isolated preparations of umbilical vessels. Placental vessels are exquisitely sensitive to vasoconstriction by angiotension II (Tulenko, 1978), but angiotension II receptor blockade in fetal lambs produces no demonstrable effects on umbilical flow or vascular resistance (Rankin and Phernetton, 1978). Alpha- and beta-adrenergic receptors are found in sheep and human placental vessels. Vasoconstrictor responses and sensitivity to alpha-adrenergic stimulation are poorly developed, whereas vasodilation in response to beta-adrenergic stimulation is a more sensitive response (Tulenko, 1978). The potential benefit to the fetus of a mechanism which causes vasoconstriction of

systemic blood vessels but not of placental vessels has been noted by Berman et al. (1978). Vasoconstriction in the systemic circulation can raise placental perfusion pressure and provide the fetus with a means of increasing umbilical blood flow or maintaining it, such as during hypoxemia.

Alpha-adrenergic blockade does not alter fetal umbilical flow (Oakes et al., 1980). Beta-adrenergic blockade with propranolol consistently reduced umbilical blood flow in the fetal lamb by 20% (Oakes et al., 1976), supporting the possibility that tonic stimulation of beta-adrenergic receptors contributes to normal regulation of flow, but it is not established whether responsive beta-receptors are located in the placental vasculature. Beta-adrenergic stimulation with intravenous isoproteronol infusion reduces umbilical vascular resistance and umbilical flow (Barrett et al., 1972; Oakes et al., 1976), but a recent study using injections into the umbilical arteries has not confirmed these actions (Berman et al., 1978). These discrepancies illustrate the common difficulties of pharmacological studies in the fetal circulation, which relate to the different distribution of venous return and ventricular outflow and to clearance by the placental circulation which can alter the effective concentration of injected drugs (Lumbers and Reid, 1978).

Recently it has been proposed that the fetal heart rate has important effects on umbilical blood flow (Berman et al., 1978). In the compliant umbilical vascular circulation, vascular impedance increases as heart rate falls and decreases as heart rate rises. Consequently, there is a direct relationship between heart rate and umbilical flow, which is quite independent of changes in the caliber of placental resistance vessels. This controversial proposal has been questioned by a more recent study (Rankin et al., 1980). Because fetal heart rate changes are common and often dramatic in fetal life, it is important that this controversial question be resolved.

Of great interest are recent estimates of umbilical vein flow in the human fetus using noninvasive Doppler ultrasound methods. These yield values of approximately 100 ml/min per kilogram of estimated body weight (Gill, 1979; Eik-Nes et al., 1980). Between 22 and 35 weeks of normal human pregnancy flow per kilogram remains constant, averaging 120 ml/min per kilogram; beyond 35 weeks a gradual decrease is seen, falling to an average of 90 ml/min per kilogram at 40 weeks (Gill et al., 1981). A lower cardiac output per kilogram in the human fetus or a different distribution favoring the head and upper body at the expense of the placenta might explain the lower flows reported in the human fetus compared to sheep, but these questions are unresolved.

### Hepatic, Portal, and Ductus Venosus Circulations

The liver of the fetus receives its blood supply from the hepatic artery, the portal vein, and the umbilical vein. In the fetal lamb, hepatic arterial flow represents 9%, portal venous flow 18%, and umbilical flow 73% of total liver flow (Edelstone et al., 1978).

In most animal species the ductus venosus serves as a bypass of the hepatic circulation, principally for umbilical venous blood, but also for portal venous blood via the portal sinus. In the equine fetus it is obliterated early in fetal development; as a consequence umbilical flow represents the major hepatic flow source in this species (Barnes et al., 1979). In the chronically catheterized fetal lamb, 50-60% of umbilical venous flow passes via the ductus venous into the inferior vena cava. This proportion is unchanged between 0.8 gestation and term (Edelstone et al., 1978) and increases only

slightly (from 57 to 65%) in acute hypoxemia. Adrenergic and cholinergic nerves are present in the fetal liver circulation and ductus venosus, but autonomic blockade (muscarinic and alpha-adrenergic) does not influence the distribution of umbilical flow to the ductus venosus, liver, or other fetal organs (Edelstone et al., 1980). These observations do not support previously held views that the ductus venosus actively regulates the distribution of umbilical and portal venous flow to the liver or to the inferior vena cava during stress (Rudolph et al., 1981).

## FETAL BEHAVIOR

### Fetal Sleep and Activity

The development of techniques for recording physiological data directly from unanesthetized fetal animals in utero has supported a surge of experimental interest in fetal kinesis and behavioral states in utero. The many advantages associated with studying the cardiovascular system of the fetus in utero have been recognized (Rudolph and Heymann, 1974), but cyclical changes in fetal behavior in utero present new challenges, because it is now recognized that in adults cardiovascular control processes are profoundly altered according to the states of sleep and consciousness (Mancia and Zanchetti, 1980).

The brain of the fetal lamb matures early in utero and the cortex might influence the cardiovascular system after approximately 0.5 gestation. In fetuses younger than 60 days (0.4) of gestation, the cortex is electrically silent (Bernhard and Meyerson, 1973). After 65 days spontaneous electrical activity appears, initially in the form of bursts which are confined to limited areas of the cortex, then becoming continuous with further growth. After 120 days (0.8) of gestation the electrocorticogram (ECoG) becomes patterned into high-voltage slow activity (HVSA) and low-voltage fast activity (LVFA). Using measurements of fetal ECoG together with other measures of fetal activity, criteria have been established for recognizing fetal behavioral states corresponding to active wakefulness (AW), rapid eye movement (REM) sleep, and quiet or non-rapid-eye-movement (NREM) sleep (Ruckebusch et al., 1977; Ioffe et al., 1980). The ECoG pattern of HVSA is recognized as NREM sleep; the pattern of LVFA is comprised of REM sleep and AW. After 0.85 gestation in the fetal lamb the average percentages of time spent in each state are the following: NREM sleep, 53-54%; REM, sleep, 40-41%; and AW, 5-6%. With advancing gestation, cyclic alternation between states is readily recognized. Cyclic changes with a period of about 30-40 min are found at term, approximately equally partitioned between REM and NREM sleep (Ruckebusch et al., 1977; Maloney et al., 1980). Fetal breathing movements occur only during REM sleep, but 35% of REM is not associated with fetal breathing (Ioffe et al., 1980).

Baroreceptor-heart rate reflex responses have been examined in relation to ECoG and fetal breathing and limb movements in mature fetal lambs (Dawes et al., 1980). It was anticipated that the previously observed variability of baroreflex responses in fetal lambs (Shinebourne et al., 1972; Maloney, et al., 1977) might be explained by changes in fetal activity state. However, only small differences were found in heart rate slowing in response to arterial pressure elevations during LVFA and HVSA, and no association with breathing or limb movements was apparent.

## Fetal Breathing

Variations in fetal cardiovascular parameters occurring in association with fetal breathing movements have been surveyed in pregnant women and in animal studies. In fetal lambs changes of the baseline heart rate at the onset of fetal breathing are completely variable; increased, decreased, and little-changed rates which are variable in their onset and their duration have been described (Fouron et al., 1975; Dalton et al., 1977). Changes of cardiac rhythm, on the other hand, are consistently found, characterized by increased beat-beat variability and slower changes. If the rate of breathing movement is slow, heart rate variation can be recognized as typical of respiratory arrhythmia seen after birth (Fouron et al., 1975). Atropine abolishes the beat-beat variability in the fetal lamb, supporting the suggestion that modulations of vagal tone or increased vagal tone might be involved. In three fetal lambs in which ECoG and fetal breathing movements were recorded, heart rate variability measured during HVSA and LVFA in the absence of breathing movements was similar (Dalton et al., 1977). Variability increased when breathing movements began in LVFA, identifying the breathing activity rather than the concomitant sleep state as the important association.

Changes in fetal intrathoracic arterial pressure measured during breathing movements have been described in detail (Fouron et al., 1975). With the initiation of a period of rapid breathing movements, systolic and diastolic pressures decrease slightly, followed by a marked increase in pressure which is not always sustained. Phasic decreases of arterial pressure timed in phase with each inspiration are reflected in the modulation of blood flow in the descending aorta. Therefore records of arterial pressure and aortic flow, like the heart rate, are clearly irregular during fetal breathing movement. Sustained increases in arterial pressure during periods of vigorous breathing movements are associated with sustained elevations of descending aortic flow and possibly umbilical flow (Dawes et al., 1972).

In human fetuses in the last trimester, umbilical flow has been measured using Doppler ultrasound techniques and related to fetal breathing movements measured simultaneously with a second ultrasound scanner operating in real time (Marsal and Eik-Nes, 1980). Influences on umbilical vein flow are found which vary with the amplitude of fetal chest movements. In 15 fetuses the mean umbilical flow increased 6-54% (with an average of 22.5%) during fetal breathing movement; in 2 fetuses with low-amplitude breathing, flow was decreased by 11-12%. Phasic changes in the umbilical venous circulation are observed with ultrasound. These include transient decreases of blood flow velocity during inspiration (Marsal and Eik-Nes, 1980) and diaphragmatic contractions (Mantell, 1980), followed by increases during expiration. The umbilical vein diameter within the fetal trunk changes transiently by approximately 10% (Gill et al., 1981). Increased mean blood flow in the descending aorta of the human fetus has been found during high-amplitude fetal breathing movements (Marsal and Eik-Nes, 1980). In the fetal lamb chronically instrumented with an electromagnetic flow probe on the aortic root, increases of flow of approximately 20% are often found in association with fetal breathing measured using a diaphragm electromyogram (Figure 3). However, the effect is variable in its appearance and the exact mechanism which increases flow is not defined. In another study of chronically instrumented lambs no change in left ventricular output in association with respiratory movements was found (Kirkpatrick et al., 1976).

At this time there are no clear explanations for the various cardiovascular changes occurring in association with fetal breathing movement. Changes in heart rate could

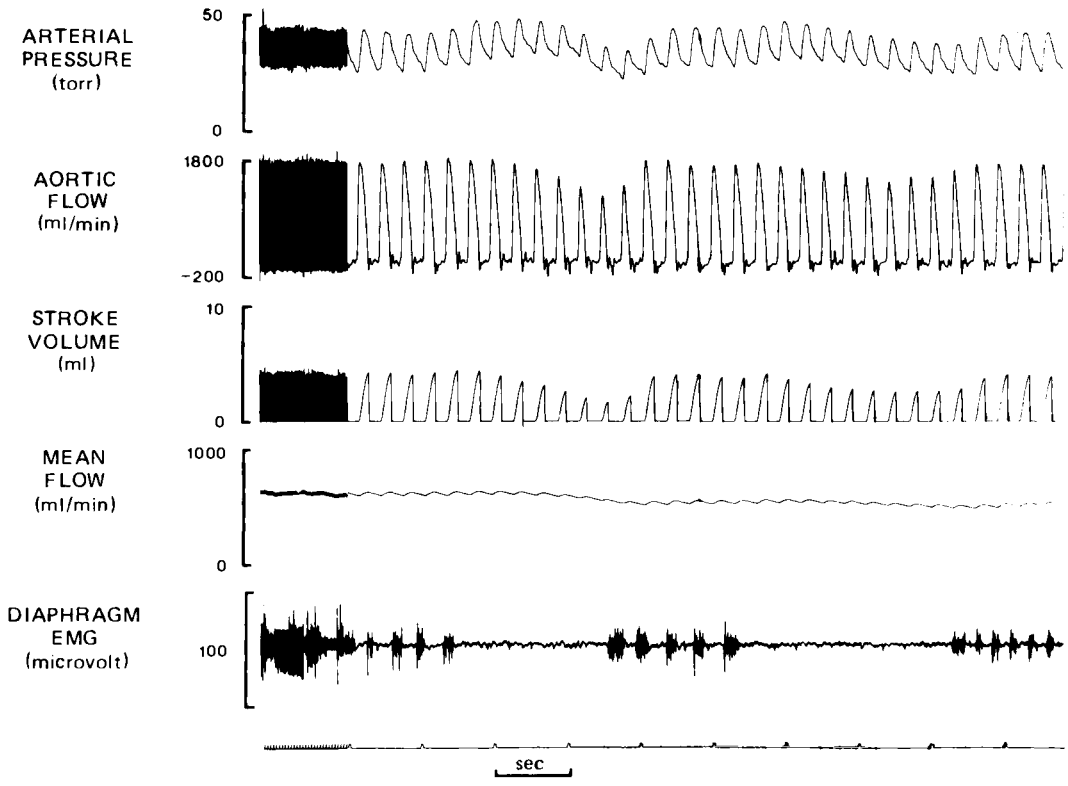


**Figure 3** Cardiovascular changes associated with fetal breathing (diaphragm electromyograph) in a fetal lamb in utero (118 days of gestation). The aortic flow (left ventricular flow output minus coronary flow) and stroke volume are increased by 20% above nonbreathing levels, and they fall abruptly when fetal breathing movements cease.

result from phasic blood pressure alterations acting via extrathoracic baroreceptors. Additionally, inhibition of the vagal outflow to the heart could be caused by antagonism of the medullary cardioinhibitory center by pulmonary stretch receptors and the brainstem respiratory center (De Burgh Daly, 1972). Because fetal breathing occurs during REM sleep, increased vagal tone, which is seen during REM sleep after birth (Mancia and Zanchetti, 1980) might amplify these phasic modulations. Blood pressure variations can be largely explained by the fall of intrathoracic pressure occurring with inspiration, as the extent of variation is proportional to the depth of the inspiratory change. Changes of blood flow are more difficult to understand because of the complex interaction of passive mechanical influences on the heart and circulation. It is tempting to suggest that increased ventricular output during fetal breathing movement is a result of increased venous return which augments ventricular filling and stroke volume via the Frank-Starling mechanism (Kirkpatrick et al., 1976). However, it is not always appreciated that just as the fall in pressure relative to the systemic veins increases right ventricular filling in the adult heart, the fall in pressure around the left heart relative to the extrathoracic arteries impedes left ventricular ejection (Summer et al., 1979). Complex pressure interactions between the heart, the pleural cavity, and the extrathoracic vessels might explain the curious phase relations observed in the human fetus, in which flow in

the descending aorta is augmented in the diastolic phase of the cardiac cycle during fetal breathing movement (Marsal and Eik-Nes, 1980).

### Maternal-Fetal Interactions

Possible rhythmic interaction between fetus and mother in human pregnancy is indicated by examples such as concomitant changes of the heart rate (Patrick et al., 1980) and the association of increased fetal movement during maternal REM sleep (Sterman, 1967). Reciprocal 24-hr rhythms exist in sheep uterine blood flow and fetal umbilical flow (Walker et al., 1977) and there is some evidence that the fetus might regulate maternal placental flow in this species (Rankin and McLaughlin, 1979). Power and Longo (1973) proposed that maternal vascular pressures in the placenta generate a tissue pressure which regulates umbilical flow via a "waterfall" phenomenon. This proposal is often cited to explain fetal cardiovascular responses to maternal uterine vascular changes, such as those associated with the supine hypotensive syndrome, and uterine contractions. However, detailed studies of the effects of altering uterine venous pressure over a wide range in the sheep placenta perfused in situ (Bissonnette and Farrell, 1973) and in fetal lamb preparations in utero (Berman et al., 1976) have not supported this concept. Recently it has been suggested that regular uterine contractions in sheep may constitute a pathway by which maternal rhythms affect fetal sleep and activity (Nathanielsz et al., 1980). This suggestion is important because it offers a focus for investigating the bewildering variety of fetal cardiovascular responses in utero.

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# 10

# Fetal Lung Maturation and the Respiratory Distress Syndrome

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## INTRODUCTION

The morbidity and potential for survival of the prematurely delivered infant is often dependent on the extent of pulmonary maturation. Satisfactory pulmonary function requires sufficient anatomic development such that gas exchange surfaces are adequate, surfactant is present in amounts sufficient to provide alveolar stability, and neuromuscular function and respiratory control are adequate to sustain an effective ventilatory effort. Lung maturity in the human is normally present after 34-36 weeks gestational age; however, clinical experience indicates that the human infant can occasionally survive with adequate gas exchange after about 25 weeks gestational age. This potential asynchrony of at least 10 weeks between functional pulmonary maturity and gestational age represents possibly the greatest variance in the timing of maturation that occurs during human fetal development. The clinical value of identifying the premature fetus with the potential for adequate lung function has been realized using amniotic fluid tests of lung maturity. The clinical use of corticosteroids to induce fetal lung maturation is accepted by many; however, the developmental mechanisms of normal and pharmacologically induced lung maturation remain to be demonstrated definitively. Empirically developed support systems and modern intensive care have allowed the neonatologist to salvage the majority of infants born with the respiratory distress syndrome (RDS); yet our understanding of postnatal lung development and surfactant metabolism in the normal infant or the infant with RDS is poor.

This chapter will briefly review the anatomy of lung development and then discuss surfactant metabolism, pharmacological agents that affect the lung, tests of lung maturity, and RDS. The references have been selected to be representative but not exhaustive and review articles have been cited when possible. The literature was reviewed through March 1980.

## ANATOMIC DEVELOPMENT OF THE LUNG

The development of the fetal lung can be divided into four periods, as shown in Figure 1 and as reviewed by Boyden (1977). The embryonic period is initiated with the appearance of the lung bud as a midline projection from the laryngotracheal sulcus. The lung bud then divides, forming primordia for the right and left lungs. Initial branching of the lung bud will not occur if the mesoderm is removed from the branching epithelium

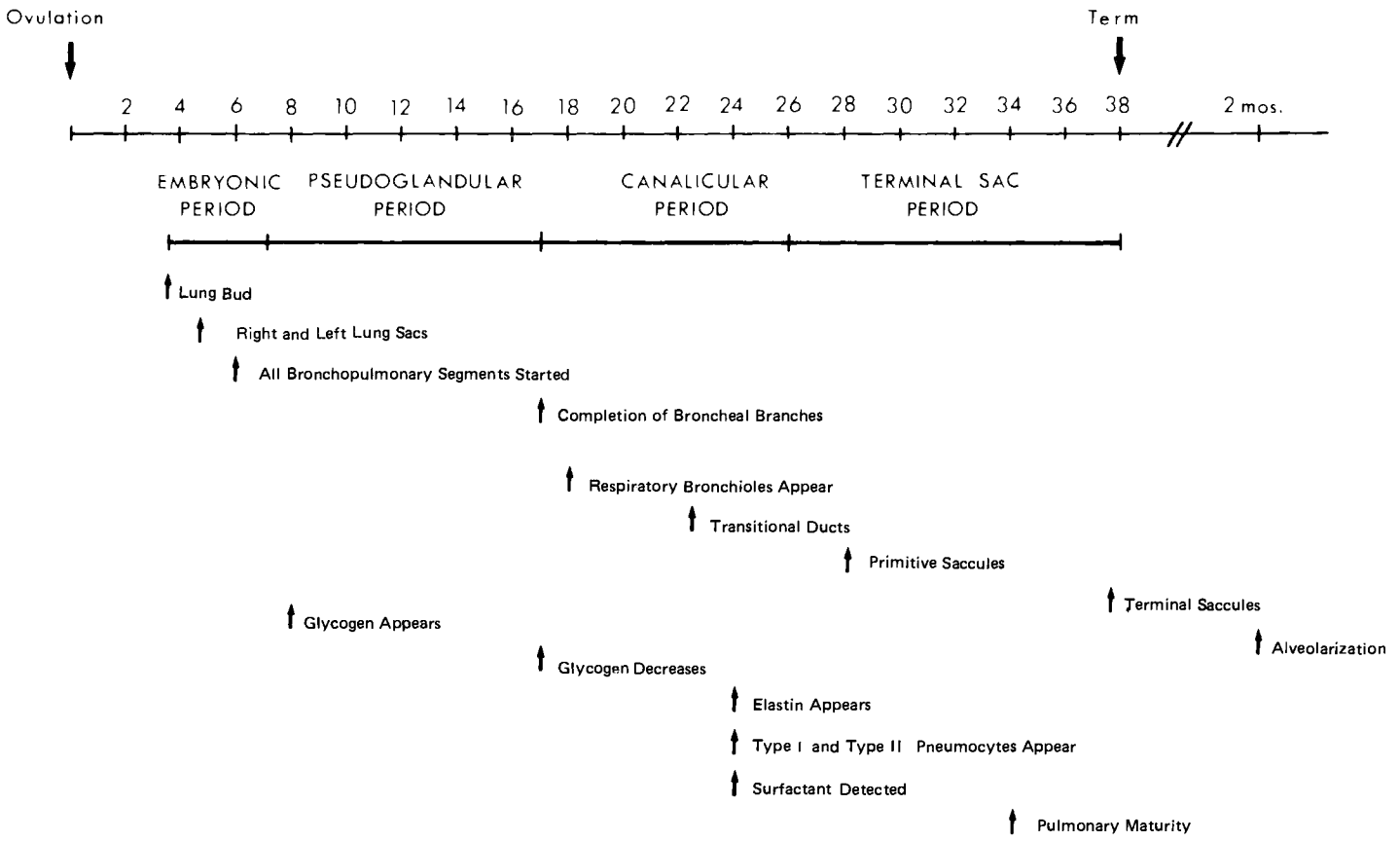


**Figure 1** Chronology of lung development. A general outline of the major events of normal human lung maturation is indicated against time in weeks following ovulation.

(Bernfield and Wessels, 1970). Further branching requires interaction of the epithelium with the specific pulmonary mesoderm, indicating very specific tissue-tissue interactions. The pattern of branching is regulated in part by a matrix of collagen fibers supplied by the mesoderm, which provides gaps through which epithelial cells can grow (Wessels, 1970). With progressive growth, all bronchopulmonary segments can be identified by 6 weeks from ovulation.

The pseudoglandular period from 7 to 17 weeks postovulation is characterized by continued branching of the cuboidal epithelium, resulting in the histological appearance of an exocrine gland. The epithelial cells have prominent rough endoplasmic reticulum and contain abundant amounts of glycogen until about 17 weeks. The glycogen stores have been proposed as precursors for later surfactant synthesis; however, this concept has not been verified (Gross, 1979). The axial branches of the bronchopulmonary segments continue to form and have reached the 15-25 generations that characterize the adult human lung by 16 weeks. The number of generations depends upon which lung segment is studied (Bucher and Reid, 1961).

The canalicular period from 17 to 26 weeks is characterized by the first appearance of potential gas exchange surfaces (the birth of the acinus) (Boyden, 1974). The acinus is the respiratory unit of the lung which includes all structures peripheral to and supplied by a terminal bronchiolus. Thus the acinus includes several generations of respiratory bronchioles, alveolar ducts, and alveoli. Three to four generations of progressive branching of the respiratory bronchioles are followed by the appearance of the transitional ducts and several generations of primitive saccules by 26 weeks (Hislop and Reid, 1974). As vascularization has followed bronchial branching in early development of the airways, vascularization by capillary invasion occurs centrifugally from the bronchi to the respiratory bronchioles. By the twenty-fourth week, some of the cells lining the developing acinar airways differentiate to be identifiable as type I pneumocytes and type II pneumocytes containing lamellar bodies (Campiche et al., 1963). The morphological identification of the storage organelle of surfactant, the lamellar body, corresponds temporally with the appearance of surfactant at about 24-26 weeks (Brumley et al., 1967). By the end of the canalicular period, the lung, while immature, possesses a gas exchange surface and a potential for surfactant synthesis sufficient to permit extrauterine survival of the 500- to 1000-g human infant.

The terminal sac period from 26 weeks to term corresponds to the final stage of airway branching, with further division of the primitive saccules, which become the terminal saccules of the acinus. The terminal airways become more completely vascularized and the epithelium thins out, bringing the airways into close apposition with the vascular channels. This period merges with the alveolarization of the acinar airways that is well established by 2 months of postnatal age (Hislop and Reid, 1974).

## FETAL LUNG FLUID

The lungs of the late gestational age fetus are not collapsed; rather, the potential airways are filled with about 30 ml/kg body weight of fluid at term. This volume is important for normal lung development and approximates the functional residual capacity of the aerated newborn lung. If lung fluid is continually removed from the fetal lamb lung, pulmonary hypoplasia results, while excess fluid stimulates pulmonary hyperplasia (Alcorn et al., 1977). The clinical correlate is the association of oligohydramnios and chronic amniotic fluid leakage with pulmonary hypoplasia. Experiments in the fetal

Table 1 Composition of Fetal Lamb Body Fluids

| | Lung liquid | Lung lymph | Plasma | Amniotic fluid |
|---|---|---|---|---|
| $[Na^+]$ (mEq/dl) | 150 | 147 | 150 | 113 |
| $[K^+]$ (mEq/dl) | 6.3 | 4.8 | 4.8 | 7.6 |
| $[Cl^-]$ (mEq/dl) | 157 | 107 | 107 | 87 |
| $[HCO_3^-]$ (mEq/dl) | 2.8 | 25 | 24 | 19 |
| pH | 6.27 | 7.31 | 7.34 | 7.02 |
| Protein (g/dl) | 0.027 | 3.27 | 4.09 | 0.10 |

*Source*: As abstracted by Strang (1977).

lamb using either nonpermeable markers mixed with the lung fluid (Olver and Strang, 1974) or systems to collect all lung fluid from the fetal trachea (Adamson et al., 1973; Mescher et al., 1975) indicate production rates from 120 days gestational age to term (150 days) of 3.9-4.5 ml/kg per hour. This flow is episodic and reflects fetal breathing movements resulting in pressure changes that can be detected in the fetal trachea (Adamson et al., 1973). The total flow of fetal lung fluid to the pharynx either to be swallowed or to enter the amniotic fluid is large; for example, a 4-kg fetus would secrete 270-430 ml of lung fluid per 24 hr; a volume sufficient to contribute significantly to amniotic fluid volume and composition. Before spontaneous birth the flow of fetal lung fluid from the lung decreases, and this decrease is not affected by atropine or section of the cervical vagosympathetic trunk (Kitterman et al., 1979); however, catecholamines infused into the fetal lamb late in gestation will acutely decrease fetal lung fluid production and increase the surfactant flux from the lung (Lawson et al., 1978; Walters and Olver, 1978).

The composition of fetal sheep tracheal fluid is different from lung lymph, plasma, or amniotic fluid (Table 1). The chloride content is high, and pH, bicarbonate, and protein are remarkably lower than in the other associated biological fluids. The only two components that seem to change with gestational age are the tracheal fluid potassium, which increases from 4.3 mEq/liter at 120 days to 8.9 mEq/liter at term (150 days), and the flux of surfactant phospholipids (Mescher et al., 1975).

Elegant experiments by Strang and his collaborators (Strang, 1977) have defined the mechanisms of fetal lung fluid secretion and the properties of the epithelium of the fetal airways. The permeability of the pulmonary capillaries can be assessed by measuring the appearance of macromolecules intravascularly injected into lung lymph and lung fluid. The plasma solutes including protein can enter the interstitium of the lung and thus are detected in lymph, but the appearance of macromolecules into fetal tracheal fluid is limited by restricted diffusion through pores predicted to be 0.6 nm in radius (Normand et al., 1971). Thus serum proteins are virtually excluded from the fetal lung fluid (Table 1), and an osmotic gradient between the vascular spaces and the fetal airways is generated by the protein. However, fetal lung fluid flows from the lung to the amniotic fluid, and not the reverse as predicted by the protein osmotic gradient. The compositional differences in chloride and bicarbonate between fetal lung fluid and plasma, lymph, and amniotic fluid indicate other nonequilibrium states. The chloride is transported actively from plasma to lung fluid, providing sufficient electrical and

osmotic forces to produce the observed volume flow of fetal lung fluid (Olver and Strang, 1974). The 10-fold lower concentration of bicarbonate in fetal lung fluid than in fetal plasma is explained by a bicarbonate pump moving the bicarbonate ion from fetal lung fluid to the plasma, or a hydrogen ion pump operating in the reverse direction and causing the dissociation of bicarbonate to carbon dioxide. The low pH of fetal tracheal fluid results from the low bicarbonate buffering the systemic and therefore fetal tracheal fluid $pCO_2$ (Adamson et al., 1969).

The scenario for the disappearance of fetal lung fluid at birth as presently understood can be summarized as follows (Strang, 1977). Shortly before birth fetal lung fluid secretion ceases, possibly by catecholamine-mediated effects on the chloride pump. The remaining disequilibrium between the plasma and the fetal lung fluid is the protein oncotic pressure (about 17 mmHg). The direction of flow of fetal lung fluid reverses, and at birth the interstitial spaces and lymphatics absorb that part of the 30 ml/kg of lung fluid not lost via the airway during labor and delivery. The majority of this fluid volume must be cleared from the lungs of the fetus delivered by cesarean section.

At birth, two further changes encourage rapid absorption of fluid from the airways. Lung expansion at birth will lower interstitial hydrostatic pressure, and an acute change in epithelial permeability permits restricted diffusion through pores estimated to be 5.2 nm in radius (Egan et al., 1975). This represents a 6- to 10-fold enlargement of the pores in the epithelium present in the fetal lung, possibly resulting from expansion of the lung.

## SURFACTANT AND THE DEVELOPING LUNG

### Biochemistry of Surfactant

A large body of scientific and clinical literature is available concerning lung development and phospholipid metabolism as reviewed by Ohno et al. (1978). I will attempt only to present information that may help us understand the dynamics of maturation of lung surfactant. Surfactant isolated from adult animal lungs by alveolar lavage is a complex mixture of phospholipids, neutral lipids, and protein that appears to be quite similar in all mammals studied to date (Ohno et al., 1978). Lipids represent about 90% of surfactant by weight, of which 80% of the total lipids are phospholipid species (Table 2). The phospholipids of surfactant are unique relative to the phospholipid constituents of the rest of the body. About 45% of the surfactant phospholipids are saturated phosphatidylcholine molecules (*phosphatidylcholine* and *lecithin* are synonyms). A saturated phospholipid carries two saturated fatty acids esterified to the 1-acyl and 2-acyl positions of the diglyceride backbone of the phospholipid. The saturated phosphatidylcholine of surfactant is predominantly dipalmitoyl phosphatidylcholine, a species found in only small amounts in other tissues. Phosphatidylglycerol represents the second most common phospholipid and is quite specific for surfactant. The proteins that copurify with the surfactant lipids contain traces of serum proteins and two proteins of 35,000 and 10,000 daltons that may be specifically associated with surfactant (King, 1979). Thus surfactant can best be quantified biochemically by measuring saturated phosphatidylcholine, phosphatidylglycerol, or possibly specific surfactant-associated proteins.

The function of surfactant to stabilize the air-alveolar gas exchange surface during the breathing cycle is dependent on the phospholipid composition. Saturated

Table 2 Composition of Purified Surfactant Isolated from Dog

| | Percentage of total |
|---|---|
| Lipid | 90 |
| saturated phosphatidylcholine | 45 |
| unsaturated phosphatidylcholine | 25 |
| phosphatidylglycerol | 5 |
| phosphatidylethanolamine | 3 |
| neutral lipids | 10 |
| other phospholipids | 2 |
| Protein | 8 |
| Carbohydrate | 2 |

*Source*: King (1979), courtesy of Mead Johnson Nutritional Division.

phosphatidylcholine alone will lower the surface tension from the 72 dynes/cm of an air-water interface to less than 10 dynes/cm. However, pure dipalmitoyl phosphatidylcholine is quite insoluble in water and will spread only slowly to form a surface film. The other phospholipids and protein interact with saturated phosphatidylcholine to permit adsorption of the phospholipid to the alveolar surface. The low surface tension of the alveolar-air interface is then regulated during the respiratory cycle by expansion and contraction of the predominantly phospholipid surface film (King, 1979).

Surfactant and surface tension considerations of course have no relevance to the fetus. To dissect the development of the surfactant system which will ultimately be essential to survival of the newborn, I will consider the synthesis, storage, secretion, and degradation of surfactant in the developing fetus and the newborn. Clements and Tooley (1977) have compiled the available data on the amount of surfactant phospholipids associated with the lung parenchyma and the amount which is recovereable from the alveoli of the fetal human and a number of other mammals (Figure 2). By 60% of gestation the saturated phosphatidylcholine content of human lung is increasing, indicating synthesis and storage of surfactant long before the increase in surfactant content in the lung tissue of other mammals. This early biochemical identification of surfactant is consistent with the anatomic maturation of human lung (Campiche et al., 1963) and the potential for the survival of the occasional very immature newborn.

As the principal surface-active lipid in surfactant is saturated phosphatidylcholine, most studies of surfactant-related pulmonary phospholipid synthesis concern this molecule. The interrelated pathways for the synthesis of lung phospholipids are given in Figure 3. These synthetic pathways are reviewed extensively elsewhere (Mason, 1976; Ohno et al., 1978). The three-carbon backbone of the phospholipid is derived ultimately from glucose and enters the pathway as glycerol-3-phosphate. The de novo synthetic pathway then proceeds through two sequential acyltransferase reactions. A saturated fatty acylcoenzyme A (usually palmitic acid) is esterified to the 1-acyl position of glycerol-3-phosphate by glycerolphosphate acyltransferase (I). 1-Acylglycerolphosphate phosphotransferase (II) then esterifies an unsaturated fatty acid to the 2-acyl position, generating diacylglycerol-phosphate (phosphatidic acid), which is the common precursor of all the phospholipids. Phosphatidic acid is then either dephosphorylated to diacylglycerol by phosphatidic acid phosphatase (III) or converted to cytidine-5'-diphosphodiacylglycerol (CDP-diacylglycerol) by phosphatidate cytidyltransferase (VIII); CDP-diacylglycerol is the common precursor

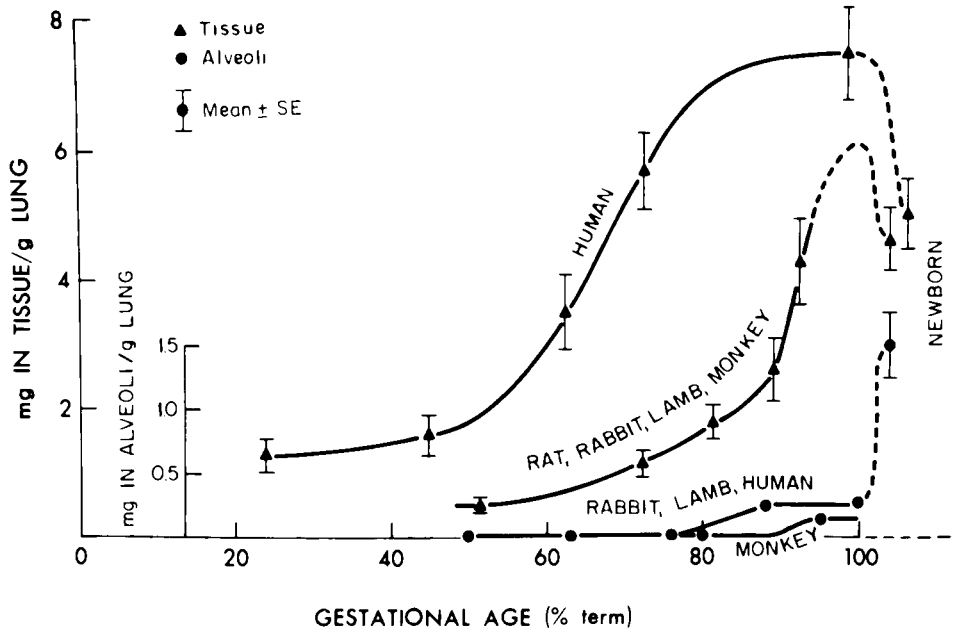


**Figure 2** Accumulation of saturated phosphatidylcholine in fetal lungs and airways. The data here have been collected from various sources and express the fetal lung tissue and airway (alveoli) contents of saturated phosphatidylcholine per gram of lung tissue versus relative gestational age for man and several other mammals. The saturated phosphatidylcholine content increases much earlier in gestation in human fetal lung than in lung tissue from other mammals. (From Clements and Tooley, 1977.)

of phosphatidylglycerol and phosphatidylinositol. Phosphatidylglycerol is synthesized by a two-step pathway involving glycerolphosphate phosphatidyltransferase (IX) and phosphatidylglycerol phosphatase (X). The diacylglycerol is the direct precursor of both phosphatidylethanolamine and phosphatidylcholine following the transfer of ethanolamine or choline from CDP-ethanolamine or CDP-choline to the diacylglycerol by the appropriate phosphotransferase (IV, XII). However, the resulting phosphatidylcholine is predominately 1-acyl saturated, 2-acyl unsaturated, and not surface active. A remolding of the phosphatidylcholine resulting from this de novo synthetic pathway apparently occurs within the type II pneumocyte before packaging of the saturated phosphatidylcholine in lamellar bodies for secretion. Two pathways for the synthesis of saturated phosphatidylcholine from unsaturated phosphatidylcholine have been proposed. Both initially involve the generation of 1-acyl-2-lysophosphatidylcholine by phospholipase $A_2$ (V). The lysophosphatidylcholine acyltransferase (VI) or two lysophosphatidylcholines can generate saturated phosphatidylcholine via reaction VII. Recent information derived from primary cultures of type II pneumocytes indicates that the direct reacylation of lysophosphatidylcholine (enzyme VI) is probably the most important pathway for saturated phosphatidylcholine synthesis (Batenburg et al., 1979).

While the synthesis of saturated phosphatidylcholine occurs with progressive accumulation of this surface-active lipid from mid-gestation in the human (Figure 2), phosphatidylglycerol is not present normally until after 35 weeks gestion (Hallman et al., 1976), indicating some immaturity of the synthetic pathways. Assuming the mix of phospholipids

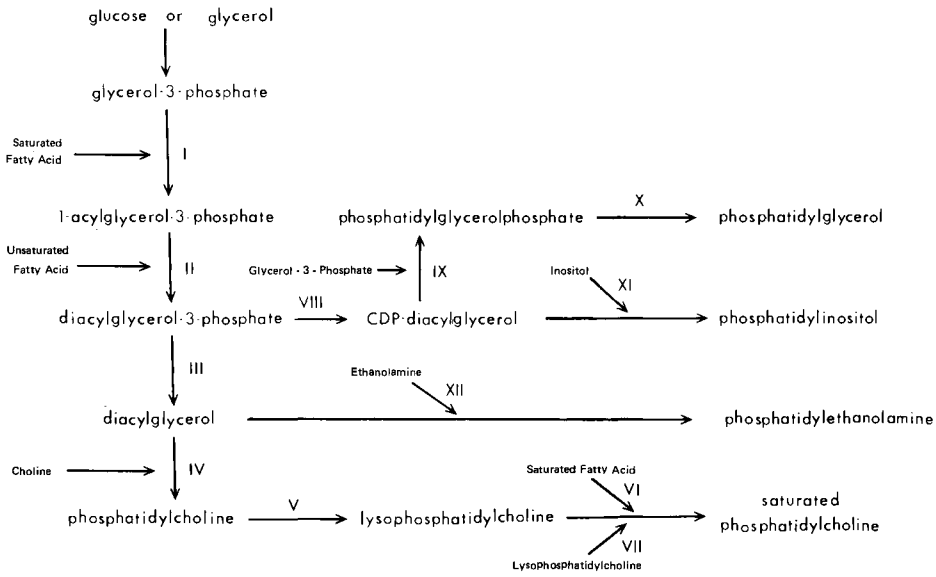


**Figure 3** Biosynthesis of lung phospholipids with an outline of the major pathways and precursors for the synthesis of saturated phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylglycerol. The enzymes specific for each step are indicated by Roman numerals: (I) glycerolphosphate acyltransferase, (II) 1-acylglycerol-phosphate phosphotransferase, (III) phosphatidic acid phosphatase, (IV) cytidine-5'-diphosphocholine diacylglycerol phosphotransferase, (V) phospholipase $A_2$, (VI) lysophosphatidylcholine acyltransferase, (VII) lysophosphatidylcholine-lysophosphatidylcholine acyltransferase, (VIII) phosphatidate cytidyltransferase, (IX) glycerolphosphate phosphatidyltransferase, (X) phosphatidylglycerol phosphatase, (XI) cytidine-5'-diphosphodiacylglycerolinositol phosphotransferase and, (XII) cytidine-5'-diphosphoethanolamine diacylglycerol phosphotransferase.

is important, the quantity of synthetic activity after mid-gestation may not reflect the quality of the surfactant synthesized and stored. Surfactant is not normally detected in amniotic fluid in large quantities until after 35 weeks gestation, at a time when large amounts of saturated phosphatidylcholine are present in the fetal lung tissue. The development of the secretory function of the type II pneumocyte is not well understood, but secretion may be the critical last link permitting extrauterine survival of the premature infant.

## Effectors of Surfactant Synthesis and Secretion

### Corticosteroids

Agents that will effect late fetal lung maturation and surfactant metabolism not only have the potential to further basic understanding of lung development, but may be used therapeutically to prevent RDS. The corticosteroids have been extensively studied as "inducers" of surfactant synthesis, as recently reviewed by Taeusch and Avery (1977) and Ballard (1979). Studies in animals have demonstrated that late in gestation corticosteroid administration to the fetus can cause anatomic maturation of lung tissue with early appearance of lamellar bodies, disappearance of glycogen, and thinning of the

alveolar capillary membranes. These anatomic changes have been correlated with increased survival of prematurely delivered animals and with increased surfactant content of lung tissue and airways in selected experiments. The possible control mechanisms that may be involved have been reviewed by Farrell and Morgan (1977). In the human a period of at least 48 hr is required between treatment and effect and the timing of treatment is critical. Thus corticosteroids may prematurely trigger the normal maturation process by the induction of an enzyme (or enzymes) of the synthetic pathway (Ballard, 1979). A number of investigators have explored this possibility in different animal models both in vivo and in vitro by measuring enzymatic activities associated with phospholipid metabolism after treatment with corticosteroids. There is a general consensus that corticosteroids stimulate phosphatidylcholine synthesis; however, different investigators have identified increases in different enzymatic activities as probably causative. Recent data suggest that the activities of phosphatidic acid phosphatase (Figure 3, III) and the enzymes responsible for saturated phosphatidlycholine synthesis from phosphatidylcholine (Figure 3, VI and VII) are increased in fetal rabbits following betamethasone treatment of the does (Possmayer et al., 1979). These enzymatic activities are attractive candidates, as they regulate precursor production (diacylglycerol) for phosphatidylcholine synthesis and synthesis of saturated phosphatidylcholine. The inconsistencies in this extensive literature are reviewed by Gross (1979).

Johnson et al. (1978) and Mitzner et al. (1979) have studied the effects of betamethasone on lung maturation in fetal rhesus monkeys in dosages similar to those used in human studies. They demonstrated no change in lung phospholipid concentrations or effects on surface tension measurements 72 hr after steroid administration. However, a similar and large increase in total lung capacity was apparent with either air or saline filling of the lungs, implying a maturation process characterized by improving compliance by structural changes in the lung and not by surfactant-related mechanisms. Thus while most animal studies document fetal lung maturation caused by steroid administration, there is as yet no consensus as to the mechanism.

Ballard and Ballard (1979) have reviewed the clinical trials of antepartum treatment with glucocorticoids to prevent RDS. Following the initial study by Liggins and Howie (1972), both retrospective and excellent prospective studies (Taeusch et al., 1979; Papageorgiou et al., 1979) demonstrate the efficacy of this therapy in decreasing the risk and severity of RDS in infants of 26-34 weeks gestation and in increasing survival of these premature infants. Delivery must be delayed at least 48 hr after treatment, and treatment is ineffective for infants weighing less than 750 g because of mortality related to general immaturity (Ballard et al., 1979). While steroid therapy decreases the incidence of RDS in premature infants approximately two- to threefold, 10-25% of these infants will still be diagnosed as having RDS. Liggins (1976) noted no increased benefit by doubling the dose of betamethasone; thus the desire to prevent RDS by giving larger and longer courses of corticosteroid therapy must be resisted (Ballard and Ballard, 1979). Similarly, the therapy should be considered only in patients with fetuses of 26-34 weeks gestational age in whom the prospects of delaying delivery 48-72 hr are realistic. Fetal lung maturity should be assessed if possible before steroid therapy is instituted, as many premature infants will not develop RDS.

The proponents of prevention of RDS by steroid therapy feel that the 12-mg dose betamethasone used to induce maturity is within the physiological rather than pharmacological range. The drug does not appreciably elevate fetal corticosteroid levels and is cleared by 3 days from the fetal circulation. The low dose and short duration of therapy

argue against serious side effects to the fetus. No long-term effects have been noted in survivors (Ballard and Ballard, 1979); however, glucocorticoids have profound effects on fetal growth. Recent studies using low doses of betamethasone in rhesus monkeys (Epstein et al., 1977; Johnson et al., 1978), rats (Frank and Roberts, 1979) and rabbits (Barrada et al., 1980) demonstrate significant decreases in fetal weight and brain weight relative to untreated controls. These multisystemic effects cannot be ignored, and while some (Ballard and Ballard, 1979) feel the benefits of prenatal treatment with betamethasone outweigh the risks, other disagree (Gluck, 1976). The use of corticosteroids to achieve fetal lung maturation is a typical medical dilemma; the benefits are real, but the risks and mechanism of action are not well defined.

### Aminophylline

Agents other than corticosteroids might be more specific and potentially safer for use in preventing RDS. Barrett et al. (1976, 1978), studied the effects of aminophylline in comparison to hydrocortisone on lung maturation of rabbits. The aminophylline-treated fetal rabbit lungs had increased concentrations of cyclic adenosine 5'-monophosphate (cAMP), decreased phosphodiesterase activity, and increased incorporation of choline into phosphatidylcholine. Similarly treated prematurely delivered rabbits survived longer, had lungs that retained more air at low pressure, and had more surfactant recoverable than either control or corticoid-treated rabbits. Both corticoids and aminophylline may stimulate phosphatidylcholine synthesis by increased cAMP levels. Hallman (1977) treated fetal rabbits directly with dibutyryl cAMP and found a gestation-dependent stimulation of phosphatidylglycerol synthesis and increased release of surfactant to the alveolar space within 1 hr of birth. Similar effects have been detected in organ culture explants of fetal rat lung (Gross and Rooney, 1977). Recently Hadjigeorgiou et al. (1979) reported that aminophylline given to women when used in conjunction with isoxsuprine to delay delivery decreased the incidence of RDS from 53 to 13% in 28-30 weeks gestational age infants. The aminophylline effect was noted at all times from 24 hr to 7 days after administration and in the presence of ruptured membranes. As aminophylline has been used with apparent safety during pregnancy and in premature infants with apnea, this agent may be an attractive choice for further human studies. However, cAMP-mediated effects on the fetus will be rather general and multisystemic, as are the corticosteroid effects, and caution is warranted.

### Beta-Adrenergic Agents

A number of investigators have investigated the beta-adrenergic agents used to inhibit labor for their effects on fetal lung. Wyszgrodski et al. (1974) noted that 3 hr after injecting 28-day-gestation fetal rabbits with isoxsuprine, the rabbit lungs were more stable to deflation when air filled. This observation was confirmed by Corbet et al. (1977) and extended to show that propranolol blocked the isoxsuprine-mediated effects on lung stability. Terbutaline, another beta-adrenergic agent, caused similar effects on fetal rabbit lung (Bergman et al., 1978). Biochemical studies showed that 1 day after a 24-hr treatment course of isoxsuprine given to pregnant does, both total lung phosphatidylcholine and the lecithin/sphingomyelin ratio of the alveolar wash was increased relative to untreated controls (Hayden et al. 1977). This result suggests that longer-term treatment may affect total synthesis, while the short-term studies support a stimulatory effect of isoxsuprine on surfactant secretion. Enhorning et al., 1979 confirmed the short-term effects of isoxsuprine administered to both fetal rabbits and

pregnant does and further observed degranulation of the type II pneumocytes and dehydration of the fetal lung. This result is in agreement with studies of fetal lung fluid in sheep. Lawson et al. (1978) demonstrated an acute decrease in the flow of fetal lung fluid and an increase in surfactant concentration during an infusion of epinephrine into the fetus. Walters and Olver (1978) also found a decrease in fetal lung fluid production during epinephrine or isoproterenol infusions.

Experiments in adult animals have demonstrated that neurocontrol mechanisms apparently control surfactant secretion. Acetylcholine infusion into the pulmonary artery of rabbits, vagal stimulation, and hyperventilation stimulate surfactant release, while atropine blocked the effects of hyperventilation (Oyarzun and Clements, 1977). Similarly, pilocarpine given to fetal rabbits 2-5 hr before sacrifice improved the pressure-volume curves of the treated fetuses, indicating surfactant secretion (Corbet et al., 1976). As catecholamine-mediated effects are generally via the cAMP second messenger system, the short-term stimulation of surfactant secretion by beta-adrenergic agents and the suggested long-term stimulation of surfactant synthesis may be mediated by mechanisms similar to those suggested for aminophylline. A recent study in adult rats suggests that dibutyryl cyclic-guanosine 5'-monophosphate may cause surfactant secretion (Klass, 1979).

The animal studies indicate that stimulators of surfactant secretion might well prepare the premature human for delivery, as the human fetus has large tissue stores of surfactant (Figure 2). The data from animals suggest that short-term therapy is sufficient to stimulate surfactant secretion. Most protocols for the use of corticosteroids to induce fetal lung maturation also involve the use of a beta-adrenergic drug to inhibit labor and thus expose the fetus to two agents that may influence lung maturation. Two clinical studies (Kero et al., 1973; Boog et al., 1975) suggest that these agents, when used to suppress premature labor, may decrease the incidence of RDS; however, the controlled studies of corticosteroid therapy also used beta-adrenergic agents to inhibit labor, and RDS was frequent in infants not treated with corticosteroids. Furthermore, infants treated with corticosteroids and born after failure of isoxsuprine therapy to inhibit labor for more than 48 hr have a high incidence of RDS. A recent study demonstrated an increased risk of hypocalcemia, hypoglycemia, and neonatal death without a decrease in incidence of RDS following maternal treatment with isoxsuprine (Brazy and Papkin, 1979).

### Thyroxine and Thyrotropin-Releasing Factor

While the lack of thyroid hormones has many detrimental effects on the newborn human, the human fetus grows and develops reasonably normally without thyroid function. However, Wu et al. (1973) demonstrated an increase in the number of lamellar bodies and decreased surface tension in the lungs of fetal rabbits following treatment with thyroxine ($T_4$). A recent anatomic study using rats noted accelerated lung development in animals receiving $T_4$, and possibly a cooperative effect of both $T_4$ and glucocorticoids in accelerating lung development (Hitchcock, 1979). Triiodothyronine ($T_3$) and $T_4$ do not cross the placenta; thus the maternal route of administration cannot be used to accelerate fetal lung maturation. However, thyrotropin-releasing factor (TRF) will cross the placenta and cause the endogenous secretion of thyroid hormones. Rooney et al. (1979) reported that while no changes in lung phospholipids are detected, alveolar phosphatidylcholine increases two to five times, suggesting that TRH may stimulate surfactant secretion rather than synthesis. A noniodinated $T_4$ analog

(3,5-dimethyl-3'-isopropyl-L-thyronine) that crosses the placenta stimulates choline incorporation into phosphatidylcholine, decreases fetal lung glycogen, and increases airway saturated phosphatidylcholine without changing tissue phospholipids or saturated phosphatidylcholine (Ballard et al., 1980b). The pattern of change of enzymatic activities of the phospholipid synthetic pathways suggests maturational effects different from those caused by corticosteroids. Possible mechanisms of action of thyroid hormones on lung maturation have been reviewed by Ballard (1979). A single uncontrolled clinical trial showed rapid fetal lung maturation as assessed by a microviscosity assay of amniotic fluid after intra-amniotic administration of $T_4$ to eight women (Mashiach et al., 1978). None of the infants had RDS at delivery. This suggestive experience is consistent with the animal studies; however, insufficient data concerning the effects of thyroid hormones are available to presently recommend anything other than carefully controlled trials.

### Estrogen

The possible effects of estrogens on lung maturation have only recently been explored systematically. Khosla and Rooney (1979) gave 17β-estradiol to pregnant does at 25 and 26 days gestational age and noted a fourfold increase in phosphatidylcholine recovered by lung lavage at 27 days gestational age and 1.6 times as much phosphatidylcholine in the washed lung tissue as in controls. The increases noted exceed those documented in similar studies using corticosteroids and suggest that 17β-estradiol stimulates both synthesis and secretion of surfactant. Estrogen may affect lung tissue directly, as similar effects were noted on explants of fetal lung in organ culture (Gross et al., 1979). Fetal female rabbit lungs have greater stability on deflation and greater anatomic maturity than do the lungs of the male littermates (Kotas and Avery, 1980). No clinical studies have reported the administration of estrogens to pregnant humans by protocols which could be expected to adequately test the hypothesis that estrogens induce pulmonary maturation. However, corticosteroid treatment for the prevention of RDS was curiously noted to be ineffective for males weighing 1250-1750 g, while females experienced a fourfold reduction in RDS (Ballard et al., 1980a).

### Prolactin

The possibility that prolactin may stimulate lung maturation was raised by Hamosh and Hamosh (1977), who showed increased phosphatidylcholine and saturated phosphatidylcholine content in lungs from fetal rabbits who were administered ovine prolactin purchased from Sigma Chemicals Co. An association of low prolactin levels in the cord blood of infants with RDS indicated that prolactin might have an effect on lung maturation (Gluckman et al., 1978); however, neither Ballard et al. (1978) nor Cox and Torday (1978) found changes in the pulmonary phospholipids of fetal rabbits following the administration of prolactin obtained from The National Institutes of Health. Also, no effect was apparent on surfactant flux of fetal lung fluid during or after 5 days of infusion of prolactin to fetal sheep (Ballard et al., 1978). Furthermore, Van Petten and Bridges (1979) showed no changes on the pressure-volume curves caused by prolactin given to fetal rabbits. Recent experiments do not support a role for prolactin in pulmonary maturation.

### Other Effectors

A number of other agents have been studied with respect to lung maturation. Insulin is of particular interest because of the well-known association between maternal diabetes

and RDS in infants. Most of the studies with insulin have involved in vitro techniques, as animal models of diabetes are difficult to study. Smith et al. (1975) showed in primary culture of rabbit fetal lung cells that insulin blocked the corticosteroid-mediated stimulation in phosphatidylcholine synthesis. Fetal lung explants in organ culture showed increased glycogen content and delay in morphological maturation when exposed to insulin (Gross, 1979). These findings suggest that insulin may delay normal lung maturation, a concept consistent with the occurrence of RDS late in gestation in diabetic pregnancies.

Epidermal growth factor is a biologically active polypeptide isolated from male mouse submaxillary glands which causes early tooth eruption and eye opening in mice. This agent will induce accelerated lung maturation when administered to fetal rabbits (Catterton et al., 1979). Smith et al. (1974) have demonstrated that medium pretreated by exposure to fetal lung fibroblasts will stimulate the synthesis of saturated phosphatidylcholine by cloned human type II cells. This factor will also induce lung maturation when given to fetal rats (Smith, 1979).

### Pregnancy-Related Stimuli

Tests of lung maturity when applied to uncomplicated pregnancies generally predict the absence of the RDS after 35 weeks of gestation. However, the majority of prematurely born infants do not develop RDS, risk being dependent on the degree of prematurity. None of the approximately 7% of pregnancies terminating before 37 weeks can be considered to be normal, and many of the causes of prematurity that have been identified are associated with decreased incidences of RDS (Table 3). Possibly it is helpful to think of the premature infant with RDS as the normal premature, while the protected infant has somehow "sensed" the impending premature delivery and lung maturation has occurred. What the physiological stimuli are remains to be demonstrated; however, the known causes of accelerated lung maturation could be said to be stressful. Placental "insufficiency" associated with infarction, maternal hypertension, severe pregnancy-induced hypertension, other maternal illnesses, and intrauterine growth retardation are associated with a decreased incidence of RDS (Morrison et al., 1977; Okada and Thibeault, 1977; Bustos et al., 1979; Skjaeraasen, 1979). Premature labor and delivery

**Table 3** Some Pregnancy-Related Conditions Affecting Lung Maturation

| |
|---|
| Accelerated maturation |
| chronic maternal hypertension |
| maternal cardiovascular disease |
| placental infarction |
| severe pregnancy-induced hypertension |
| prolonged rupture of membranes |
| incompetent cervix |
| intrauterine growth retardation |
| racial differences |
| hemoglobinopathies |
| Delayed maturation |
| diabetes mellitus |
| Rh isoimmunization with hydrops fetalis |
| nonhypertensive maternal renal disease |

may also help stimulate lung maturation, consistent with the decrease in the occurrence of RDS in vaginally delivered infants. Labor has been shown to decrease lung water content (Bland et al., 1979) and stimulate surfactant synthesis and secretion in rabbits (Rooney et al., 1977). Prolonged premature rupture of membranes remains a controversial maturation stress for the premature fetus, being statistically associated with a decrease in the incidence of RDS in some studies (Kulovich and Gluck, 1979) but not in others (Schreiber and Benedetti, 1980). The concept of stress suggests elevated fetal catecholamine and corticoid levels, agents that can cause accelerated lung maturation; however, many agents seem to effect lung maturation and no agent to date can predictably prevent RDS in all infants.

## TESTS OF FETAL LUNG MATURATION

### Physiology

The tests of fetal lung maturation depend upon amniotic fluid composition reflecting the status of the fetal lung. As discussed above, the lung secretes lung fluid and any surfactant released into that lung fluid throughout late gestation. From studies in fetal sheep, the flow of fluid out of the lung is episodic (Dawes, 1973) and the swallowing or release of this fluid to the amniotic cavity is controlled by the larynx (Adams et al., 1967). Clements and Tooley (1977) aptly described the amniotic fluid as the fetal cesspool, containing all fetal excretions as well as desquamated cells and other biological matter whose volume and composition are variable and dependent upon the gestational age of the fetus. Therefore the pulmonary surfactant must contribute appreciably to whichever aspect of surfactant biochemistry or physical properties are to be utilized to assess pulmonary maturation. A number of pieces of evidence indicate that surfactant-associated properties can be distinguished from the complex mixture that is amniotic fluid. The amniotic fluid of mature fetuses contains a high proportion of saturated phosphatidylcholine relative to the unsaturated species of phosphatidylcholines that are from nonpulmonary sources (Helmy and Hack, 1962). The amniotic fluid also contains an astonishing amount of saturated phosphatidylcholine late in gestation, estimated to be 100-200 mg, an amount comparable to the lung tissue surfactant-saturated phosphatidylcholine of the human fetus (Clements and Tooley, 1977). Another surfactant-specific phospholipid, phosphatidylglycerol, is present in amniotic fluid late in gestation (Bustos et al., 1979). The amniotic fluid also contains lipid aggregates similar to the lamellar bodies identified within the type II pneumocytes of fetal lung (Jiminez and Johnston, 1976), and a high level of the lamellar body-associated enzyme phosphatidic acid phosphatase (Figure 3, III) (Herbert et al., 1978). The surfactant apoprotein appears synchronously with surface-active phospholipids in the amniotic fluid of humans, and this protein is not found in human tissue other than lung (King et al., 1975). Thus there remains little question that the amniotic fluid can reflect the status of fetal lung maturation.

### The Lecithin/Sphingomyelin Ratio

The first successful use of amniotic fluid to predict lung maturity was the measurement of the content of lecithin (phosphatidylcholine) relative to sphingomyelin (L/S ratio) proposed by Gluck et al. (1971). Since that time a large number of tests for lung maturity measuring either biochemical or surface-active characteristics of amniotic

fluid-associated surfactant have been proposed. O'Brien and Cefalo (1980) recently reviewed pulmonary maturity testing and cited 54 references, these references representing only a part of the vast literature that appears to be exponentially accumulating concerning evaluations of fetal lung maturity. This discussion will selectively refer to this literature.

Biochemical methods of assessing pulmonary maturity emphasize aspects of surfactant unique among components of amniotic fluid. The L/S ratio was best reviewed by Gluck et al. (1974). This measurement relies on a cold acetone precipitation step to selectively separate predominately saturated species of phosphatidylcholine (surface-active species) and some other phospholipids from the unsaturated phosphatidylcholines in amniotic fluid. The precipitated phospholipids are then separated by thin-layer chromatography, and either the reflected densities or the areas of the phosphatidylcholine (lecithin) and sphingomyelin spots are expressed as a ratio. Sphingomyelin content of amniotic fluid changes little during late gestation and is used to normalize the measurement of phosphatidylcholine against a convenient internal standard. This convention is said to automatically correct for variations in amniotic fluid volume, amniotic fluid concentration, sample size, and losses during the procedure. The use of the ratio increases the reliability of the measurement, as no absolute measurement is required. The normal change in the L/S ratio with advancing gestation is shown in Figure 4; empirically a ratio greater than 2 as measured by reflectance densitometry indicates lung maturity, a situation that normally occurs after 35 weeks of gestational age. Published reports of a mature L/S ratio in well over 4000 pregnancies predicted the absence of RDS correctly (independently of gestational age) between 95 and 98% of the time, the inaccurate predictions primarily involving infants of diabetic mothers, infants with Rh disease, and asphyxiated infants (O'Brien and Cefalo, 1980). This remarkable record and vast experience makes the L/S ratio the gold standard against which all other tests must be compared. The methodology of the L/S ratio has stimulated a search for a simpler test. The measurement requires meticulous attention to the details of the procedure, thin-layer chromatography, and a reflectance densitometer or other equipment for area measurements. This sort of organic chemistry is not routine in most laboratories and is not automated. Anyone attempting the measurement should consult Gluck et al. (1974).

### Other Biochemical Measures of Lung Maturity

Other biochemical assessments of lung maturity are measurements of total phospholipid phosphorus (Sbarra et al., 1977), amniotic fluid phosphatidylcholine (Lindback et al., 1974), and the amount of palmitic acid present in amniotic fluid (Lindback, 1976). These estimates rely on the large pulmonary maturation-dependent increase in saturated phosphatidylcholine entering the amniotic fluid and have little advantage over the L/S ratio. Total phospholipid phosphorus, phosphatidylcholine, and palmitic acid content will all indirectly reflect the quantity of saturated phosphatidylcholine. Others have proposed measuring the quantity of saturated phosphatidylcholine per milliliter of amniotic fluid as a more specific test. The saturated phosphatidylcholine can be selectively recovered from amniotic fluid by oxidation procedures and will predict lung maturity (Curbelo et al., 1978; Torday et al., 1979). As phospholipid measurements are unpalatable to many clinical laboratories, measurements of phosphatidic acid phosphatase by enzymatic assay (Herbert et al., 1978) or quantitation of the surfactant

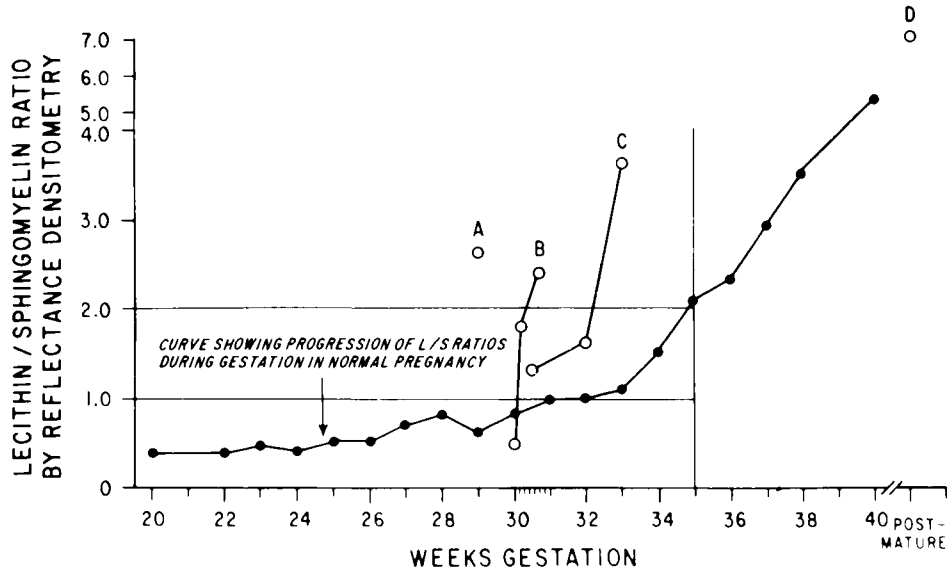


**Figure 4** The L/S ratio versus gestational age. The curve for the normal change in the L/S ratio with gestation is shown in contrast to ratios measured from complicated pregnancies: (A) L/S ratio following an early case of chronic abruptio placentae, (B) L/S ratio following premature rupture of membrane, and (C) L/S ratio in a case of repeated early intrauterine death. (D) L/S ratio with postmaturity. (From Gluck et al., 1974.)

specific apoprotein by radioummunoassay (King et al., 1975) have been suggested. Much more experience with both normal and complicated pregnancies is needed before tests other than the L/S ratio should be applied generally in the clinical setting.

### Measurements of Surface Activity

The most useful measurement of surface properties of amniotic fluid to assess lung maturity is the shake test or foam stability test proposed by Clements et al. (1972). This bedside test measures the stability of surface bubbles formed by shaking 95% ethanol and amniotic fluid mixed in specific proportions. The ethanol inactivates foaming agents in the amniotic fluid other than the surface-active phospholipids. The scoring of the persistence of the bubbles roughly quantifies the overall surface-active properties and quantity of the fetal lung-derived phospholipid. A positive test will predict fetal lung maturity with great reliability; however, a negative test is poorly correlated with fetal lung maturity. The shake test is an ideal screening test which can be performed within minutes to help decide which amniotic fluids should be further studied. Other tests of surface activity requiring direct measurements of surface tension will predict lung maturity (Shelley et al., 1973; Goldkrand et al., 1977). The accurate measurement of surface activity requires care and special instrumentation and is unlikely to achieve general clinical acceptance. Recently a simple observation of the behavior of amniotic fluid in a capillary tube has been used to assess pulmonary maturity (Sing, 1980).

### Biophysical Measures of Lung Maturity

An intriguing approach to amniotic fluid measurements is to assess the fluorescence depolarization caused by the phospholipids suspended in amniotic fluid. A microviscosimeter (FELMA, Elscint, Ltd., Haifa, Israel) measures the degree of fluorescence depolarization (p) of an amniotic fluid sample containing a diphenylhexatriene dye; the p value is correlated with lung maturity. Different authors have reported various critical p values for the assessment of lung maturity (Gonen et al., 1978; Elrad et al., 1978). The p value also appears to be influenced by phospholipids other than sphingomyelin and phosphatidylcholine; both phosphatidylinositol and phosphatidylglycerol decrease p toward mature values (Blumenfeld et al., 1979). The advantages of this test are that it is simple, requires very little amniotic fluid, and is quick to perform once the instrument is purchased; however, further clinical trials seem warranted.

Sbarra et al. (1978) proposed that a simple measurement of the optical density of amniotic fluid at 650 nm was useful for the prediction fetal lung maturity. Subsequent reports indicated that optical density measurements at either 400 or 650 nm were no substitute for the L/S ratio in assessing lung maturity (Arias et al., 1978; Spellacy et al., 1979).

This has not been an exhaustive review of the tests for assessing fetal lung maturity; others and variations of each of the tests described continue to appear in the search for the perfect test. This ideal test should be simple to perform and inexpensive and should reliably predict infants who can be safely delivered without risk of RDS, as well as identify precisely which infants will develop RDS. Infants with immature L/S ratios or shake tests will develop RDS only 20-60% of the time (O'Brien and Cefalo, 1980). Furthermore, the ideal test should accurately predict pulmonary maturity in complicated pregnancies, as this is often the clinical situation where such information is most useful. The L/S ratio is said to be less reliable in diabetic pregnancies; as many as 18% of these pregnancies with an L/S ratio greater than 2 resulted in infants with the respiratory distress syndrome (Meuller-Heubach et al., 1978). Others have noted only a 5% incidence of respiratory disease in infants of diabetic mothers with L/S ratios greater than 2 (Lowensohn and Gabbe, 1979).

### The Lung Profile

Kulovich et al. (1979) have proposed the lung profile as a refinement of the L/S ratio that will provide much more information to the clinician. The lung profile will be described in some detail because of its apparent high reliability and its content of information about lung maturity. Phospholipids other than saturated phosphatidylcholine are in surfactant and are important for function (Table 2). The composition of the phospholipids in the surfactant of developing experimental animals and humans changes as maturity approaches (Hallman and Gluck, 1976). These changes are reflected in the human by changing phospholipid patterns in amniotic fluid (Figure 5) and are very well correlated with clinical assessments of lung maturity. The two phospholipids other than phosphatidylcholine that are of interest are phosphatidylglycerol and phosphatidylinositol. Phosphatidylglycerol is present in appreciable amounts only in lung tissue and surfactant; elsewhere trace amounts are detectable as a precursor of mitochondrial cardiolipin. Phosphatidylglycerol is absent from the amniotic fluid of infants who

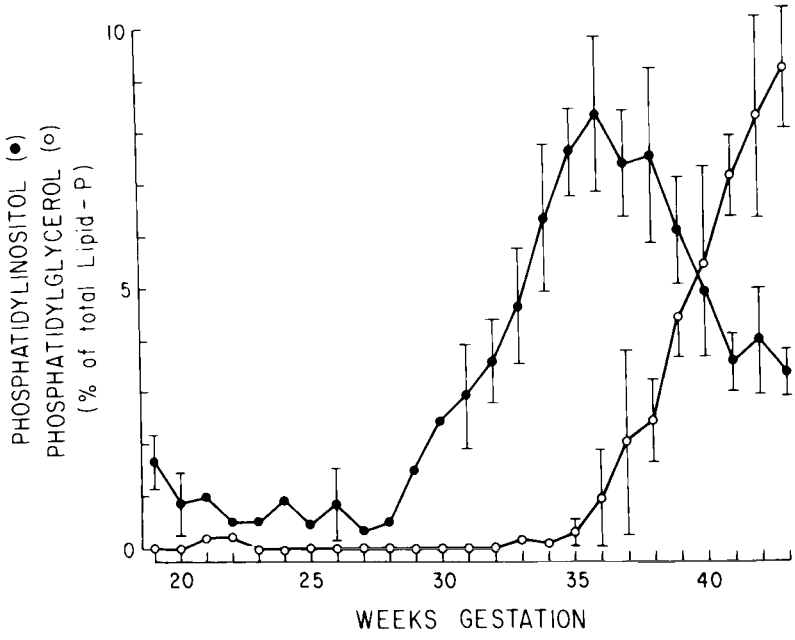


**Figure 5** Changes in the content of phosphatidylglycerol and phosphatidylinositol in amniotic fluid during late gestation. The percentage of phosphatidylinositol and phosphatidylglycerol as measured by phosphate content in amniotic fluid samples from normal pregnancies is expressed versus gestational age (values are mean ± SD). (From Hallman et al., 1976.)

develop RDS and appears in the tracheal aspirate of these infants as the disease resolves (Hallman et al., 1977). Thus the presence of phosphatidylglycerol in amniotic fluid will accurately predict fetal lung maturity even when the samples are heavily contaminated with blood or meconium. Phosphatidylinositol elevations in amniotic fluid precede an L/S ratio greater than 2 and the appearance of phosphatidylglycerol. A high amniotic fluid phosphatidylinositol is correlated with a maturing lung and a decreased risk of RDS in spite of a low L/S ratio. A complete lung profile gives the L/S ratio, the percentage of saturated phosphatidylcholine, and the relative content of phosphatidylinositol and phosphatidylglycerol in the amniotic fluid specimen. Using this test, Kulovich et al. (1979) reported that while an L/S ratio greater than 2 predicted the occurrence of RDS correctly only 69% of the time, the lung profile was correct 93% of the time. No mature lung profiles were associated with RDS, and similar predictability has been confirmed by others (Golde and Mosley, 1980). The lung profile also has been evaluated in diabetic pregnancies, and the presence of phosphatidylglycerol indicates that no RDS will occur after delivery (Kulovich and Gluck, 1979). This more elaborate test uses multiple pieces of information about the maturation of the mammalian lung.

The cost of the excellent reliability of the lung profile is increased complexity of the determination. The test requires two-dimensional thin-layer chromatography on 20-X-20-cm silica gel plates and some experience in interpreting and quantifying the

spots–certainly not a test for occasional use in a general hospital laboratory. One-dimensional thin-layer chromatographic separations of phosphatidylglycerol, phosphatidylcholine, and sphingomyelin have been described and may give the essential information contained in the lung profile (Gotelli et al., 1978; Tsai and Marshall, 1979). The L/S ratio is adequate for the assessment of lung maturity for most obstetric situations; however, the extra information available from the lung profile may be particularly useful for large high-risk delivery services.

### Reasons for Incorrect Assessment of Pulmonary Maturity

The L/S ratio progressively increases in normal pregnancies from about 26 weeks of gestation (Figure 4). A significant delay between an assessment of pulmonary maturity and delivery would be expected to be associated with a decrease in the incidence of RDS. Figure 6 summarizes some of the effectors of pulmonary maturity that have been discussed. Four steps between surfactant synthesis by the fetal lung and appearance of that surfactant in amniotic fluid are identified. The time relations for these events are not accurately known in man. The time from surfactant synthesis to maximal labeling of phosphatidylcholine in the fetal lung fluid as an assessment of surfactant secretion occurs many hours after synthesis in normal premature and term rabbits (Jobe et al., 1978b). Amniotic fluid is downstream from the fetal lung fluid and changes in amniotic fluid should occur later. If betamethasone is given by intra-amniotic injection to premature baboons, a significant increase in the L/S ratio is noted 48 hr later (Kotas et al., 1978). Metyrapone, an 11β-hydroxylase inhibitor of corticoid synthesis, does not prevent normal lung maturation in baboons; however, the L/S ratio fails to rise, indicating that the maturation has occurred (Kotas and Kling, 1979). This result encourages caution in interpreting tests of lung maturation in pharmacologically treated pregnancies. Experiments in sheep indicate a dramatic increase in surfactant flux following corticosteroid treatment (Platzker et al., 1975). Human studies generally involve complicated pregnancies at risk for premature delivery and the use of tocolytic agents. While pulmonary maturity is often achieved 48 hr after betamethasone therapy, the L/S ratio is inconsistently elevated to mature values by such therapy. While Zuspan et al. (1977) reported an increase in the L/S ratio in 12 of 17 pregnancies treated 48-72 hr previously with hydrocortisone succinate, Caritis et al. (1977) measured only minimal changes after treatment of Rh-sensitized pregnacies with betamethasone. In a controlled study, Arias et al. (1979) noted a

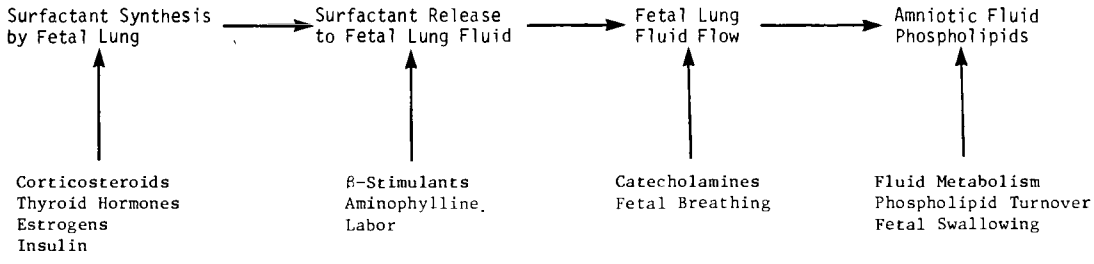


**Figure 6** Fetal lung-amniotic fluid relationships, with a few of the possible effectors that may influence how the amniotic fluid will reflect fetal pulmonary surfactant metabolism.

small increase in the L/S ratio 1 week following therapy in 11 patients treated with betamethasone. However, only 1 of 11 reached a mature value. These clinical studies suggest that the delay between fetal lung maturity and the detection of that maturity may be a number of days. Clements and Tooley (1977) used the data from sheep to suggest that 4-8 days might be required for the L/S ratio to increase from 1 to 2 following maturation. The lag between fetal lung maturation and sufficient surfactant accumulation in the amniotic fluid to reflect that maturation probably explains many of the pregnancies with immature tests which result in infants without RDS.

Another clinical situation is the rapidly increasing L/S ratio early in gestation. Such a case is illustrated in Figure 4, where following premature rupture of membranes at 30 weeks the L/S ratio changed from 0.5 to 1.8 within 48 hr. One can propose an explanation by referring to Figure 6. Premature rupture of membranes or the events causing it are a stress to the fetus, causing lung maturation (via corticosteroids) and surfactant secretion (elevation of fetal catecholamines) to a now small residual amniotic fluid pool, resulting in an acute elevation of the L/S ratio. The point of this hypothetical explanation is to illustrate the multiple inputs that may ultimately affect any measurement of pulmonary maturity. We do not know the stimulus-response times for any of the possible effectors of human fetal lung maturation. Each stimulus may effect not only the synthesis of surfactant, but also secretion, fetal lung fluid flow, or the metabolism of amniotic fluid. In fact, the site of amniocentesis either close to or away from the fetal head may affect the L/S ratio (Worthington and Smith, 1978). It is surprising that so many different assessments of pulmonary maturity are reasonably accurate, even in complicated pregnancies.

Furthermore, incorrect predictions can be anticipated that relate to the obstetric management and evaluation of the newborn. Infants with marginal lung maturity may develop severe respiratory disease if asphyxiated at birth. This experience has increased the use of cesarean section delivery for tiny premature infants. The premature infant has few ways to respond to stress and mishandling, and respiratory distress predominates all symptoms. Infants with the sepsis-pneumonia syndrome caused by the group B streptococcus, with "shock lung" secondary to asphyxia, or with heart failure may be very difficult to distinguish from RDS caused by surfactant deficiency. As neonatal and obstetric care has improved, smaller and smaller infants are surviving. Some of these infants have respiratory distress that is of late onset and quite atypical for RDS. These tiny infants may be difficult to classify into traditional disease entities, as our understanding of pulmonary disease in tiny infants is still evolving. Accurate follow-up of fetal pulmonary maturity testing demands a close scrutiny of the infant's chart, using appropriate criteria for diagnosing RDS.

## THE RESPIRATORY DISTRESS SYNDROME

### The Disease

Recent advances in the obstetric care of premature labor together with the neonatal care of the premature infant and tests of fetal lung maturity have affected the incidence of RDS. A recent report could not document an effect of glucocorticoids on the incidence of RDS when the medical management was designed to assure unstressful labor, atraumatic delivery, and excellent neonatal care. The incidence of RDS in both treated and comparison groups was only about 15% (Quirk et al., 1979). However, iatrogenically

terminated pregnancies resulting in sick premature infants remain common. In a series of 1000 infants admitted to a neonatal center, 32 prematures were delivered electively without adequate assessment of fetal gestational age or lung maturity. Of these infants, 24 developed RDS (Flaksman et al., 1978). Neonatal mortality statistics for the United States from 1968 to 1974 indicate falling mortality rates but an increased incidence of death due to RDS, which accounts for about 12,000 deaths per year (Manniello and Farrell, 1977). The relative increase in incidence was attributed to increased time of survival of premature infants, resulting in the diagnosis of death being RDS rather than asphyxia, as no increase in the incidence of prematurity was identified. Respiratory distress syndrome will remain a common disease in neonatal units; however, the preceding obstetric histories and infant size will change, no doubt.

Since Avery and Mead (1959) demonstrated decreased surface activity of lung extracts from infants who died with hyaline membrane disease, the importance of surfactant deficiency in the pathogenesis of RDS has been appreciated. I will define RDS as respiratory failure that is progressive from birth in the premature infant caused by inadequate surfactant function superimposed upon a structurally immature lung. This definition stresses many of the aspects of the disease which will help the clinician identify affected infants. While aspects of the syndrome depend somewhat on the size and gestational age of the premature infant, the severity of respiratory failure can be related to time after birth. The disease can be divided into five stages (Figure 7): (1) The premature birth is often accompanied by some tachypnea, cyanosis, and mild asphyxia, and most infants who develop RDS are distressed shortly after birth. (2) The infant develops progressive respiratory failure characterized by tachypnea with expiratory grunting, chest retractions, nasal flaring, and increased hypoxemia and acidosis. A progressively worsening situation requiring more supplemental oxygen and/or respiratory support characterizes the first 24-48 hr of life. (3) The next 24-48 hr of the clinical course is often remarkable for the prolonged stability of the clinical situation at the most severe level of the infant's disease. (4) The severe respiratory failure then rapidly and progressively improves over several days until respiratory support and oxygen are no longer required. (5) The final healing of the lungs probably occurs over many weeks. This clinical course is seen most characteristically in large, more

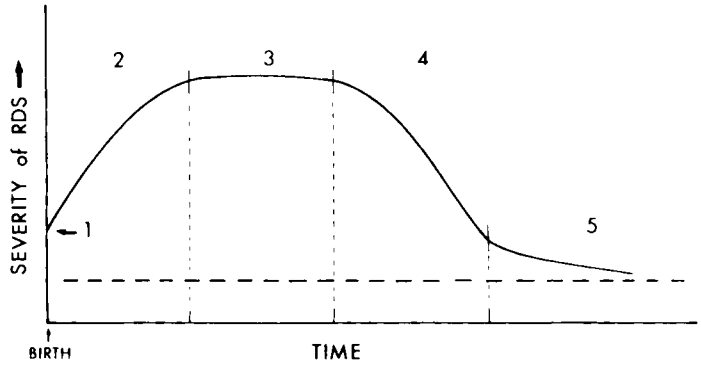


Figure 7 The clinical course of respiratory distress syndrome (RDS). This curve shows the severity of the clinical syndrome versus time. The time scale of the abscissa is discussed in the text. Normal lung function for age is indicated by the dashed line.

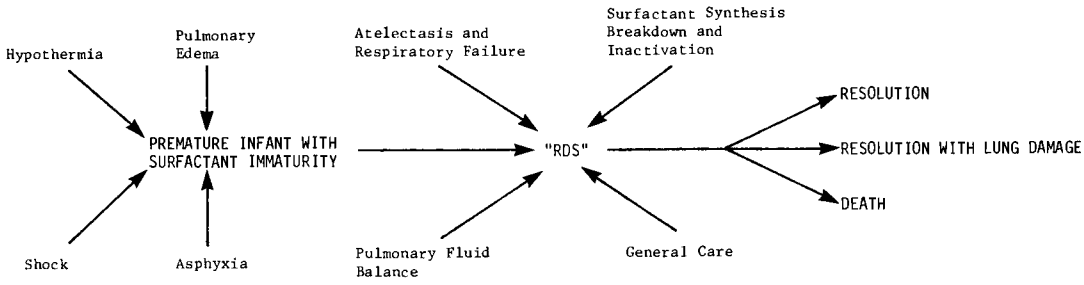


**Figure 8** Effects on the respiratory distress syndrome (RDS), with a schematic illustration of some of the inputs that may effect the clinical course and outcome of RDS.

mature infants with RDS and may be influenced by therapeutic interventions. My definition includes "inadequate surfactant function" rather than surfactant deficiency, because the incidence and severity of RDS can be appreciably influencd by the management of the premature infant during labor, delivery, and the first few hours of life (Jones et al., 1975). Some of these influences are illustrated in Figure 8. Not only may the surfactant of the premature infant be deficient in quantity, but also the phospholipid composition and surface-active properties of that surfactant are abnormal (Hallman and Gluck, 1976). Apparently premature infants with marginal surfactant reserves are very susceptible to such insults as asphyxia, shock, hypothermia, and pulmonary edema and will present with a syndrome which may be clinically indistinguishable from RDS. These insults must affect surfactant function.

A pattern of atelectasis of the majority of the gas exchange airways combined with overdistension of those airways remaining aerated is responsible for the ground-glass appearance of the chest film of infants with RDS. The progressive atelectasis results from increasingly inadequate surfactant function over the first day or two of life, implying that synthesis and secretion of surfactant are inadequate to meet the needs of the infants. The eventual stabilization and resolution of the atelectasis signals the secretion to the airways of surfactant with the appropriate surface activity properties. Is there experimental evidence for such a scenario relating surfactant metabolism to the sourse of RDS? Phospholipid analyses of tracheal aspirates of infants with the respiratory distress syndrome indicate high phosphatidylinositol levels, no phosphatidylglycerol early in the disease, and the appearance of phosphatidylglycerol as the disease resolves. The surface activity of the phospholipids also improves as the phospholipid composition of the surfactant "matures" (Hallman et al., 1977). However, no data exist concerning the kinetics of surfactant synthesis and secretion in infants with RDS. Experiments with premature and term newborn rabbits (Figure 9) indicate that the time from synthesis to secretion of surfactant phospholipids is longer in newborn than in adult rabbits, indicating a difference in surfactant secretory mechanisms between adult and newborn animals (Jobe, 1977; Jobe et al., 1978a).

Inactivation or metabolic breakdown of surfactant could aggravate a surfactant deficiency state. No measurements of surfactant half-life have been made in the human, and while the half-life of surfactant phospholipids is about 16 hr in small rodents (Tierney et al., 1967; Jobe, 1977), the half-life of surfactant-associated phosphatidylcholine in newborn rabbits is much longer (Jobe et al., 1978a). These

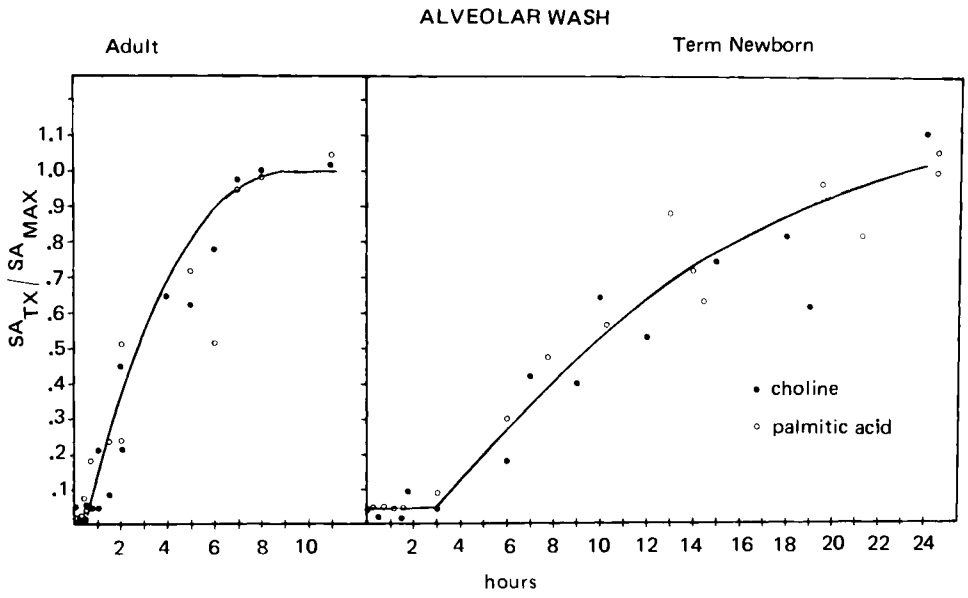


**Figure 9** Increase in specific activity of phosphatidylcholine recovered from the airways by alveolar wash. Newborn rabbits and adult rabbits received radiolabeled choline or palmitic acid intravenously. These radiolabeled precursors were incorporated into lung phosphatidylcholine and secreted to the alveolar space. The two curves demonstrate the time in hours required for the newly secreted phosphatidylcholine to saturate the alveolar pool. The data are expressed as normalized specific activities (SA) by dividing the specific activity at a time x (t = x) by the maximal specific activity in the alveolar wash (SA/max). (Data for newborn from Jobe et al., 1978a, and data for adult rabbits from Jobe, 1977.)

biological half-life values can be anticipated to be much longer in larger animals and man (Jobe et al., 1979). Inactivation of surfactant may play a role in the pathophysiology of RDS. While the air spaces normally exclude plasma proteins, the hyaline membranes identified pathologically contain plasma proteins as well as fibrin (Gitlin and Craig, 1956). The changes in pulmonary perfusion and pulmonary hypertension characteristic of RDS superimposed on atelectasis and pulmonary damage secondary to therapy may cause a transudation of fluid and proteinaceous material into the alveolus and inhibit surfactant function. While direct experimental evidence for this hypothesis is lacking, the effects of asphyxia and hypothermia on RDS support such an idea. Strang (1979) has recently discussed the possible effects of pulmonary fluid balance on the clinical course of RDS.

These multiple "potentiating influences" on the severity of RDS are most important in determining the ultimate outcome of the tiny infants now being salvaged. As parental, obstetric, and neonatal expectations have risen, many neonatal units are experiencing a large increase in viable admissions weighing less than 1000 g. Our clinical experience indicates that an asphyxic or hypothermic episode during neonatal care can convert mild lung disease to severe and often fatal lung disease in the tiny infant. Another source of cardiopulmonary stress for the tiny infant is the continued patency of the ductus arteriosis after birth. While all infants with RDS may develop pulmonary

edema and heart failure during resolution of RDS because of a patent ductus arteriosus, the tiny infant may present with symptoms clinically indistinguishable from RDS which are primarily related to increased pulmonary flow caused by the open ductus arteriosus (Jacob et al., 1980). Indomethacin, a prostaglandin synthetase inhibitor, will medically close the patent ductus arteriosis in many of these infants and simplify management (Friedman et al., 1976). The safety of this potent agent for the treatment of the patent ductus arteriosus syndrome is under evaluation. Indomethacin has been suggested as an agent to be used for the inhibition of premature labor. A number of case reports suggest that such usage may cause closure of the fetal patent ductus arteriosis, resulting in fetal death or the persistent fetal circulation syndrome (Levin et al., 1978). This experience should discourage the use of prostaglandin synthetase inhibitors for the inhibition of labor.

### Therapy for RDS

This section will briefly outline and discuss some of the strategies presently used for the treatment of RDS. Recent reviews describe the myriad of details and cautions necessary to optimize neonatal respiratory care (Strang, 1977; Thibeault and Gregory, 1979). Mild to moderate degrees of atelectasis in a vigorous baby with good respiratory drive will result primarily in low $paO_2$ values in the infant, without significant hypercarbia or acidosis. Sufficient therapy simply may be to increase inspired oxygen concentrations in the incubator or in a hood surrounding the infants's head. Infants with the RDS generally will require more therapy if more than 50-60% oxygen is required. Oxygen concentations must be accurately and frequently regulated by analysis of the infant's blood gases. The use of a simple oxygen hood does not decrease the monitoring the infant requires or the risk of retrolental fibroplasia if prolonged hyperoxia occurs.

Gregory et al. (1971) first applied continuous pressure (continuous positive airway pressure) to the airways of infants with RDS to reverse the diffuse atelectasis and thus improve oxygenation. This pressure is commonly applied via nasal prongs or an endotracheal tube to infants heavier than 1200-1500 g who require more than 50-60% oxygen and who have a strong respiratory drive. The administration of positive pressure to the properly resuscitated infant will often result in a dramatic improvement in oxygenation, a decrease in respiratory frequency and effort, and a stabilization of the clinical course. Continuous positive airway pressure (CPAP) does not adversely affect cardiovascular function in infants with RDS unless excessive pressures or hypovolemia exists. The apparatus for the administration of CPAP can be constructed easily from parts used for anesthesia, and with experience, CPAP can be very effective therapy for selected infants with RDS (Gregory, 1979). Rather than applying positive pressure to the airway, negative pressure can be applied around the thorax, with similar results.

Recently both the design of infant ventilators and the techniques of artificial ventilation have significantly improved the survival of infants with RDS. These changes have most benefited infants weighing less than 1500 g. Early attempts to ventilate infants made use of volume ventilators to mimic the ventilatory pattern of the distressed infant, using rapid rates and high pressures, with little success. Several problems are unique to the ventilation of infants, as reviewed by Reynolds (1979). The desired tidal volume of 7-10 ml/kg represents a very small volume that cannot be accurately and predictably delivered by the circuitry of the volume ventilator to infants with uncuffed endotracheal tubes, which generally leak. Infants with distress breathe with very rapid shallow respiratory efforts, a pattern that is not effective for tripping a

patient-controlled ventilator. Total control of ventilation of an infant by a ventilator is not desirable, as the infant needs to exercise to prevent "disuse atrophy" in anticipation of his needs to support ventilation with abnormal lungs for some time as RDS resolves. These problems are minimized with the use of continuous flow, pressure-limited ventilators. A resistance to the continuous flow generates positive-end expiratory pressure (PEEP) within the system. The infant thus receives intermittent mandatory ventilation (IMV) of the characteristics, PEEP, and the rate desired by the physician. The infant can assist the ventilatory support by breathing as he desires to supplement the IMV. Tidal volume is not known; however, a pressure is selected which will cause "adequate" chest excursions as assessed clinically. The resulting effectiveness of ventilation which represents the sum of the IMV plus the respiratory efforts of the infant is assessed by blood gas analysis. This use of pressure limitation measured at the connector of the ventilator to the infant's endotracheal tube permits consistent pressure delivery to the infant during IMV; however, the volume delivered may vary, depending upon when during the infant's respiratory cycle the IMV occurs. The pressure delivered by the ventilator may not reflect the pressure transmitted to the distal airways.

The selection of the maximal pressures, PEEP, inspiratory times, and rates for the ventilation of infants with RDS continues to evolve (Mannino and Gluck, 1979). The goal is to assist ventilation while using minimal supplemental oxygen, pressure, and rates to minimize the risk of damage to the lung, such as pneumothorax and other air leaks or bronchopulmonary dysplasia. Slow rates and low maximal pressures when coupled with prolongation of the inspiratory time to 1 sec or more have been used effectively by Reynolds (1979). Variations of this protocol are in common use for the routine therapy of infants with RDS who require ventilator assistance. Initial ventilator settings used in our unit with Baby Bird or Bourns BP-200 infant ventilators are given in Table 4. The selection of PEEP, rate, and inspiratory times varies little between infants; however, maximal pressure is based on the size of the infant. Smaller infants go into respiratory failure early in the course of RDS. They have more compliant chest walls, less respiratory musculature, and less respiratory drive, and thus respiratory failure can usually be treated with less pressure. The evaluation of the correctness of the pressure selected requires a rapid clinical evaluation of the resulting chest excursions and blood gas analysis. Successful management of infants with RDS also requires meticulous attention to cardiovascular status, fluids, nutrition, and temperature.

The strategy of therapy is to support the infant sufficiently until such time as he can synthesize adequate amounts of qualitatively normal surfactant to mature his own lungs. Pharmacological agents such as steroids administered postnatally have not benefited infants born with RDS. If surfactant deficiency is the problem, treatment of infants with RDS with surfactant should improve the symptoms. Early studies

**Table 4** Initial Ventilator Settings for Infants with RDS

| | |
|---|---|
| $FiO_2$ | As needed to keep $pO_2$ between 50 and 80 mmHg |
| PEEP | 2-4 $cmH_2O$ |
| Rate | 20-30 breaths per minute |
| Inspiratory time | 1.0 sec |
| Peak pressure | |
| infants <1000 g | 12-16 $cmH_2O$ |
| 1000-1500 g | 16-20 $cmH_2O$ |
| >1500 g | 20-25 $cmH_2O$ |

reported little effect of dipalmitoyl phosphatidylcholine on lung function when administered as a mist to infants (Chu et al., 1967). This material probably did not reach the distal airways and pure dipalmitoyl phosphatidylcholine has only some of the properties of surfactant. If natural surfactant (i.e., material isolated by alveolar wash procedures) is instilled in a water solution into surfactant-depleted adult rat lungs, the compliance and surface properties of the lungs return to normal (Ikegami et al., 1979). If natural surfactant is used to treat premature lambs at birth, improvements in survival, blood gases, and pH are noted (Adams et al., 1978). Fujiwara et al. (1980) recently treated 10 neonates with severe RDS with a partially artificial surfactant which was a mixture of an acetone extract of surfactant from bovine lung, dipalmitoyl phosphatidylcholine, and phosphatidylglycerol. The blood gas values and clinical status of the patients improved. The concept of surfactant replacement for a surfactant deficiency disease is attractive; however, much experimental work needs to be done to better understand how this replacement therapy can be best administered. For example, we have shown that surfactant therapy given after respiratory failure is established in 120-day premature lambs can dramatically improve the blood gases, pH, and lung compliance, but the effect only lasts 3-4 hr (Jobe et al., 1981).

## CONCLUSIONS

The complexities of the interrelationships that characterize fetal lung development are not yet fully understood. Fetal adaption to extrauterine life demands anatomic and biochemical maturation sufficient to support this essential transition, which normally has occurred by about 35 weeks of gestational age. However, the fetal human lung has the potential to mature approximately 10 weeks before the normal maturational timing if the fetus receives the appropriate stimuli. The possibility of the dissociation of lung maturity and gestational age means, of course, that tests of pulmonary maturity do not assess fetal size or gestational age. While the prevention of lung immaturity may be a primary goal in the management of the tiny immature infant, the numerous other problems of severe prematurity may dictate the ultimate outcome of such infants. Fetal lung maturity tests must be used in conjunction with assessments of fetal age and status to assure the optimal outcome for a prematurely delivered infant. The pharmacology of fetal lung maturation remains to be better defined in both the laboratory and the clinic. The physiological "inducers" of early fetal lung maturation have not been identified; however, the agents discussed in this chapter have been proposed as either the primary inducers or agents that prepare the lung for another maturation signal. The many agents that may affect surfactant synthesis and/or secretion suggest that the phenomenon of early lung maturation may be a very complex response to multiple stimuli. Maternal corticosteroid therapy does not induce lung maturity in all treated cases and may ultimately prove to be harmful to the fetus. The most effective and safest methods to pharmacologically mature the fetal lung may presently be unknown.

Most of the reliable tests of pulmonary maturity utilize the presence of surfactant in amniotic fluid to signal fetal lung maturity. The lung profile, the test that most carefully assesses the surfactant phospholipids, is presently the most predictive test; however, no test can be expected to be entirely accurate, as the amniotic fluid only indirectly reflects the fetal secretion of surfactant. The delays between a fetal response and the detection of that response in the amniotic fluid are unknown. Furthermore, obstetric and early neonatal management of the premature infant will significantly

influence the clinical course and incidence of respiratory distress. A close working relationship between the obstetrician and pediatrician is essential for an optimal outcome. The obstetrician must be aware of the possibilities for neonatal care in his own hospital and referral centers. The local infant mortality statistics based on size and gestational age are essential information for the obstetrician who must decide how to proceed with a complicated premature delivery. Similarly, the pediatrician must be skilled at neonatal resuscitation and early neonatal stabilization and recognize that obstetric outcomes are occasionally unpredictable.

RDS remains a major cause of morbidity for premature infants. Recent progress in care has greatly decreased mortality in infants weighing more than 1 kg. The timely use of pulmonary maturity tests has decreased the incidence of iatrogenic RDS caused by elective induction and cesarean sections. Respiratory distress syndrome continues to occur in spite of the use of corticosteroids for lung maturation. Further understanding of the biochemical and physiological aspects of RDS can be anticipated to improve therapy and outcome.

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# 11
# Maternal and Fetal Infection

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## INTRODUCTION

The great majority of infants born at term to apparently healthy women are free of readily identifiable infection. Preterm infants, on the other hand, are at greater risk, for their birth is sometimes preceded or accompanied by low-grade genital infection in the mother which rarely affects her well-being and is thus unsuspected. Despite recent advances in laboratory diagnosis, we are still unable to determine with any accuracy the true extent to which maternal transmission occurs throughout pregnancy. It is also clear that factors determining the fate of an individual infected fetus are imprecisely known, and the end result may be abortion, stillbirth, neonatal death, a living child severely or mildly damaged, or one apparently healthy and capable of normal development. Dizygotic twins may be affected to different degrees in a variety of maternally derived infections, suggesting that genetic differences in fetal immune response may be important. There are a number of other variables to be considered, including geographical and socioeconomic disparity; the number, virulence, and route of entry of the infecting organisms; and the timing of the maternal infection and hence the state of fetal organ differentiation and maturity of the immune system. Changing maternal habits and obstetric practice may also contribute to subtly changing patterns of infection. This chapter will try to record some of the infective risks to the fetus and the impact of these various features.

## GENERAL ASPECTS

### Defense Mechanisms

A complete understanding of the reasons behind the survival of the genetically incompatible "fetal allograft" has proved elusive, though much relevant research followed Medawar's hypotheses (1953). It seems likely that there are several mechanisms, both specific and nonspecific (see reviews by Rocklin et al., 1979, and Harkness, 1980). These are of particular importance to the subject of maternal and fetal infection, since they involve an alteration in the maternal immune state that is subtle enough to allow the human race to continue.

Microbial organisms—bacterial, viral, and other—may variously invoke responses from the humoral and cellular immune systems, from phagocytes, both polymorphonuclear and mononuclear, and from interferon, for these are the main pillars of host defense. Depressed cell-mediated immunity (T-cell activity) in pregnancy (St. Hill et al., 1973) may be one of the reasons for a more severe illness in the course of some systemic

viral diseases (Frucht and Metcalfe, 1954; Greenberg et al., 1958; Hardy, 1965) and for a changing susceptibility to localized viral genitourinary infection (Reynolds et al., 1973). Then there is an increase in the number of polymorphonuclear leukocytes in pregnancy. In vitro these cells show decreased activity of the hexose monophosphate shunt (Bodel et al., 1972), but enhanced bactericidal activity associated with increased myeloperoxidase activity during phagocytosis (Jacobs et al., 1973).

Antimicrobial properties have been demonstrated in amniotic fluid. A low molecular weight fraction consisting of a family of polypeptides–with zinc dependent bacteriostatic activities–has been isolated (Schlievert et al., 1976; Sachs and Stern, 1979). Bacteriolytic activity, mainly due to lysozyme, increases threefold from 25 weeks of gestation to term (Bratlid and Lindback, 1978). Subtle changes may occur in the bacterial flora of the birth canal throughout gestation so that it becomes progressively more benign as the end of pregnancy approaches (Larsen and Galask, 1980). On the other hand, in vitro studies have shown that *Escherichia coli* multiplies more rapidly in the urine of pregnant women than in that of nonpregnant women (Roberts and Beard, 1965). Suggestions that many changes of the pregnancy immune state are mediated at least in part by hormones, perhaps most importantly estrogens, have gained ground in recent reviews (Rocklin et al., 1979; Harkness, 1980) and have some experimental backing.

The development of fetal defense mechanisms is described in detail in Chapter 3 of this volume by Adinolfi and Stern. It is so organized that at birth the normally grown mature infant of a healthy mother is tolerably well equipped to meet microorganisms for the first time, provided that they only reach him in modest numbers and are those of a clean environment. But it will become clear that the preterm infant may have been deprived of much of the active placental transfer of maternal IgG normally taking place in the last trimester, and will have much less complement than even 50% of the adult levels he might have achieved by full term. He may also have to pass through a birth canal that is, bacterially speaking, a little more hostile than it would have been at term.

### Geographical and Socioeconomic Factors

Variations in pregnancy infections are to be expected in different parts of the world, depending on the medical services available to the population, the immune status of women of child-bearing age, and the differing strains and types of infecting organisms. Densely populated countries such as the United Kingdom may generally expect a high degree of immunity among their citizens. Vaccination and immunization policies have now removed fear of certain diseases in this country, but the scope of modern international travel cannot allow one to be too parochial in one's outlook. Elsewhere such maternal diseases as malaria, cholera, and syphilis continue to take their toll in perinatal morbidity and mortality. Stray-Pedersen and Lorentzen-Styr (1980) found that Norwegian women who often ate raw meat or traveled abroad, especially to countries with a high infection risk such as France, were more likely to acquire *Toxoplasma* infections than others. Unlike women in urban communities, those in rural areas appear to encounter the organism at a younger age so that they are immune by the time they enter the reproductive years.

There seems to be increasing evidence that women of poor social and economic status are more likely to have certain pregnancy infections such as bacteriuria and chorioamnionitis and to harbor cytomegalovirus (CMV), herpes simplex virus (HSV), and other sexually transmitted microorganisms than those who are more privileged. Maternal malnutrition may impair immune defense. Thus antibacterial activity of

amniotic fluid against certain organisms was found in only half as many samples from African women as from white women, and was absent in 75% of Ethiopian women at term (Appelbaum et al., 1977; Tafari et al., 1977). Malnourished mothers often produce undernourished newborns, and these infants have in turn been shown to be relatively immunoincompetent (Chandra, 1975).

### Mechanisms of Fetal Damage

Miscarriage, fetal death, or preterm live birth undoubtedly occurs in the course of certain maternal infections without serological or cultural evidence that the conceptus itself is infected. The extent to which this happens and the underlying mechanisms for it are speculative. When the conceptus passes through a birth canal colonized by microorganisms, positive cultures are still difficult to interpret. The fetus is probably not invaded by *Vibrio cholerae* when the mother develops the infection and maternal pyrexia, toxemia, and profound acidosis are thought to precipitate miscarriage or preterm labor (Hirschhorn et al., 1969). Similar mechanisms could be responsible in less dramatic maternal infections. As pregnancy progresses, the most important factors determining fetal damage are the timing of the maternal infection, the special properties of the infecting organisms themselves, and the routes by which they reach the fetus. The two most important paths of entry—the transplacental route and that ascending from the birth canal—may not always be easy to distinguish. In the former placental infection is thought to be invariably present first, and in some cases it may be quite extensive without obvious fetal involvement.

If organ formation in the first trimester is disturbed by infecting organisms, congenital defects may follow. Such defects are known to occur with rubella, CMV, HSV, varicella zoster, and *Toxoplasma* infections. The rubella virus inhibits or retards cell growth, and malformations may also occur, though the predilection of the virus for infecting vascular endothelium, with resulting vessel thrombosis and tissue necrosis, as well as its chronic persistence account for many of the effects seen (Menser and Reye, 1974). Reasons put forward to explain the persistence of rubella virus excretion after birth in the presence of high antibody titers are that the antibody-forming cells (B lymphocytes) are only partly damaged; that because mitotic division of infected thymic lymphocytes is impaired, they are less capable of destroying infected cell clones; and that the rubella antibody produced by uninfected B lymphocytes is stimulated in utero without the development of tolerance. As unaffected clones of T lymphocytes become available, they destroy infected cells, the B lymphocytes then neutralizing the released virus (Plotkin, 1975). Cytomegalovirus (CMV) and HSV may exert their damaging effect by cell lysis; in the first trimester of pregnancy this will cause morphological disruption, being most serious in the brain, without evidence of inflammation. The lesions following infection with the varicella zoster virus, which is well known for its neurotropic properties, may result from damage to motor nerves (Savage et al., 1973). *Mycoplasma hominis* type 1 has been shown to increase abnormalities of chromosome structure in cell cultures (Allison and Paton, 1966), but there is as yet no evidence to incriminate it in vitro, though the authors suggested that a possible relationship between mycoplasmal infection and chromosomal aberrations in abortuses should be sought.

It was originally thought that the Langhan's layer of the placenta acts as a barrier to spirochetal invasion in the first trimester because *Treponema pallidum* is rarely responsible for abortion. However, it is now realized that the organism may be present

Table 1 Neonatal Signs of Prenatally Acquired Infection[a]

| Possible clinical involvement | Infecting organisms |
|---|---|
| Central nervous system | |
| meningitis, meningoencephalitis (may lead to microcephaly, hydrocephalus, hydranencephaly, abnormal central nervous system signs, fits, and in some cases cerebral calcifications) | Bacteria, *Candida albicans*, coxsackievirus, CMV, echovirus, HSV, *M. hominis*, poliovirus, rubella virus, *Toxoplasma gondii*, *T. pallidum*, varicella zoster virus, variola virus |
| Special sensory organs | |
| eye | |
| cataracts | Rubella virus, *T. gondii* |
| choroidoretinitis | CMV, HSV, rubella virus, *T. gondii*, varicella zoster virus |
| microphthalmia | HSV, rubella virus, *T. gondii* |
| keratitis and corneal opacity | HSV, rubella virus, *T. pallidum* |
| purulent ophthalmia | *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *M. hominis*, *and other bacteria* |
| ear | |
| eighth nerve damage (may not be detected even with special techniques) | CMV, rubella virus, *T. pallidum* |
| otitis media | *Mycobacterium tuberculosis* and other bacteria |
| Cardiovascular system | |
| congenital heart disease (patent ducts arteriosus, pulmonary artery stenosis, pulmonary valve stenosis, ventricular septal defect, aberrant subclavian vessels) | Rubella virus |
| peripheral arterial stenoses | Rubella virus |
| myocarditis | Coxsackie B virus, poliovirus, T. gondii |
| pericarditis | Bacteria |
| Respiratory system | |
| pneumonia | Bacteria, *C. albicans*, *C. trachomatis*, coxsackievirus, CMV, HSV, *M. tuberculosis*, *M. hominis*, poliovirus, rubella virus, *T. gondii*, *T. pallidum*, vaccinia virus, varicella zoster virus, variola virus |
| Skeletal system | |
| periostitis and/or defective mineralization and growth disturbance | CMV, HSV, rubella virus, *T. gondii*, *T. pallidum* |
| reduction deformities of limbs | Varicella zoster virus |
| osteomyelitis, septic arthritis | Bacteria |
| Gastrointestinal system | |
| hepatosplenomegaly with or without jaundice | Coxsackie virus, CMV, ECHO virus, hepatitis B virus, HSV, *M. tuberculosis*, *Plasmodium*, rubella virus, *T. gondii*, *T. pallidum*, vaccinia virus, varicella zoster virus, variola virus |

Table 1 (continued)

| Possible clinical involvement | Infecting organisms |
|---|---|
| Gastrointestinal system (continued) | |
| enteritis | enteropathogenic *E. coli*, Shigellae, *Campylobacter fetus*, Salmonellae |
| Genitourinary system | |
| nephritis, nephrotic syndrome | *T. gondii, T. pallidum* |
| vaginitis | *Trichomonas vaginalis* |
| urinary infection | Bacteria |
| Hematopoietic system | |
| anemia, sometimes hemolytic with jaundice | Bacteria, CMV, HSV, rubella virus, *T. gondii, T. pallidum* |
| purpura, with or without disseminated intravascular coagulation (some hemorrhagic skin nodules are erythropoietic in nature) | Coxsackie virus, CMV, ECHO, HSV, rubella virus, *T. gondii, T. pallidum* |
| Lymphatic system | |
| lymphadenopathy | *M. tuberculosis, T. pallidum* |
| Skin and mucous membrane | |
| vesicular lesions, single, grouped or scattered, sometimes unilateral | HSV, varicella zoster virus, *T. pallidum* |
| large umbilicated lesions | Vaccinia and variola viruses |
| macular or maculopapular lesions | *T. pallidum* |
| mouth—"milk curd" lesions leaving raw area when removed | *C. albicans* |
| skin—papulovesicular lesions and scaling, pustules, abscesses | *C. albicans*<br>Bacteria, *M. hominis* |
| Intrauterine growth retardation | CMV, *Plasmodium*, rubella virus, *T. pallidum* |

[a]*Maximum* involvement listed.

*Source*: Adapted from Davies et al. (1972).

then, but that no inflammatory response occurs until the development of fetal antibody-producing cells in the second trimester (Plotkin, 1975; Harter and Benirschke, 1976). Plasma cell infiltration then proceeds and the recognized lesions of congenital syphilis (see Table 1) are the results of this inflammation (Benirschke, 1974).

That fetal damage of the order mentioned so far could also be caused by other bacteria remains a theoretical and largely uninvestigated possibility (see also below). Occasional case reports document proven bacterial infection—for instance, hydrocephalus following intrauterine meningitis (Crosby et al., 1951)—but they are few and far between. In pregnancy bacteriuria there is at least suggestive evidence that the bacteria do not confine themselves to the maternal urinary tract but perhaps enter the maternal circulation and reach the intervillous space. Thus Wallach et al. (1969) were able to report that the lymphocytes of newborn infants of bacteriuric mothers showed *E. coli* antigen-induced mitosis when grown in cell culture. This indicates previous contact with the antigen, for similar ability was only very rarely found in lymphocytes from control infants of nonbacteriuric mothers, though at 2-3 weeks of age it was universally acquired, presumably due to normal bacterial colonization after birth (Brody

and Oski, 1967). Studying the influence of endotoxin on the placenta-fetus "barrier" in rabbits using Trypan Blue, McKell et al. (1960) showed that in endotoxin-treated animals the dye reached the lumen of vessels in various fetal organs, especially those of the brain, choroid, liver, and kidneys. Whether this could have any parallel in the human is uncertain.

### Possible Association Between Fetal Infection and Subsequent Malignancy

There may be a link between viral disease in pregnancy and malignant disease in the child. Fedrick and Alberman (1972) found that children whose mothers had had "influenza" during pregnancy had an unexpectedly high incidence of leukemia and neoplasms of lymphatic and hematopoietic tissue. (This was not serologically confirmed influenza, but the illness occurred at the time of a recognized pandemic). Others have produced data that are not as striking but which nevertheless suggest a significant similar association (Bithell et al., 1973; Hakulinen et al., 1973). On the other hand, in certain parts of the United Kingdom and in Connecticut these findings have not been confirmed (Curnen et al., 1974; Leck and Steward, 1972; Donovan et al., 1974). Bithell et al. (1973) postulated as well an association between chickenpox infection in pregnancy and later central nervous system tumor in the child, an association also apparent in the data of Donovan et al. (1974). Lack of laboratory confirmation of maternal infection must constitute a very large variable in these studies and, as several of the authors pointed out, even if these suspicions were to be confirmed on a larger scale, the number of cases of childhood cancer and leukemia attributable to maternal infection is probably very small. The hepatitis B surface antigen (HBsAg) may be transferred from mother to infant in certain circumstances (see below). There is an association between HBsAg-positive cirrhosis and primary hepatocellular carcinoma (hepatoma) in areas of the world where malnutrition and certain toxins are also rife, though a direct link has not yet been established. Nevertheless, there is growing interest in the idea of an increased incidence of neoplasia with impairment of immunological functions, and the latter is known to exist following at least some of the congenital intrauterine infections.

## BACTERIA

Bacteria can reach the fetus by several routes: ascending from the cervix, vagina, and perineum; transplacentally from the maternal circulation; from the peritoneal cavity via the fallopian tubes; and from an infected uterine wall (Benirschke, 1960; Blanc, 1961). In practice the ascending route of spread is presumed to be by far the most frequent. Intact membranes are not a perfect safeguard, for their biopsy near the os at term may show degeneration and necrosis (Bourne, 1962). Furthermore, whether the mother is in or out of labor, bacteria have occasionally been recovered from amniotic fluid before rupture occurs (Gosselin, 1937; Miller et al., 1980), though after rupture, their numbers increase during the interval to delivery. Thus bacterial infection is most likely to be acquired, probably by only a small minority of fetuses, shortly before or during labor and delivery, and *any* organism colonizing the genitourinary tract and rectum of the mother may be responsible.

Unfortunately, surveys of the microbial flora of the birth canal at various stages of pregnancy, using techniques now considered appropriate for the recovery of aerobic and

anaerobic bacteria, are few and far between; even fewer have included nonpregnant controls or attempted to quantify bacterial numbers. Larsen and Galask (1980) have reviewed much of the available work and believe there is just enough evidence to suggest a gradual increase in colonization by *Lactobacillus,* with a parallel decline in *E. coli* and anaerobes such as *Bacteroides* species, peptococci, and peptostreptococci as pregnancy progresses to term. This flora may presumably be modified in subtle ways in individual women by factors such as immune status, trauma to the genital tract, number of consorts, and pelvic inflammatory disease. Infection caused by intrauterine devices is relevant because of the small but constant number of women who become pregnant with a device in situ. There is some evidence that women who are pregnant with a device in situ have a higher incidence of infection (Whyte et al., 1982), and studies have shown that women who carry a device are much more likely to develop pelvic inflammatory disease than those who do not (see review by Eschenbach, 1980).

In the sections below only the Group B streptococcus, *Chlamydia trachomatis,* and *T. pallidum* have been selected for detailed mention. The size of the streptococcal problem in relation to other organisms has led to further work on maternal genital colonization, antibody status, and immunity, the implications of which have more general application. *Chlamydia trachomatis* is also dealt with in some detail, as it is only relatively recently that improved laboratory techniques for its recognition have become available.

### Risk to the Fetus in Bacterial Infection

It is quite impossible to quantify the risk of bacterial infection in general in pregnancy. It must vary according to gestational age, the socioeconomic status of the mother, and the infecting organisms involved. Large prospective surveys of the sort carried out for viral infections are lacking, and there are many unanswered questions. For instance, if a fetus is retained following threatened abortion associated with chorioamnionitis and subsequetly turns out to be a mentally handicapped child, there is at present no way of knowing whether bacteria or their toxins played any causative role. Prospective laboratory investigation of this sort of problem presents enormous difficulties; yet isolated case reports suggest that if it could be overcome, the cause of some major handicap in childhood, at present unknown, might be discovered. Thus Dungal (1961), reporting a case of congenital listeriosis, wondered whether three previous children in the family who were considered to have congenital cerebral malformation could all be victims of listeriosis of the central nervous system. After treatment with oxytetracycline in the fifth pregnancy the mother delivered her first normal, healthy child. Lang (1955) investigated a group of mentally retarded children; antilisteria titers were significantly higher in those children in whom the cause of retardation was uncertain compared with those in whom it was known. Although perinatal listeriosis has always been rare in the United Kingdom, it is conceivable that other bacteria found in the birth canal could contribute to prenatal brain damage.

### Chorioamnionitis

Benirschke and Driscoll (1967) showed *histologically proven* chorioamnionitis to be present in 11% of consecutive unselected pregnancies, a prevalence greater than all perinatal mortality and morbidity combined, and certainly far in excess of that likely to be suspected on clinical grounds. The *clinical* diagnosis may be considered when

maternal fever, marked peripheral neutrophil leukocytosis, and maternal or fetal tachycardia (the latter greater than 160 beats per minute) cannot be shown to have another infective or noninfective cause. A foul smell to the liquor clearly increases suspicion, but the condition may be present with intact membranes. In that case the demonstration of bacteria on direct Gram stain and later by culture from an aspirated specimen of amniotic fluid might confirm the diagnosis, though a negative culture would not exclude it (Miller et al., 1980).

Data from the U.S. Collaborative Perinatal Project which investigated births at a number of centers in the United States from 1959 to 1966 has been analyzed by Naeye and colleagues with respect to amniotic fluid infection and reported in a series of publications (Naeye and Blanc, 1970, 1973; Naeye et al., 1971, 1977; Naeye and Peters, 1978). The diagnosis was made histologically by demonstrating acute inflammation throughout the subchorionic plate of the placenta. Among 6613 preterm singletons–all born before 37 weeks gestation–amniotic fluid infection was found to be twice as common when the fetal membranes ruptured just before the onset of preterm labor than when they ruptured just after its onset. This suggested that infection may have been the cause of membrane rupture (Naeye and Peters, 1978). Data from over 26,000 singleton births from the same survey have shown that amniotic fluid infection occurred very significantly more commonly when mothers reported coitus once or more per week during the month before delivery than when none was reported during this time. Furthermore, the percentage of infants dying with congenital pneumonia in the "coitus-associated" group was nearly five times as great as among those whose mothers did not report coitus (Naeye, 1979a). The same author has also reported that seasonal changes in maternal coital activity were correlated with seasonal variations in perinatal mortality (Naeye, 1980b), and that premature delivery was four times more likely, and spontaneous membrane rupture preceding labor nearly twice as likely, when recent coitus was reported and chorioamnionitis was present than when they were not (Naeye and Ross, 1982). Naeye (1979a) suggested that sperms' motile activity and semen's proteolytic enzymes could facilitate bacterial penetration of cervical mucus, and that if too many bacteria entered the amniotic fluid, its antimicrobial activity could be inactivated, allowing free growth. However, Mills et al. (1981) could demonstrate no deleterious effect of coitus in a study involving 10,081 low-risk pregnancies, and their conclusion still held when, challenged by Naeye (1981), they added high-risk cases to the total (Mills et al., 1982). Herbst (1979) pointed out that neutrophil infiltration of the placenta, upon which Naeye's index cases were based, is not equated by all with the amniotic infection syndrome, and definition becomes a crucial issue in the interpretation of Naeye's data. The question of coitus in pregnancy must always be a difficult area to investigate with accuracy; it would seem that the question of whether or not it is harmful can still be debated.

Other analyses of chorioamnionitis have shown a direct association with poverty, amniotic fluid infection occurring more commonly in blacks than in Puerto Ricans and whites in New York City (Naeye and Blanc, 1970). The same relation with poverty was shown in Ethiopia, where, in addition, an inverse connection with water usage and the amniotic infection syndrome was demonstrated; when water usage was high, the prevalence of the condition was low, even in the poorest sections of the community. This might suggest some connection with perineal hygiene, but as it is not known how the water was used, no firm concludions can be drawn (Naeye et al., 1977). The fetal and infant deaths associated with amniotic fluid infection in the U.S. Collaborative

Perinatal Project occurred in an era when perinatal mortality was higher than at present. The congenital pneumonia described as the cause of death in many was often merely passive aspiration of maternal leukocytes, squamae, and amniotic debris into the air spaces (Osborn, 1962; Davies and Aherne, 1962). Infiltration and destruction of bronchopulmonary tissue may not be seen, though present in the fetal membranes. Improved antenatal care with careful watchfulness during labor and prompt resuscitation at birth would now allow many of these infants to live (Koh et al., 1979). Certainly perinatal deaths from pneumonia in this country decreased strikingly from 1.3 to 0.1 per 1000 total births between the two national surveys of 1958 and 1970 (Claireaux, 1975), though to what extent "congenital pneumonia," as defined by Naeye, took part in this fall must remain unknown. The American and Ethiopian data is important for underlining the association of ascending infection with preterm labor and poverty, for drawing attention to the possible importance of coitus during pregnancy, and for its hints of the importance of perineal hygiene.

Management of membrane rupture before the onset of labor poses a dilemma for the obstetrician. The uncertainties and conflicting views have been summarised by Ledger (1977), while Gibbs (1977) has reviewed the diagnostic criteria for a clinical diagnosis of amniotic infection. The salient points seem to be as follows. The likelihood of ascending infection increases with the duration of membrane rupture, but is greatest with low gestational age and socioeconomic status, and guidelines laid down for a prosperous middle-class population (Varner and Galask, 1981) may not be generally applicable. The membrane rupture itself may be a manifestation of already established chorioamnionitis. At a gestational age of less than 32 weeks, however, fetal immaturity may still lead infection as the major cause of perinatal morbidity and mortality. If at rupture the liquor is foul smelling and purulent, delivery should be effected whatever the gestational age. If at rupture the liquor is clear, therapeutic attempts to increase fetal lung maturity which include the arrest of labor are justified at gestations under 32 weeks, given careful monitoring of the fetus. Transvaginal monitoring of itself, though, may increase the risk of ascending infection; however, this should be reduced to a minimum if scrupulous care is taken with catheter placement, and other measures taken to reduce bacterial motility, as suggested by Roberts and Steer (1977). Attempts to relate the number of amniotic fluid polymorphonuclear cells per high-power field to clinically significant amniotic fluid infection have not given clear-cut answers. Amniotic fluid cultures with colony counts of more than 1000/ml may show a better correlation, but clearly cannot be arrived at in time to be useful. However, maternal swab, amniotic fluid, and blood culture results will always be of value to those looking after the infant later. When some time has elapsed after membrane rupture, further samples of amniotic fluid may be difficult to collect, either via abdominal puncture, which may carry a risk for the fetus, or via the transvaginal pressure-monitoring catheter.

### Group B Streptococcus (*Streptococcus agalactiae*)

In some parts of the world there has been a growing awareness since the 1960s that maternal transmission of the Group B streptococcus (GBS) may cause intrapartum death or early and fulminating neonatal infection (Hood et al., 1961; Franciosi et al., 1973; Lloyd and Reid, 1976). The late Rebecca Lancefield recognized the organism as colonizing the adult vagina at the time of her streptococcal grouping studies (Lancefield and Hare, 1935). This is not the place to discuss whether GBS perinatal infection was occurring unrecognized over the ensuing 30 years or whether a genuine change in the

organisms's virulence or—more likely perhaps—a subtle change in the habits of mothers, obstetricians, and pediatricians took place, but these possibilities merit attention. The maternally transmitted early-onset neonatal illness frequently presents as respiratory distress shortly after birth (Franciosi et al., 1973). In this it may be no different from other maternally transmitted intrapartum bacterial infections, but it does seem to differ in occurring more commonly and in having a swifter downhill course, with death frequently resulting in the first 12-24 hr (Jeffery et al., 1977). It was predicted in 1977 that in the following 12 months, 12,000-15,000 infants in the United States would develop infection due to this organism shortly after birth, and that approximately half of them would die (Baker, 1977). It is, however, possible that its impact may now be on the wane.

Colonization studies show differences in the frequency with which GBS is isolated from the pregnant woman, ranging from fewer than 5 to 35%. This is due to variations in sites cultured, variations in microbiological techniques, and possibly variations in socioeconomic and racial samples. Group B streptococcus is recovered from the rectum and periurethral area more commonly than from the vagina; swabbing from more than one site certainly leads to increased isolation rates (Patterson and Hafeez, 1976; Badri et al., 1977; Mhalu, 1977; Christensen and Christensen, 1979). Carriage may be transient, intermittent, or chronic (Anthony et al., 1980), so that a woman colonized in the first trimester of pregnancy may not be so in the third, and vice versa. Although found in prepubertal children (Maurer et al., 1979), the organism can be sexually transmitted (Patterson and Hafeez, 1976) and there is work to suggest it is isolated more commonly from those with venereal disease (Finch et al., 1976) and from those under 20 years of age, and less commonly in fourth and later pregnancies (Anthony et al., 1980). In one study Mexican-American women (Anthony et al., 1980), in a second Asian women (Ross, 1980), and in a third women of Mediterranean origin (Gerard et al., 1979), were found to be significantly less frequently colonized with GBS than American, British, and Belgian women, respectively.

Strains of GBS capable of causing neonatal illness do not invariably show beta-hemolysis (Roe et al., 1976); thus if possession of the latter is used as a criterion for further identification of streptococci by laboratories, the organism's presence may go unsuspected. Failure to use selective media will also lead to underrecognition (Baker and Barrett, 1973). Five major types of GBS are known, Ia, Ib, Ic, II, and III, all of which have been proved to cause maternally transmitted early neonatal illness, though type III is nearly always responsible for later-onset meningitis. All members of a single sterotype are not identical and phage typing may be necessary for epidemiological work (Stringer and Maxted, 1979).

A significant association between maternal colonization with GBS and the possession of blood group B has been found by Regan et al. (1978). This has not been confirmed by K. Farmer (personal communication 1978) or by Reid and Lloyd (1980); the latter did, however, find, as Regan did, that infants of blood group B mothers had an increased susceptibility to GBS septicemia. The blood group of the infant does not appear to be significant where infant colonization is concerned (Blanc, 1980). The group B streptococcus may possess a receptor site for B surface antigens, or cross-reacting antigens with blood group B may possess a B-like antigen, making those who lack anti-B antibodies at increased risk for colonization (Regan et al., 1978). It has been suggested that neonatal susceptibility to GBS infection is correlated with a lack of maternal antibody to the organism (Klesius et al., 1973; Baker and Kasper, 1976;

Baker et al., 1977); yet the finding that IgG antibody against GBS serotypes Ia, Ib, II, and III was detectable in only a minority of pregnant women, regardless of their colonization status, makes this unlikely (Vogel et al., 1980). In vitro work suggests that adherence of GBS to human vaginal epithelial cells is not related to either group-specific or type-specific antigens (Zawaneh et al., 1979). It still has to be explained why for every 100 colonized mothers only 1 infant is likely to develop invasive disease, though at least 75 of them will themselves become colonized with the organism (Baker, 1977). Preliminary work suggests that it is the *numbers* of the organism colonizing a given mother that are important, and when high, and when many infant sites are colonized, neonatal illness is more likely to result (Bobitt and Ledger, 1976; Anthony et al., 1979; Ancona et al., 1979; Pass et al., 1979; Bobitt et al., 1980).

The high perinatal mortality associated with GBS infection has led to various recommendations such as identification and treatment of colonized mothers and their consorts, active or passive immunoprophylaxis of antibody-deficient women, prophylactic treatment of all infants with respiratory distress, and single-dose antibiotic prophylaxis for all newborn babies. Women colonized with GBS in the third trimester were given either one week's oral ampicillin or no treatment in a controlled trial. Although the organism appeared to be eradicated in 80% of patients 3 weeks after stopping treatment, at delivery most of the women were again colonized and there was no difference in carriage rates among treated and control mothers or their infants (Hall et al., 1976). In another trial 44 women colonized with GBS at 38 weeks gestation, together with their consorts, were randomly assigned to treatment with oral penicillin or erythromycin, continued until delivery, or to a control group. There was a significant reduction in both maternal and infant colonization rates in the treated group (Merenstein et al., 1980). Others reserved maternal antibiotic therapy until the onset of labor and gave 34 colonized women intravenous ampicillin. None of their infants were similarly colonized at birth, whereas the infants of 14 out of 24 colonized but untreated mothers were themselves colonized at birth or by 48 hr (Yow et al., 1979). These latter two trials involved relatively small numbers, but imply that routine screening of all pregnant women to identify carriers is desirable, a work load many routine microbiology laboratories could not accept. Furthermore, identification well before term would be essential, as many of the affected infants are born prematurely, yet intermittency or transience of carriage makes a single culture unreliable. If antenatal screening is to be carried out at all, it may have to be restricted to women at high risk—such as those with premature rupture of membranes before the onset of labor, those in premature labor, and those about to undergo surgical induction of labor. It should be noted that GBS has been recovered from the domes of intrauterine pressure transducers used in fetal monitoring, the resulting contamination of the liquor causing an increased rate of colonization among infants over several months (Davis et al., 1978). Any maneuver such as transcervical fetal monitoring or surgical induction of labor increases the risk of carrying organisms from the vagina into the amniotic fluid.

An alternative approach to treatment of identified maternal carriers of GBS is that of single-dose prophylaxis of all live-born infants, as suggested and practiced by Steigman (Steigman et al., 1975, 1978), or of all infants of low birth weight. The latter approach resulted in a report of only 1 case of nonfatal GBS septicemia among 983 low birth weight infants born between 1974 and 1977, who were given prophylactic penicillin treatment within 2 hr of birth, continuing for 48 hr until cultures proved negative (Lloyd et al., 1979). In the 5 years immediately preceding this period, 11 of

1208 low birth weight infants developed GBS septicemia and 10 died. At Hammersmith Hospital five cases of GBS bacteremia occurred among inborn infants during the years 1968-1975 inclusive, and none during the years 1976-1982 inclusive, without such antibiotic prophylaxis being used and without any change in the criteria for diagnosis (Jeffery et al., 1977; Battisti et al., 1981 and unpublished data). A randomized controlled trial involved 520 infants weighing less than 2000 g at birth; one-half received 100,000 units of benzyl penicillin intramuscularly once within 90 min of birth and one-half were left untreated. There were no significant differences between the groups where colonization, septicemia or death due to GBS were concerned (Ramamurthy et al., 1979). These authors also reported a yearly GBS infection rate varying between 1.14 and 3.58 per 1000 live births over an 8-year period. Similar variations in prevalence have been recorded elsewhere. A large controlled trial involving 32,058 infants born in one Texan hospital over a 41-month period–1977-1981–has been recently completed (Siegel et al., 1982). Half the infants were given a single dose of penicillin intramuscularly within 1 hr of birth and the other half had tetracycline ointment applied locally to the eyes (both acceptable as adequate prophylaxis for gonococcal ophthalmia). In the penicillin-treated group, the incidence of early-onset disease caused by GBS and other susceptible pathogens was significantly reduced; but an increased rate of illness caused by penicillin-resistant organisms (nonsignificant) occurred during one year of the trial. It was concluded that while penicillin prophylaxis might be justifiable in nurseries experiencing a high rate of illness due to streptococci and other susceptible bacteria, it was unlikely to prevent infection acquired in utero or affect the incidence of late-onset Group B streptococcal disease; close surveillance would have to be kept for possible adverse effects.

Present evidence suggests that immunoprophylaxis of antibody-deficient mothers might be ineffective in protecting the fetus and newborn, even supposing laboratories had the resources to identify all such women. Thus it can be seen there is no easy answer to the prevention of intrapartum GBS infection; the possible danger of an allergic reaction to penicillin occurring in a mother (albeit apparently less with oral than with parenteral therapy) and the large work load entailed in effectively detecting carriers make any routine treatment of all pregnant women, or even of all colonized women, impracticable. While penicillin allergy has not been recognized in the infant, the prophylactic treatment of all newborns does not seem warranted on present evidence, particularly in communities where the infection rate may be as low as 0.4 per 1000 live births (Jeffery et al., 1977). It would seem preferable for obstetricians to have a very selective attitude to surgical induction, a preference for transabdominal rather than transcervical monitoring (Ledger, 1977), and to identify GBS carriers before surgical induction, or among those presenting in preterm labor, particularly when rupture of the membranes precedes the labor, and to treat accordingly.

### Chlamydia trachomatis

A report from the second annual summary of the United Kingdom data on sexually transmitted disease suggests that genital chlamydial infections and genital chlamydial ophthalmia neonatorum are increasing in frequency, though this could be apparent rather than real owing to increased availability of microbiological tests and reports (Anonymous, 1981a). *Chlamydia trachomatis* is thought to account for about half the cases of nonspecific genital infection (Dunlop et al., 1972), a condition reported nearly twice as commonly as gonococcal infection in the United Kingdom in 1979 (Anonymous, 1981a), though the two may frequently coexist. As well as causing neonatal conjunctivitis, which may occasionally lead to pannus formation and conjunctival

scarring, the organism has been associated with pneumonitis in the newborn (Schachter et al., 1975; Beem and Saxon, 1977). It may also colonize the infant vagina and rectum (Schachter et al., 1979b).

### Pregnancy and Neonatal Involvement

As with the other sexually transmitted organisms, *C. trachomatis* will be found most often in the genitourinary tract of those with many sexual partners, and in clinics for sexually transmitted diseases it may be recovered from one-third of women. Its recovery from pregnant women is likely to vary with socioeconomic status; thus it was not recovered at all from a sample of 143 pregnant women from Harrow of predominantly high social class (Ross, 1980). In a prospective study the organism was present in 4% of 900 pregnant Californian women tested: 20 infants born to chlamydia-positive women and 18 born to chlamydia-negative women were followed. The organism was recovered from half the exposed infants, though seroconversion could be shown in nearly three-quarters of them, whereas none of the unexposed infants showed evidence of chlamydial infection. The authors concluded that their figures suggested there would be 14 cases of conjunctivitis and 8 cases of pneumonia per 1000 live births, and considered it a major health problem (Schachter et al., 1979a). In a Boston, Massachusetts, sample of 322 pregnant women, 2% were found to be infected with *C. trachomatis.* The 6 infants born to infected women, the 61 born to culture-negative but local antibody-positive women, and the 28 control infants of culture- and antibody-negative women were followed for up to 6 months. These three groups produced four, three, and no infected infants, respectively. Antibody appeared to be placentally transferred and was found in 79% of all the infants (Hammerschlag et al., 1979).

A fourth prospective survey was reported from Denver, Colorado. Over 340 women considered at low risk for pregnancy complications and presenting at less than 32 weeks gestation were cultured. The rate of infection with *C. trachomatis* was 8.8% and the organism was recovered significantly more frequently from those who were under 20 years, unmarried, or black. In the same sample the recovery of HSV, CMV, and *Neisseria gonorrhoeae* occurred in 3, 0.9, and 1.8%, respectively. A total of 18 infants of chlamydia-positive women and 16 of chlamydia-negative women were followed; 11 of the study group developed evidence of infection with *C. trachomatis* and only 1 of the controls did (Frommell et al., 1979).

### Diagnosis

As *C. trachomatis* is an obligate intracellular parasite, special methods are necessary for its isolation. These include demonstration of typical intracellular inclusion bodies from epithelial cells of the maternal genitourinary tract or infant conjunctiva, using Papanicolaou or Giemsa stains, respectively; culture on special media such as McCoy's; and immunofluorescence testing for chlamydial antibodies. Recovery of the organism from the mother will be enhanced by the inclusion of urethral as well as cervical or vaginal swabs. The collection of specimens and their transport should only be carried out under the direct supervision of the laboratory concerned. Few laboratories are as yet able to offer a routine service.

## Treponema pallidum

A pregnant woman with primary or secondary syphilis is likely to transmit the infection to her fetus at any stage in the pregnancy. Prompt treatment of the mother should

effect a cure in the majority of infants. Failure to cure the fetus while successfully treating the mother, judged by florid lesions in the newborn, has been reported following erythromycin therapy of a penicillin-allergic mother (Fenton and Light, 1976). In such cases cephaloridine appears to be a more effective alternative where the fetus is concerned (Holder and Knox, 1972). In 1980, 143 new cases of congenital syphilis were seen at clinics in Britain (Anonymous, 1982).

### Diagnostic Tests

The scope of diagnostic tests available has not greatly changed since it was reviewed by Wilkinson (1972), and his recommendations are given below. The tests measure the presence of antibodies and are of two main kinds.

*Antitreponemal antibody* (reagin, the antibody, is both IgG and IgM, the latter predominating in early infection) is regularly produced by syphilitic infection, but also by some other diseases, and it may be measured by the Wasserman reaction, the Veneral Disease Research Laboratory (VDRL) slide test, the rapid plasma reagin test, or the automated reagin test. Biological false positives have been found in autoimmune diseases such as disseminated lupus erythematosus, thyroiditis, and acquired hemolytic anemia; following enterovirus infection; following vaccination; and in drug addicts.

*Antitreponemal antibody tests* can be further subdivided into group and specific tests. *Treponema pallidum* has antigen in common with various commensals, particularly those in the mouth. The Reiter protein complement fixation test detects *group antitreponemal antibody,* which appears at about the same time as reagin. Specific tests include the treponemal immobilisation (TPI) test, the absorbed fluorescent treponemal antibody (FTA-ABS) test (mainly IgG but also IgM and IgA), and the treponemal hemagglutination test. A modification of the FTA-ABS test, the FTA-ABS IgM test, has been used for the diagnosis of congenital syphilis.

### Use and Interpretation

In the past 5 years much work on the treponemal hemagglutination test has led to its use, either alone or with the VDRL or rapid plasma reagin test, as a first-line screening procedure; the FTA-ABS and/or TPI test is then generally used as a confirmatory test (Notowicz and Menke, 1981). False positive reactions due to abnormal macroglobulins or antinuclear factor have very occasionally been reported with the FTA-ABS test. Both the TPI and FTA-ABS tests may be positive for many years unless treatment is first given during the primary or secondary stage, and thus cannot be used to assess progress after treatment. Positive TPI and FTA-ABS tests are an indication of past or present syphilitic infection, but the VDRL, particularly if reactive at a dilution of 16 or more, and with a rising titer, may be a better guide to activity. There are no tests yet available which differentiate syphilis from yaws (Wilkinson, 1972).

The FTA-ABS IgM test has to be used cautiously, for an infected fetus produces a good deal of IgM-anti-IgG, but much less IgM antibody to *T. pallidum.* Maternal IgM in the infant's circulation following a maternal-fetal transfusion may also cause confusion. These and other snags of the FTA-ABS IgM test in giving both false negatives and false positives have been discussed by Notowicz and Menke (1981). Negative maternal tests early in pregnancy may give a false sense of security. Congenital syphilis has been reported following primary infection acquired after such tests (Al-Salihi et al., 1971) and it has been suggested that in high-risk cases serology should be repeated at the beginning of the third trimester, and even at the time of admission

for delivery. In certain communities this may be sound advice, but in general laboratories could not be asked to do this as a routine.

Finally, *T. pallidum* may be demonstrated in material from early lesions by using conventional dark-ground microscopy or indirect or direct immunofluorescence. They are sometimes difficult to find in the placenta, the examination of which is now often neglected; however, focal villitis, endovascular and perivascular proliferation in villous vessels, and relative immaturity of villi may be found (Russell and Altshuler, 1974), and placental weight increased, sometimes considerably, above normal.

### Other Bacteria

The United Kingdom data on sexually transmitted diseases suggests that between 1977 and 1979 there has been a slight decline in the number of reported cases of gonorrhoea among women. Before that there had been a steady rise since 1965 among those less than 25 years of age. Infections due to beta-lactamose-producing gonococci, however, have increased considerably since 1977, and the report states that this poses serious problems of control which are now global, with increasing importation of such strains into Europe and North America from the endemic foci of West Africa and the Far East (Anonymous, 1981a). The risk of neonatal ophthalmia caused by *N. gonorrhoeae* is well known. There have been occasional case reports of systemic infection such as arthritis (Kohen, 1974) and more recently of scalp infection secondary to fetal monitoring leading to gonococcal bacteremia in the infant (Thadepalli et al., 1976). The Gram stain of genital secretions–urethral, cervical, as well as vaginal for increased recovery–remains the most important means of immediate diagnosis in the acute phase; however, recent improvements in transport media, selective culture media, and identification procedures now mean that between 95 and 98% of infected women should be diagnosed at their first attendance (Young, 1981). It should be remembered that certain vaginal lubricants on specula or surgical gloves are known to have bactericidal effects and may inhibit the growth of *N. gonorrhoeae.*

Practically every other bacterial genus which may be found colonizing a mother's genitourinary tract or bowel, normally or abnormally, has been recorded at some time or other as transmitted to the fetus during the last part of pregnancy, usually during labor, causing early illness. Some individual bacteria such as *Streptococcus pneumoniae,* other streptococci, *Haemophilus influenzae,* and *Listeria monocytogenes* may give rise to serious illness shortly after birth similar to that caused by GBS. Anaerobes have been underdiagnosed in the past, but with improved techniques they may be increasingly recognized. Maternal carriers of *Salmonella, Shigella,* and the various enteropathogenic and enterotoxic *E. coli* strains have been responsible for infecting their infants, a situation which may lead to epidemics in newborn nurseries.

### Pregnancy Bacteriuria

Kass and Zinner (1973) stated that bacteriuria (defined as 100,000 or more bacteria per milliliter of urine) occurs in the female at the rate of about 1% for each decade of life from at least the age of 5 onward. It is, however, more commonly present in pregnancy and, depending on age, parity, and social class, may be found in 3-8% of pregnant women. Significant pregnancy bacteriuria was detected in as many as 21% of rural Guatemalan women (Urrutia et al., 1980). Criteria for the collection and processing of urine specimens should be strict: the labia should be spread, the introitus

washed with a soap solution from front to back twice, and the urine voided into a sterile dish. If not cultured immediately, the urine should be refrigerated at 4-6°C until this is possible (Kass, 1962). Kass reported that an excess of low birth weight infants were born to bacteriuric women, and showed that this could be corrected if sterilization of the urine with antimicrobial therapy was achieved. This has remained a controversial topic, for certain authors have reproduced Kass' findings while others have not. Bacteriuria, perinatal mortality, low birth weight, smallness for gestational age, and preterm birth (the latter three terms often imprecisely included as "prematurity" in previous years) are all inversely related to socioeconomic status, and it is likely that this has confounded the issue in samples of varying size. The conflicting evidence on the subject has been summarized by Sweet (1977), who concluded that there *is* an increased incidence of preterm births among bacteriuric women, and that more specifically this increased risk occurs in those with underlying renal involvement as judged by serum antibody studies, defect of maximal urine-concentrating ability, and radiological evidence of chronic pyelonephritis.

Naeye (1979b) has further underlined the importance of maternal urinary tract infection (diagnosed when bacteriuria and leukocyturia—15 or more white cells per high-power field—or leukocyturia alone were present in clean-catch or catheter specimens) in over 50,000 women in a report from the U.S. Collaborative Perinatal Project. Preterm birth was more common. The combined perinatal mortality rate for eight common placental and fetal disorders (amniotic fluid infections, congenital malformations, umbilical cord compression, large placental infarcts, abruptio placentae, growth-retarded placentae, Rhesus isoimmunization, placenta previa) was 42 per 1000 in the infected as opposed to 21 per 1000 total births in the uninfected. Naeye showed that all the excess mortality occurred when the urinary tract infection was found within 15 days of delivery. Death rates were highest when maternal hypertension, which might lead to inadequate uteroplacental perfusion, and acetonuria were present. Elsewhere he has drawn attention to work showing that fasting pregnant women become acidotic more rapidly than nonpregnant women, and he has suggested that the supply of glucose to the fetus may be reduced at this time, making reliance on fatty acids and ketone bodies necessary as substitutes, though not all areas of the brain may be able to use the latter (Naeye, 1980a). Zinner (1979) pointed out that the U.S. Collaborative Perinatal Project was not specifically designed to study the effect of bacteriuria on the outcome of pregnancy, that leukocyturia alone is not generally accepted as a definition of urinary tract infection, and that urine collection was not necessarily standardized, much less performed in all women. Nevertheless, Zinner drew attention to the independent analysis of other data from the project by Sever et al. (1979) which confirmed the findings of increased incidence of low birth weight infants and stillbirth among mothers with symptomatic urinary tract infection, as compared with matched noninfected controls. Acute symptomatic pyelonephritis will develop in up to a third of pregnant women with asymptomatic bacteriuria, and this can be largely avoided if the bacteriuria is dealt with by antimicrobial therapy (Sweet, 1977). As it is not easy to identify those with underlying renal involvement, it is surely important to seek out and treat all pregnant women with asymptomatic bacteriuria. Screening at the first antenatal visit will find the majority (Sweet, 1977) and a short course of treatment and a repeat screening to ensure continued sterility of the urine should benefit mother and fetus. As Zinner (1979) said, in identifying and treating pregnancy bacteriuria, doctors have "unique opportunity to practice effective preventive medicine."

## MYCOPLASMA

The mycoplasmas are neither bacteria, because they lack a rigid cell wall, nor viruses, because they contain both DNA and RNA.

### Maternal Involvement

*Mycoplasma hominis* and *Ureaplasma urealyticum* (formerly T mycoplasmas) are to be found in the genital tract of over 50% of sexually experienced adults; and in a thorough and critical recent review, Taylor-Robinson and McCormack (1980) have pointed out that past studies have often been unsatisfactory because they have ignored the role of associated microorganisms and have eschewed controls comparable in sexual experience. Thus in vaginitis and cervicitis, *M. hominis* is frequently isolated with *Trichomonas vaginalis, Haemophilus vaginalis, N. gonorrhoeae,* and *Candida albicans,* so that its importance is uncertain. They concluded that *M. hominis* is responsible for an uncertain proportion of cases of pyelonephritis. They found weak evidence linking *U. urealyticum* with repeated spontaneous abortion and stillbirth, and much stronger evidence associating it with both chorioamnionitis and low birth weight, though in none of these three instances has a *causal* relationship been proved.

### Risk to the Infant

Low birth weight and preterm birth have obvious disadvantages to the infant, but it is quite clear that these cannot be ascribed to the mycoplasmas only. *Mycoplasma hominis* has been cultured from a supraclavicular abscess in a 7-day-old infant and from her mother's vagina (Sacker et al., 1970). Neonatal meningitis due to *M. hominis* has also been recently reported (Gewitz et al., 1979; Hjelm et al., 1980). The same organism has been isolated from some infants with neonatal ophthalmia (Jones and Tobin, 1968), but it seems likely that it could also be cultured from the conjunctival sac in the absence of eye infection.

### Diagnostic Tests

The organisms are recovered more easily from the vagina and periurethral area than from the cervix (McCormack and Lee, 1973) and special culture media are necessary for their growth. Serological evidence of *Mycoplasma* infections can be sought by radioimmunoprecipitation, mycoplasmacidal metabolism inhibition, and indirect hemagglutination tests (Taylor-Robinson and Csonka, 1981).

## VIRUSES

Serological techniques have burgeoned in the last decade or so, and such tests as radioimmunoassay and enzyme-linked immunosorbent assay are being increasingly used by specialist laboratories in the diagnosis of viral and other illness. Monoclonal antibodies too are likely to play on important role. Thus while hemagglutination inhibition (HI) complement fixation (CF), and neutralization tests are mentioned in the text, their importance is lessening. Clinicians should always seek the advice of their individual laboratories about the relevant tests before investigating illnesses thought to be infective, and work in the closest cooperation with them, especially where the accurate reporting of clinical details is concerned.

## Coxsackievirus

The largest prospective survey of coxsackievirus available is that of Brown and Karunas (1972), who studied 22,935 pregnant women with serological testing in the first trimester and at delivery. Their results suggest a significant association between infection with certain coxsackie types during pregnancy and congenital malformations in offspring, as compared with controls. First-trimester coxsackie B4 infection was associated with an excess of urogenital defects. Infection with coxsackie B3 and B4 viruses throughout pregnancy was associated with cardiovascular defects, but of a wide variety; similarly, coxsackie A9 infection, again throughout pregnancy, showed a link with digestive tract anomalies. The wide range of anomalies and the fact that the infections were not necessarily limited to the first trimester make this study difficult to assess. Others have suggested that intrauterine infections with coxsackievirus B, clinically occult at birth, might be responsible for some forms of heart disease in later life. Immunofluorescent antibody methods and routine histological techniques revealed coxsackievirus B antigen in about a quarter of 41 stillbirths and neonatal and infant deaths in whom myocardial inflammatory tissue reactions were demonstrated. In those dying beyond the perinatal period in the first year, cardiac involvement had not been suspected (Burch et al., 1968). Spontaneous abortion occurring in the course of hand, foot, and mouth disease caused by coxsackievirus A16 was recently reported in two women (Ogilvie and Tearne, 1980). In one the products of conception were available for study, and the virus successfully recovered. Infection later in pregnancy is now well recognized as a cause of pneumonia, meningitis, and myocarditis in the newborn, though one cannot quantify the risk. As with so many perinatal infections, the neonatal component may range from inapparent to severe and fulminating. A few fatal cases have been recorded (Baker and Phillips, 1980).

## Cytomegalovirus

### Maternal Involvement

The epidemiology of acquired as opposed to congenital CMV infection is not fully known. It is geographically widespread, but unlike rubella, it appears to be more common among those of poorer socioeconomic status and the incidence may vary between racial groups. In a study in the northwest of England, the infants of young unmarried mothers were found to be infected six times more frequently than those of married ones (Collaborative Study, 1970). Stern and Tucker (1973) found that two-thirds of 1040 London women in a prospective pregnancy survey possessed antibody to CMV, and the authors demonstrated its presence in 58% of native white English women and in 90% of immigrant Asian women. A total of 4% of women developed a primary infection during the course of pregnancy. Although a much greater number of the Asian women were without antibodies in the first trimester and thus susceptible, the rate of primary infection among them was considerably higher than among the white women. Fewer than 1% of 5575 women in another prospective London survey developed a primary CMV infection in the second or third trimester (Griffiths et al., 1980). Virus has been recovered from the cervix more frequently than from urine during pregnancy, and in the third trimester of pregnancy more often than in the second or first. In a prospective pregnancy survey of white and black women from Pittsburgh, 58% were found to have antibodies to CMV, but the virus was recovered from the cervix in only 4% (Montgomery et al., 1972). Women known to have antibodies to CMV before the

onset of pregnancy may start to excrete CMV in pregnancy (urine and cervix), presumably because of their temporary alteration of immunity. Hanshaw and Dudgeon (1978), in a review of the literature, found CMV recovered from the urine of between 3 and 6% of pregnant women, and from the cervix of between 2 and 28% of pregnant women from varying parts of the world.

### Risk to the Fetus

Both the placenta and the fetus may be infected by the viremia occurring with primary infection, though the fetus is not always infected when the placenta is. Stern and Tucker (1973) found the overall incidence of fetal infection to be almost 50% following primary infection in the mother, and noted that it was higher in early pregnancy. Only 25% of infants born to mothers infected in the second and third trimesters were themselves infected (Griffiths et al., 1980). Stern and Tucker calculated that about 4000 CMV-infected infants would be born yearly in England and Wales. Prospective American surveys have shown that CMV can be recovered from the urine of between 0.5 and 3.0% of newborn infants (Birnbaum et al., 1969; Feldman, 1969), but the majority of them appear normal at birth and are thus presumably affected late in the second or third trimester, for clinical manifestations are primarily a reflection of the duration of intrauterine infection (Monif et al., 1972). Weller (1970) has pointed out that positive cultures are higher by 3-4 months of age, and it is probable that this increase represents infections acquired at birth during passage through the birth canal. Another possibility is that transmission through breast milk could be the reason for the frequent acquisition of CMV among breast-fed infants of seropositive mothers (Stagno et al., 1980); others, however, have not found this correlation (Levinsohn et al., 1969).

It used to be said that reactivation of latent infection in pregnancy did not appear to involve the fetus (Montgomery et al., 1972; Stern and Tucker, 1973); however, there are now some well-documented case reports which show that successive fetuses may be involved (Embil et al., 1970; Stagno et al., 1973). This has occurred despite substantial levels of preconceptual antibodies (Stagno et al., 1977). The extent of this mode of infection may have been underestimated, for it has never been studied on a prospective scale. It may happen when the interval between pregnancies is short. Intrauterine transfusion of donor blood infected with CMV may be a hazard for erythroblastotic babies (King-Lewis and Gardner, 1969). Children born to mothers on immunosuppressive drugs, such as following renal transplantation, may also be at risk (Evans et al., 1975). The severe manifestations of CMV infection in the newborn, seen in only a tiny minority of infants—less than 5% according to Hanshaw and Dudgeon (1978)—are shown in Table 1.

The realization that apparently normal infants excreting virus at birth greatly outnumber those who also excrete virus but who already have obvious central nervous system and other damage has been relatively recent, and the exact prognosis for these children is not yet accurately known. Cytomegalovirus was thought to account for 10% of mental retardation in children under 6 living at home in the London area (Stern et al., 1969). On the other hand, others could find no significant association between microcephaly and evidence of CMV infection (Baron et al., 1969), and a follow-up study of children congenitally infected yet normal at birth showed no difference in their mean intelligence quotient compared with controls (Kumar et al., 1973). Hanshaw (1966) found CMV antibodies in 43.9% of microcephalic children, compared with 3.9% in normocephalic controls, but showed that 16 of 22 children infected with

CMV at birth were developing normally, while 3 of the remaining 6 were deaf. Subtle neurological abnormalities may of course only be apparent in the school years and Hanshaw has said that 36% of 44 children excreting virus at birth would have school failure, compared with 14% of matched controls (Hanshaw and Dudgeon, 1978).

### Diagnosis

The lack of an obvious clinical illness in most cases of primary CMV infection means that a rising antibody titer is unlikely to be detected during pregnancy unless a prospective survey is being made. Specific IgM antibody is normally present for 6-12 weeks after primary infection and may be more useful. Tobin (1973) did not believe that routine cervical culture to identify maternal virus excretors was a helpful measure.

Newborn screening which is based only on raised cord blood IgM levels (greater than 0.2 g/liter) will miss a significant proportion of CMV urine excretors, and children severely damaged at birth with raised levels at birth may be seronegative after a few years. Viral excretion in the urine may persist for many months and is probably the most useful means of diagnosing congenital infection, along with the detection of CMV-specific IgM antibody. Infected cells with a greatly increased diameter (which gives the virus its name) may be found in freshly passed urine.

## Hepatitis B Virus

Although there are now known to be several types of viral hepatitis, the discovery of the hepatitis B surface antigen (HBsAg) in 1965, previously called Australia or hepatitis-associated antigen, has made the epidemiology of hepatitis B virus (HBV) more clearly understood, though there are still unanswered questions. The surface and later the core (HBcAg) and "e" (HBeAg) antigens and their respective antibodies (anti-HBs, anti-HBc, and anti-HBe) have proved to be valuable markers. All blood donors and many pregnant women in the United Kingdom are screened for the presence of HBsAg, which appears in the blood during the incubation period of hepatitis B as early as 6 days after exposure (Krugman et al., 1979) and remains there during the acute phase. It is also present in a number of apparently healthy carriers—about 200 million of them—throughout the world. In the United Kingdom HBsAg is present in only about 0.5% of the population; the incidence is highest, sometimes up to 10%, in certain racial groups, particularly African and Asian groups, in drug addicts, and in homosexuals. It may be present in their body secretions and, most relevant for consideration here, in semen, amniotic fluid, vaginal secretions, and breast milk. Possession of the e antigen may be a marker of liver damage and is thought to be correlated with HBV replication in the host (Zuckerman, 1979). A total of 6% of HBsAg-positive people volunteering as blood donors and 10% of those with acute hepatitis may carry it (Takahashi et al., 1976; Nielsen et al., 1974).

### Risk to the Fetus

This is greatest if the mother develops hepatitis during the latter part of pregnancy, itself a rare event in the United Kingdom. According to Cossart (1974), only five maternal pregnancy HBV cases were confirmed at the Virus Reference Laboratory during 1969 and 1970. She followed six such mothers and found that half the infants were HBsAg positive at birth or shortly after, and that clinical or biochemical hepatitis was present in two of the three. Skinhøj et al. (1972) reported that two of eight

infants exposed in utero to maternal hepatitis themselves developed hepatitis, and there have been similar scattered reports. The mode of spread is presumably transplacental.

The risk to infants of asymptomatic carrier mothers in the United Kingdom is less easy to quantify, because it varies according to ethnic background. Transmission largely, though by no means exclusively, occurs through the HBeAg-positive mothers, who are most commonly Asian, particularly Chinese and Japanese. A Birmingham study recorded that only 14% of 122 infants of carrier mothers were still HBsAg positive at 3 months, although half of them had had positive cord bloods. The transmission rate was highest among the Chinese but also occurred in Afro-Caribbean infants, no carriers being detected among European babies. Possession of e antigen among the former two groups of mothers was well correlated with transmission (Derso et al., 1978). Of 110 infants, 14 (13%) born to HBsAg carrier mothers in two West London hospitals were HBsAg positive after the first week of life—10 of them at birth—and all were well. Only one-third of the mothers were Asian, but two-thirds of the HBsAg-positive infants belonged to them, the remainder belonging to the African and white mothers, and eight of the nine positive infants born to Asian mothers were Chinese. Maternal HBeAg was significantly correlated with transmission; anti-HBe did not necessarily prevent it (Woo et al., 1979).

Transmission rates of 0-6% have been reported from Denmark, the United States, Greece, and Belgium (Skinhøj et al., 1972, 1976; Schweitzer et al., 1973; Papaevangelou et al., 1974). From Taiwan and Hong Kong, on the other hand, the figure is nearer to 50% (Stevens et al., 1975; Lee et al., 1978). The Hong Kong authors have reported finding HBsAg in liquor in 33% of samples obtained by amniocentesis, in 71% of breast milk samples from HBsAg carrier mothers, and in 95% of gastric fluid aspirates from their newborn infants (Lee et al., 1978). They believe that maternofetal transfusion during labor might be reponsible for fetal acquisition of HBsAg, for they showed the presence of HBsAg in cord blood to be correlated with the length of labor, a linear relationship between the latter and maternofetal bleeding being accepted (Wong et al., 1980). They also reported that there was a correlation between HBsAg in gastric fluid at birth and HBsAg positivity at 3 months of age. If amniotic fluid HBsAg was not due to blood contamination following amniocentesis, it is possible that it could have arrived there by the ascending route from the cervix. Not all HBsAg-positive infants of carrier mothers are well; acute hepatitis, sometimes fatal, has occasionally developed (Dupuy et al., 1975; Fawaz et al., 1975; Mollica et al., 1977; Shiraki et al., 1980).

### Diagnosis

The numerous methods now available for the detection of surface, core, and e antigens have recently been reviewed by Zuckerman, (1979), and their description is beyond the scope of this chapter. Contamination of cord blood with maternal blood probably occurs on occasion, for HBsAg detected in cord blood by sensitive radioimmunoassay has not always been confirmed in specimens drawn directly from the infant on the day of birth.

### Management of the HBsAg-Positive Pregnancy

Careful precautions against transmission of the antigen from pregnant women to the nursing and medical personnel responsible for their care are necessary. The major risk appears to be from blood contamination, though theoretically urine, feces, amniotic

fluid and vaginal secretions are also possible sources. Particular care is necessary with vaginal bleeding at any time through pregnancy, venipuncture, and disposal of excreta. There is some controversy about the advisability of HBsAg mothers breast-feeding their infants, but most believe the advantages to outweigh theoretical dangers (Krugman, 1975; Woo et al., 1979).

The prevention of the chronic HBsAg carrier state in infants of HBsAg-positive mothers may be possible by the administration of hepatitis B immunoglobulin (HBIg). A total of 21 such children were given 0.5 ml/kg HBIg within 48 hr of birth and subsequently 0.16 ml/kg every month for 6 months. None of them became HBsAg positive, compared with 5 out of 20 untreated controls (Reesink et al., 1979).

## Herpes Simplex

### Maternal Involvement

Herpes simplex infections in man are due to two antigenic types, 1 and 2. Type 2 isolates are those responsible for genital tract infections in 95% of cases, whereas most strains from nongenital sites such as the mouth, eyes, and central nervous system are type 1. The socioeconomic state of the population is an important influence on incidence, as with CMV infection, and antibodies at relatively young ages are found most commonly among the less privileged. The incidence of type 2 antibodies is greatest among the sexually promiscuous, for the virus is believed to be venereally transmitted. Primary and recurrent herpetic disease can involve the external genitalia in males and females, but in the latter the cervix and vagina may also be involved, the cervix usually being the principal site of infection. Cervicitis and vaginitis frequently cause no symptoms (Kibrick, 1973). Female herpetic infection (HSV type unspecified) has occurred in up to 2.5% of pregnant women, as demonstrated by a fourfold rise in CF titers (Sever et al., 1963). The incidence of genital infection is three times that in the nonpregnant, increasing as pregnancy advances to term (Nahmias et al., 1971). Even this, however, may well be an underestimate, and the depressed cellular immunity of pregnancy again presumably accounts for this recurrence. Genital herpes is now considered one of the most important of the sexually transmitted diseases and, in the United States at least, cases are numbered "in the millions" (Brunell, 1980).

### Risk to the Fetus

A study of 283 women with genital herpes found that the abortion rate during the first 20 weeks in those with primary infection was five times higher than in noninfected women and three times higher when all types of herpetic infection—primary, recurrent, and undetermined—were considered together (Nahmias et al., 1971). It should be noted that histological examination of the abortuses did not reveal evidence of viral invasion, but two-thirds showed evidence of fetal death prior to abortion. Genital infections detected after 20 weeks of gestation were not associated with an increased rate of preterm birth compared with the uninfected group. When HSV was detected after 32 weeks, the fetus became infected in 10% of cases, and the later in pregnancy the infection was found, the greater the risk to the infant. Primary infections were associated with a higher risk than recurrent infections, perhaps because of greater persistence of the virus. Genital infection at term appeared to offer the greatest risk, with at least 40% of fetuses involved. However, as in so many other maternal infections, not all infected fetuses are damaged, and the overall risk of a dead or affected infant

appears to be 20% when maternal infection occurs at term. One case has been reported in which HSV was actually recovered from amniotic fluid without clinical or immunologically proven infection occurring in the child, followed to 18 months of age (Zervoudakis et al., 1980). There are, so far, few large surveys of this problem, and as more figures are collected, it may well be that the risk will eventually prove lower still. We do not yet know with accuracy the long-term prognosis, particularly where neurological handicap is concerned, for the brain, along with the liver and adrenals, is frequently involved. Skin vesicles are another feature of congenital infection and these may become recurrent and, with the other lesions, not develop until after birth. Although the common mode of infection is thought to be ascending, transplacental infection has been very rarely reported in the first trimester, leading to central nervous system damage such as microphthalmia, microcephaly, and choroidoretinitis (Nahmias et al., 1970).

The management of pregnancy complicated by genital herpes has been reviewed by Hanshaw and Dudgeon (1978). They felt that the risk of intrauterine infection is too small to be considered a serious deterrent to pregnancy, but that cesarean section should be considered for delivery if virus can be shown to be present. They pointed out, however, that there is no information as yet that this procedure will reduce the likelihood of neonatal disease; the recommendation is merely based on the hypothesis that the fetus should be protected from the inevitable exposure to the high titers of virus on passing through the birth canal. Cesarean section would be pointless, however, if the membranes had been ruptured for more than 4 hr. A case of disseminated HSV infection thought to have followed introduction of the virus at the site of a fetal monitoring electrode has been recorded (Parvey and Ch'ien, 1980).

Males diagnosed as having active genital herpes should be warned of the risk to the fetus if intercourse occurs during pregnancy, particularly during the latter part of it.

### Diagnosis

Detection of a fourfold rise in neutralizing antibody titer in maternal serum in paired specimens would be considered significant of recent infection, as would the finding of HSV-specific IgM antibody. Characteristic cellular changes can be seen in Papanicolaou-stained cervicovaginal smears in a high proportion of virologically proven cases of genital HSV infection (Ng et al., 1970). Herpes simplex virus can be isolated from the maternal urine and cervix and from skin lesions, throat swabs, and urine in the infant. Immunofluorescent techniques have been used for rapid identification of HSV in the infant's nasopharynx, but are not generally available.

## Rubella

### Maternal Involvement

Gregg (1941) first drew attention to the association between fetal defect and maternal pregnancy rubella. The isolation of the virus in 1962 and the widespread epidemic of 1963-1964 in the United States have made further advances in knowledge possible. It is a disease of comparatively low communicability, and approximately 20% of women who have not been given rubella vaccine reach reproductive age having escaped it in childhood (Public Health Laboratory Service, 1967). The infection may present atypically or may be subclinical, and rubella-like rashes may be induced by other viruses, for example, the enteroviruses. A diagnosis of rubella, therefore, particularly in

pregnancy, should not be made on clinical grounds alone, but should be confirmed (or refuted) serologically. Substantial immunity exists after naturally occurring primary rubella infection, and reinfection, though reported, is rare. It is nearly always subclinical and often diagnosed only by a boost in IgG antibody titer.

## Risk to the Fetus

It has been estimated, since the Abortion Act of 1966, that rubella can maim or terminate, directly or indirectly, the lives of over 1000 fetuses per year in this country (Broadbent et al., 1980). There has though been discrepancy between the high recovery rate of virus from the aborted fetus and the prevalence of defects in survivors. Genetic factors may be involved, for Australian studies have suggested a significant increase in the prevalence of some histocompatibility antigens in children with defects (Honeyman et al., 1975). Hanshaw and Dudgeon (1978) have summarized the risk of congenital defect from six prospective studies from five countries (Sweden, New Zealand, Great Britain, Australia, and the United States). The figures given below, though, may underestimate the risk, as all but one of the studies were carried out before laboratory diagnosis was available; furthermore, follow-up may not always have been long enough to detect late deafness. Among infants exposed to rubella, 15.3% had congenital defects following maternal infection in the first month of gestation. For the second, third, fourth, and fifth months of gestation the figures were 24.6, 17.5, 6.4, and 1.7%, respectively. Thus, as the authors pointed out, the risk, though greatest in the first 8 weeks, does not stop at the twelfth week of gestation, though congenital heart disease, cataract, and other eye defects are very unlikely after this time. The main risk after the first trimester is of deafness, developmental delay, and neurological deficit. Persistence of virus in the child or reactivation may be the cause of some late-appearing disease. Maternal rubella occurring in the weeks *before* conception has been reported as responsible for congenital defect, though Hanshaw and Dudgeon (1978) believed the risk to be rare. Reinfection with rubella during pregnancy following a previous natural infection is also rare, and even if it occurs, it is unlikely to be a hazard for the fetus. This is because rapid maternal IgG production should prevent a viremic phase, and there are no families known with more than one affected child, other than in twins (Hanshaw and Dudgeon, 1978).

## Diagnosis

Theoretically the diagnosis of rubella could be established in a number of ways, but in practice, particularly when the patient is a pregnant woman, speed, reliability, and reproducibility of results are essential. The HI antibody test, long used in the diagnosis of rubella, has certain disadvantages as a screening test because nonspecific inhibitors of rubella hemagglutination are increased in pregnancy and, unless removed by pretreatment of the serum, may give false positive results. Many laboratories are now using the single radial hemolysis (SRH) technique in its stead because of this; it also has the advantage of being easier to perform (Banatvala et al., 1981). Banatvala et al. recommended that both mothers previously tested by HI and considered rubella immune and those vaccinated but not retested, particularly if exposed to rubella or a rubella-like illness during pregnancy, should be retested by SRH. Banatvala's (1972) clear rules for investigation during pregnancy are still valid and are given below.

### Exposure Only to Rubella

(1) Blood should be withdrawn for testing of serum HI antibodies to rubella as soon as possible after exposure. If antibodies are present in high titer (and confirmed by SRH) well within the incubation period, the patient can be considered protected by previously acquired infection. (2) If the antibody titer is low, probably particularly important when contact is within the family, a second sample should be taken 7-10 days later and, if time allows, also 21-25 days after first exposure, and HI and CF tests performed to ensure that there has been no rise in antibody titer. A significant rise in titer (say, fourfold) confirms recent infection. Allowance should be made if the patient has been given gammaglobulin, which may prolong the incubation period. (3) If the patient first presents at a stage when termination of pregnancy, if it is to be done at all, must be done immediately (say, approaching 18-20 weeks of gestation), determination of rubella-specific IgM antibody may be the most helpful, for if present, it suggests recent infection (see below though).

### Development of Rubella-Like Illness

Confirmation of true rubella is most often required urgently. Blood should be taken as soon as possible after symptoms develop and, if time permits, 4-5 days later. The serum samples should be tested in parallel for a rise in HI antibody titer. If the situation is as in (3) above, the sample should be tested for rubella specific IgM antibody.

### Interpretation of Results

Banatvala (1972) has stressed that considerable care in conducting tests and interpreting their results is essential, and that the titers obtained by one laboratory may not be applicable to another, so that it is preferable for one laboratory to conduct all tests on an individual patient. The situation has been simplified, however, by the use of a standard control serum containing 15 IU of rubella antibody. Where HI tests are concerned, a titer above this level is regarded as immune, and one below it as seronegative. For the SRH technique, a zone of hemolysis greater than that of the control serum indicates immunity, whereas one smaller than it shows the patient to be seronegative. Improved techniques for rubella-specific IgM now show that it may persist for 8-12 weeks after the initial illness (J. E. Banatvala, personal communication); however, it can persist for a year or more after the acute illness (Al-Nakib et al., 1975; Pattison et al., 1975); or following rubella vaccination (Al-Nakib et al., 1975). Thus the finding of rubella-specific IgM cannot guarantee rubella virus infection in the current pregnancy as was first thought, reemphasising the need always to consider laboratory results in the light of historical and clinical findings.

### Rubella Vaccination and the Fetus

Trials of different live attenuated rubella virus vaccines are still in progress. It is as yet uncertain whether the immunity conferred by a single dose of vaccine will be lifelong, as is the natural infection. Locally produced rubella-specific IgA in the nasopharynx, which may be important in preventing viral multiplication at sites of entry, appears to be less efficient and long-lasting with at least three of four vaccines when compared with the natural infection (Al-Nakib et al., 1975). The United Kingdom policy, started in 1970, of vaccinating 11- to 14-year-old girls will, it is hoped, safeguard women

during their reproductive years. Booster doses may eventually be shown to be necessary.

Immediate postpartum vaccination of seronegative women is also practiced, but it cannot protect the firstborn, who constitute 40% of births (*British Medical Journal,* 1972). It is important to check the seroconversion rate if large blood transfusions or anti-D-immunoglobulin has also had to be given, to ensure that it has not influenced the serological response.

Reinfection may be more common in those with vaccine-acquired rather than natural immunity. Rubella virus has been recovered from the placenta, decidua, and various organs of therapeutically aborted conceptuses of seronegative women who had been vaccinated (*Lancet,* 1973); however, the risk of defect following vaccination early in a pregnancy continuing to term has been calculated at less than 5% in the United States (Hayden et al., 1980). Differing vaccines used in this country nevertheless suggest that vaccination should still be avoided during pregnancy, and, similarly, pregnancy avoided, probably for several months, after vaccination until more data is gathered. An injectable "depot" progestogen, given for contraceptive purposes at the same time as the vaccine in postpartum cases, is said to have proved effective in this respect (Sharp and MacDonald, 1973).

### Use of Gammaglobulin

Trials of passive protection of the mother and fetus against rubella with 700-1500 mg of immunoglobulin (Public Health Laboratory Service, 1970) did not suggest that the incidence of rubella was affected. Peckham (1974) was later able to examine the children of those women involved in this trial who subsequently showed serological evidence of the infection despite immunoglobulin prophylaxis. She found the incidence of congenital defects to be diminished when the mother had subclinical rubella as opposed to clinical rubella. Although it was not possible to rule out reinfection in the subclinical cases, she nevertheless felt that immunoglobulin could be of limited usefulness, particularly in those who do not wish pregnancy terminated, since it may suppress the clinical manifestations of the disease. Although some controversy still surrounds its use, Dudgeon (1974) advocated the administration of 1500 mg of immunoglobulin as soon as possible after contact and after a blood sample has been taken. If the latter does not show HI antibodies, a further similar dose is given 3-4 days later (about 6 days after contact) and presumptively just before the viremic phase. As it may be possible for the incubation period to be prolonged a few days by immunoglobulin, a second sample is tested between 21 and 28 days later for seroconversion.

## PROTOZOA

### Toxoplasma Gondii

*Toxoplasma gondii,* found in many parts of the world and infecting a large number of warm-blooded animals, including man, is the protozoan parasite responsible for toxoplasmosis. It exists in three forms—trophozoite, tissue cyst (containing many trophozoites), and oocyst. The mode of human infection and the life cycle of the parasite—now known to involve the domestic cat—have been reviewed by Beverley (1973).

### Maternal Involvement

Congenital toxoplasmosis may occur if a woman acquires her primary infection in pregnancy, with temporary parasitemia leading to placental involvement. Her infection is most likely to be subclinical, though occasionally it may present as an infectious mononucleosis-like illness. Parasitemia has been demonstrated as long as 14 months after the onset of illness (Miller et al., 1969). The risk of contracting the illness in the United Kingdom is still uncertain and may vary geographically. A London survey tested 3187 mothers at the first antenatal visit, again at the twentieth and thirtieth weeks, and finally at delivery. Seven contracted toxoplasmosis during pregnancy, a further two developing it either shortly before or during early pregnancy (Ruoss and Bourne, 1972). In an American series serological tests were made at the first antenatal visit and at delivery in 4048 women; 2765 were initially negative, of whom 6 converted to positive; 1283 had positive first tests, 17 showing a substantial rise in titer (Kimball et al., 1971). In Oregon, 1 in every 200 pregnant women contracts the infection during pregnancy (Beach, 1979); on the other hand, in a recent French study, the rate of pregnancy infections was 6.3% (Desmonts and Couvreur, 1974). The national penchant for eating undercooked meat is generally held to blame for this higher risk.

### Risk to the Fetus

The role *T. gondii* plays in abortion has been much debated, and the large literature has been ably and critically reviewed by Remington (1973). His own studies in California, where the prevalence of infection in the childbearing age group is low (30%), and in El Salvador, Central America, where it is more than twice as high, led him to the following conclusions: The parasite can definitely cause abortion in chronically infected women (California study); it is not a significant cause of perinatal mortality in Central America; and serological surveys are inadequate to answer the question of whether or not *Toxoplasma* can be transmitted from a chronically infected woman to her fetus, for the parasite has been reported to have been isolated from an abortus and placenta in a woman with a negative dye test. The recent finding of *T. gondii* demonstrated by immunofluorescence from endometrial biopsies in habitual aborters who had negative serological tests confirms this latter view (Stray-Pedersen and Lorentzen-Styr, 1977).

The prevalence of congenital toxoplasmosis is always difficult to determine accurately, for lesions attributable to the disease, particularly those affecting the eye, and to a lesser extent the brain, may be inapparent at birth and only develop gradually (Wilson et al., 1980). The most accurate figures for the United Kingdom may come from Scotland, where diagnostic tests have been centralized in one laboratory, and a congenital infection rate of 1 in 2000 live births has been given (Williams and Williams, 1979). Follow-up, though, has been acknowledged as incomplete, so this is almost certainly an underestimate. In a thorough review of the literature, Remington and Desmonts (1976) gave the following incidence figures for congenital toxoplasmosis, calculated per 1000 live births: Netherlands, 6.5; New York City, 1-3; Birmingham, Alabama, 1.3; Paris, 3; Gottingen, 5; Vienna, 6-7; and Mexico City, 2. They suggested that most of these figures are too low. They believe that 3000 of the 3 million annual births in the United States have the congenital infection. In the prospective British survey already cited, 8 out of 10 infants born to mothers with a primary pregnancy infection were followed for 2 years and showed no evidence of infection; 1 was stillborn and 1 died in the neonatal period, but there was no definite evidence that *Toxoplasma* played a contributory role in either of these deaths (Ruoss and Bourne, 1972). In the prospective

Table 2 Possible Effects of Some Viruses and Other Organisms not Described in Text

| Infecting organism | Fetal and/or neonatal involvement |
|---|---|
| Viruses[a] | |
| echovirus | Serological and virological confirmation of placental passage exists, but no evidence to suggest congenital defects caused by early maternal infection (Hanshaw and Dudgeon, 1978). Spectrum of neonatal illness ranges from inapparent to overwhelming and fatal (see also Table 1); hepatic necrosis and disseminated intravascular coagulation may occur with latter (Modlin, 1980). |
| influenza (most studies relate to A) | Possible risk increased abortion. No consistent pattern of congenital anomalies in different parts of the world; evidence in favor of increased teratogenic risk is inconclusive. Increased perinatal mortality during recent epidemic year reported (Department of Health and Social Security, 1971). Possible association with later development of leukemia and neoplasms of lymphatic and hematopoietic tissue (see text). |
| lymphocytic choriomeningitis | Placental passage occurs (Komrower et al., 1955). Cases very rare in United Kingdom; occasional report elsewhere associated with maternal contact with infected golden hamsters, with severe central nervous system involvement in infants (Ackermann et al., 1974). |
| mumps | Excessive number of abortions associated with first trimester mumps (Young, 1976). Postulated association with endocardial fibroelastosis (Noren et al., 1963) and risk of condition in offspring of a woman developing mumps in pregnancy is approximately 2% (Hanshaw and Dudgeon, 1978). Mumps virus certainly able to infect placenta (Aase et al., 1972) and fetus (Garcia et al., 1980; Kurtz et al., 1982). Clinically apparent mumps may occur in infant following maternal perinatal infection, but rare and usually benign (Young, 1976). |
| poliovirus | Increased risk of abortion (Cherry, 1976); no increase of congenital defects, inconclusive evidence of preterm birth. Neonatal poliomyelitis most frequently seen with paralytic maternal illness occurring just before delivery (Siegel and Greenberg, 1956). |
| rubeola | No evidence of teratogenic effect, doubtful increased risk of abortion, probable risk of preterm labor; congenital measles not inevitable with perinatal maternal infection, but usually severe (Young, 1976). |
| vaccinia | No harmful effect first trimester vaccination in prospective study involving 4172 women (Greenberg et al., 1949). Virus can involve placenta and fetus quite extensively; isolated case reports describe abortion, stillbirth, and live birth with generalized vaccinia (lesions often unusually large). Vaccination should be avoided |

Table 2 (continued)

| Infecting organism | Fetal and /or neonatal involvement |
|---|---|
| Viruses[a] (continued) | |
| | in pregnancy; if essential, give specific antivaccinal gammaglobulin (Central Public Health Laboratory, London) at same time. |
| varicella zoster | Teratogenic effect probably established beyond doubt, but risk very small (Hanshaw and Dudgeon, 1978). Lesions include hypoplasia of limbs, skin scarring, choroidoretinitis, and meningoencephalitis (Hanshaw and Dudgeon, 1978). Varicella ocasionally and herpes zoster more rarely may occur in newborn following maternal varicella late in pregnancy (see also the section on prevention). |
| variola | High fetal loss, increased risk premature delivery, live or stillborn infants may bear old scars or fresh lesions, often unusually large. Congenital infection not inevitable (Hanshaw and Dudgeon, 1978). |
| western equine encephalitis | Infection not invariable, risk unknown, central nervous system damage to infant may be severe |
| Protozoa | |
| *Plasmodia* | Congenital malaria very rare among indigenous populations of endemic areas who have substantial immunity, but not uncommonly found when mothers are poorly immune and inadequately treated or untreated. Placental involvement can be massive without fetal infection. |
| *Trichomonas vaginalis* | Reports of infection involving infant girls in the United Kingdom are rare (Postlethwaite, 1975), but more common elsewhere, probably in association with untreated maternal infection. |
| *trypanosomes* | Transplacental infection shown to occur with African and South American trypanosomiasis, but precise risk unknown. Difficult to know to what extent maternal infection itself may predispose to probable increase in abortion and stillbirth (Bittencourt, 1976). |
| Fungi | |
| *Candida albicans* | Intrauterine infection of fetus, placenta, and membranes recorded in a few isolated cases, and organism demonstrated on fetal surface placenta in 0.8% cases (Maudsley et al., 1966). May be increasing with use of intrauterine contraceptive devices (Whyte et al., 1982). Incidence oral and perineal moniliasis in offspring of women with inadequately or untreated monilial vulvovaginitis is high (Shrand, 1961). |
| *Coccidioides immitis* | Very rare. Maternal disease may disseminate in pregnancy (McCoy et al., 1980). Placenta is usually effective barrier, but transmission to fetus has occurred (Bernstein et al., 1981). |

[a]Evidence for maternofetal transmission of Epstein-Barr virus is still hypothetical, but see Icart and Didier (1981).

American survey, only 3 of the 19 women involved transmitted the infection to their fetuses, and only 1 of the 3 showed any abnormality (Kimball et al., 1971). Desmonts and Couvreur (1974) reported that 17% of fetuses were infected when maternal toxoplasmosis occurred in the first trimester, 25% in the second trimester, and 65% in the third. However, the risk to the fetus of severe manifestations was far greater the earlier in pregnancy the infection occurred. 80% were severely involved in the first trimester, 30% in the second, whereas third-trimester infection appeared to give subclinical or no fetal involvement. The offspring of successive and closely spaced pregnancies of infected mothers have rarely been reported to be involved (Langer, 1963; Garcia, 1968).

Treatment with spiramycin during pregnancy reduced the overall frequency of the fetal infections, but not of obvious disease (Desmonts and Couvreur, 1974). There is, however, no general agreement on the treatment of toxoplasmosis acquired in pregnancy, and other regimens such as pyrimethamine and sulphonamide have their advocates, though the possibility of any of the drugs having a teratogenic effect must be borne in mind.

### Serological Diagnosis

An enzyme-linked immunosorbent assay has recently been adapted to detect low concentrations of *T. gondii* antigen. The test was successful in finding blood antigens in 65% of cases of recently acquired acute toxoplasmosis. Antigen was also detected by this method in amniotic fluid (Araujo and Remington, 1980). The diagnosis of primary toxoplasmosis can also be established by the demonstration of rising antibody titers. All too often, a stable high titer, which may persist for several years after infection, may have been reached when the patient is first tested, and a conclusive diagnosis may not be possible (Remington, 1973). The most commonly used tests have been the Sabin-Feldman dye test, the CF test, the HI test, and the *Toxoplasma*-specific IgM fluorescent antibody test. If dye test or IgM fluorescent antibody titers are already high when first seen (greater than 1:512), a negative CF test turning positive or increasing CF titers together with stable high dye test titers are indicative of active infection. The indirect fluorescent antibody test has occasionally given false positive results with some sera containing antinuclear antibodies. Thus in patients with disorders such as lupus erythematosus, a dye test or HI test should be performed to confirm a positive indirect fluorescent antibody test (Remington, 1973).

In the newborn, the *Toxoplasma*-specific IgM fluorescent antibody test will be positive in those infected but asymptomatic, as well as in those who have signs of disease. Remington (1973) demonstrated that several infants with false positive tests showed no serological evidence of congenital toxoplasmosis in later life. It must also be remembered that a majority of infants in whom congenital infection is later proved have *no* antibodies demonstrated by the IgM fluorescent antibody test at birth, so that their absence then, or even during the first months of life, does not rule out congenital infection, and suspect infants should be carefully followed clinically and serologically. The reasons for these occasional uncertainties of the specific IgM test are similar to those already discussed for *T. pallidum.*

### Histological Diagnosis

Trophozoites and tissue cysts can be demonstrated in tissues and body fluids such as cerebrospinal fluid. If placental evidence is sought, the organ should be fresh and not formalin fixed. Parasites were isolated from the placenta in 25% of the French series (Desmonts and Couvreur, 1974).

The possible effects on the fetus of some other microorganisms not discussed in the text are shown in Table 2.

## PREVENTION

Many of those who transmit certain infections to the fetus in pregnancy or during the process of birth are of low socioeconomic status. The remedies are complex and are social as much as medical. A vicious cycle often exists which it is difficult to break. The selective rubella vaccination program introduced in the United Kingdom in 1971 may now be having a favorable impact on the immune status of women of reproductive age (Clarke et al., 1979). This is not, however, a universal view (Hambling, 1980; Broadbent et al., 1980), and the numbers of children registered with congenital defects following the epidemic of 1978 (Anonymous, 1981b) mean that further efforts must be made to reach those still susceptible. Simple explanation of the medical facts of congenital rubella given directly to schoolgirls eligible for vaccination has been shown to be the most effective form of recruitment (Jones, 1980).

Whether the detection of pregnancy toxoplasmosis, serologically speaking, and the treatment of infected mothers in the hope of preventing fetal infection is cost effective is difficult to ascertain because of the uncertainties surrounding the true extent of congenital toxoplasmosis. There may be more to be said for the health education of mothers with regard to the disease: They should avoid eating or handling raw or undercooked meat, avoid contact with the domestic cat's litter boxes, and beware of contamination of uncooked vegetables. There seems every reason to give immune globulin (HBIg) to infants born to mothers who have hepatitis due to HBV, or to those born to HBsAg-positive carrier mothers who are also HBeAg positive, in view of the increased risk they run of becoming infected. The question of a vaccine for CMV and the problems of possible oncogenicity and attenuation associated with a live tissue culture-adapted viral strain have been discussed by Hanshaw and Dudgeon (1978); it appears the questions are still unsolved. Passive immunization with zoster-immune globulin, if available, may be offered to susceptible pregnant women who have come into contact with varicella or zoster in early pregnancy (Hanshaw and Dudgeon, 1978). It may also be given to newborn infants whose mothers develop varicella 5 days or less before delivery, or to those whose mothers develop varicella at or shortly after birth. In these cases the infants should be isolated from their mothers at delivery, and, of course, the mothers isolated as well (Hanshaw and Dudgeon, 1978).

Overseas travel is now common, and obstetricians may be faced with decisions regarding the protection of their patients with vaccine and the safety of such protection for the fetus. Killed vaccines are probably not important, though live vaccines may pose a hazard. In a review of the available evidence, it has been concluded that immunization against poliomyelitis and yellow fever is indicated in women traveling to affected zones. As has already been stated, rubella vaccination is contraindicated in pregnancy; this also applies to mumps and smallpox vaccination (should need for the latter ever again be necessary). Measles vaccination is not indicated (Levine et al., 1974). Malarial prophylaxis in the form of 50 mg of pyrimethamine given as a single dose once monthly to pregnant African women has been considered safe and effective and has resulted in a greater fetal weight gain during pregnancy compared with untreated controls (Morley et al., 1964), though resistance to antimalarial drugs is becoming increasingly common.

## CONCLUSIONS

It has to be reiterated that the true extent to which maternal infection is transmitted to the fetus is still unknown. New serological techniques, improved cultural methods, and epidemiological surveys are gradually making a clearer assessment possible. Viral and protozoal infections acquired in the first trimester due to rubella, CMV, HSV, and *Toxoplasma* can be devastatingly crippling for the individual child. Numerically speaking, however, such early damaging infections are in the minority and, with the possible exceptions of rubella early in the second trimester and HSV at any time, acquisition of viral and protozoal infections later in pregnancy or even during delivery leads to mild or inapparent clinical involvement of the child. Only really long-term follow-up studies involving sizable numbers of children matched with noninfected controls of the same gestational age and social class can determine the true significance of these individual infecting microorganisms. Future work could also profitably explore genetic differences between dizygotic twins who are found to be involved to very differing degrees following transplacental infection.

Many early and later spontaneous abortions may be accompanied by chorioamnionitis, and ascending bacterial infection may have a causal role. When the pregnancy continues in the face of bacterial infection in early or mid-pregnancy, such an infection's possible contribution to fetal brain damage in particular is unknown and largely uninvestigated. There is a growing awareness that a mother in the least advantageous social and economic circumstances is most likely to put her fetus in jeopardy. At present there are all too few completely comprehensive surveys in which the flora of the birth canal at various stages of pregnancy have been studied with accurate counts of individual microorganisms and compared to that in nonpregnant controls matched for sexual experience and social class. It seems likely that these, together with further study of factors of local immunity which influence the numbers of bacteria adhering to epithelial surfaces, will lead to a greater understanding of fetal risk. It is already known that women with many sexual partners and of poor social background are more likely to be colonized with a number of microorganisms such as CMV, HSV, GBS, *C. trachomatis,* and *N. gonorrhoeae,* which may be transmitted to the fetus in labor. Surgical induction and transvaginal monitoring may occasionally increase the risk of ascending infection.

Finally, health education of schoolgirls entering puberty and of young women of reproductive age in general would seem to have a definite role to play if preventive vaccines are to be used to their fullest advantage. It could also be used to explain in more general terms the ways in which infective risks to the fetus could be reduced.

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# 12

# Factors Influencing Perinatal Wastage

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## INTRODUCTION

Trends in perinatal and infant mortality rates in recent years have demonstrated that a larger proportion of such deaths are preventable than we had previously thought. However, with the steep falls in mortality rates that are occurring in all developed countries, the causes of fetal wastage are increasingly those most difficult to prevent, particularly those acting before or throughout pregnancy. In spite of this, there is as yet no evidence of an irreducible level below which these rates will not fall, even from that most privileged of countries, Sweden.

There is therefore a continuing need to identify the remaining resistant causes, particularly those likely also to be causes of nonlethal damage, so that specific preventive actions can be instituted. There has been a continuing development and refinement of epidemiological tools to meet this need, and in this account these methods and recent results from their use will be described. These include the study of mortality rates over time and contemporary national and regional comparisons allowing for population differences. They also include attempts at distinguishing between deaths and damage preventable by medical care alone, and those whose prevention demands measures of a more widespread social nature, often depending on political priorities. Of more fundamental interest is the study of the basic pathways through which social and demographic disadvantages can be transmitted to the fetus, and this too will be discussed briefly.

## DEFINITIONS

The eighth revision of the *Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death* defined a perinatal death as one occurring at any time from the twenty-eighth week of gestation to the seventh day of life (World Health Organization, 1967). However, many problems of international comparison persisted, particularly in regard to the definition of *viability* (Weatherall, 1977). For instance, the weight of a fetus is used by some countries, but not others, to place a lower limit to viability. The ninth revision of the *Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death* (World Health Organization, 1977) has recommended that *national* perinatal statistics should include all fetuses and infants of a weight of 500 g or more or, where birth weight is unavailable, of the corresponding gestational age (22 weeks) or of a crown-heel length of 25 cm.

Standard perinatal statistics for *international* comparisons should use only those infants with birth weights of 1000 g and above or, where birth weight is unavailable, of the corresponding gestational age (28 weeks) or with a body length of 35 cm. The use of such limits is only possible where birth weight or the other parameters are registered at birth, and therefore cannot be applied even in some developed countries. In England and Wales the birth certificate included no information on birth weight until very recently (Office of Population Censuses and Surveys, 1980a) and it still includes none on gestational age.

With increasing expertise in perinatal care, some neonatal deaths may now be shifted from the first week of life to later in the first month of life. It has therefore been suggested (Macfarlane et al., 1980) that it would be appropriate to include all first-month deaths in perinatal statistics, as is already being done in some countries, for instance, Australia.

Although international comparisons can be misleading because of differences in definitions, provided that the latter have not changed within countries, alterations in rank order of countries by mortality rates are likely to reflect real differences. The World Health Organization and the United Nations try to present comparable data in their statistical reports wherever possible and carry out standardized reviews of mortality from time to time. Examples of these data will be given later in this account.

## DIFFERENCES BETWEEN AND WITHIN COUNTRIES AND TRENDS OVER TIME

Figure 1 shows the trends in the rates of stillbirth and early neonatal mortality (first-week death) recorded since 1935 in England and Wales. Each of these has fallen continuously, and the rate of fall has accelerated in recent years (Macfarlane, 1979). Moreover, neonatal (first month) deaths comprise an increasing proportion of all infant (first year) deaths, as the more easily preventable postneonatal deaths have been reduced

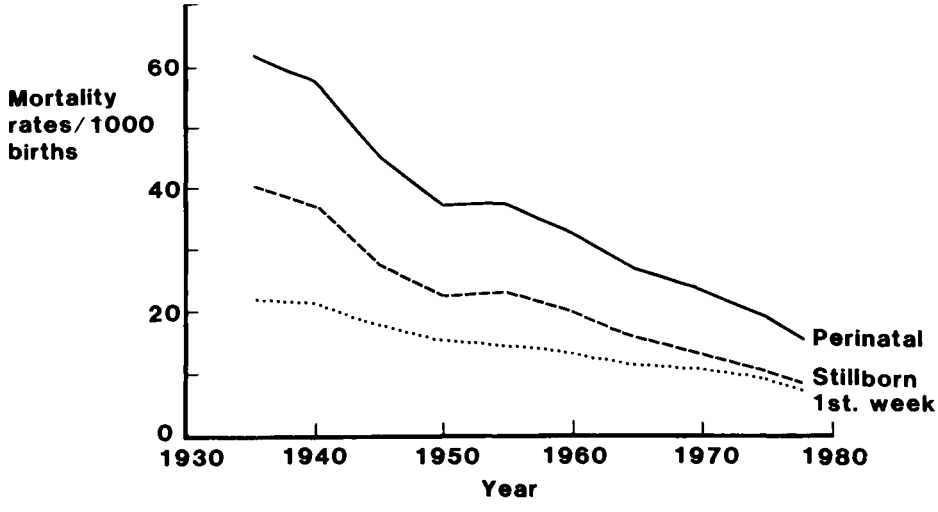


Figure 1 Components of perinatal mortality rate in England and Wales, 1935-1978. (Data from the Office of Population Censuses and Surveys, 1980b.)

**Table 1** Infant and Early Neonatal Mortality Rates in 1977

| Country | Mortality rates per 1000 live births | | Early neonatal mortality rate / Infant mortality rate × 100 |
|---|---|---|---|
| | Infant | Early neonatal | |
| Chile | 47.5 | 14.7 | 31 |
| Cuba | 25.0 | 12.8 | 51 |
| United States | 14.1 | 8.4 | 60 |
| Sweden | 8.0 | 5.0 | 63 |

*Source*: Data from World Health Organization (various years).

**Table 2** Regional Mortality Rates in England and Wales

| Region[a] | Perinatal mortality rates per 1000 total births | | Mortality rates at ages 45-54 per 1000 males |
|---|---|---|---|
| | 1968 | 1978 | 1978 |
| East Anglia | 21.1 | 13.3 | 5.6 |
| South East | 23.0 | 14.2 | 5.8 |
| South West | 22.6 | 14.6 | 5.9 |
| England and Wales | 24.7 | 15.5 | 6.8 |
| East Midlands | 24.3 | 15.6 | 6.5 |
| Yorkshire and Humberside | 26.2 | 15.7 | 7.4 |
| Wales | 27.5 | 16.8 | 7.9 |
| North West | 28.3 | 17.0 | 8.2 |
| West Midlands | 25.2 | 17.2 | 6.7 |
| North | 24.7 | 17.6 | 8.7 |

[a]Ranked by 1978 perinatal mortality rates.

*Source*: Data from the General Register Office (1970) and the Office of Population Censuses and Surveys (1980b,c).

**Table 3** International Secular Trends in Perinatal Mortality Rate

| Year | France | Japan | England and Wales | Singapore | Sweden |
|---|---|---|---|---|---|
| 1967 | 27.2 | 26.3 | 25.8 | 24.6 | 18.9 |
| 1971 | 22.8 | 20.5 | 22.5 | 21.2 | 15.7 |
| 1977 | 15.8 | 14.1 | 17.1 | 16.2 | 10.1 |

*Source*: Data from the World Health Organization (various years), using 1000 live births as a denominator in the calculation of perinatal mortality rates.

Table 4 High Parity, Teenage Pregnancy, Low Birth Weight, and Perinatal Mortality in 1973

| Country | Percentage of births to mothers of parity 4 and above | Percentage of births to mothers under 20 years | Percentage of births 2500 g or less | Perinatal mortality per 1000 total births |
|---|---|---|---|---|
| Sweden | 1.8 | 7.5 | 3.9 | 12.6 |
| United States (part) | 9.7 | 17.6 | 6.0 | 14.9 |
| Japan | 0.8 | 0.9 | 5.3 | 17.0 |
| New Zealand (legitimate births only) | 7.6 | 14.6 | 5.2 | 17.3 |
| England and Wales (legitimate births only) | 3.9 | 11.0 | – | 18.9 |
| Austria | 7.7 | 14.5 | 5.7 | 21.4 |
| Cuba | 17.7 | 22.3 | 10.8 | 26.9 |
| Hungary | 4.1 | 16.5 | 10.8 | 29.1 |

*Source*: Data from the World Health Organization (1978).

(Pharoah and Morris, 1979). The same feature is shown by comparing international statistics (Table 1), the more developed countries having the highest proportion of early deaths.

Within England and Wales, as in most other countries, there are marked and consistent variations in perinatal mortality rates by region, the rates in the north of England being higher than in the south. This pattern closely parallels the north-south gradient of mortality rates at other ages (Table 2), in women as well as in men, a fact which underlines the necessity to study perinatal wastage within the framework of the health status of the entire community in which it occurs.

Table 3 shows the falls which have been recorded in some countries and the changes in ranking order of perinatal mortality rates where these accelerated or decelerated in relation to each other. Such changes in ranking may reflect changes in health care, environmental factors, or population demographic characteristics and may be helpful in identifying important agents of change. Thus the relative improvement of the perinatal and infant mortality of France since 1973 has been made the subject of many studies to identify the factors responsible. These have proved to be difficult to isolate, but the massive publicity given to the French government's initiative to reduce perinatal mortality in 1972 was probably one of the major reasons for the fall. Sweden, for many years the leader in the league of perinatal mortality rates, has also often been studied in a search for ways of emulating their success and it is felt that their good result may stem in part from the strong regional organization of the Swedish obstetric and pediatric services.

However, it has long been recognized that an essential part of interpreting such comparisons between or within countries must be to allow for the effect of such important variables as the maternal health, age, parity, and socioeconomic compositions of the populations to be compared, and the related risks of reduced birth weight and certain congenital abnormalities in the disadvantaged groups. The marked differences between countries in such respects is illustrated in Table 4.

## IMMEDIATE CAUSES OF DEATH IN ENGLAND AND WALES AND TRENDS OVER TIME

The identification of causes is necessary in order to further reduce these deaths and can be approached in several different ways. First, one can study immediate causes of death as certified by the medical attendant, and their trends over time.

A classification of 14 categories of perinatal wastage, based on the P list of the eighth revision of the *Manual of the International Statistical Classification of Diseases, Injuries, and Causes of Death,* has been used in the evaluation of recent trends in the causes of perinatal mortality in England and Wales (Edouard and Alberman, 1980). Nearly 60% of perinatal deaths in 1978 were due to four causes (Table 5): congenital malformation, prematurity, placental insufficiency, and anoxia of unspecified cause. The three most common causes of stillbirths were placental insufficiency, congenital malformation, and placental hemorrhage, corresponding causes for early neonatal deaths being prematurity, congenital malformation, and anoxia of unspecified cause. Although hemolytic disease and infection are numerically few, they are important in view of their known favorable response to treatment.

An analysis of secular trends in the certified causes of perinatal mortality revealed sharp decreases for anoxia of unspecified cause, congenital malformation, and prematurity from around 1973 (Table 6). The fall in perinatal mortality rate from congenital malformation can be only partly due to the antenatal program aimed at the secondary prevention of conditions such as neural tube defects, Down syndrome, and certain inborn errors of metabolism. In 1977, 1870 children in England and Wales were notified as having been born with a malformation of the central nervous system, but only 124 legal abortions were carried out on the grounds that this was a suspected abnormality in the fetus (Bradshaw et al., 1980). Although the aim of this program is the prevention of handicap and not perinatal death, one can predict that if total population screening, for neural tube defects only, were to be fully implemented, the overall perinatal mortality rate would fall by about 8%, even with no other change occurring.

**Table 5** Percentage Distribution of Perinatal Deaths in England and Wales in 1978 by Certified Cause Groups

| Certified cause group | Stillbirths | First-week deaths | Perinatal deaths |
|---|---|---|---|
| Congenital malformation | 20.6 | 24.4 | 22.3 |
| Prematurity | 1.4 | 31.1 | 14.9 |
| Multiple pregnancy | 2.7 | 6.0 | 4.2 |
| Hemolytic disease of the newborn | 1.7 | 0.8 | 1.3 |
| Umbilical cord complication | 7.8 | 0.9 | 4.7 |
| Birth injury | 0.6 | 7.6 | 3.8 |
| Difficult labor | 2.7 | 1.3 | 2.1 |
| Anoxia of unspecified cause | 6.5 | 13.0 | 9.5 |
| Placental hemorrhage | 10.8 | 0.9 | 6.3 |
| Placental insufficiency | 21.0 | 0.4 | 11.7 |
| Preeclampsia syndrome | 8.4 | 0.6 | 4.9 |
| Maternal and fetal infection | 0.4 | 3.4 | 1.8 |
| Medical condition of mother | 5.2 | 0.4 | 3.0 |
| Miscellaneous | 10.3 | 9.2 | 9.8 |

*Source*: Adapted from Edouard and Alberman (1980).

Table 6 Perinatal Mortality Rates per 1000 Total Births in England and Wales

| Year | Certified causes of perinatal mortality | | | |
|---|---|---|---|---|
| | Congenital malformation | Anoxia of unspecified cause | Prematurity | Hemolytic disease of the newborn |
| 1968 | 4.3 | 2.1 | 3.8 | 0.88 |
| 1973 | 4.2 | 2.2 | 3.4 | 0.45 |
| 1978 | 3.5 | 1.5 | 2.3 | 0.19 |

*Source*: Adapted from Edouard and Alberman (1980).

The decrease in perinatal mortality rate associated with anoxia of unspecified cause coincided with, but is not necessarily due to, the introduction of electronic intrapartum fetal heart rate monitoring into routine clinical practice.

The category of hemolytic disease of the newborn has shown the steepest fall in perinatal mortality rate since 1968 (Table 6). Knox (1976) showed that parity changes and general improvements in the quality of medical care were major factors leading to the decline in mortality rate from hemolytic disease, specific technological advances such as intrauterine transfusion and anti-D prophylaxis having had only little influence up to 1976, although this may have changed since.

## EFFECT OF MATURITY AT BIRTH

The most important factor in determining the risk of mortality from almost any cause is the maturity of the fetus at delivery, and in our present state of knowledge it appears that little in the way of medical care or technology can influence this, except insofar as delivery can be induced early. Because of difficulties in defining and reporting gestational age, birth weight is commonly used as the main indicator of maturity. Its overwhelming importance is illustrated by the fact that neonates of birth weight 2500 g and under accounted for only 7.2% of births in England and Wales in 1979, but 65.1% of perinatal deaths (Table 7). The improved fetal outcome with increasing birth weight, even within gestational age groups, is shown in Figure 2. The rise in rates at the upper end of the birth weight scale may be due to the association of maternal diabetes mellitus and labor dystocia with heavy babies.

The joint effect of birth weight and gestation on perinatal mortality can also be illustrated by a three-dimensional presentation with the use of contour charts. These have been used very effectively by Hoffman and his colleagues (1974). Bakketeig et al. (1979) used the same technique to show that siblings often have similar birth weights and gestational lengths, so that there is a tendency for mothers to have repeated small-for-dates or just low-birthweight pregnancies.

Differences in birth weight distribution account for a large proportion of the variation seen between and within countries, and several workers have tried to allow for such variations by different methods of standardization or by the use of birth weight-specific rates (Macfarlane et al., 1980).

Thus in a comparative study of social and biological effects on perinatal mortality (World Health Organization, 1978) 10.8% of all births in Hungary, as compared to only 3.9% in Sweden, had a birth weight of less than 2500 g. Standardization for birth weight improved the position of Hungary and reduced the variation in perinatal

Table 7 Birth Weight and Perinatal Mortality in England and Wales in 1979

| | Birthweight | | |
|---|---|---|---|
| | Up to 2000 g | 2001-2500 g | Over 2500 g |
| Percentage distribution of total births | 2.4 | 4.8 | 92.8 |
| Perinatal mortality per 1000 total births | 319.0 | 39.1 | 5.5 |
| Percentage distribution of perinatal deaths | 52.1 | 13.0 | 34.8 |

*Source*: Data from the 1979 Community Health Services Statistics of the Department of Health and Social Security (personal communication) and the Office of Population Census and Surveys, (1980a).

mortality rates between this and other countries. Similarly, the ranking of administrative areas within England and Wales by perinatal mortality rate has changed with standardization for the birth weight distribution (Chalmers et al., 1978; Mallet and Knox, 1979).

Nevertheless, birth weight-specific mortality rates do reflect the quality of medical care (Wigglesworth, 1980). A Norwegian study relating perinatal mortality rates to the provision of facilities showed that the most sensitive indicator of the quality of care was the outcome for births weighing above 2500 g (Bakketeig et al., 1978). The excess of observed over expected perinatal deaths of babies of 2500 g or more found by Alberman (1980) by applying 1978 Swedish birth weight-specific mortality rates to the corresponding births in England and Wales in 1978 may well reflect a real difference in the quality of medical care between the two countries, although this possibility needs to be re-examined within narrower birth weight groups.

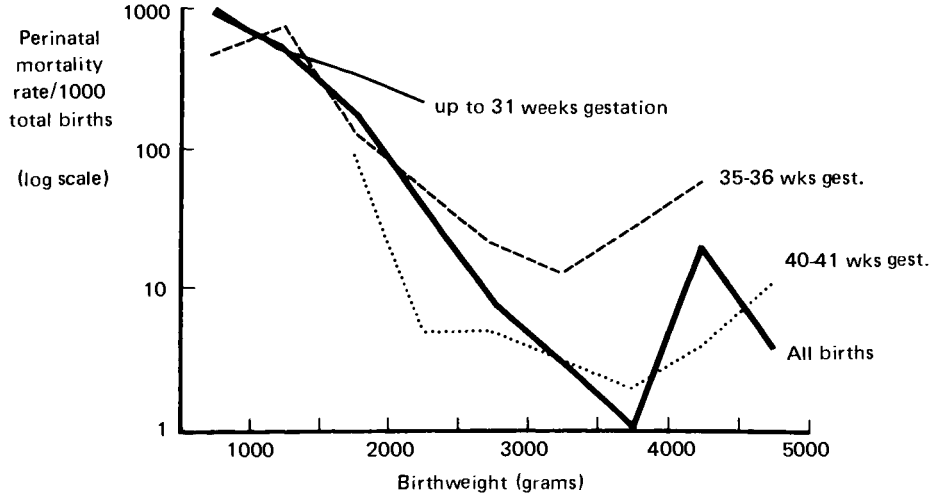


Figure 2 Perinatal mortality rates by birth weight (grams) and gestational age (in weeks). (Data from McIlwaine et al., 1979.)

## SOCIAL AND DEMOGRAPHIC FACTORS

### Social Class

Table 8 summarizes some of the most important effects of certain social and biological factors on perinatal mortality, derived from information obtained by linking birth and infant death certificates for England and Wales. Similar effects have been reported consistently from studies over many years and in various countries.

One of the most important effects is that of social class, however measured, although it probably reflects an interaction between many other factors. Table 8 shows the steady rise in perinatal mortality from the most advantaged to the most disadvantaged groups within the legitimate births, and the even higher rate in illegitimate births, twice that of the lowest rate for legitimate births among the highest social class mothers. This factor alone explains much of the regional differences that are found in England and Wales and their positions in international comparisons of perinatal mortality rates.

### Maternal Age and Parity

Social class gradients in perinatal mortality can be explained, to a large extent, by the accompanying differences in demographic and biological factors. The effects of such biological factors as maternal age and parity on perinatal mortality are themselves extremely complex. On cross-sectional analysis the typical pattern of risk with each tends to be J shaped, with the minimal risk in England and Wales in 1977 being in second pregnancies of mothers aged between 25 and 29 (Figure 3). In 1977 in England and Wales such babies made up 18.9% of legitimate births in the professional and managerial classes, but only 8.3% of those among unskilled manual workers, and this alone would account for a proportion of the observed difference in perinatal mortality between the social classes.

However, the results of longitudinal analyses suggest that this J-shaped pattern is an artifact. Studies within sibships reveal that perinatal risk appears to fall consistently with increasing parity. There are, however, differences in risk between mothers of sibships of different sizes. Even the *first* pregnancy of mothers who go on to become of high parity, and who allow close spacing between their pregnancies, is at higher risk of mortality (Figure 4) and of lower birth weight than those of mothers who limit and space out their families; and this consistently raised risk of high-parity mothers accounts for the apparent rise in rate after the second pregnancy (Bakketeig and Hoffman, 1979). It is still not clear to what extent this pattern is determined by the self-selection of mothers into high- and low-parity groups, whether for reasons of compensating for perinatal loss or because of poor education, or whether birth order itself has an independent effect on perinatal risk. Similarly, it seems that advancing age alone may not have the deleterious effect that has long been assumed, for a falling risk with birth order within sibships must imply a risk falling with maternal age, or at least without an important rise in age.

### Maternal Stature

There are other, probably more important differences between mothers of different social class. One of the most easily measured is stature, and a consistent finding in all parts of the world is that the height of adults in the socially advantaged groups is greater than that in disadvantaged groups. Data from the 1958 National Birthday Trust Study

**Table 8** Social and Biological Effects on Perinatal Mortality in England and Wales in 1977[a]

| Legitimacy | Perinatal mortality rate | Parity[b] | Perinatal mortality rate | Maternal age | Perinatal mortality rate | Social[b] class | Perinatal mortality rate | Birthplace of mother | Perinatal mortality rate |
|---|---|---|---|---|---|---|---|---|---|
| Legitimate | 16.2 | 0 | 18.1 | <20 | 23.0 | Professional and managerial | 11.6 | Continental Europe | 14.1 |
| Illegitimate | 23.3 | 1 | 13.0 | 20-24 | 16.8 | Supervisory | 13.0 | United Kingdom | 16.5 |
| | | 2 | 16.2 | 25-29 | 14.8 | Skilled non-manual workers | 14.1 | West Indies | 21.9 |
| | | 3 or more | 22.4 | 30-34 | 15.8 | Skilled manual workers | 17.1 | India/ Bangladesh | 22.0 |
| | | | | 35 and above | 24.2 | Semiskilled workers | 18.8 | Africa | 23.7 |
| | | | | | | Unskilled workers | 22.0 | Pakistan | 25.2 |

[a]Perinatal mortality rate for all births, 16.9 per 1000.

[b]Data available for legitimate births only.

*Source*: Adapted from Adelstein et al. (1980).

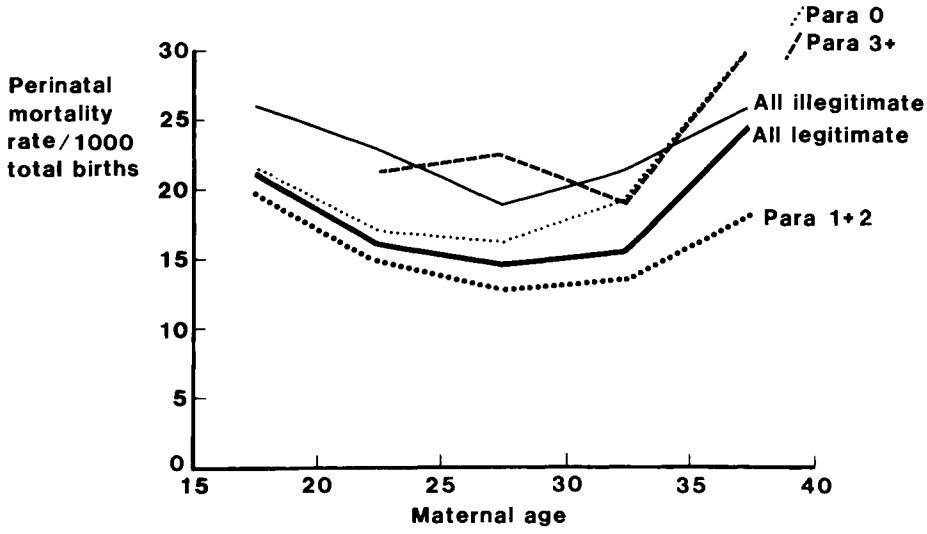


Figure 3 Perinatal mortality rates for England and Wales in 1978 by maternal age, parity, and legitimacy. (From Adelstein et al., 1980.)

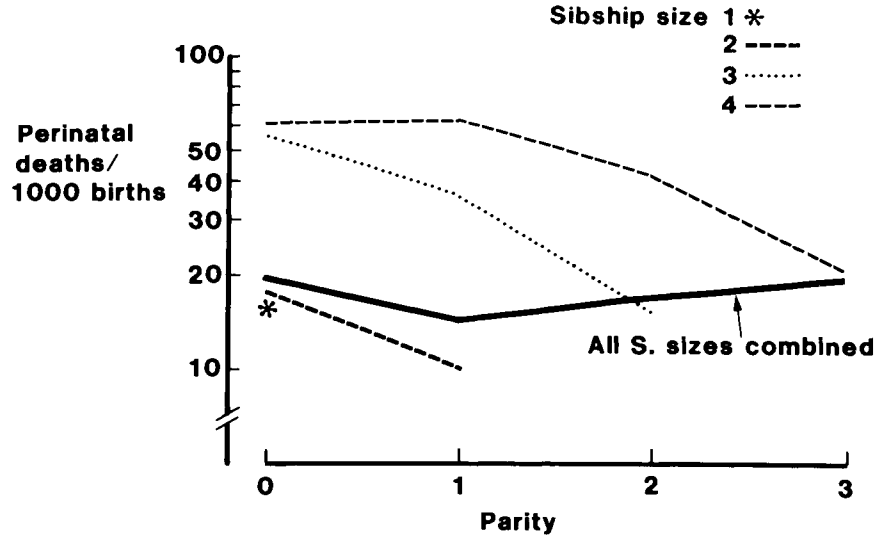


Figure 4 Perinatal mortality by parity and sibship size. (Data from Bakketeig and Hoffman, 1979.)

Table 9 Relation of Perinatal Mortality Ratio[a] (MR) to Socioeconomic Class and Height Distribution (%) in Maternal Population

| | Maternal height | | |
|---|---|---|---|
| | 65 in. and over | 62-64 in. | Under 62 in. |
| Professional | | | |
| % | 40.9 | 45.6 | 13.5 |
| MR | 61 | 73 | 87 |
| Nonmanual | | | |
| % | 33.4 | 45.5 | 21.1 |
| MR | 72 | 80 | 90 |
| Skilled | | | |
| % | 27.8 | 48.9 | 23.3 |
| MR | 83 | 91 | 105 |
| Semi- and unskilled | | | |
| % | 26.0 | 48.6 | 25.4 |
| MR | 88 | 106 | 140 |

[a]100 = average perinatal mortality ratio.

*Source*: Adapted from Illsley and Kincaid (1963).

(Illsley and Kincaid, 1963), given in Table 9, shows how maternal height varies with socioeconomic group and how perinatal mortality varies with maternal height within each socioeconomic group. The difference in stature by social class is self-perpetuating, for Illsley and Kincaid showed in the same study that the tallest women in each social class tended to marry men in a higher social class than their fathers, whereas the shortest were more likely on marriage to move socially downward. The variation of stature with socioeconomic circumstances is partly due to a stunting of growth associated with childhood nutritional status, rather than a genetically determined characteristic. However, the tendency is for adult growth to increase over generations with improvements in economic circumstances (Van Wieringen, 1978). The effects of short stature on perinatal risk are mediated in several different ways, partly through an increased risk of disproportion or otherwise difficult delivery, but largely through increased risk of fetal growth retardation with all its hazards.

### Ethnic Groups

It is well established that in countries like the United States or South Africa the black indigenous populations have considerably higher perinatal mortality rates than the white populations. The disadvantage observed in these and many ethnic minorities must, however, be seen in the context of social class differentials, as a greater proportion of such mothers are in the lower social classes. Studies of recent immigrant groups to the United Kingdom (Adelstein et al., 1980) have also shown some groups to have higher than average perinatal mortality rates (Table 8).

Nutritional deficiencies and excesses of certain congenital malformations (MacVicar, 1981) certainly play some part in causing the excess deaths, and underuse of medical care facilities may also play a part (Robinson et al., 1982).

Summary

These social and demographic effects result in the overall level of mortality being made up of the weighted average of the subgroups at different risk. Thus a population which includes only a small proportion of high-parity mothers or illegitimate births will have a lower mortality rate than one with a large proportion of such mothers. The confounding effects of such demographic variations in populations which are to be compared can be overcome statistically by standardizing for the proportion of mothers in different risk groups, for instance, by parity or social class. This was the method adopted by Hellier (1977) to demonstrate that changes in social class, maternal age, and parity together accounted for nearly a quarter of the fall in perinatal mortality seen between 1950 and 1973 in England and Wales.

## NUTRITION IN PREGNANCY

The relationship of maternal nutrition in pregnancy to placental function and fetal growth is far from clear. It has been reviewed in great detail by Metcoff (1978) and more recently in the report of a symposium on the subject (Dobbing, 1981). Much of our current knowledge rests on past experience during wartime conditions and current observations in developing countries. Stein and her colleagues (1975) studied the birth weight of infants born in Holland during and after the hunger period of 6 months up to May 1945 which caused acute generalized undernutrition. The effect of reduced maternal energy intake upon fetal weight seemed negligible in infants in the first two trimesters of pregnancy at the time, but a limitation of maternal diet in the last trimester was associated with a fall in birth weight.

Lechtig and his colleagues (1975) found that in a poor community in Guatemala supplementation of calorie levels in pregnancy was associated with an increase of birth weight; however, Susser and Stein (1977), in a randomized control trial of high-protein supplementation in pregnancy in black mothers in New York, found evidence of an increased risk of premature labor in the supplemented group. The picture is one of great complexity. Interactions between the constitution of the diet consumed and the characteristics of the mothers seem to lead to as yet unpredictable effects on birth weight when supplementation is attempted (Rush, 1983).

Recent findings resulting from trials of periconceptional vitamin supplementation in mothers who have had previous pregnancies with neural tube defects suggest that this treatment may reduce the incidence of such malformations (Smithells et al., 1981) and raise new questions about the role of specific nutritional factors in reproduction.

## ENVIRONMENTAL HAZARDS

Closely related to social and demographic factors are environmental and sometimes occupational hazards, which together with other contributory factors may increase fetal wastage; often through an effect on fetal growth. At present the most important and best documented of these is maternal smoking, although it is possible that alcohol consumption may prove to be a hazard of the same order (Kaminski et al., 1978). Occupational hazards such as those posed by anesthetic gases have been shown to increase the risk of spontaneous abortion, and possibly the risk of fetal defects, and decreased fetal growth rate (Vessey and Nunn, 1980). As an example of how such hazards may effect the fetus, an account will be given of the current state of knowledge in relation to maternal smoking.

### Maternal Smoking

Because the most important effect of maternal smoking in pregnancy is on fetal growth rate (Table 10), its effect on mortality risk is determined by the presence or absence of other factors acting on fetal growth. Thus in a pregnancy in which fetal growth is proceeding at a normal or faster than normal rate, the limitation imposed by maternal smoking may not be enough to appreciably increase the risk of mortality. However, where growth is already constrained, for whatever reason, an additional check such as that imposed by maternal smoking may be sufficient to shift the birth down to a high-mortality-risk weight group. The effect of this can be seen from population studies. In populations at low risk, as in California (Van Den Berg, 1977) or Finland (Rantakallio, 1978), maternal smoking is not associated with a statistically increased risk of perinatal mortality, whereas in populations at higher overall risk there is a significantly increased risk in babies of smokers (Butler and Alberman, 1969; Meyer and Tonascia, 1977).

Maternal smoking has certain specific effects on the placenta of exposed pregnancies It appears to be associated with a thinner placenta, but one spread over a wider area than normal, and with effects on the blood vessels at both the cellular and macroscopic level (Naeye, 1978; Christianson, 1979). It has also been shown to be associated with an excess risk of antepartum hemorrhage, possibly from the placental changes. The effect on birth weight seems to be mediated partially through placental vascular anomalies, and acute vasoconstriction during smoking, and partially through a direct effect of the products of cigarette smoke on the fetus. The cigarette smoking habit and drinking are associated with each other, and in Great Britain smoking is also associated with low social class, young maternal age, and high parity (Butler and Alberman, 1969). Both smoking and drinking are more common in the high-risk north than in the south of England and Wales (Office of Population Censuses and Surveys, 1975, 1980e). The general effect is a clustering together of adverse factors which leads to a cumulative adverse effect on the fetus. It is probable that other environmental chemical hazards may act in similar ways.

Infections during pregnancy are also important environmental causes of perinatal wastage, but this and the question of congenital malformations are discussed in Chapter 11 of this volume.

**Table 10** Mean Birth Weight (Grams) by Gestational Age and Smoking Status

| | Gestational age | | | | |
|---|---|---|---|---|---|
| Smoking status | Under 30 weeks | 30-33 weeks | 34-37 weeks | 38-41 weeks | 42 weeks and over |
| Current | 1154 | 1872 | 2793 | 3267 | 3351 |
| Never | 1645 | 2159 | 2869 | 3428 | 3497 |

*Source*: Adapted from Chamberlain et al. (1975).

## CONSTITUTIONAL FACTORS IN THE MOTHER

Constitutional maternal factors which increase the risk of perinatal loss or damage include such obvious disadvantages as uterine anomalies, maternal hormone or immunological disturbances, and also other less well understood tendencies such as may increase the risk of preeclampsia or multiple pregnancy.

### Preeclampsia

Preeclampsia is a syndrome within the spectrum of the hypertensive disorders of pregnancy and has an epidemiological pattern as interesting as that of nonpregnancy essential hypertension. Although it is difficult to define closely, analyses of groups of mothers with different degrees of hypertension and proteinuria in pregnancy in surveys from many parts of the world and at different times have produced fairly consistent results.

In the 1970 British births study (Chamberlain et al., 1978) 25% of mothers had some degree of preeclampsia, with 15.8% classified as mild, 3.9% as moderate, and 5.3% as severe. The prevalence varied with smoking habit and oral contraceptive use, having been slightly less common in mothers who smoked and in those who had used oral contraception. It also varies with maternal age and parity, being most common in primiparae and least in parities one through three, but rising steadily with age in each parity (Butler and Alberman, 1969). There was no significant difference between the overall incidence of hypertension recorded between the 1958 and the 1970 British national birth surveys, despite a significant fall in severe hypertension from 5.6 to 4.9%, but there was a drop of 50% in perinatal deaths associated with maternal hypertension. This confirms the clinical impression of a general reduction in the most severe forms of hypertension over the years, although much of this may be accounted for by demographic changes, such as a sharp fall in the proportion of births to older mothers. It has also long been known that preeclampsia is more common in association with essential hypertension, diabetes mellitus, chronic renal disease, rhesus isoimmunization, hydatidiform mole, and multiple pregnancy.

As well as variations in the incidence of the condition, there are marked variations in the perinatal mortality associated with severe preeclampsia in different demographic subgroups. Table 11 shows how this varies with maternal age, the lowest perinatal loss being in affected mothers of 25-29 years, and Table 12 shows this by maternal height, the lowest loss being in the tallest mothers.

### Antepartum Hemorrhage

The causes of antepartum hemorrhage also are many and ill-understood. A description of the epidemiology of such hemorrhage is also to be found in the report of the 1970 British births. Of all births in this study, 88.7% were reported as having no form of bleeding, 0.5% were described as having placenta previa, and 1.2% accidental hemorrhage. Another 4.2% had had a threatened abortion only. The incidence of each tended to rise with maternal age. Perinatal mortality in cases of antepartum hemorrhage was always considerably higher than that in mothers who had not bled, was least in mothers aged between 25 and 29 years and highest in mothers of 35 years or more.

### Multiple Pregnancy

There were 10.0 multiple maternities for every 1000 maternities in England and Wales in 1978. Duncan (1866) showed that "mothers rising in age were more prolific in

Table 11 Incidence of and Perinatal Mortality in "Severe" Preeclampsia and Maternal Age

| Maternal age | Total number in group | Percentage with severe preeclampsia | Perinatal mortality rate in severe preeclampsia |
|---|---|---|---|
| Under 19 | 1,647 | 5.6 | 35.3 |
| 20-24 | 5,961 | 5.7 | 46.0 |
| 25-29 | 5,136 | 4.9 | 21.5 |
| 30-35 | 2,533 | 5.9 | 35.7 |
| 35-39 | 1,094 | 6.9 | 43.5 |
| 40 and over | 341 | 11.7 | 157.9 |
| All including not known | 16,815 | 5.3 | 41.2 |

*Source*: Adapted from Chamberlain et al. (1978).

Table 12 Incidence of and Perinatal Mortality in "Severe" Preeclampsia and Maternal Height

| Maternal height (in.) | Total number in group | Percentage with severe preeclampsia | Perinatal mortality rate in severe preeclampsia |
|---|---|---|---|
| Under 62 | 3958 | 5.4 | 70.4 |
| 62-64 | 7313 | 5.3 | 38.6 |
| 65 and over | 5372 | 5.3 | 24.4 |

*Source*: Adapted from Chamberlain et al. (1978).

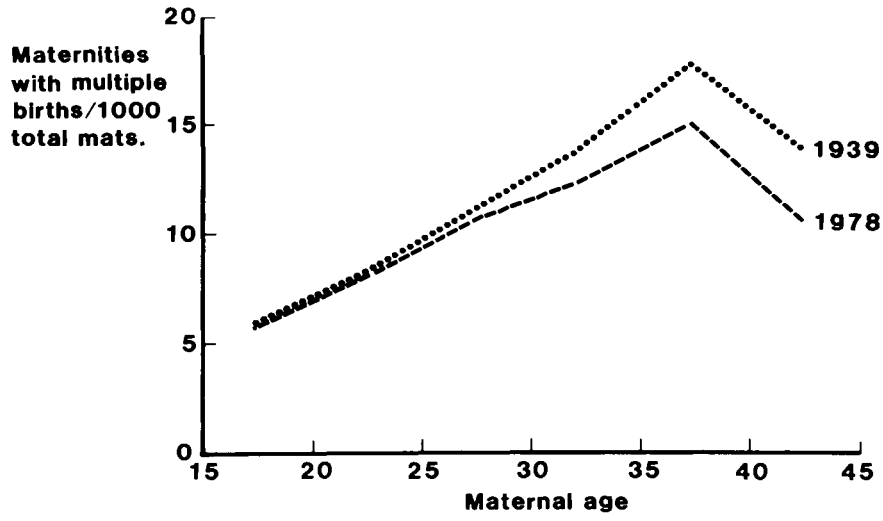


Figure 5 Incidence of multiple maternities per 1000 maternities in England and Wales. (Data from the Office of Population Censuses and Surveys, 1980d.)

twins, till at the age of from thirty-five to thirty-nine years the climax of fertility in twins was reached." This pattern has continued into the twentieth century but has been accompanied by a decrease in the age-specific incidence of multiple maternities (Figure 5). The variations with maternal age and time are small compared to racial differences: Yorubas in Western Nigeria have an incidence around 53 multiple maternities per 1000 as compared to 6.4 per 1000 maternities in Japan (Parkes, 1969).

As the perinatal mortality rate of 71.4 per 1000 multiple births in England and Wales in 1977 compares unfavorably with high mortality in other risk groups in terms of maternal age, parity, social class, and ethnic group (Table 8), perinatal deaths in multiple pregnancy account for 8.1% of all perinatal mortality. The worst perinatal outcome is mainly related to low birth weight, immaturity, placental insufficiency, and malpresentation. Multiple pregnancies carry an increased risk of congenital malformation, an apparent association existing between risk of neural tube defects and dizygotic twinning (Stevenson et al., 1966; Knox, 1974).

Monozygotic twinning seems to have a remarkably constant incidence, around 4.0 per 1000 maternities, and is not influenced by any known factor. Dizygotic twinning is largely responsible for variations in the incidence of multiple pregnancies with characteristics such as race, age, and abnormal ovulation. Gonadotrophin therapy for the induction of ovulation carries a risk of dizygotic twinning which is minimized through close monitoring of the biochemical profile. Superfecundation and superfetation are interesting rarities which do not contribute significantly to the numbers of dizygotic twins.

## THE RELATIONSHIP BETWEEN PERINATAL MORTALITY AND LONG-TERM MORBIDITY

There is general agreement that many of the immediate causes of perinatal death will also, in a less severe form, inflict damage on potentially normal fetuses, leaving them alive but permanently impaired. However, the relationship between death and handicap of perinatal origin is far from simple.

Two main types of relationship exist. Where the *primary* cause of death or handicap is removed or rendered less severe, the risk of both will be reduced together. For example, with the fall of the number of babies affected with Rh disease, both deaths and handicap in survivors due to this cause will be reduced. On the other hand, where the primary cause remains, as in the onset of premature labor, but the treatment of the resulting low birth weight babies is improved enough to reduce mortality, there is always the possibility that the number of survivors with some impairment may increase. The situation in England and Wales over the past 20 years, as in most developed countries, has been that the proportion of births of very low weight has changed very little, but that the birth weight-specific mortality has fallen very quickly (Figure 6), and there has been considerable discussion about the effect of the latter on morbidity rates in survivors (Chalmers and Mutch, 1981; Stewart et al., 1981; Paneth et al., 1981; Hagberg et al., 1982).

While it is not yet possible to estimate with any confidence the overall balance currently achieved between the overall reduction of perinatal mortality and the level of long-term morbidity, one can make an estimate in the case of infants of low birth weight. The improvement which has occurred in the first-day mortality rate of low birth weight infants born in England and Wales between the 1950s and 1979 means

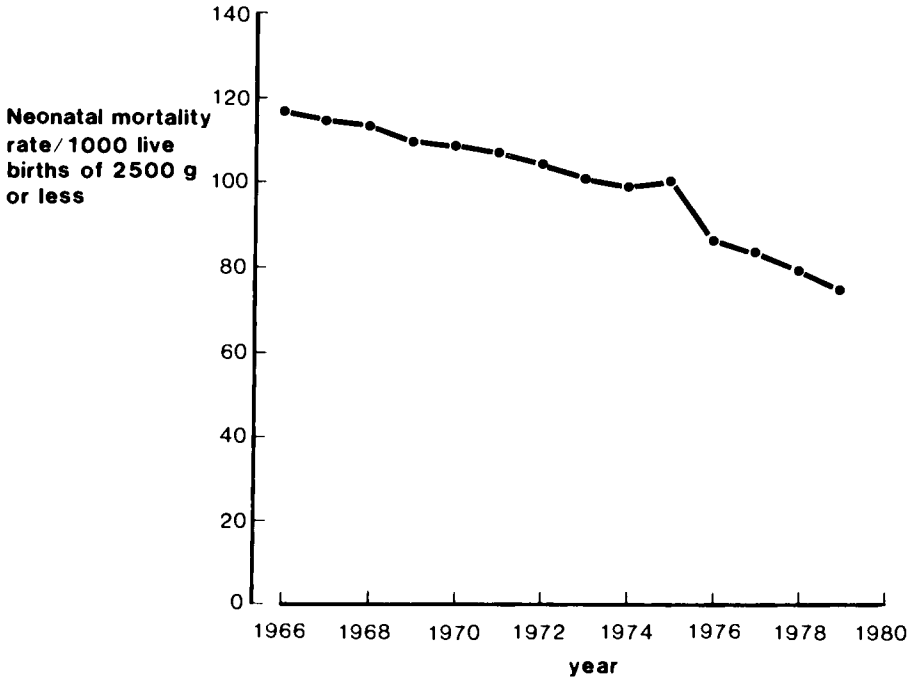


Figure 6 Neonatal mortality rate in births of 2500 g or less in England and Wales 1966-1979. (Data from Pharoah and Alberman, 1981.)

that of infants of 1500 g or less born in the latter year, 800 babies of 1500 g or less survived who would have died 20 years ago. Similarly, 630 of those of 1501-2500 who were surviving in 1979 would previously have died. Reports of severe handicap rates in babies of these birth weights suggest that currently 10-15% of survivors of 1500 g or less, and perhaps 8% of those of 1501-2500 g, are severely handicapped. Thus an extra 150 or so low birth weight children with severe handicaps survived in 1979 who would previously have died. This should be viewed in relation to an extra 1100 or so survivors without handicaps in 1979, and an overall severe handicap rate in all births of about 1%, about 6000 in all in 1979 (Pharoah and Alberman, 1981).

Such calculations do not take into account the likelihood that with increasing expertise in the treatment of sick neonates, the risk of handicap in survivors appears to fall with decreasing mortality rate, although there may be an interim period in which the handicap rate rises (Stewart et al., 1978; Stanley, 1979).

Such estimates underline the increasing necessity to monitor the morbidity of survivors as well as the early mortality of all births in order to evaluate the total effect of improvements in medical care.

## FUTURE DEVELOPMENTS

The perinatal mortality rate is clearly a most important indicator of social "well-being" and of the organization and quality of medical care. Moreover, the balance of the existing evidence suggests that a very low mortality rate also implies a low rate of impairment,

mild or severe, of survivors, so that they get the best possible start in life. This being so, any marked variations within a country may reflect the beginning of persisting health differences between social or regional groups, differences which will be further reinforced unless positive efforts are made to raise social and medical standards in the disadvantaged areas.

Future developments in the reduction of perinatal mortality must include good information and well-organized audit systems in order to raise all standards to those of the best available. This must comprise well-thought out and internationally compatible systems of birth and early death registration and considerable development in the field of morbidity data collection. Clinicians must become involved and familiar with these, and routine data should be presented in an easily assimilable form.

Innovations in obstetric and perinatal care must be examined critically, not only to determine their safety, effectiveness, and cost, but their acceptability to mothers. Not even the most effective medical breakthrough will succeed in reducing mortality if mothers will not accept it, and a general rejection of all medical technology is a real threat in many countries at present. The profession ought to take note of the wishes of consumers and adapt their care accordingly. Mothers will almost certainly accept well-proven innovations if they are fully informed and treated as individuals. Evaluation of perinatal care must comprise a method of ascertaining consumer satisfaction and the medical establishment must take this fully into account.

Another function of a good information system is its use in guiding political priorities. First in France and now in England and Wales, obstetric and neonatal care has been discussed at the parliamentary level (House of Commons, 1980), much use being made of national and international data. A political commitment to the reduction of early death and disability is an essential part of an effective national program.

However, no amount of professional or political activity will succeed without a further understanding by each prospective parent of the problem of perinatal mortality and morbidity. The reproductive behavior of individuals, their habits of smoking and drinking, their exposure to occupational hazards, and lifelong nutritional status have all been shown to bear importantly on individual risk of perinatal death or disability and together on the national risk. As the more easily preventable causes are reduced, risks determined by personal behavior become more important, and no program of prevention will succeed now without a greater degree of involvement of the individual citizen.

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# 13

# Effect of Hypoxia on Fetal Brain

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## INTRODUCTION

Asphyxia either during gestation or the intrapartum period remains one of the principal causes of brain injury and death of the fetus and newborn (Brown, 1976; World Health Organization, 1978). The high frequency and serious consequences of asphyxia make it a condition meriting the close attention of both the obstetrician and pediatrician. Recent years have markedly changed our understanding of both the causation and consequences of asphyxial brain injury. The present chapter focuses primarily upon recent advances in brain biochemistry and pathology that have given rise to new interpretations of the causation of brain injury. It attempts to place these new findings in a broad perspective. Almost all of these recent advances are the result of animal experimental studies; however, the general principles evolved are likely to prove substantially the same in man.

## EARLY EXPERIMENTAL STUDIES OF PERINATAL ASPHYXIA

### Fetal Exposure to Anoxia (Total Asphyxia)

Windle and his associates were the first to use animal models to investigate the clinical and pathologic effects of perinatal asphyxia. Their primary effort was to understand the pathogenesis of cerebral "birth injury" in man. They initially exposed fetal guinea pigs (Bailey and Windle, 1959) and later rhesus monkeys (Ranck and Windle, 1959) to asphyxia by delivering them in the intact amnion. Exposure of term monkey fetuses to such total asphyxia produced precisely definable clinical neurologic abnormalities and a pattern of brain injury that affected gray matter nuclei located in the brainstem. Having produced definite neurologic abnormalities and brain injury in monkey newborn, Windle believed he had succeeded in reproducing those unique pathologic circumstances that cause "birth injury" and cerebral palsy in man.

We also have asphyxiated term monkey fetuses by delivering them surgically, clamping their umbilical cord, and preventing breathing (Myers, 1969b, 1972) using a technique first developed by Dawes et al. (1960). These studies largely duplicated the experimental conditions and produced the same pathologic findings as described by Windle earlier. However, the findings we obtained were perplexing. The clinical neurologic deficits produced consisted largely of somatic sensory disturbances leading to clumsiness in the

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use of the hands and feet. These overall sensory deficits failed to resemble the motor deficits that are so characteristic of cerebral palsy in man.

We were also disturbed that in the monkey the pattern of brain injury was largely restricted to gray matter nuclei located in the brainstem. This specific and reproducible pattern of injury failed to resemble any of the commonly described patterns of perinatal brain damage in man. The patterns of brain injury produced in man by asphyxia at birth emphasize destruction of structures located in the hemispheres, including the cerebral cortex, the hemispheral white matter, and various parts of the basal ganglia, in different combinations. Only a few cases of damage restricted to nuclei located in the brainstem have been described in the human newborn and these only recently after this pattern of injury had been pointed out in experimental studies with the rhesus monkey. Furthermore, these uncommon instances of injury to the brainstem in man are always associated with extensive injury elsewhere. Thus even these human cases differ from those in the monkey, where the damage remains largely restricted to nuclei in the brainstem. Is it possible that the hemispheral patterns of brain injury typical of perinatal asphyxia in man result from the fact that human fetuses tend to experience marked hypoxias rather than anoxias?

### Fetal Exposure to Hypoxia (Asphyxia)

We exposed term monkey fetuses of food-deprived mothers to marked hypoxia by infusing the mothers with large doses of oxytocic agents to increase uterine activity and reduce intervillous space perfusion (Myers et al., 1969a), by mechanically constricting the maternal abdominal aorta to diminish uterine artery blood flow (Adamsons and Myers, 1977), by lowering the maternal blood pressure by respiring the mother with high levels of halothane (Brann and Myers, 1975), by respiring the mother with carbon monoxide (Ginsberg and Myers, 1976, 1974b), and by exposing the mothers to marked physologic stress causing sympathetic nervous system stimulation and constriction of her uterine blood vessels (Myers, 1975b, 1979c; Myers and Myers, 1979). All these methods of treatment produced a graded fetal hypoxia rather than a fetal anoxia.

Fetuses exposed to hypoxia developed entirely different patterns of brain injury than fetuses exposed to anoxia. Fetuses (and newborn) exposed to marked hypoxia generally developed marked brain edema with an associated hemorrhagic or nonhemorrhagic necrosis affecting widespread regions of their brain. These fetuses typically survived exposure to 1 hr of carefully controlled, marked hypoxia, only to die many hours later after delivery, having passed through a period of progressive neurologic deterioration. During the period of neurologic deterioration associated with the development of brain edema, these newborn evolved a marked hemorrhagic retinopathy. At the same time, their cranial sutures separated widely. Most of these fetuses or newborn also developed a paralytic ileus characterized by loss of bowel sounds, abdominal distension with tympany, and hematemesis. These gastrointestinal findings, like the changes in the brain, became fully evident only 4-24 hr after the animals' exposure to hypoxia and reoxygenation.

The asphyxiated newborns were maintained and closely monitored in the intensive care unit throughout their survival. All were killed if and when they showed signs of an impending death from cardiogenic shock, that is, their mean arterial blood pressure declined to values lower than 30 mmHg, or if they developed the blood chemical evidences of an impaired respiratory gas exchange, their arterial blood oxygen content declined to values lower than 4-6 vol % despite full mechanical ventilation and oxygen administration.

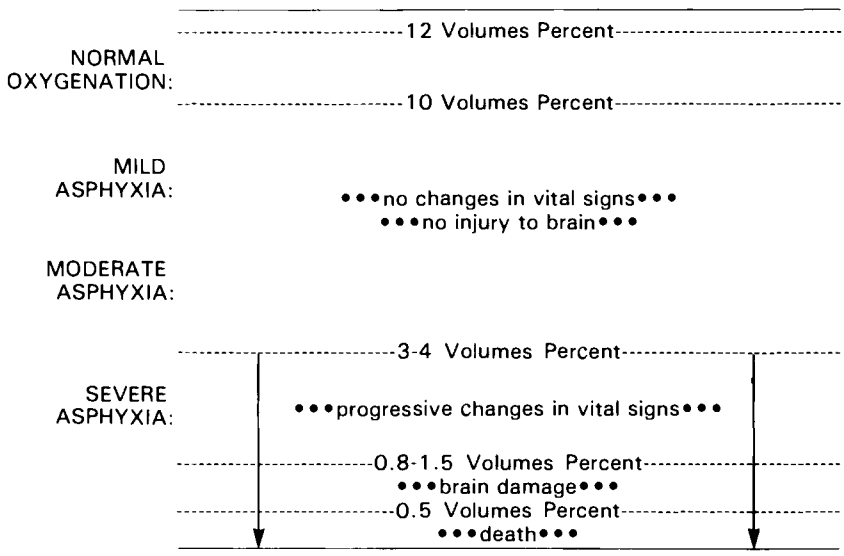


**Figure 1** Spectrum of oxygen deprivation required to produce (1) changes in vital signs, (2) damage to the brain, and (3) death of term monkey fetuses (gestational age range, 154-160 days). Volume percents represent the fetal abdominal aortic arterial blood oxygen content.

All brains were examined following perfusion fixation and 2 weeks of submersion in formaldehyde solution. The brains of all the newborn which evidenced a neurologic deterioration leading to flaccidity, opisthotonus, and apnea exhibited a moderate or marked edema that could be identified by a flattening of the cerebral convolutions and a herniation of the cerebellar tonsils and vermis (Myers, 1969b, 1972). Depending on how long the animals survived, many brains also showed gross morphologic evidence of softening. Nissl-stained cross sections examined microscopically consistently demonstrated the presence of a widespread tissue necrosis which often affected the entire cerebrum. The general postmortem examination confirmed the presence of multiple retinal hemorrhages and wide separation of the cranial sutures. The latter were often associated with adjacent subperiosteal hemorrhages. The intestinal tract showed both the gross and microscopic findings of a widespread hemorrhagic necrosis largely restricted to the mucosa.

The most important characteristic of that fetal asphyxia required to produce injury to the fetal brain or fetal death is its marked severity (Myers 1972, 1973a). Figure 1 summarizes the relation between the magnitude of hypoxia to which mature monkey fetuses (154-160 days gestation) have been exposed and the physiologic and pathologic changes that such exposure produces. Anesthetized term monkey fetuses maintain an arterial blood oxygen content (as sampled from their abdominal aorta) of 10-12 vol %. When such fetuses are exposed to magnitudes of hypoxia that reduce their arterial blood oxygen content through the entire range from the normal down to 3-4 vol % for several hours, they fail to show any changes in their vital signs or any injury to their brains following their delivery and survival. To produce neurologic changes the anesthetized fetus must be exposed to more marked hypoxia. When their arterial blood oxygen contents are reduced to values lower than 3-4 vol %, their cardiovascular function is depressed and they exhibit bradycardia and a fall in mean arterial blood

pressure. Below the threshold value of 3-4 vol %, the more marked the hypoxia the greater the impairment of fetal cardiovascular function. Eventually the animals undergo total circulatory collapse. Over the range from 3-4 vol % to the circumstances of a near anoxia, the reductions in fetal heart rate and blood pressure reflect the magnitude of oxygen deprivation (Myers, 1972, 1973a).

Anesthetized term monkey fetuses respond to asphyxia with a slight depression in cardiovascular performance only when arterial oxygen content has been reduced through 60-80% of its entire range (Myers, 1973a). However, they must experience a reduction of their arterial blood oxygen content through a range of 90% or more or to values in the range of 0.8-1.5 vol % to risk brain injury or intrauterine death. Generally the marked hypoxia must last for longer than 25-30 min to produce brain injury. Term monkey fetuses must be exposed to less than 0.5 vol % to run the risk of immediate intrauterine death. Such marked hypoxia or near anoxia promptly brings about circulatory failure which requires immediate intrauterine resuscitation. Without intrauterine resuscitation such fetuses succumb within minutes.

Exposing term monkey fetuses to a marked hypoxia that ultimately leads to an anoxia or near anoxia due to a late-developing circulatory failure produces clinical findings consistent with brain edema and increased intracranial pressure associated with extensive cerebral necrosis. Typically, the accompanying neurologic findings first appear and then subsequently evolve several to many hours following the initial exposure to marked hypoxia or near anoxia and after the newborn have been transferred to the newborn intensive care unit. The clinical picture these monkey newborn develop closely resembles that in asphyxiated human newborn, who experience a marked neurologic deterioration and die within the first few days following delivery.

## BRAIN EDEMA AS A CONSEQUENCE OF PERINATAL ASPHYXIA

### Pathologic Evidence for the Occurrence of Brain Edema in Late Fetuses and Newborn

Our work has clearly established the occurrence of brain edema as a critical aspect of the neuropathology of asphyxia in term monkey fetuses (Myers, 1972; Myers et al., 1969; Selzer et al., 1972b). Despite the clarity of this work with primate fetuses, some neuropathologists and clinical neurologists working with human newborn do not accept the view that the fetal or newborn brain can develop an edema under these circumstances. Thus F. H. Gilles indicated that he generally fails to see any of the gross morphologic changes typical of brain edema in asphyxiated human newborn in The Boston Children's Hospital (personal communication). However, he indicated that he does observe such findings occasionally in cases with massive cerebral necrosis. He concluded that "animal models, while giving important leads, are inadequate to justify the extrapolation of results to the human neonate" (Gilles, 1977). Volpe stated that brain edema has yet to be demonstrated as a pathologic entity in the human newborn who has died of "hypoxic-ischemic" encephalopathy. He concluded that "there is no definite human correlate for the early brain swelling described in . . . asphyxiated fetal monkeys" (Volpe, 1977).

Several studies of the brains of human fetuses or newborn who have died following exposure to perinatal asphyxia contradict the long-established and traditional view that the brain of the fetus and newborn cannot develop edema. Pryse-Davies and Beard (1971), stimulated by our studies with rhesus monkeys, examined this critical question in their own human autopsy material. They found abundant gross morphologic evidence

of brain edema, particularly in those infants who died after the thirty-fifth week of gestation. The changes they observed included convolutional flattening, grooving of the uncal gyri of the temporal lobes, and coning of the cerebellar tonsils and vermis. Pryse-Davies and Beard, who performed full autopsies, also measured the volume of cerebrospinal fluid still contained within the cisterna magna. Nearly half of the infants older than 35 weeks of gestation showed significant reductions in the volume of cisternal cerebrospinal fluid as a consequence of herniation of brain tissue and displacement of cerebrospinal fluid.

Larroche (1968) has described the brain pathologic findings in 15 term newborn who developed cerebral necrosis as a result of asphyxia. The great majority of these infants exhibited the gross morphologic changes indicative of a marked brain edema. These findings, most clearly evident when the brain was removed from the skull, consisted of marked reductions in the extent of space observed between the surface of the brain and the inner table of the skull. During brain removal, Larroche also noted a reduction in subarachnoid cerebrospinal fluid on the surface of the brain, a flattening of the cerebral convolutions, and a narrowing of the cerebral sulci. She further commented upon a tightness of the cerebellum within the posterior cranial fossa, a process which distinctly elevated the tentorium cerebri and, in some cases, displaced or herniated the cerebellar tonsils and vermis downward through the foramen magnum. Pryse-Davies and Beard (1971) were so impressed by the downward displacement of the brainstem in the infants of their study that they specifically emphasized the downward displacement of the inferior colliculi behind the superior surface of the cerebellum.

Anderson and Belton (1974) have reported upon the brain pathologic changes exhibited by 13 liveborn near-term ($>$ 32 weeks) human infants who were exposed to marked intrapartum asphyxia. Of these 13 infants, 4 showed gross morphologic evidences of brain edema at the time their brains were removed from the skull. The changes observed included an increased tension of the dural vault overlying the brain, a pallor and flattening of the cerebral gyri, and a reduction in the size of the ventricles. Only one brain showed definite zones of tissue softening. Anderson and Belton (1974) also documented the changes in brain water and electrolytes. The brains of 10 of the 16 infants who experienced severe asphyxia exhibited increased water and sodium and decreased potassium contents. These results agree fully with our own experimental findings from studies on the changes in tissue water and electrolyte content produced in the brains of term monkey fetuses (Selzer et al., 1972b) and adults (Selzer et al., 1973) by exposure to severe asphyxia. Pryse-Davies and Beard (1971) also documented the changes in the brain water of infants who died in the newborn period. Paradoxically, they found that the brains of those infants who showed the gross morphologic findings of brain edema demonstrated lower rather than higher water contents when compared to the remaining babies in the study who showed no evidence of brain edema. This paradox is readily explained by the fact that the brains that showed gross morphologic evidences of brain edema were largely brains of near-term babies, the water contents of which are normally greatly reduced when compared to the water contents of the brains of premature babies, who largely constituted the comparison group. For a fuller discussion of the normal changes in brain water content that take place during fetal development see the maturational studies of Selzer et al. (1972a).

De Courten and Rabinowicz (1981) analyzed the brain weights of 100 human newborn dying of a variety of causes, excluding only cases of intracranial hemorrhage. The older premature and the term babies showed a significant number of heavy, edematous

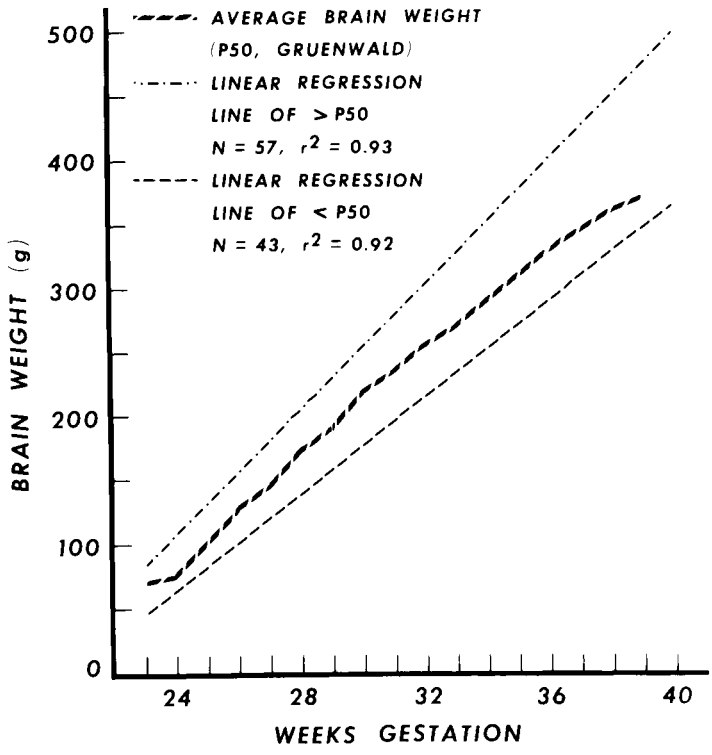


**Figure 2** Comparison of the linear regression lines of all brain weights *above* and *below* the fiftieth percentile of the brain weights of normal human newborn of different gestational ages (as defined by Gruenwald and Minh, 1960) of 100 human newborn who died of miscellaneous causes but with no intracranial hemorrhages as analyzed by de Courten. The departure of the heavier of the pathologic brains from the expected course parallel to the fiftieth percentile of the normal brains after 32 weeks of gestation indicates the presence of edematous brains in this age group.

brains (Figure 2). The regression lines which separately depict the brain weights that fall above and below the fiftieth percentile course parallel to and remain at similar distances from the curve which depicts the fiftieth percentile up to 32 weeks of gestation. Thereafter the regression line which depicts the brain weights below the fiftieth percentile continues to parallel the fiftieth percentile at a regular interval until term. In contrast, the regression line which depicts the brain weights heavier than the fiftieth percentile departs progressively from its expected parallel course beginning about the thirty-second week of gestation. It is inferred that the brains which weigh more than the fiftieth percentile include a number of brains with an edema that augments their weights out of proportion in comparison to the weights of the corresponding brains of the fiftieth percentile. The fiftieth percentile weights utilized in the present comparison follow the values defined by Gruenwald (Gruenwald and Minh, 1960). The 100 newborn brains analyzed and presented in Figure 2 include only stillborn and newborn who died before the fourteenth postnatal day and born between the twenty-third and fourtieth week of gestation, as described elsewhere (de Courten and Rabinowicz, 1981).

Since all cases of intracranial hemorrhage are excluded, the fresh brain weights depicted reflect the true parenchymal brain weights. The divergence of those brains which weigh more than the fiftieth percentile suggests that the brains of infants who are older than 32 weeks of gestation are able to gain weight by inhibition of water.

### Importance of In Situ Brain Examination for Demonstration of Brain Edema in Late Fetuses and Newborn

T. Rabinowicz viewed the brains of human newborn as fully capable of undergoing edema (personal communication). He emphasized the importance of examining the brains of fetuses and newborns in situ within the skull at the time of postmortem examination to gain specific insights as to whether brain edema is present. Particular attention should be paid to the tenseness of the fontanelle and dura and to the size of the space between the brain and skull. The infant brain is extremely soft and has a high water content. It therefore evidences and retains less well than the adult brain the surface deformation produced by its compression against the bony edges of the skull or tentorium after it has been removed from the skull and placed in formaldehyde solution. Rabinowicz additionally indicated that the fetal or newborn brain can swell to a considerable extent before it shows any external indication of its compression or deformation by the skull because of its large subarachnoid space and the elasticity of its bony envelope. This complex of factors may explain why pathologists who remove the brains of fetuses and newborn themselves as an integral part of the postmortem examination more often describe brain edema in the newborn than do those pathologists who examine only fixed brain specimens.

### Clinical Evidence for Occurrence of Brain Edema in Newborn

Neonatologists frequently document clinical signs that indicate the presence of an increased intracranial pressure and brain edema in newborn following exposure to asphyxia (Craig 1950; Fitzhardinge, 1977; Amiel-Tison, 1976; Souza and Richards 1978; Brown et al., 1974). Such signs appear particularly frequently in infants exposed to an asphyxia who develop neurologic abnormalities, including increased or decreased muscle tone, decreased levels of consciousness, or seizures. Infants usually develop clinical signs of increased intracranial pressure and brain edema during the first day or two after exposure to asphyxia (Fitzhardinge, 1977). They develop a tense fontanelle and split their cranial sutures, often at about the time that they begin to show increased irritability and experience their first seizures. Brown and associates observed suture separation and a tense anterior fontanelle in one-third of all asphyxiated newborn who subsequently developed definitive neurologic abnormalities (Brown et al., 1974). One of five asphyxiated infants who developed neurologic abnormalities also show a tense fontanelle (Souza and Richards, 1978). Almost all such infants experienced seizures and, though initially apathetic, became hyperirritable. Infants with these manifestations constitute 75% of those who survive and later suffer one form or another of handicap. Hypertonus of the neck extensors is a further sign of an increased intracranial pressure (or of meningeal irritation) in the newborn (Amiel-Tison, 1976). Extensor hypertonus of the neck appeared in two-thirds of newborns with neurologic abnormalities which lasted longer than 1 week.

Many monkey and human term fetuses and newborns exposed to asphyxia who die in the first few days after birth develop brain edema and show an acute cerebral necrosis

if they survive sufficiently long. A major problem in our earlier studies was that few animals exposed to a carefully controlled marked hypoxia for prolonged periods survived long enough to show the neurologic and neuropathologic findings typical of cerebral palsy in man (Myers, 1972, 1977). Such animals tend either to die within the first several days with brain edema and widespread cerebral necrosis or to survive and remain entirely brain intact. The few animals that survived asphyxia to show the long-term clinical abnormalities and the specific patterns of brain injury simulating human cerebral palsy almost all experienced a marked hypoxia followed by a brief period of anoxia [see, for example, our case showing an atrophic cortical sclerosis and status marmoratus (Myers, 1969a)]. The anoxia or near anoxia they experienced generally developed as a consequence of a circulatory failure that appeared late during exposure to marked hypoxia.

We shall describe some early empirical studies that characterized changes in cardiovascular function, clinical chemistry, and the development of brain pathology that helped to explain the development of asphyxial brain injury. We shall then discuss the findings of recent studies which have provided new insights into the fundamental cellular mechanisms that account for asphyxial brain injury and a better understanding of why different patterns of brain pathology result from exposure to different types of asphyxia.

## MAGNITUDE OF HYPOXIA FAILS TO DETERMINE DEVELOPMENT OF BRAIN INJURY

Asphyxia generally implies both a reduction in oxygen delivery to tissue and an accumulation of carbon dioxide in blood and body fluids. Of these two it is likely that it is the reduced oxygen delivery to tissue that alters tissue metabolism and brings about that tissue circumstance that causes brain injury. Incriminating the oxygen deprivation component as the cause of brain injury agrees closely with widely accepted and traditional views (Opitz and Schneider, 1950; Thews, 1963).

Earlier we accepted the view that tissue injury from asphyxia results from a failure in tissue oxygen delivery. Furthermore, we believed that if we could define in detail the extent of oxygen deprivation to brain throughout exposure to hypoxia, we should be able to reliably predict the brain pathologic consequences. However, exposing young adult rhesus monkeys to 25 min of carefully controlled marked hypoxia (3.5% oxygen in nitrogen) produced either (1) intact survival or (2) development of brain edema and death of the animal many hours after it was reoxygenated as a result of failure of its vital brainstem mechanisms and circulatory collapse. These results confronted us with the enigma as to why exposing a group of animals to the same marked hypoxia led to two such widely divergent outcomes.

We investigated this question by comparing the two outcome groups (intact survival as against death from brainstem failure or shock) with respect to their changes in blood composition and cardiovascular function during exposure to hypoxia. Figure 3 compares these two groups with respect to their $paO_2$, $paCO_2$, and pH values. The $paO_2$ fell from control values close to 100 to 14 and 16 mmHg at 5 min. Their $paO_2$ values thereafter remained largely unaltered throughout their exposure to hypoxia; that is, those animals which were to survive intact and those which were to die in the early hours from brainstem failure or cardiogenic shock generally experienced closely similar $paO_2$ values throughout their exposure to hypoxia.

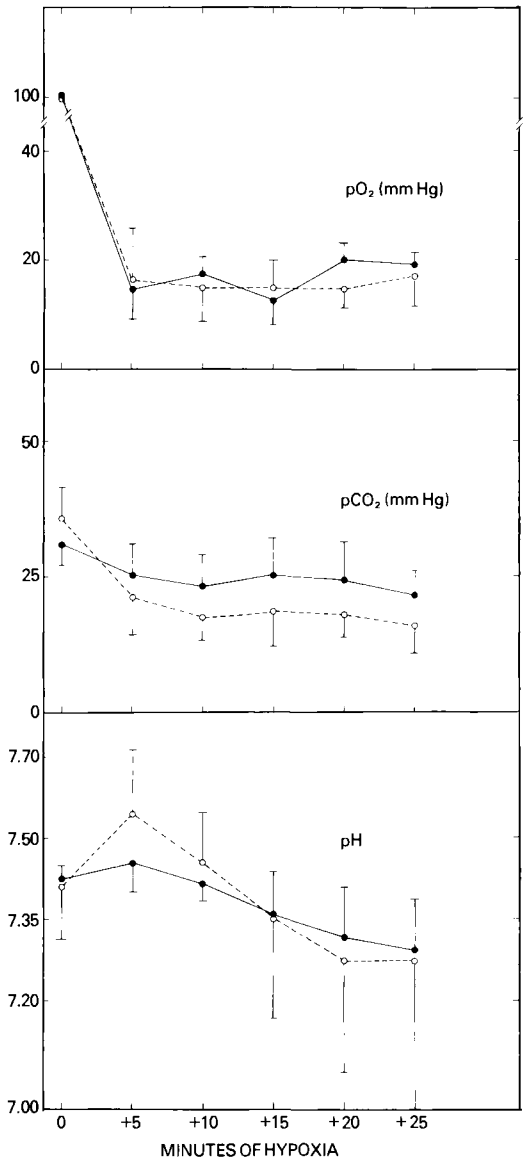


Figure 3 Comparison of mean $paO_2$, $paCO_2$, and pH values ± 1 standard deviation of two groups of young adult monkeys during exposure to 25 min of marked hypoxia. The animals of the first group (edema; ○---○), following their reoxygenation, experienced a progressive neurologic deterioration and at the time of their sacrifice after several hours survival showed a brain edema and a diffuse injury to brain tissue. The animals of the second group (nonedema; ●——● ), survived long term and failed to show neurologic or pathologic abnormalities.

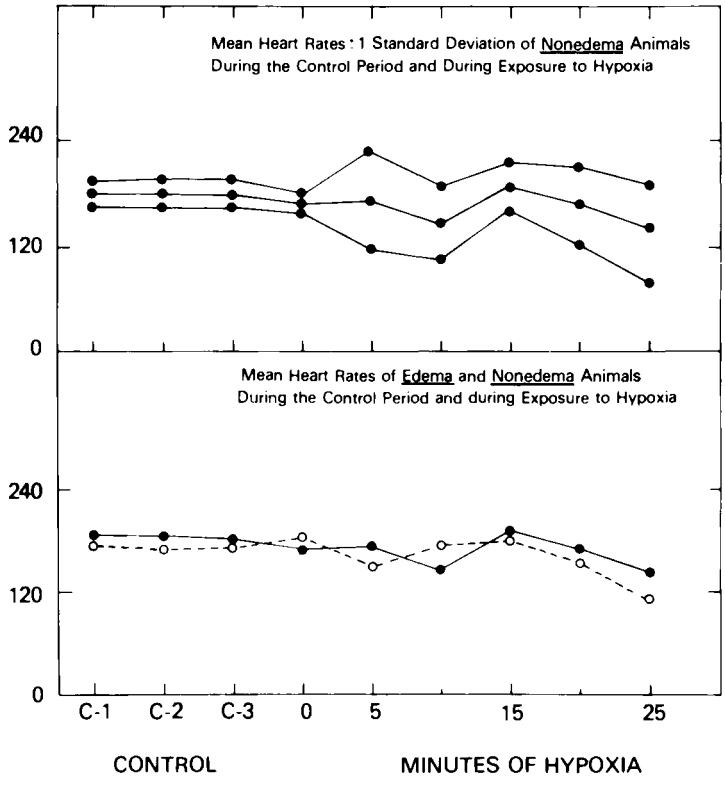


Figure 4 Heart rates of the two groups of rhesus monkeys during exposure to 25 min of marked hypoxia (for details see Figure 3). The mean heart rates ± 1 standard deviation of the animals of the nonedema group are presented on top. The mean heart rates of the animals of the two groups are presented below (edema group, ○---○; nonedema group, ●——●).

The animals of the two outcome groups reduced their $paCO_2$ values from 31 and 36 to 26 and 22 mmHg during the first 5 min of hypoxia. Once again, the $paCO_2$ values then remained largely unchanged from these values throughout the remaining 20 min of exposure to hypoxia. However, the animals which were to develop brain edema and/or die in cardiogenic shock generally reduced their $paCO_2$ values more markedly than did the animals which were to remain intact, although the differences failed to reach statistical significance. The animals of the two groups also elevated their mean arterial blood pH from 7.43 and 7.41 to 7.45 and 7.55 during the first 5 min of hypoxia. The animals which were to remain intact showed less elevation of blood pH than those which were to develop brain edema. After the peak values were reached, the animals of both groups then progressively reduced their blood pH to values that reached 7.29 and 7.27 at 25 min. Again, the differences in the values of the animals of the two groups remained nonsignificant throughout the entire period of exposure.

These results demonstrate that the monkeys of the two widely divergent outcome groups behaved in a closely similar way with respect to changes in the respiratory gas composition and pH of their arterial blood during hypoxia. This finding was difficult

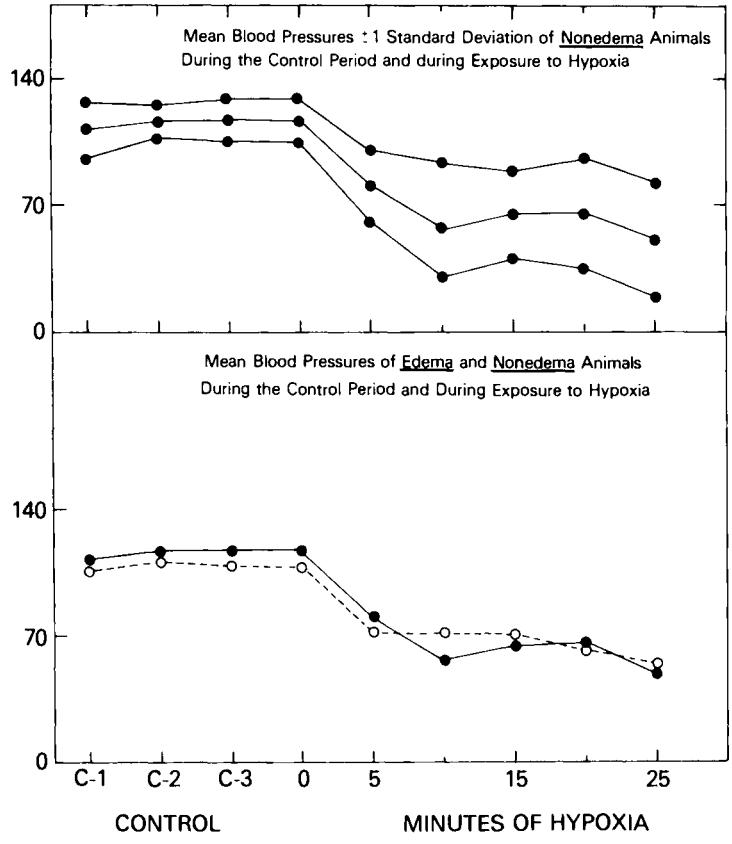


**Figure 5** Blood pressures (mmHg) of two groups of rhesus monkeys during exposure to 25 min of marked hypoxia (for details see Figure 3). The mean blood pressures ±1 standard deviation of the animals of the nonedema group are depicted on top. The mean blood pressures of the animals of the two groups are directly compared below (edema group, ○---○; nonedema group, ●——●).

to reconcile with the view that the details of reduced oxygen delivery to tissue precisely define the brain pathologic response to asphyxia. However, differences in oxygen delivery to tissue might still have taken place in these animals, based not on differences in arterial blood composition, but on differences in their cardiovascular response to hypoxia in their reductions in cerebral blood flow.

This possibility was investigated by analyzing the animals' cardiovascular performance during hypoxia. Figure 4 depicts the heart rates of the animals of the two outcome groups during hypoxia. The animals of the two outcome groups behaved similarly, in that hypoxia provoked only slight or no changes in the mean heart rate. The large standard deviation values in the animals of the two groups reflects the marked heart rate oscillations that many animals of both groups exhibited during hypoxia.

The mean arterial blood pressure (MABP) values of the animals of the two outcome groups are depicted in Figure 5. The animals of both groups experienced preexposure MABP values in the range of 110-120 mmHg. At the beginning of hypoxia the animals

increased their MABP to 130-150 mmHg, the peak values appearing during the second or third minute. This early augmentation of heart action (not depicted in Figure 5 because of its short duration) probably reflects a hypoxic stimulation of the sympathetic nervous system (Korner, 1959). Subsequently MABP values fell rapidly to close to 70 mmHg until further reduced to 45-55 mmHg after 15-20 min of exposure. This latter late reduction in MABP probably reflects the early stage of a cardiovascular collapse, the full extent of which was prevented by the animals' reoxygenation at that time. Many of these monkeys showed a delayed development of brain edema and many died hours later of cardiogenic shock, even though none had experienced any total collapse of systemic circulation but only a late-developing accentuation of the fall in MABP leading to brief periods where their MABP was as low as 45-55 mmHg during hypoxia. Many animals of both groups experienced wide oscillations in MABP during hypoxia, even though the composition of the inspired gas mixture remained constant. These blood pressure oscillations again accounted for the large standard deviation values observed.

These findings failed to support the supposition that the animals of the two outcome groups would segregate according to differences in the magnitudes of the depression of their cardiovascular performance during hypoxia. Rather, the overall findings indicate that the animals of the two outcome groups behaved similarly not only with respect to changes in arterial blood pH and respiratory gas values, but also with respect to changes in cardiovascular performance. These results forced us to conclude that one or more variables other than the magnitude of the reduction in oxygen delivery to tissue determines whether brain injury develops and animals survive.

In contrast to animal studies, investigations of patients suffer from a dearth of information that might define the magnitudes of oxygen deprivation that they experience during exposure to asphyxia. Nonetheless, Brown and associates have succeeded in documenting the respiratory gas and acid-base status of a number of human newborn who experienced a postpartum asphyxia as a result of the respiratory distress syndrome. These authors described findings in human newborn that agreed closely with the findings in monkeys. Markedly depressed $paO_2$ values were compatible with an intact survival in some infants, while it killed others (Brown et al., 1974). These results with human newborn also indicate that additional factors beyond the magnitude of depression of arterial blood oxygen content and reduction in blood pressure play a role in the development of brain injury and of infant death from asphyxia.

## CRITICAL IMPORTANCE OF SERUM GLUCOSE CONCENTRATION IN DETERMINING BRAIN PATHOLOGIC RESPONSE TO OXYGEN DEPRIVATION

A 1975 investigation in our laboratory identified a previously unsuspected variable that critically determines whether or not brain injury occurs and whether or not an animal will survive exposure to anoxia or marked hypoxia (Myers, 1977). The remainder of the present discussion will detail the considerable evidence we have developed that has established the pivotal role that serum glucose concentration or substrate availability plays in determining asphyxial brain injury and animal survival. We shall also delineate some of the important tissue metabolic and biochemical consequences of oxygen deprivation and trace their implications for brain pathology both in the fetus and in the adult.

## Evidence from Studies of Brain Pathology

We used food-deprived young adult rhesus monkeys to define the brain pathologic consequences of exposure to circulatory arrest (Miller and Myers, 1970, 1972; Myers, 1973b, 1974; Myers and Yamaguchi, 1977). These studies led to two astonishing findings: Firstly, 12-14 min of circulatory arrest are required to cause first brain injury and, secondly, brain injury, when it occurs, affects gray matter nuclei located in the brainstem. A similar extended tolerance of dogs (Marshall et al., 1956; Kaupp et al., 1960; Brockman and Jude, 1960; Neely and Youmans, 1963) and monkeys (Kaupp et al., 1960; Wolin et al., 1971; Nemoto et al., 1977) to circulatory arrest has also been described by others. These results disagree entirely with contemporary clinical teachings which state that periods of circulatory arrest that last for so short a time as 2-4 min regularly devastate the brain (Mandel and Berry, 1959; Steegman, 1969) and that brain injury, when it occurs, predominately affects the cerebral cortex and other structures located in the hemispheres (Mandel and Berry, 1959; Neubuerger, 1954; Grenell, 1946). For a considerable time, the basis for these striking differences in outcome between our animal experimental studies and those described as typical of man exposed to circulatory arrest remained entirely unknown. However, our discovery of the remarkable effects of the infusion of glucose solutions in monkeys on their brain pathologic response to circulatory arrest for the first time fully clarified the critical issues involved.

Food-deprived juvenile rhesus monkeys infused with glucose solutions dramatically accentuated their brain pathologic response to 14 min of circulatory arrest when compared to similarly food-deprived monkeys that were infused with saline rather than glucose solutions (Myers, 1976, 1977, 1979b).

The glucose-infused animals all died during the early hours following their resuscitation. In contrast, the saline-infused animals, otherwise treated the same, survived for lengthy periods if successfully resuscitated. Furthermore, the food-deprived monkeys pretreated with infusions of saline solutions regularly survived exposure to 14 min of cardiac arrest and showed either no or only trivial injury to their brain restricted to nuclear structures located in their brainstem. Similarly food-deprived animals pretreated with infusions of glucose rather than saline solutions (elevating their serum glucose concentrations to 150-450 mg %) that were exposed to episodes of cardiac arrest of the same duration nonetheless all experienced a progressive neurologic deterioration that started several hours following the time of resuscitation. All of these animals died many hours later of failure of their brainstem mechanisms and of the work performance of their heart. The brains of the glucose-pretreated animals, instead of showing trivial damage to nuclei located in their brainstems, all showed the gross morphologic findings of a mild to moderate edema and the microscopic changes of a diffuse tissue necrosis. They all also exhibited a striking breakdown in blood-brain barrier function when barrier integrity was tested late during the phase of neurologic deterioration. None of these changes took place in the saline-infused animals that were exposed to identical periods of circulatory arrest. These results clearly identified the level of serum glucose concentration as a crucial variable that affects the brain pathologic response to exposure to circulatory arrest. It determines whether an individual animal will survive with an intact nervous system or whether, instead, it will undergo an inexorable neurologic deterioration leading to death many hours later with brain edema and massive brain injury.

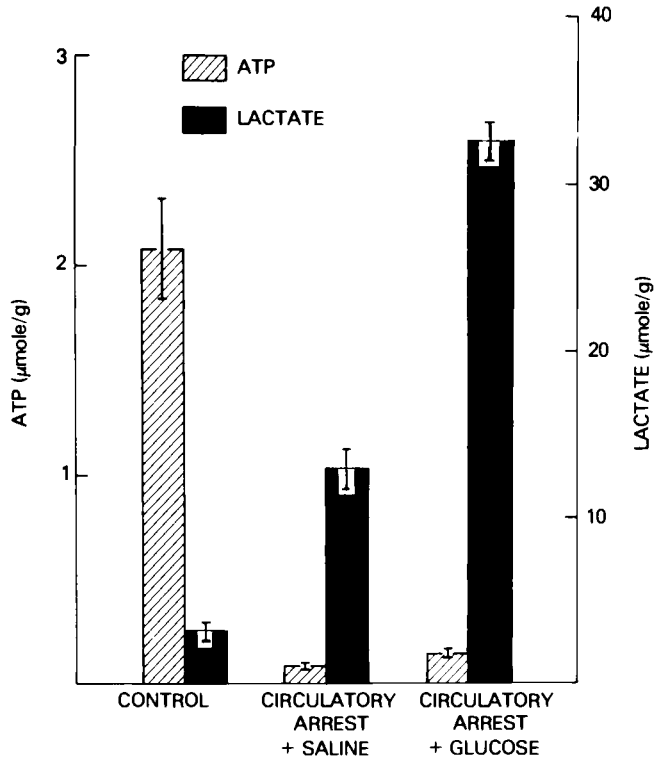


Figure 6 Adenosine triphosphate (ATP) and lactic acid concentrations of the cortex of the postcentral gyrus of food-deprived control animals and of animals exposed to 10 min of circulatory arrest following their pretreatment with infusions of saline or glucose. Each vertical bar and line represents the mean and the standard deviation, respectively, of values from four animals. (From Myers and Yamaguchi, 1976a).

### Evidence from Studies on Brain Metabolism

We also evaluated the effects of saline versus glucose pretreatment on the biochemical response of the brain to circulatory arrest (Myers and Yamaguchi, 1976a; Myers, 1979b). Comparison of the changes in composition of the brain tissue produced by exposure to circulatory arrest under these two circumstances should define the tissue metabolic basis for the development of brain edema and the occurrence of tissue necrosis from oxygen deprivation. Tissue samples were taken from such animals after 10 min of stasis of blood flow (circulatory arrest) and analyzed for the activities of a number of enzymes, as well as for the concentrations of various metabolites. In general, animals of the two treatment groups failed to show any differences in the activities of the various enzymes examined or in the concentrations of the various metabolites tested. Typical of such a negative finding was the behavior of adenosine triphosphate (ATP) of brain tissue (Myers and Yamaguchi, 1976a). Exposing the animals of the two treatment groups to circulatory arrest reduced their cortical tissue ATP concentrations to markedly low values (Figure 6). There was no significant difference in the final ATP concentrations of the animals of the two groups. This similarity in behavior of the cortical tissue ATP of the

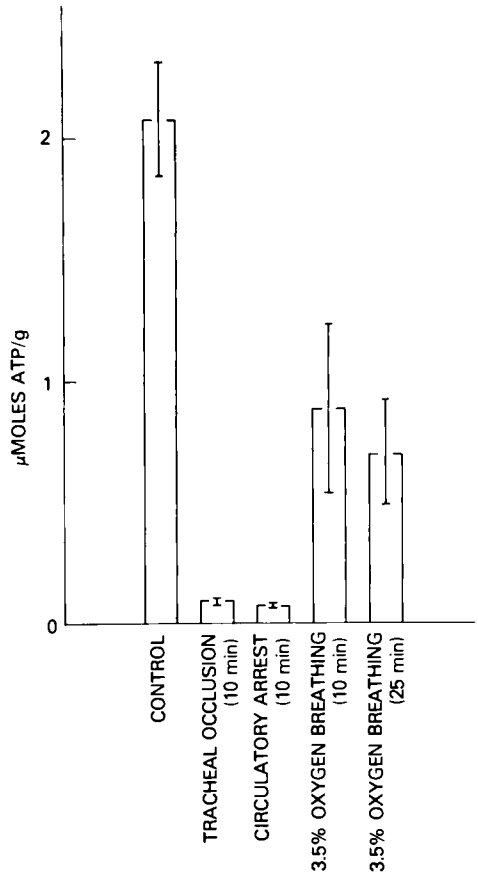


**Figure 7** Adenosine triphosphate (ATP) concentrations of the cortex of the postcentral gyrus of young adult control rhesus monkeys and of monkeys following exposure to 10 min of tracheal occlusion (anoxia), 10 min of circulatory arrest (anoxia), and 10 and 25 min of 3.5% oxygen breathing (marked hypoxia). Each vertical bar and line represents the mean and standard deviation, respectively, of values from four to five animals. (From Yamaguchi and Myers, 1976.)

animals of the two dramatically different outcome groups during exposure to circulatory arrest clearly indicates that the startling differences in the animals' brain pathologic responses and their survivals according to differences in pretreatment cannot be explained on the basis of differences in the availability of high-energy phosphate to sustain cellular processes during exposure to anoxia.

The brains of the animals of the two experimental groups did differ markedly in their concentrations of lactate (Figure 6). The cortex of the food-deprived saline-infused control animals contained lactate at a concentration of 3.01 ± 0.44 μmol/g (N = 4). The food-deprived, saline-infused animals exposed to 10 min of circulatory arrest accumulated lactate in cortical tissue to a concentration of 12.08 ± 1.10 μmol/g (N = 4), while the food-deprived but glucose-infused animals accumulated it to a concentration of 32.50 ± 1.24 μmol/g (N = 4). This remarkable difference demonstrated a strong correlation between the accumulation of lactic acid at high concentrations in brain tissue and the

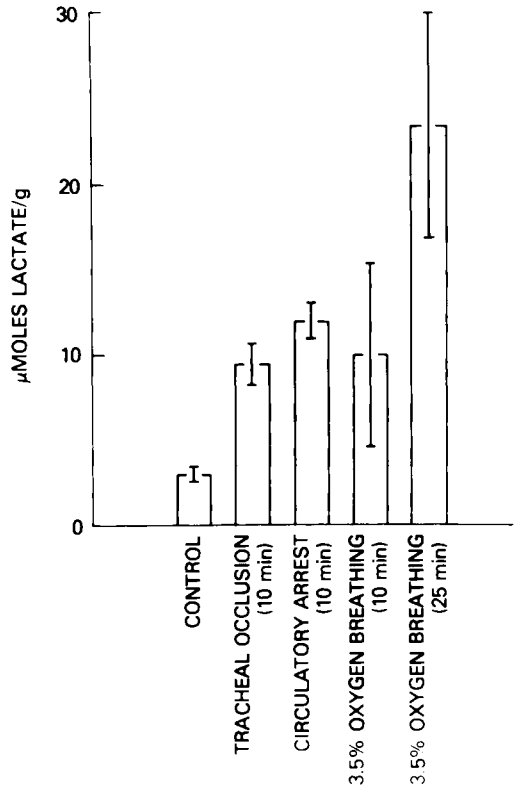


**Figure 8** Lactate concentrations of the cortex of the postcentral gyrus of young adult control rhesus monkeys and of monkeys after exposure to 10 min of tracheal occlusion (anoxia), 10 min of circulatory arrest (anoxia), and 10 and 25 min of 3.5% oxygen breathing (marked hypoxia). Each vertical bar and line represents the mean and standard deviation, respectively, of values from four to five animals. (From Yamaguchi and Myers, 1976.)

development of tissue injury. However, the animals of the two groups also differed significantly with respect to a second biochemical factor. The glucose-infused animals exposed to circulatory arrest showed a higher activity of their cortical tissue alkaline phosphatase than did the control animals or those that were saline infused and exposed to the same duration of arrest.

A second study also demonstrated a close correlation between the accumulation of lactate at high concentrations in brain tissue and the development of brain injury (Yamaguchi and Myers, 1976; Myers and Yamaguchi, 1976b). We investigated the brain biochemical findings of five rhesus monkey groups, including a normally oxygenated control group, two groups exposed to 10 min of two types of anoxia, and two groups exposed to two durations of a marked hypoxia. In every instance except the controls the cerebral cortex was sampled at the end of exposure to oxygen deprivation. All tissue samples were subsequently analyzed for the same biochemical and metabolic parameters as already described.

The tissue ATP and lactate concentrations were again of prime interest (Figures 7 and 8). The tissue ATP content of the control animals was 2.08 ± 0.22 μmol/g (N = 4).

The animals exposed to 10 min of tracheal occlusion (the first experimental group) and to 10 min of systemic circulatory arrest (the second experimental group) markedly reduced their cortical tissue ATP concentrations to 1.10 ± 0.03 (N = 5) and 0.08 ± 0.01 (N = 4) μmol/g, respectively. Prior studies in our laboratory have indicated that if the animals of these two groups had been resuscitated, they would have survived with no brain injury. The combination of marked reduction in concentration of the brain tissue ATP with, nonetheless, a failure of development of brain injury demonstrates a clear lack of correlation between a markedly low tissue availability of high-energy phosphate and development of tissue injury.

The animals of the third and fourth experimental groups were exposed to 10 and 25 min of marked hypoxia by ventilating them with 3.5% oxygen in nitrogen. These animals also depressed their cortical tissue ATP contents, but to less marked values than the animals exposed to the two types of anoxia. The animals exposed to 10 min of marked hypoxia reduced their cortical tissue ATP content to 0.88 ± 0.35 μmol/g (N = 4), and those exposed to 25 min to 0.71 ± 0.23 (N = 5) μmol/g. Thus the animals of both hypoxic groups reduced their brain tissue ATP contents only moderately and to similar final concentrations. However, despite this similarity in behavior of cortical tissue ATP concentrations, about half of the animals exposed to 25 min and none exposed to 10 min of marked hypoxia would be expected from our own prior studies to develop brain injury. Thus we again found no correlation between the quantity of high-energy phosphate available to the brain at the termination of exposure to oxygen deprivation and the occurrence of brain injury.

The food-deprived monkeys exposed to the five types of experimental manipulations accumulated lactate in their cortical tissue over a wide range of concentrations (Figure 8). Animals that were exposed to tracheal occlusion and to circulatory arrest for 10 min increased their cortical tissue lactate to 9.46 ± 1.28 μmol/g (N = 5) and 12.08 ± 1.10 μmol/g (N = 5), respectively. The animals exposed to 10 min of marked hypoxia accumulated lactate to about the same concentrations (9.46 ± 1.28 μmol/g) (N = 5). Finally, the animals exposed to 25 min of marked hypoxia accumulated lactate to considerably higher concentrations (23.45 ± 6.60 μmol/g) (N = 5) than did the animals of any other experimental group. Corresponding to this marked accumulation of brain lactate, it is uniquely this group of animals that would be expected to develop brain injury as a result of their experimental exposure, as already described above. This second study confirms a close correlation between the accumulation of lactate in brain tissue at high concentrations and the development of brain injury, but using a different model of brain damage.

These two brain biochemical studies lead to two important conclusions. Firstly, no correlation exists between the brain tissue ATP concentration during exposure to marked hypoxia or anoxia and the development of brain injury. Whatever effects on brain tissue a reduced high-energy phosphate may have, no cause-and-effect relation between such reductions and development of brain injury can be demonstrated. Secondly, among the various changes in tissue metabolism and biochemistry, only the accumulation of lactate in brain tissue at concentrations in excess of 17-20 μmol/g is correlated with later development of brain injury. All categories or varieties of exposure to oxygen deprivation demonstrated by our laboratory to cause brain injury also cause the accumulation of lactate in concentrations greater than 17-20 μmol/g. At the same time, all categories of exposure that fail to injure the brain also provoke lactate accumulation, but, in all instances, to concentrations that fall short of the above-described

**Table 1** ATP Concentrations (μmol/g) of Brain Structures in Goats Exposed to 20 min of Circulatory Arrest

| | |
|---|---|
| Hemispheres | |
| cortex | |
| superior parietal (crown) | 0.18 ± 0.03 (4)[a] |
| occipital (crown) | 0.23 ± 0.04 (4) |
| superior parietal (sulcus) | 0.19 ± 0.04 (4) |
| inferior parietal (crown) | 0.16 ± 0.03 (3) |
| white matter | |
| superficial gyral | 0.68 ± 0.10 (4) |
| deep gyral | 0.50 ± 0.11 (4) |
| centrum semiovale | 0.50 ± 0.08 (4) |
| corpus callosum | 0.56 ± 0.08 (4) |
| hemispheral gray matter | |
| hippocampus[b] | 0.21 ± 0.02 (4) |
| caudate nucleus | 0.32 ± 0.12 (4) |
| globus pallidus[b] | 0.30 ± 0.12 (4) |
| thalamus | |
| central thalamus | 0.21 ± 0.05 (4) |
| ventral thalamic nucleus | 0.16 ± 0.02 (4) |
| lateral geniculate | 0.18 ± 0.02 (4) |
| Brainstem | |
| cerebellum | |
| cerebellar cortex[b] | 0.25 ± 0.02 (4) |
| brainstem | |
| oculomotor nucleus[b] | 0.57 ± 0.14 (6) |
| superior colliculus | 0.34 ± 0.06 (4) |
| inferior colliculus[b] | 0.22 ± 0.05 (4) |
| central gray | 0.49 ± 0.08 (4) |
| substantia nigra[b] | 0.15 ± 0.04 (4) |
| reticular formation | 0.18 ± 0.02 (4) |
| superior olive[b] | 0.37 ± 0.12 (3) |
| vestibular nuclei | 0.43 ± 0.07 (5) |
| cuneate nucleus[b] | 0.39 ± 0.08 (3) |
| facial nerve nucleus[b] | 0.34 ± 0.13 (3) |

[a]Values are means ± SEM of the number of animals in parentheses.
[b]Vulnerable to injury.

threshold values. The results of these and other studies have committed us to the view that the critical tissue change that precedes and leads to brain edema and tissue injury is the accumulation of lactate (and its associated hydrogen ions) at suprathreshold concentrations (Myers, 1977, 1979a,b, 1981).

### Evidence from Studies of Topographic Brain Chemistry

This interpretation, emphasizing the importance of lactate and hydrogen ion accumulation as the cause of brain injury, has been further strengthened by a new study of the

compositional changes produced in brainstem and hemispheral structures by exposing food-deprived monkeys and goats to 20 min of circulatory arrest (Wagner and Myers, 1979b, Myers and Wagner, 1980). Both species of animals exposed to circulatory arrest reduced their ATP concentrations to markedly low values (0.15-0.68 μmol/g) throughout all brain structures, including the "vulnerable" gray matter nuclei in the brainstem and also in other structures not defined as "vulnerable" to injury under these circumstances (see Table 1). Comparing the ATP concentrations of the grouped "vulnerable" and "nonvulnerable" gray matter structures showed no significant differences between the two.

Exposure of the same animals to circulatory arrest increased the lactate concentrations of all brain structures, as Figure 9 indicates. Twenty minutes of stasis of blood flow increased the lactate contents of gray matter (15.2-24.9 μmol/g) more than white matter

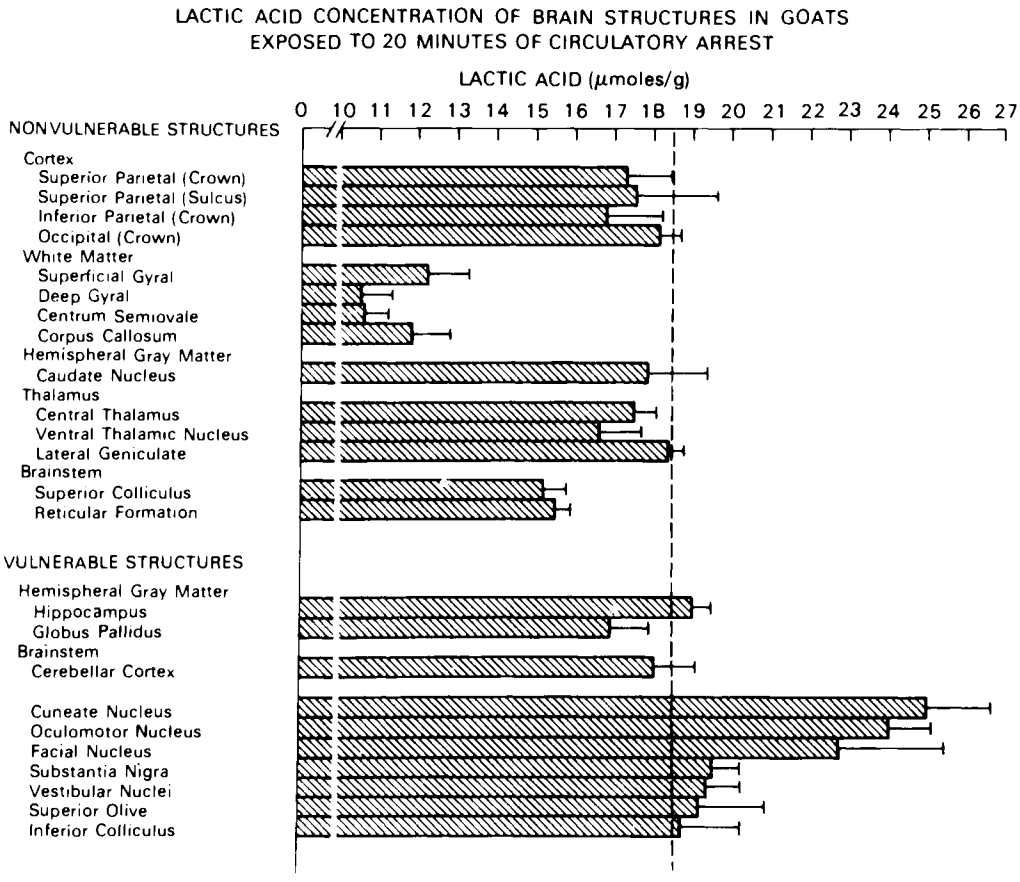


**Figure 9** Lactic acid concentrations of "vulnerable" and "nonvulnerable" brain structures in food-deprived goats exposed to 20 min of circulatory arrest. Each horizontal bar and line represents the mean and standard deviation, respectively, of three to seven samples. The vertical dashed line (---) denotes the hypothetical threshold value of lactic acid concentration (18.5 μmol/g) required to produce tissue injury. The lactic acid concentrations of the structures highly vulnerable to injury from circulatory arrest differ significantly from that of the nonvulnerable structures ($P < 0.001$).

(10.6-12.4 μmol/g) structures. More importantly, exposure to circulatory arrest increased the lactate contents of the "vulnerable" gray matter nuclei located in the brainstem to values that generally exceeded those observed in "nonvulnerable" structures. The behavior of the cuneate, oculomotor, and facial nerve nuclei are prototypic of the "vulnerable" brainstem nuclei and, as can be seen, their lactate values generally exceeded the threshold values required to produce injury. These results clearly support our thesis of a cause-and-effect relation between the accumulation of lactic acid at high concentrations in brain tissue and injury to that tissue.

Exposing animals to circulatory arrest elevated the lactate contents of most of their "nonvulnerable" gray matter structures to values that approach closely the threshold values we have demonstrated injures the tissue. Thus it is clear that any circumstance that increases the glucose contents of the various structures in the brain, even though only slightly, immediately before an animal is exposed to circulatory arrest increases the concentrations to which lactic acid accumulates throughout all these structures during exposure. Because so many structures already approach the threshold concentration of lactate required for their injury in food-deprived animals exposed to anoxia, any process that increases the brain glucose content prior to exposure to anoxia will convert the brain pathologic response from one of injury restricted to gray matter nuclei in the brainstem to one affecting widespread regions of the brain. Actual clinical circumstances that increase the glucose concentrations of the various structures of the brain and, in the process, that predispose the animal to diffuse brain injury in the event of exposure to near anoxia or anoxia (circulatory arrest or marked asphyxia) include food ingestion during the several hours beforehand and the infusion of glucose solutions.

We have examined both the glucose and glycogen concentrations of various structures in the brains of food-deprived animals to determine their relative availabilities for conversion to lactic acid during exposure to circulatory arrest (Wagner and Myers, 1979a; Myers et al., 1980c,d). The results presented in Figure 10 show that glucose appears at similar concentrations in large numbers of brain structures, though many gray matter structures located in the cerebrum contain glucose at higher concentrations than do the various nuclei in the brainstem or thalamus. Gray matter structures also contain glucose at higher concentrations than do white matter structures. These results show no clear relation between the concentration of free glucose in the various structures of the brain and the distribution of brain injury from circulatory arrest in food-deprived animals. Thus the behavior of free glucose in the brain does not account for the vulnerability of brainstem nuclei in food-deprived animals.

The distribution of glycogen in the various brain structures of the normally oxygenated control animals was of greater interest. The various brainstem nuclei that we have shown to be "vulnerable" to injury from circulatory arrest in food-deprived animals generally contained significantly more glycogen than did the other "nonvulnerable" brain structures.

The vulnerable cuneate and oculomotor nuclei stood out as structures particularly high in their glycogen contents. Thus, as Figure 10 illustrates, the glycogen contents of the various brain structures generally follow the distribution of brain injury provoked by exposing food-deprived animals to circulatory arrest. The high glycogen contents of the vulnerable brainstem nuclei seems to provide a full explanation for their vulnerability to injury from anoxia in food-deprived animals.

Circulatory arrest constitutes a unique biochemical "locked-in" state during which nothing is transported to and nothing is removed from the brain and various other organs.

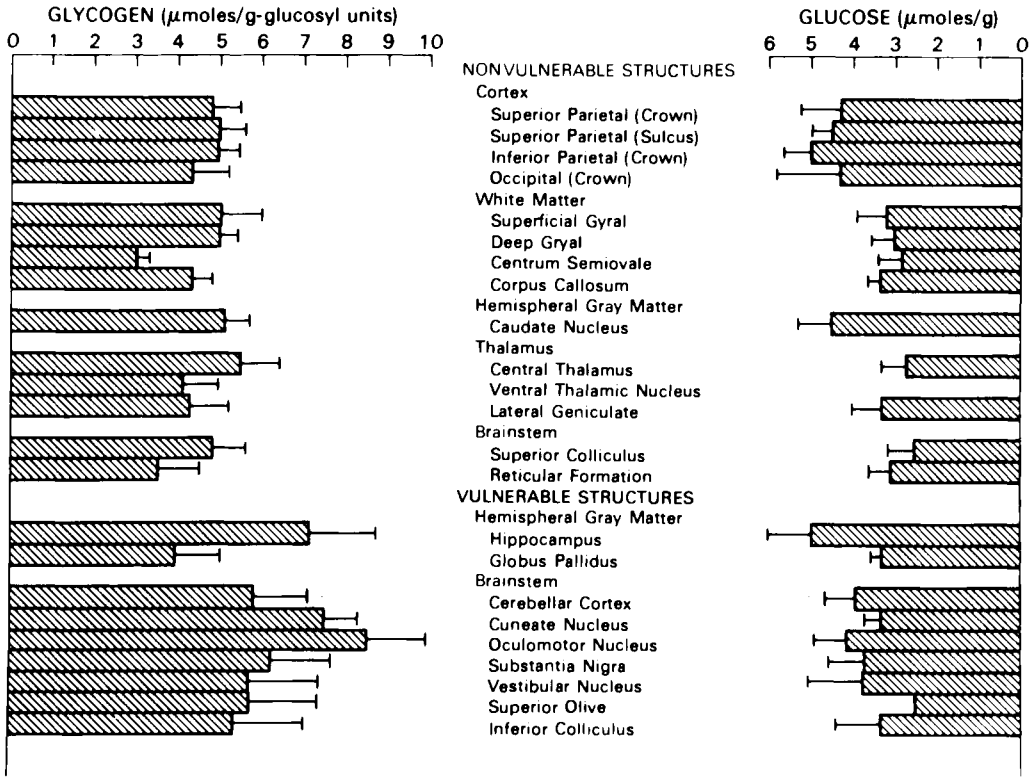


**Figure 10** Glycogen and glucose concentrations of "vulnerable" and "nonvulnerable" brain structures in normally oxygenated control goats. Each horizontal bar and line represents the mean and standard error of the mean, respectively, of three to six samples.

Under these circumstances, the quantity of lactic acid generated by a given structure during its anaerobic breakdown of available carbohydrate is determined almost entirely by its glycogen and glucose contents. Thus if the glycogen and glucose contents of a given brain structure at the time circulatory arrest develops is known, the concentration of lactic acid that will be generated in that structure when the reactions have been carried out to completion can be predicted with accuracy. Knowing the concentration to which lactic acid will accumulate in a given brain structure in turn provides all the information necessary to predict whether this structure will be injured as a consequence of the exposure. The propensity of the nuclei of the brainstem to be injured as a consequence of exposure to circulatory arrest (or total asphyxia) is determined by their normally high glycogen contents, while the extent of injury as more and more structures beyond the most vulnerable ones are affected is determined by the serum glucose concentration at the time of exposure and by the brain's content of free glucose.

## SIGNIFICANCE OF BLOOD PRESSURE TROUGHS OR CIRCULATORY FAILURE IN DEFINING LONG-TERM OUTCOME FROM EXPOSURE TO HYPOXIA

The biochemical and brain pathologic consequences of exposure to *hypoxia* are quite different from those incurred as a result of exposure to *anoxia*. Early work in our

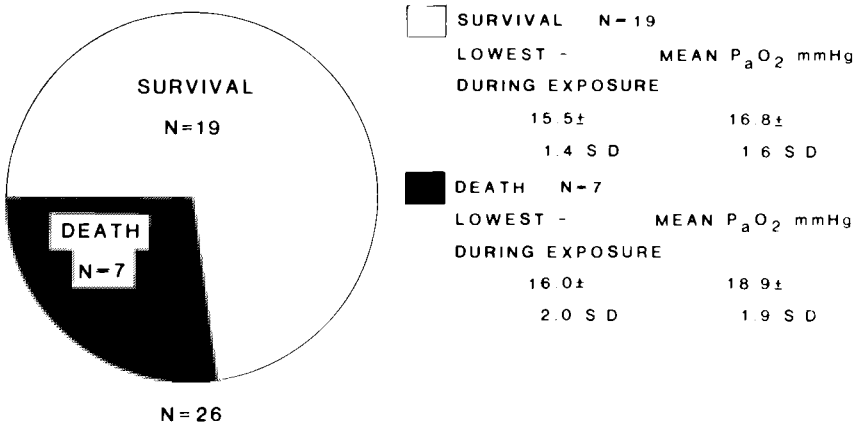


**Figure 11** Frequency of occurrence of fatal cardiogenic shock developing hours after cats were exposed to 25 min of marked hypoxia ($FiO_2$, 3.4%), and reoxygenated. Neither the duration nor the magnitude of hypoxemia explains this striking difference in outcome, since they were similar in both outcome groups.

laboratory has shown that exposing food-deprived monkeys to an anoxia such as is incurred during circulatory arrest or total asphyxia injures nuclear structures located in the brainstem, while exposing otherwise similar animals to a marked hypoxia damages structures located primarily in the hemispheres. Our more recent studies of the effects of *hypoxia* and *anoxia* have provided a full explanation for these striking differences in outcome (Myers, 1972; Myers et al., 1980c,d; de Courten et al., 1981a; Myers et al., 1980a).

We exposed adult cats to 25 min of marked hypoxia by mechanically respiring them with 3.4% oxygen in nitrogen. The majority of animals survived long term, while a smaller proportion died of cardiogenic shock early during the recovery period (see Figure 11). The animals of these two outcome groups exhibited mean lowest $paO_2$ values during hypoxia of 15.5 ± 1.4 mmHg (N = 19) and 16.0 ± 2.0 mmHg (N = 8), respectively. Of the 26 animals exposed to marked hypoxia, slightly more than half showed no brain injury, while the remainder showed either focal or diffuse brain injury when the brains were examined after animal survival for 3 hr to 2 weeks. The animals that remained brain intact and those that developed focal or diffuse brain injury experienced mean lowest $paO_2$ values during hypoxia of 15.2 ± 1.4 mmHg (N = 4) and 16.2 ± 1.6 mmHg (N = 4), respectively.

These further studies with cats greatly strengthen the view already described above in relation to studies with monkeys, that the absolute values of the oxygen content or oxygen partial pressure of arterial blood during exposure to hypoxia fail to determine whether the animals will develop brain injury or even whether or not they will survive. Instead, these results pinpoint the operation of a second factor other than the magnitude of the depression of the oxygen content of arterial blood that critically determines each animal's pathologic response to marked hypoxia.

Exposure to hypoxia, when sufficiently marked, reduces the MABP throughout exposure (except for brief early elevations brought about by sympathetic nervous system stimulation). During hypoxia monkeys, as a species, first stabilize their MABP at a

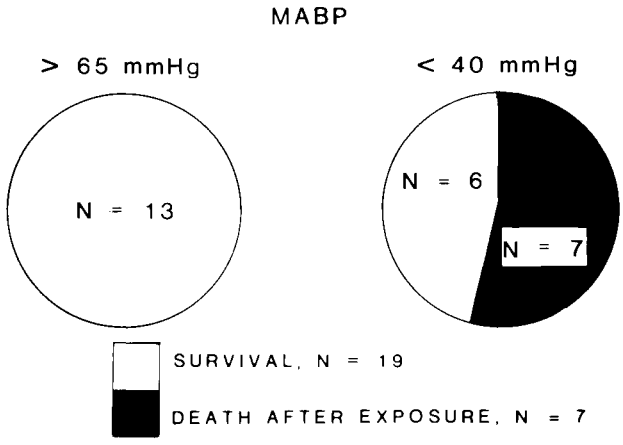


Figure 12 Influence of cardiovascular function during exposure to 25 min of marked hypoxia ($FiO_2$, 3.4%) on the late development of fatal cardiogenic shock. A circulatory collapse was required for the cats to later die from cardiogenic shock.

lower value and then, late on, suffer a circulatory collapse, provided that the hypoxia is sufficiently marked. In contrast to this behavior, cats, after an initial elevation, progressively reduce their blood pressure throughout exposure to marked hypoxia. In many instances they reduce their MABP to tissue pressure levels by the end of 25 min of exposure. In both species, reductions in blood pressure beyond a critical level lead first to slight and later to marked reductions in cerebral blood flow. The combination of a circulating blood that contains oxygen at extremely low concentrations and a marked decline in cerebral blood flow progressively reduces

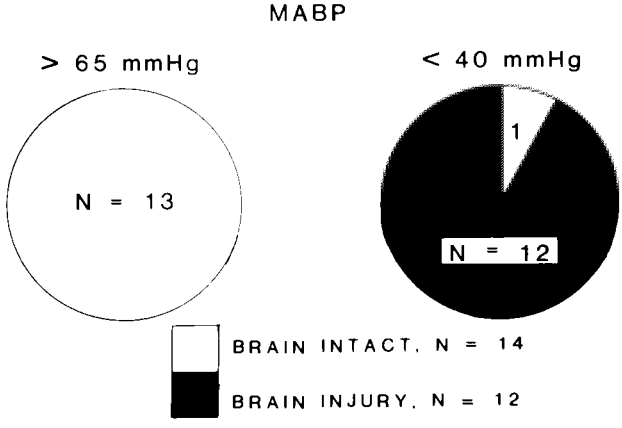


Figure 13 Influence of cardiovascular performance during exposure to 25 min of marked hypoxia ($FiO_2$, 3.4%) upon the development of brain injury. Cats that maintained their MABP above 65 mmHg failed to injure their brains, while cats that experienced a circulatory failure (MABP $<$ 40 mmHg) during hypoxia suffered brain injury in large proportion.

the net oxygen delivery to the brain and, ultimately, leads to a tissue anoxia or near anoxia if the process is not interrupted. About half of the cats in our hypoxia study experienced such a marked lowering of blood pressure that it reached in many instances tissue pressure levels and resulted in a generalized stasis of blood flow. Reductions in blood pressure to values lower than 40-60 mmHg greatly affected outcome, as Figures 12 and 13 demonstrate. All cats that maintained their MABP above 65 mmHg throughout exposure to a marked hypoxia survived long term, while more than half of those that experienced a 3-10 min MABP decline below 40 mmHg died of cardiogenic shock several hours into the recovery period after they were re-oxygenated. Exposure to a hypotension below 40 mmHg or to a circulatory collapse during hypoxia also provoked brain injury in these cats, as Figure 13 illustrates. While no cat which maintained its MABP above 65 mmHg during exposure to marked hypoxia injured its brain, almost all cats which experienced brief reductions in their blood pressure below 40 mmHg developed one or another form of brain injury.

The findings of these studies with hypoxia may be summarized in other terms. Exposure to a marked hypoxia by itself fails to injure the brain, while exposure to a marked hypoxia in combination with a near anoxia or anoxia can damage the brain and may also cause a delayed death in cardiogenic shock secondary to a direct injury to the myocardium in a high proportion of cases. These conclusions confirm those drawn much earlier in describing the response of term monkey fetuses to asphyxia (Myers, 1975a).

## EFFECTS OF CARBOHYDRATE STATE ON CARDIOVASCULAR AND BRAIN PATHOLOGIC RESPONSE TO HYPOXIA

### Effects of Cardiovascular Failure on Response to Hypoxia

Exposing cats and monkeys to marked hypoxia significantly reduces their MABP. Rhesus monkeys exposed to a marked hypoxia establish a new blood pressure plateau that may remain stable for 10-15 min. This is often followed by a circulatory collapse. Cats exposed to a marked hypoxia progressively reduce their blood pressure throughout exposure and often experience a terminal failure of circulation. These hypoxia-induced reductions in blood pressure result from reductions in cardiac contractility or in work output of the heart, rather than from reductions in peripheral vascular resistance (Myers et al., 1980b).

The cardiovascular effects just described appear as a direct consequence of exposure to marked hypoxia and are generally dramatically reversed by reoxygenating the animals if the circulatory failure has not lasted too long. As we have seen, these acute depressions of cardiovascular performance produced by marked hypoxia are critically important because they play a central role in provoking brain injury. Exposing animals to a marked hypoxia or to an anoxia may cause a separate critically important cardiovascular effect that, in many instances, determines actual animal survival. Monkeys and cats exposed to marked hypoxia or anoxia and resuscitated commonly develop the cardinal findings of cardiogenic shock that may begin to appear starting several to many hours into the recovery period (Miller and Myers, 1970; Myers, 1972; Gamache and Myers, 1975; Ginsberg and Myers, 1974a; Selkoe and Myers, 1979). Even though all animals involved have been reoxygenated and their circulatory performances fully restored, and even though they have maintained a near-normal

cardiovascular function for many hours into the recovery period, they may nonetheless, subsequently develop progressive reductions in their blood pressure and may die in cardiogenic shock. During the development of this delayed cardiogenic shock they again sustain significant reductions in their cardiac contractility associated with marked reductions in their blood pressure and stroke volume and they increase their heart rate. These objective signs of heart failure then progress, ending in death several hours later. For a fuller description of the striking alterations in cardiovascular performance produced both (1) during exposure to marked hypoxia and (2) during the period of delayed cardiovascular decompensation afterwards, see the study of Myers et al., (1980b).

### Effects of Sympathetic Nervous System Stimulation on Response to Hypoxia

An animal's history of recent food intake and its serum glucose concentration at the time of exposure to hypoxia and various forms of anoxia critically affect both its cardiovascular and brain pathologic responses. Cats or monkeys food-deprived for 48 hr both reduce their serum glucose concentrations and markedly deplete their liver glycogen contents (Rivera and Martinez-de Jesus, 1974; Myers et al., 1980d; de Courten et al., 1981a). Animals food-deprived for 24 rather than 48 hr also deplete their liver glycogen, but to less marked degrees. Animals exposed to the stress of marked hypoxia experience a major stimulation of their sympathetic nervous system. Such stimulation causes catecholamine release from the adrenal medulla (Cannon and Hoskins, 1911-12) and from sympathetic nerve terminals throughout the body. The catecholamines transported in blood directly affect the liver, stimulating glycogen breakdown and glucose release into the bloodstream (Young and Landsberg, 1977). Splanchnic nerve discharge as a component of sympathetic nervous system stimulation also activates liver phosphorylase, catalyzing the breakdown of glycogen and liver glucose-6-phosphatase and freeing glucose to pass directly from hepatic cells into the bloodstream (Shimazu and Amakawa, 1975).

The glucose outflow from the liver stimulated by the stress of hypoxia and sympathetic nervous system stimulation is limited in monkeys and sheep and leads to peak serum glucose concentrations that generally remain below 300-350 mg %. Rats respond variably, depending on the severity of hypoxia: the lower the $paO_2$ values (below 35 mmHg), the lower the peak values of serum glucose concentration provoked (MacMillan and Siesjo, 1972; Kogure et al., 1977). At $paO_2$ values below 16 mmHg rats utilize glucose at a faster rate than it is released from liver, reducing rather than increasing their serum glucose concentrations.

### Effects of Carbohydrate State on Cardiovascular Response to Hypoxia

Cats respond still differently to the stress of hypoxia. Normally fed cats exposed to marked hypoxia elevate their serum glucose concentrations to 759 ± 97 mg % (N = 6), with peak values reaching as high as 980 mg %. Cats food deprived for 1 and 2 days elevate their peak serum glucose concentrations to 494 ± 164 mg % (N = 17) and 240 ± 82 mg % (N = 11), respectively. Depriving cats of food markedly reduces the peak serum glucose concentration provoked by exposure to marked hypoxia (see Figure 14).

Cats' striking differences in metabolic response to marked hypoxia according to the duration of food deprivation profoundly affect the animal's pathologic response. Cats exposed to 25 min of marked hypoxia were evaluated with respect to their death rates from delayed cardiogenic shock and/or occurrence of brain injury in relation to their peak serum glucose concentrations developed during hypoxia, as described in Figures 15

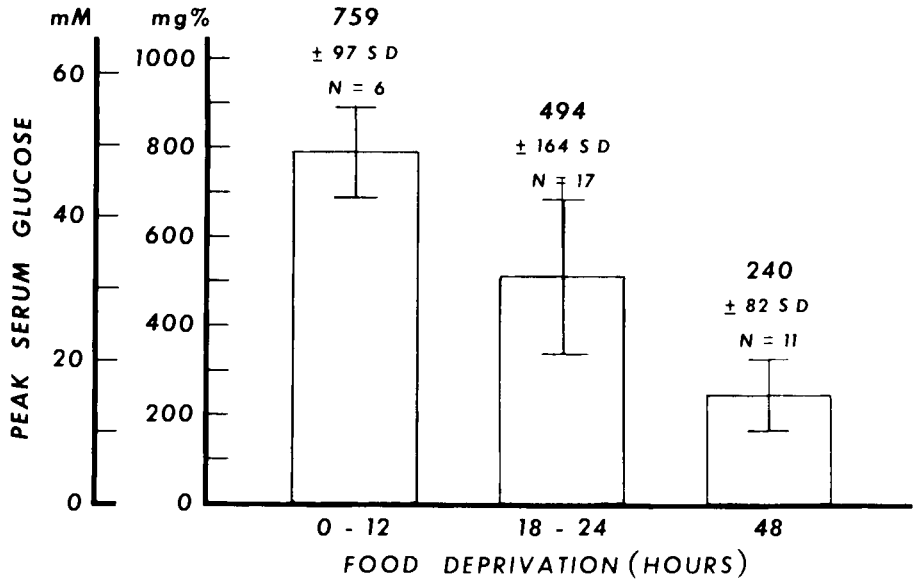


**Figure 14** Relation between duration of food deprivation and magnitude of hyperglycemic response provoked by 25 min of marked hypoxia ($FiO_2$, 3.4%) in cats (values are the mean ± SD).

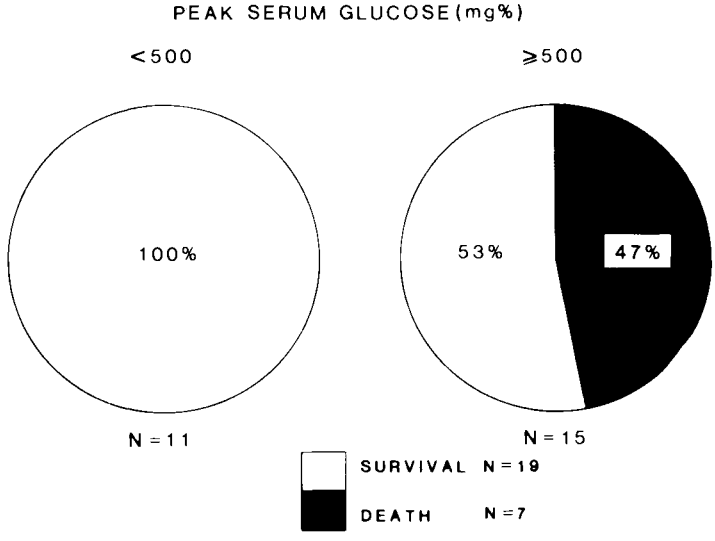


**Figure 15** Effects of magnitude of hyperglycemia provoked by exposure to 25 min of marked hypoxia ($FiO_2$, 3.4%) on the incidence of fatal cardiogenic shock developing hours later in the recovery period. Elevation of the serum glucose concentrations to values greater than 500 mg % during hypoxia greatly increased the frequency with which cats died in delayed cardiogenic shock.

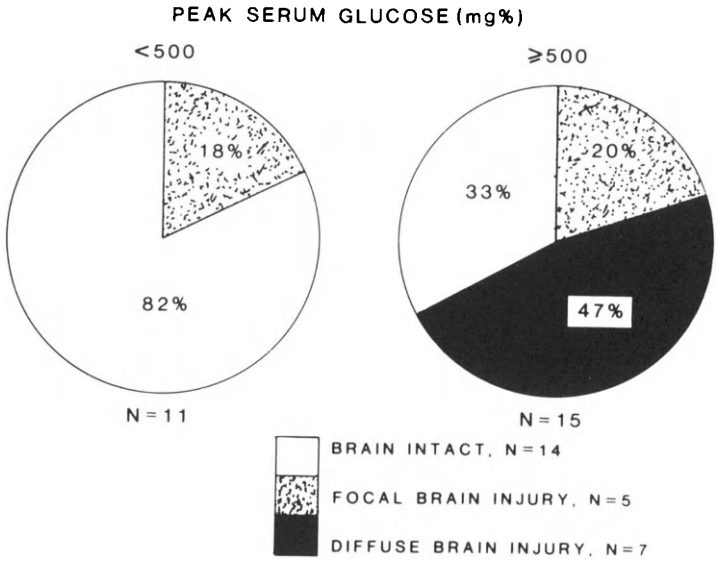


**Figure 16** Effects of magnitude of serum glucose concentration during exposure to 25 min of hypoxia ($FiO_2$, 3.4%) on the development and severity of brain injury in cats.

and 16. Those cats that experienced a combination of both high serum glucose concentrations and a brief period of marked blood pressure lowering during hypoxia died of cardiogenic shock many hours after they were reoxygenated in much higher proportion than did those animals that failed to fulfill one or the other of these two conditions (see Figures 12 and 15). A similar adverse effect of high serum glucose concentrations (in excess of 500 mg %) was observed with respect to the development of either focal or diffuse brain injury (Figure 16).

Cats normally fed, and hence rendered markedly hyperglycemic during exposure to hypoxia, experienced earlier and more rapid declines in their blood pressure during hypoxia than did cats food deprived for 24 or 48 hr. As a result of the sparing effect of lower levels of hyperglycemia on the development of circulatory collapse during hypoxia, it was necessary to provoke a late-developing circulatory collapse in many of the food-deprived animals by ventilating them briefly with 100% nitrogen toward the end of exposure to hypoxia. The results of the next study, defining the relation between the level of glycemia during hypoxia throughout the entire range accompanied by a brief period of circulatory failure (hypotension $< 40$ mmHg), are depicted in Figure 17. The higher the serum glucose concentration provoked by exposure to hypoxia in animals that experienced a concomitant circulatory collapse, the greater the frequency and the more marked the brain injury.

The cats that experienced high peak serum glucose concentrations also experienced more rapid declines in blood pressure and more frequent occurrences of circulatory collapse during exposure to marked hypoxia. The pathophysiologic mechanism accounting for such depressions in cardiovascular function *during* hypoxia has been identified. The normally fed cats that early on markedly elevate their serum glucose concentrations also elevate the concentrations of serum lactate during hypoxia. These cats generally

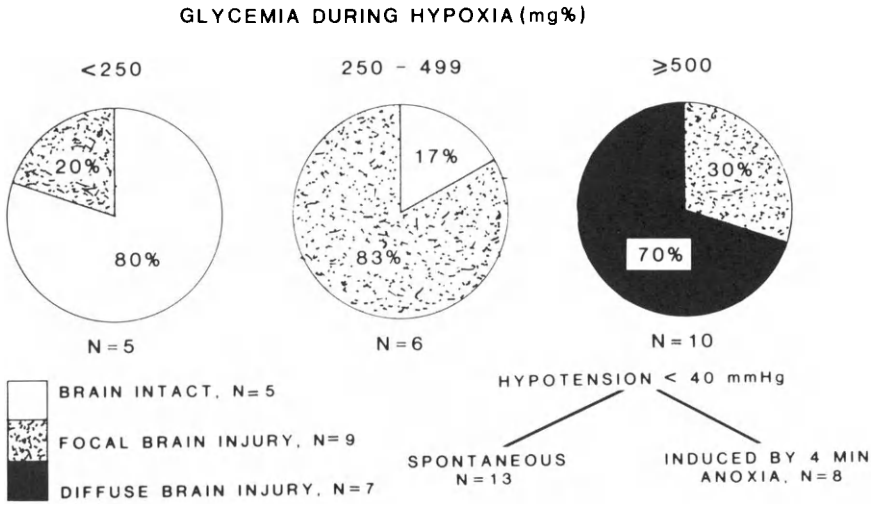


**Figure 17** Effects of the level of glycemia during 25 min of marked hypoxia ($FiO_2$, 3.4%) with hypotension < 40 mmHg MABP (spontaneous or induced by 4 min of anoxia) on the development and severity of brain damage in cats.

achieved peak concentrations of serum lactate early during the "washout" period after they were reoxygenated and their circulations restored. Cats that experienced serum glucose concentrations in excess of 500 mg % due to recent intake of food prior to exposure experienced peak serum lactate concentrations of 16.5 ± 2.8 mM (N = 15), with maximal values reaching as high as 20.7 mM. Such marked elevations of serum lactate concentrations were accompanied by marked reductions in blood pH (as low as 6.59). Food-deprived cats exposed to hypoxia also elevated their serum lactate concentrations, but to much lower values. Thus food-deprived cats which, under the stimulation of hypoxia, elevated their serum glucose concentrations to values lower than 500 mg % experienced mean peak serum lactate concentrations of 11.2 ± 2.9 mM (N = 12) and generally maintained their blood pH above 7.00.

The normally fed animals' marked elevation of serum lactate concentration and their production of an associated marked acidosis during hypoxia favored an early collapse of cardiovascular function. These more marked changes in blood composition during hypoxia (particularly with respect to increases in hydrogen ion activity) also caused the normally fed animals to die in high proportion in cardiogenic shock several hours into the recovery period. This relation between occurrence of a marked systemic acidosis during hypoxia and delayed death in cardiogenic shock can be seen in Figure 18. The great majority of cats that experienced minimal pH values lower than 6.80 later died in cardiogenic shock, while all cats that maintained their pH values above this level failed to develop delayed circulatory problems and all survived long term. These results indicate that a marked systemic acidosis caused by exposure to hypoxia or anoxia impacts upon the heart and significantly increases the incidences of (1) collapse of cardiovascular function during actual exposure to hypoxia and (2) death from the delayed development of cardiogenic shock taking place hours later in the recovery period. An increase in hydrogen ion activity in the range provoked in our cats has been demonstrated by others to alter the electrical properties of the heart (Vaughan Williams and Whyte, 1967; Skinner and Kunze, 1976; Marrannes et al., 1979) and to initiate heart failure (Williamson et al., 1976; Steenbergen et al., 1977).

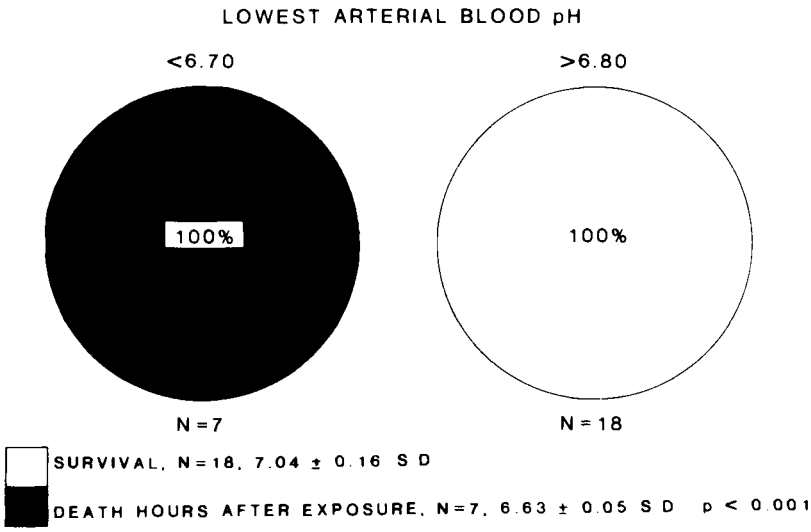


**Figure 18** Influence of lowest arterial blood pH in cats exposed to 25 min of marked hypoxia ($FiO_2$, 3.4%) on the incidence of fatal cardiogenic shock developing hours after reoxygenation.

Information that allows an accurate prediction of the lactate concentration of serum as a result of exposure to hypoxia also permits an accurate prediction of long-term outcome with respect to the occurrence of delayed death from cardiogenic shock. However, the prediction of long-term outcome can be made with still greater accuracy by following the animal's serum lactate concentrations during the early hours following exposure to hypoxia, as Figure 19 points out. Animals that survived exposure to 25 min of marked hypoxia restored their serum lactate concentrations to the normal range by 2 hr into the recovery period. In contrast, animals that later died from cardiogenic shock many hours into the recovery period still experienced significant elevations of their serum lactate concentrations 2 hr into recovery ($P < 0.001$).

### Effects of Carbohydrate State on Brain Pathologic Response to Hypoxia/Anoxia

The level of serum glucose concentration during marked hypoxia plays a critical role in determining the brain pathologic response, as has already been described. The animals that survived intact and those that survived but with focal brain injury all behaved alike and both of these groups differed from those that died of delayed cardiogenic shock with diffuse brain injury with respect to provoked plasma lactate concentrations, as Figure 20 demonstrates. The normally oxygenated control cats exhibited a serum lactate concentration of 1.2 ± 0.9 mM (N = 28). The animals that survived intact and those that survived but with focal brain injury following marked hypoxia increased their serum lactate concentrations during hypoxia to similar peak values, though the latter animals trended toward slightly higher values. The animals that developed diffuse brain injury also increased their serum lactate concentrations, but to significantly higher values than did the animals of the other two outcome groups. Furthermore, while the animals of the first two outcome groups reduced their serum lactate concentrations to near normal values within the first 2 hr following reoxygenation, the

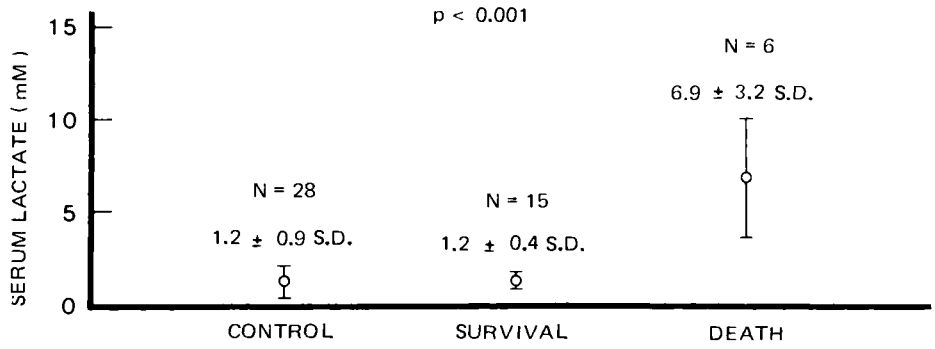


**Figure 19** Comparison of serum lactic acid concentrations 2 hr after exposure to 25 min of marked hypoxia ($FiO_2$, 3.4%) in cats that survived long term and in those that died many hours into the recovery period of cardiogenic shock. The animals that survived had restored their serum lactic acid concentrations to normal by this time, while those that later died still experienced elevated values.

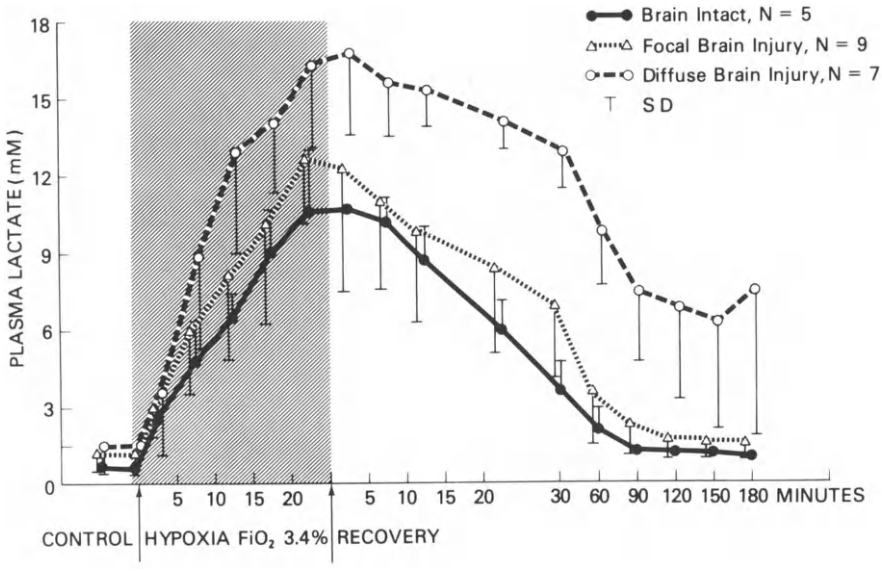


**Figure 20** Lactic acid concentrations of serum during and following exposure to 25 min of marked hypoxia ($FiO_2$, 3.4%) in cats that developed focal or diffuse brain injury and in those that survived intact. The animals that later developed diffuse brain injury experienced higher serum lactic acid concentrations than did those that survived brain intact or which developed focal brain injury.

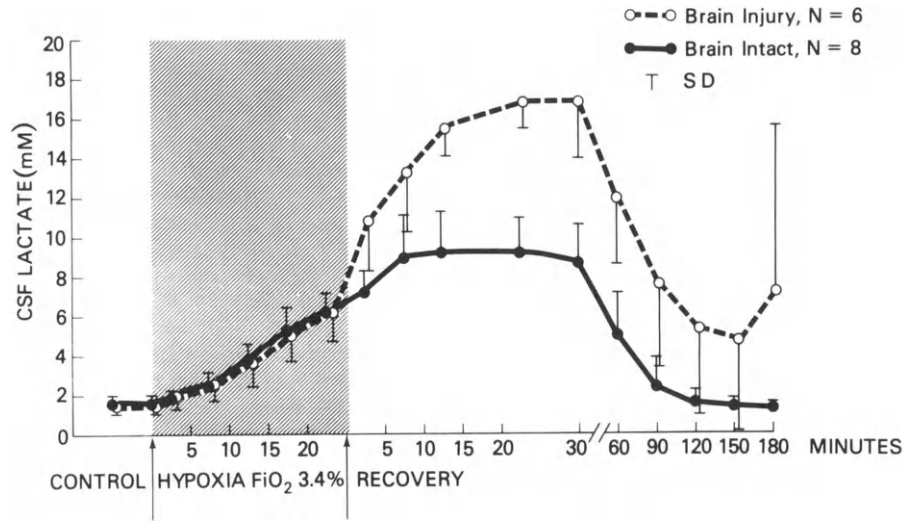


**Figure 21** Lactic acid concentrations of cisterna magna CSF during and following exposure to 25 min of marked hypoxia ($FiO_2$, 3.4%) in cats that developed focal or diffuse brain injury and in those that survived intact. The animals that later developed brain injury experienced markedly higher CSF lactic acid concentrations than did those that survived brain intact.

animals that developed diffuse brain injury and which also later died in cardiogenic shock experienced continued elevations of their serum lactate concentrations (to 3-8 mM) up until they died or required euthanasia.

All cats exposed to 25 min of marked hypoxia that maintained their MABP above 65 mmHg failed to develop brain injury. During hypoxia, they experienced glycemias from 200 to 820 mg %. Thus animals that maintained their blood pressure above a critical level throughout exposure to hypoxia failed to show an effect of serum glucose concentration on the development of brain injury; rather, only those cats that reduced their MABP to values lower than 40 mmHg or which were respired with 100% nitrogen for 4 min at the termination of hypoxia responded to level of serum glucose concentration with an effect on occurrence of brain injury. In these animals the serum glucose concentration critically determined both the development and the extent of brain damage.

The metabolic basis for brain injury from hypoxia/anoxia was clearly evident from studies on the changes in the composition of cerebrospinal fluid (CSF) sampled from the cisterna magna during hypoxia and the recovery period (see Figure 21). The animals that suffered focal or diffuse brain injury developed higher CSF lactate concentrations than did the animals that remained brain intact, both following exposure to hypoxia. After the animals achieved peak lactate concentrations in their CSF, they slowly reduced these concentrations. However, the animals that suffered brain injury did so at a later time than did those that remained brain intact. The animals that developed either type of brain injury maintained elevated CSF lactate concentrations throughout the 150 min of postexposure examination, while those that remained brain intact restored their CSF lactate to baseline values by 90 min. The cats that developed

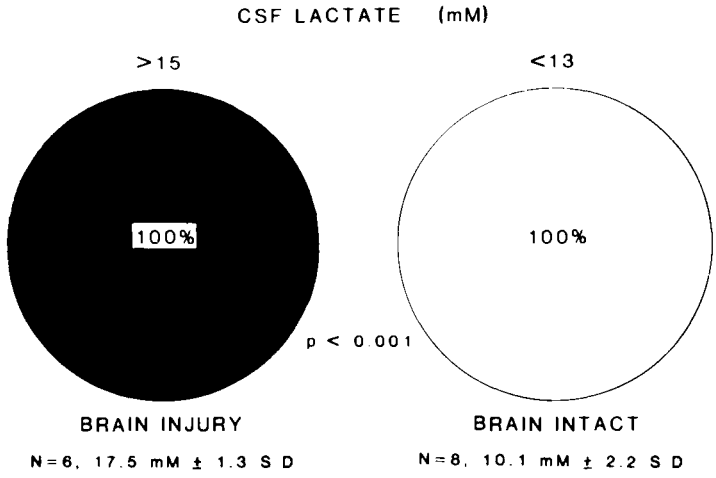


**Figure 22** Influence of *peak* cisterna magna CSF lactic acid concentrations in cats following 25 min of marked hypoxia ($FiO_2$, 3.4%) on the occurrence of brain injury. All animals that developed brain injury experienced CSF lactic acid concentrations greater than 15 mM for variable times after they were reoxygenated, while all animals that survived intact experienced it at concentrations below 13 mM.

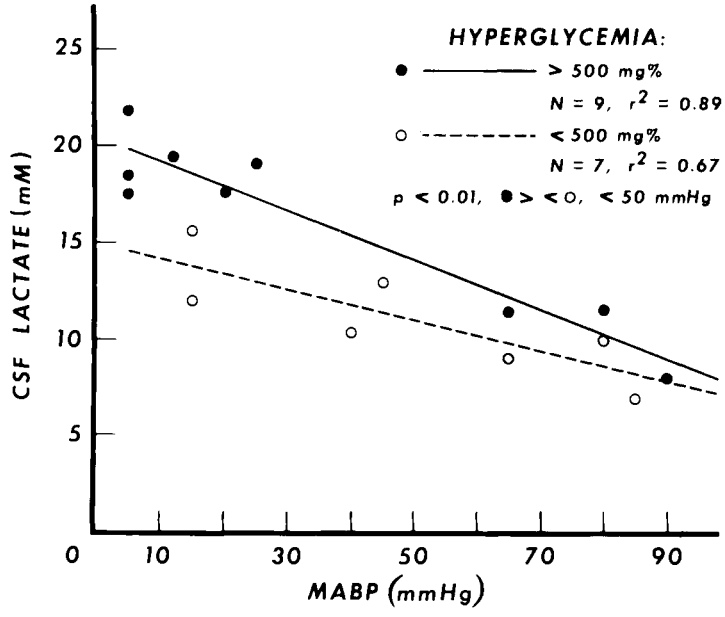


**Figure 23** Effects of lowest MABP and of serum glucose concentration on the *peak* cisterna magna CSF lactic acid concentration in cats exposed to 25 min of marked hypoxia ($FiO_2$, 3.4%). Reductions in MABP below 40-60 mmHg increased the cisterna magna CSF lactic acid concentrations above those observed at higher MABPs. Differences in serum glucose concentration affected the *peak* CSF lactic acid concentrations only when the MABPs were reduced to low values (below 40-60 mmHg).

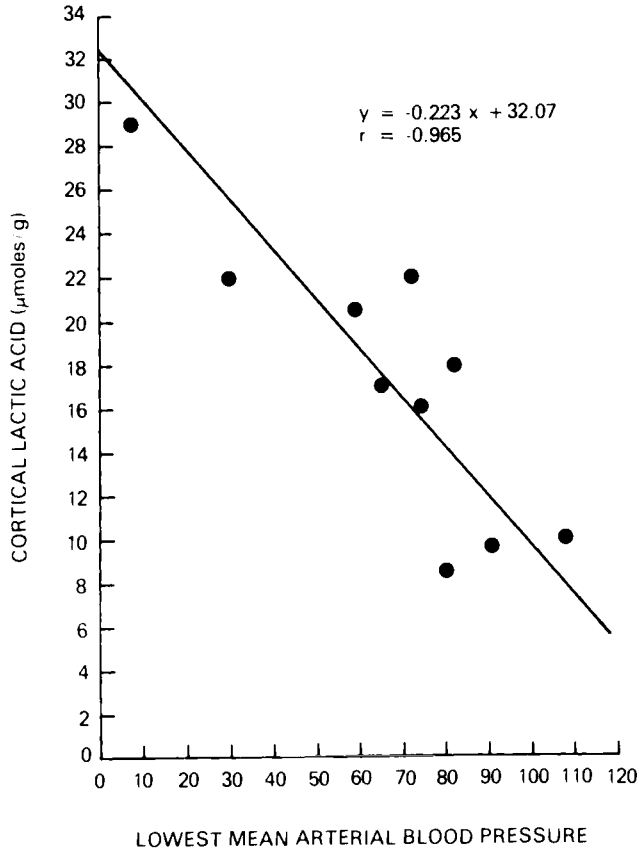


Figure 24 Correlation between the lowest MABP in rhesus monkeys exposed to marked hypoxia and the lactic acid concentration of the superior parietal cortex. Throughout the range examined, the lower the animals reduced their blood pressure, the more they accumulated lactic acid in their brain tissue.

either type of brain injury elevated their CSF lactate concentrations to peak values of 17.5 ± 1.3 mM (N = 6), while those that remained brain intact experienced the lower *peak* values of 10.1 ± 2.2 mM (N = 8). Determining the cisterna magna CSF lactate concentration at any time during the first 2 hr following exposure to hypoxia permitted the development of brain injury to be predicted with high accuracy, as can be seen from Figure 21. The *peak* CSF lactate values divide the animals into the brain-injured and the brain-intact categories with 100% accuracy (see Figure 22).

The influences of the carbohydrate state (serum glucose concentration) and the lowest MABP on the peak value of lactate in cisterna magna CSF are described in Figure 23. Several points emerge: (1) The concentration of lactate in CSF is not influenced by the serum glucose concentration as long as the animals maintain an MABP during hypoxia above 65 mmHg; (2) a decline in MABP below 40 mmHg greatly augments CSF lactate concentrations, significantly more in animals with elevated than in those with low serum glucose concentrations; and (3) the combination of both a marked fall in MABP and a high serum glucose concentration during hypoxia is required

to provoke CSF lactate values that are sufficiently high as to be associated with brain injury.

We have examined the brain metabolic consequences of exposing food-deprived rhesus monkeys to marked hypoxia (Wagner et al., 1980). These animals sometimes sustained marked reductions in blood pressure during exposure to hypoxia and often experienced a genuine circulatory collapse late during exposure. Monkeys exposed to 10-25 min of marked hypoxia that maintained their MABP above 80 mmHg elevated their cortical tissue lactate concentrations to 8.82 ± 0.59 mol/g (N = 4). In contrast, monkeys that experienced MABP reductions to values lower than 65 mmHg during hypoxia accumulated lactate in their cortex to values of 23.88 ± 2.62 μmol/g (N = 3), an almost threefold increase. Figure 24 presents the relation between the lowest MABP recorded during 20 min of marked hypoxia and the concentration to which lactate accumulated in the cerebral cortex. The monkeys showed a significant relation between the two such that the more reduced the MABP during hypoxia below 80 mmHg, the greater the quantity of lactate they accumulated in the brain. It was necessary that the animals experienced MABP reductions below about 80 mmHg to increase their brain lactate concentrations to the values required to produce brain injury.

All of the monkeys that experienced decreases in MABP below 65 mmHg during exposure to marked hypoxia accumulated lactate in high concentrations in brain tissue. These high concentrations resulted from a marked stimulation of both glycolysis and glycogenolysis. The food-deprived animals that maintained their MABP above 80 mmHg showed cortical tissue glucose and glycogen concentrations of 3.9 ± 0.71 and 5.9 ± 1.05 μmol/g, respectively (N = 4). By contrast, the food-deprived animals that reduced their MABP below 65 mmHg showed cortical tissue glucose and glycogen concentrations of 1.17 ± 0.38 and 2.57 ± 0.59 μmol/g, respectively (N = 3).

The studies just described show that adult monkeys or cats exposed to a specific marked hypoxia may (1) develop brain edema, widespread brain tissue necrosis, and die of delayed brainstem failure or cardiogenic shock; (2) survive and show focal brain injury; or (3) survive, but with no brain injury. Which of these outcomes obtain in individual cases depends on whether the animal sustains a circulatory failure during hypoxia and, if it does, what the serum glucose concentration is at the time of circulatory failure. Similar results and conclusions have been drawn for fetuses and newborns. Mid-gestational sheep fetuses that we exposed to 2 hr of marked hypoxia experienced the same three clinical outcomes: delayed death in circulatory failure, survival but with widespread focal cerebral necrosis, and intact survival (Ting et al., 1983). The fetuses that survived intact and those that survived but with marked brain injury experienced similar arterial blood $paO_2$ values throughout exposure to hypoxia. Thus fetuses, like adults, can respond differently to a marked hypoxia with respect to whether they develop brain injury and whether they survive, and these differences do not depend on differences in the oxygen composition of arterial blood during hypoxia.

We also compared the brain-intact and brain-damaged surviving sheep fetuses with respect to differences in cardiovascular function during hypoxia. The fetuses that survived intact maintained their blood pressure well, while those that suffered extensive cerebral softening reduced their MABP slightly but significantly by the end of 2 hr of exposure to hypoxia. The serum glucose and lactate concentrations were also measured throughout exposure to hypoxia. The fetuses of all outcome groups showed

similarly low serum glucose concentrations during hypoxia. However, the fetuses that suffered widespread cerebral necroses accumulated lactate in their serum to much higher concentrations toward the end of exposure to hypoxia than did those that survived intact. These results with mid-gestational sheep fetuses agree well with those already described with adult animals irrespective of species. These results demonstrate that (1) reductions in blood pressure leading to more marked reductions in oxygen delivery to the brain play an important role in determining whether brain injury develops from exposure to hypoxia and (2) similar mechanisms operate in fetuses as in adults to produce hypoxic/anoxic brain injury.

## SUMMARY AND CONCLUSIONS

The brain biochemical and pathologic findings described in the present chapter provide a new understanding of the cause-and-effect relation between oxygen deprivation and development of brain injury. The accumulation of lactate (and its associated hydrogen ions) in brain tissue above the threshold concentration of 17-20 μmol/g accounts for the development of brain edema and tissue necrosis. The concentrations to which lactate (and hydrogen ions) accumulates is determined by (1) the magnitude of reduction in oxygen delivery to the brain and (2) the availability of glucose and its storage form, glycogen, in brain tissue.

A stasis of blood flow (circulatory arrest) produces a metabolic "locked-in" state in which no substances are transported to or taken away from the brain. Exposure of food-deprived animals to circulatory arrest injures only those brain structures that contain glycogen in high concentrations (e.g., gray matter nuclei located in the brainstem). These structures accumulate lactate and hydrogen ions locally at high concentrations, while the cerebral cortex and other hemispheral structures that contain glucose at similar concentrations but which are significantly lower in their glycogen contents generate these same metabolites, but at below threshold concentrations and therefore escape injury. Exposure of normally fed animals to the same circulatory arrest injures widespread areas of the brain because their (1-2 mM) higher serum glucose concentrations elevate the glucose concentrations of brain tissue to a degree sufficient to lead to a lactate and hydrogen ion accumulation above the threshold values required to injure widespread areas of the brain. Animals infused with glucose solutions elevating the serum and brain glucose concentrations to even greater extents develop a marked serum and tissue lactic acidosis and sustain, for this reason, widespread brain edema and tissue necrosis. Thus both the localization and the extent of brain injury brought about by circulatory arrest depend on the quantity of glucose available to tissue at the time of arrest and on the distribution of glycogen across brain structures.

Exposure of animals to marked hypoxia when failure of circulation or episodes of marked hypotension is avoided does not injure the brain. This result is entirely in keeping with the results of our studies on the brain metabolic effects of exposing rhesus monkeys to marked hypoxia. Either food-deprived or glucose-infused monkeys exposed to marked hypoxia which maintained their MABP above 80 mmHg showed brain tissue lactate concentrations that remained well below the 17-20 μmol/g threshold required to injure brain tissue. However, animals that sustained even a brief period of marked hypotension or circulatory failure during exposure to hypoxia developed lactate concentrations in their brain well in excess of the 17-20 μmol/g required to produce injury. A significant reduction in blood pressure or a failure of

circulation during hypoxia further reduces oxygen delivery to tissue to a critical near anoxia or anoxia and, in the process, significantly stimulates glycolysis and glycogenolysis. Only under this last circumstance does the serum glucose concentration significantly determine the occurrence of brain injury, because only with a marked stimulation of glycolysis does the serum glucose concentration determine the concentration to which lactate and hydrogen ions accumulate in the brain. Animals with a low serum glucose concentration which experience a failure of circulation either fail to injure their brains entirely or injure only restricted focal brain regions, while animals with a high serum glucose concentration which experience the same circulatory failure inevitably damage widespread regions of their brains.

It is now possible to understand the basis for our previous observation of the necessity to expose fetuses to both a marked hypoxia combined with a brief anoxia or near anoxia to produce the clinical and brain pathologic abnormalities of cerebral palsy. Exposure of fetuses of food-deprived mothers to a brief pure anoxia or to a marked hypoxia not associated with an episode of near anoxia or anoxia (hypotension or circulatory failure) generally fails to elevate the brain lactic acid concentrations to values sufficiently high to cause widespread injury to the brain. However, their exposure to an antecedent marked hypoxia significantly stimulates the sympathetic nervous system and, through this mechanism, elevates the serum glucose concentration. The subsequent development of an anoxia or near anoxia brought about by a circulatory failure provoked by the marked hypoxia then, for the first time, stimulates glycolysis and initiates glycogenolysis. The stimulated glycolysis in the presence of a plentiful supply of glucose from the stress-induced hyperglycemia combined with a tissue glycogenolysis all markedly augment the local accumulation of lactic acid in the brain. In the fetus, the end result of this process is to produce the characteristic clinical manifestations and the classical brain lesions of cerebral palsy.

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# 14

# Renin-Angiotensin System in Early Life

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## INTRODUCTION

As the fetus swims in its private pool, it is, like the diver working at depth, totally dependent upon the supply from the surface of all the essentials for the maintenance of life. For the fetus, pressure head is thus maternal blood pressure and the maintenance of an adequate pressure-flow relationship in the uteroplacental circulation is of preeminent importance. It is therefore perhaps surprising that so little attention has been paid to the possibility of fetal manipulation of the maternal circulation to maintain a favorable "milieu intérieur." On available evidence it appears that the prime homeostatic role of the phylogenetically ancient renin-angiotensin system may be prior to and around birth and during pregnancy. This chapter reviews such evidence and also suggests the hypothesis that the fetoplacental renin-angiotensin system is implicated in the pathogenesis of pregnancy-induced hypertension.

## THE RENIN-ANGIOTENSIN SYSTEM

Renin is an enzyme (EC 3.4.99.19) that cleaves the angiotensin decapeptide (angiotensin I) from an $\alpha_2$-globulin substrate (Figure 1). The angiotensin I is rapidly converted to an octapeptide, angiotensin II (A II), by a peptidyl dipeptidase-converting enzyme (EC 3.4.15.1) primarily situated in the lung. The A II has a half-life of the order of one circulation time and is broken down to various fragments by a group of enzymes, collectively referred to as angiotensinases. One of these fragments, the angiotensin (2-8) heptapeptide (A III) has less pressor effect than A II, but is a highly effective stimulus to aldosterone secretion (for references see Peach, 1977). The various stimuli thought to control renin release in the adult have been recently reviewed (Davis and Freeman, 1976).

Renin exists partly in an inactive form of molecular weight ~55,000 which is converted to the active form in vitro below pH 3.0 (Lumbers, 1971; Leckie and McConnell, 1975; Derkx et al., 1978). The proportion of active to inactive renin in plasma can be altered by maneuvers such as sodium depletion (Weinberger et al., 1977), but the physiological agent involved has yet to be ascertained. Tissues other than the kidney have been shown to be capable of the synthesis of renin; those relating to pregnancy and the fetus are discussed below. Renin substrate may also be present in more than one form (Eggena et al., 1978).

The physiological actions of the renin-angiotensin system are believed to be mediated via A II, although it is likely that A III also plays a part in the regulation of aldosterone synthesis. On a molecule-for-molecule basis intravenous A II is 40-50 times as active as

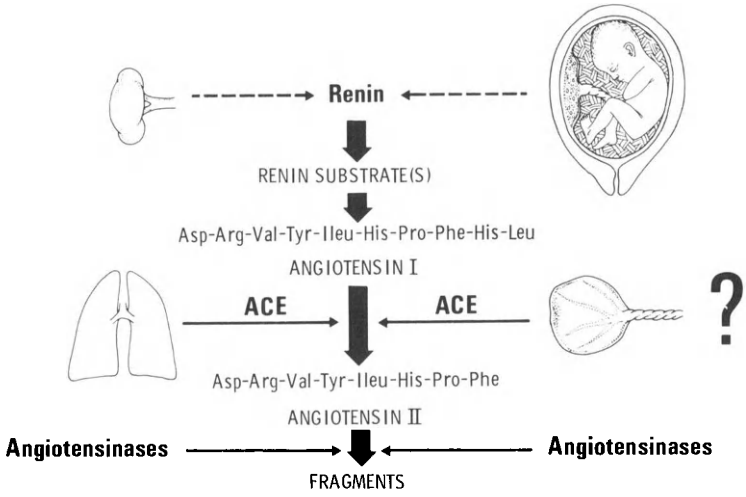


Figure 1 Schematic diagram of the renin-angiotensin system in the pregnant human (ACE, angiotensin-converting enzyme).

noradrenaline as a vasoconstrictor agent in man. The evoked rise in arterial blood pressure is proportional to the log dose (de Bono et al., 1963; Chinn and Düsterdieck, 1972). Cardiac output is usually reduced, although stroke volume is unchanged; pulmonary arterial and wedge pressure rise, probably due to constriction of pulmonary resistance vessels (de Bono et al., 1963). Angiotensin II administered via the vertebral artery has been found to exert a consistently greater pressor response in the conscious human than the same dose given intra-aortically (Ueda et al., 1969). Similar effects have been observed in anesthetized animals, and it is suggested that this greater effect may in part be mediated via the sympathetic nervous system. At least some of the renal effects of A II in man also appear to be related to its vasoconstrictor actions; for example, following the administration of A II the glomerular filtration rate and effective renal plasma flow fall, with a consequent decrease in sodium, potassium, and chloride excretion (Brown and Peart, 1962). The interested reader is referred to the recent review by Peach (1977) for an extensive consideration of the various actions of angiotensin II.

Having presented an extremely brief outline of the renin-angiotensin system in the adult, the remainder of this chapter is devoted to the fetus and newborn. For obvious reasons, only minimal information is available concerning the human fetus. The very processes of labor and delivery markedly affect the renin-angiotensin system, so that it is not possible to describe accurately samples obtained at such delivery as being representative of those pertaining in utero. In consequence, much of the fetal data is derived from animal experiments.

A description will first be given of the basal fetal concentrations of the various components of the system, followed by a consideration of some of the stimuli found to affect these basal levels. Anesthesia and laparotomy both alter renin levels. Discussion of such stimuli will therefore be confined to work carried out in the unanesthetized, chronically cannulated fetus unless otherwise stated, although much of the earlier work, which markedly affected the course of subsequent investigation, was

carried out in the acute preparation. In Section IV the renin-angiotensin system in the human newborn and some of the factors found to affect it will be discussed.

## THE RENIN-ANGIOTENSIN SYSTEM IN FETAL LIFE

### The Enzyme

#### Renal

It is now more than 40 years since Kaplan and Friedman (1942) showed a substance with the characteristics of renin to be present in hog mesonephros from as early as 22 days gestation (term, ~114 days). Juxtaglomerular cells were not histologically identifiable in the mesonephrons studied. As mesonephric degeneration occurred, with the development of the metanephros, so the mesonephric renin content per gram of kidney declined. However, all metanephric kidneys contained "renin" in considerable amounts, and this concentration increased progressively with fetal age. Plasma renin concentration, measured in anesthetized fetal piglets, increases progressively from 55 to 95 days gestation and declines thereafter to term (Broughton Pipkin et al., 1981).

Renin has been extracted from the kidneys of a 40-day fetal lamb (Wintour et al., 1977) but in lambs of 90-104 days gestation (term, ~147 days), fetal plasma renin levels were lower than maternal levels (Carver and Mott, 1975). Plasma renin concentration was significantly higher in fetal lambs than in their mothers in the last quarter of gestation, increasing progressively with gestation length (Broughton Pipkin et al., 1974a, Broughton Pipkin and O'Brien, 1978).

Renin-containing cells have been found using immunohistochemical techniques in the early metanephros of 5-week-old human fetuses, close to the prospective vascular pole of the developing glomeruli (Phat et al., 1981). As in the hog fetus, direct measurement of renal renin reveals increasing activity with gestation age (Molteni et al., 1974). The oldest fetus examined was of ~107 days gestation. Bowie-positive granules were only seen spasmodically, and only in the kidneys of older fetuses. High plasma concentrations of both active and inactive renin have been reported in four fetuses following termination of pregnancy by hysterectomy at 16-20 weeks gestation (Franks and Hayashi, 1979).

#### Extrarenal

It is highly unlikely that maternal renin crosses the placental barrier, since its molecular weight is of the order of 48,000-55,000. However, reninlike enzymes have been extracted from fetal tissues other than the kidney, the most notable being the chorion. Large concentrations of renin have been measured in human chorion, both in tissue homogenates (Skinner et al., 1968) and in in vitro culture (Symonds et al., 1968). Reninlike enzymes have also been measured in extracts of cat placenta (Stakemann, 1960) and rabbit placenta (Gross et al., 1964). High concentrations of activatable renin were first demonstrated in amniotic fluid by Brown et al. in 1964, an observation subsequently confirmed by numerous investigators.

It seems possible that the fetal genital tract may also be capable of producing renin. Cultured myometrium from a 20-week fetus produced considerable amounts of both active and inactive renin (D. J. Craven and A. Y. Warren, personal communication). Symonds and Furler (1973) reported measurable levels of acid-activatable renin (~10% of normal newborn levels) in the cord blood of a female infant with congenital renal agenesis. Interestingly, in this fetus no plasma renin activity (see below) was detected, although

substrate levels were higher than in any of the four normal fetuses reported for comparison. However, in another anephric female fetus, plasma renin concentration, plasma renin activity, and angiotensin II were all within the low normal range for neonates (D. J. Craven, A. Y. Warren, and F. Broughton Pipkin, unpublished data). Measurable levels of renin and angiotensin II have been found in both male and female patients studied for up to 2 years after bilateral nephrectomy (Medina et al., 1972). Since the human uterus is capable of the production of renin in cell culture (Symonds et al., 1968) and since considerable concentrations of renin have been measured in human semen (D. J. Craven and A. Y. Warren, unpublished data), it does not seem unreasonable to suppose that the renin in the anephric fetuses originated in the genital tract. This is perhaps not surprising in view of the close embryological links between the renal and genital systems.

### The Substrate

It has been suggested (Skinner et al., 1975) that renin substrate concentration is as important as the concentration of renin in determining the rate of production of angiotensin. It is therefore perhaps surprising that so little is known about renin substrate production in utero. Measurable concentrations of renin substrate are present in fetal pig plasma from 55 days gestation (Broughton Pipkin et al., 1981), but are lower than those in the nonpregnant adult and show no consistent trend with gestation age.

Carver and Mott (1978), using ewes and their fetal lambs of 103-141 days of gestation, found consistently lower substrate levels in the fetus than in the ewes in stable preparations. Lambs nephrectomized in utero had significantly higher substrate levels than intact lambs. No correlation was found between simultaneously measured fetal and maternal levels, nor did fetal levels show any consistent change with age.

### Plasma Renin Activity

Plasma renin activity (PRA) is a measure of the rate of generation of angiotensin I by a plasma sample under controlled conditions. It is thus a crude measure of the endogenous velocity of the enzyme-substrate reaction. Plasma renin activity has been found to be consistently higher in fetal lambs than in the ewe in the chronically cannulated state (Broughton Pipkin et al., 1974a; Smith et al., 1974; Fleischman et al., 1975; Broughton Pipkin and O'Brien, 1978). Plasma renin activity has also been measured in the "cord blood" of three abortuses delivered by hysterotomy at 16-20 weeks of gestation (Franks and Hayashi, 1979). In two of the three, plasma renin activity was higher than in simultaneously obtained maternal blood.

### The Hormone

Few direct measurements of fetal angiotensin II concentrations are available, since most radioimmunoassay methods require considerable volumes of plasma. However, it was found in a small series of lambs that although fetal plasma renin concentration in the last quarter of gestation was some 12-fold higher than that of the ewe, angiotensin II concentrations in the undisturbed state were very similar (Broughton Pipkin et al., 1974a). A subsequent, much larger study found fetal basal angiotensin II levels to be some 70% higher than maternal concentrations (Broughton Pipkin and O'Brien, 1978). There is thus indirect evidence that angiotensin-converting enzyme must be present in utero.

Lungs of fetal rabbits of 22 days of gestation have been found to extract only one-third of infused angiotensin I, while lungs from term and newborn rabbits extracted more than 80%. Converting enzyme was present in the lungs from 17 days gestation (0.54) and in the placenta from 0.67 of gestation (Stalcup et al., 1978).

Hébert et al. (1972) in acute experiments suggested that pulmonary converting enzyme activity in the fetal lamb was only about half that in the adult. Interestingly, converting enzyme activity has also been demonstrated in cultured human umbilical endothelial cells (Johnson and Erdos, 1977).

Angiotensinase activity in the placenta has been inferred from the fact that A II given via the umbilical artery to anesthetized lambs resulted in a smaller systemic pressor response than did the same dose given via the umbilical vein (Lumbers and Reid, 1978). It has also been shown chromatographically that iodinated A II does not pass intact from the fetal to the maternal circulation in the anesthetized guinea pig, again suggesting the presence of placental angiotensinases (Broughton Pipkin et al., 1977).

## Physiological Actions

### Vasoconstrictors

It is not possible to compare directly fetal and adult responsiveness to vasoconstrictor agents because of the large, low-resistance circuit of the placenta. Various investigations on the fetal response itself have, however, been carried out.

The first investigations of the fetal effects of renin and "angiotonin" were made in 1942 (Burlingame et al., 1942). One can only marvel at the technical expertise required to cannulate umbilicoplacental vessels in the 15½-day rat fetus and maintain an apparently stable preparation. Both renin and "angiotonin" were found to exert a marked pressor effect in the fetus.

Assali et al. (1962) found in the anesthetized preparation that although the fetal lamb was sensitive to the vasoconstrictor effect of angiotensin II, the effect was less in the lamb than in the ewe. They were, however, using extremely high doses of angiotensin. Lumbers and Reid (1978) found angiotensin II to be a more potent pressor agent on a molecule-for-molecule basis than either noradrenaline or adrenaline in the fetus. The combined administration of phenoxybenzamine and propranolol failed to affect consistently the pressor response to A II.

Infused A II evoked a positive chronotropic effect in chronically cannulated fetal lambs from at least 120 days of gestation, as has been reported in adult animals (Iwamoto and Rudolph, 1981a).

Berman et al. (1978) found that A II given via the hypogastric artery resulted in an elevation of systemic blood pressure and an increase in umbilicoplacental vascular resistance. However, since the administered doses of angiotensin II were very high (160-360 $\mu$g/kg), it is difficult to assess the physiological significance of these findings.

Hyman et al. (1975a) found angiotensin II to be a potent vasoconstrictor in the fetal lamb's pulmonary circulation, an effect which was blocked by prior infusion of the competitive angiotensin antagonist saralasin. However, the infusion of saralasin during hypoxemia failed to block the evoked increase in vascular resistance. Broughton Pipkin et al. (1974a) also concluded that angiotensin II was not involved in the response to moderate hypoxemia in utero. However, Moutquin and Liggins (1981) found severe asphyxia and asphyxia leading to fetal death to be associated

with marked activation of the renin-angiotensin system in chronically catheterized fetal lambs. The area requires further study.

The effect of acute hypercapnia on the renin-angiotensin system has not been studied in the fetus. It is, however, interesting to note that Sill et al. (1973) found acute hypercapnia in unanesthetized human volunteers to be associated with a significant rise in PRA. It thus seems possible that the renin-angiotensin system may play a role in maintaining fetal pulmonary vasoconstriction, since the fetus, relative to the adult, has a much higher $pCO_2$.

Behrman and Kittinger (1968) reported equal doses of angiotensin II to evoke a similar absolute pressor response in the anesthetized fetal rhesus monkey (*Macaca mulatta*) and its mother. However, since the fetus only weighed ~6% of the mother, the concentration given to the fetus was some 16-fold higher, and since the concentrations given were in any case pharmacological (up to 25 μg/kg to the fetus), it is difficult to attach much significance to these findings.

In addition to its effects on the fetal systemic vasculature, A II has been found to exert a marked contractile effect on human villous stem arterioles (Tulenko, 1979). There appears to be a graded increase in response from the minimal effects observed on the umbilical artery to a moderate effect on chorionic plate arteries and a very marked effect on the deep placental vessels. Angiotensin II may thus be actively contributing to placental vascular tone.

### Effect on Fetal Arterial Blood Pressure

From what has been said, it appears that the chronically cannulated fetal lamb is sensitive to the pressor effects of angiotensin II in at least the last 6 weeks of gestation. It has been calculated (Chinn and Düsterdieck, 1972) that circulating levels of angiotensin II are, in the adult human, very close to those capable of affecting the arterial blood pressure. The concentration of angiotensin II is higher in the fetal lamb than in the ewe, which itself has high levels relative to the nonpregnant animal. Thus the question arises as to whether these high levels of pressor hormone can alter fetal arterial blood pressure.

Broughton Pipkin and O'Brien (1978) found a highly significant inverse relationship between fetal diastolic blood pressure and plasma angiotensin II concentrations in the fetal lamb. The infusion of the specific angiotensin receptor-blocking agent saralasin to seven lambs resulted in a marked fall in blood pressure in the two with the highest initial level of angiotensin II, and in a small pressor response in that with the lowest level, with intermediate responses in between. Similar findings have been reported by Iwamoto and Rudolph (1979), who also showed a fall in fetal blood flow to the umbilicoplacental circulation following the administration of saralasin. Furthermore, the maternal administration of SQ14,225 (Captopril), which is both an angiotensin-converting enzyme inhibitor and a bradykinin-potentiating factor, resulted in a rapid fall in fetal blood pressure in each of five lambs studied. This fall was sustained in four lambs for up to 3 days, (Broughton Pipkin et al., 1982). It thus seems likely that, at least in lambs younger than ~125 days (0.85 gestation), the renin-angiotensin system may be playing a part in the maintenance of fetal blood pressure. Since a similar inverse relationship exists in the human newborn between blood pressure and angiotensin II concentration (see below), it is tempting to postulate a similar role for the hormone.

Further evidence that the renin-angiotensin system may be involved in the regulation of fetal blood pressure is found in the work of Lumbers and Lee Lewes (1979).

These authors found an inverse correlation between fetal arterial pressure and PRA in anesthetized lambs similar to that demonstrated in the chronic preparation for A II. Fetal hypotension, evoked by the intravenous infusion of sodium nitroprusside, was associated with a significant increase in PRA, while fetal hypertension, evoked by intravenous phenylephrine, was associated with a significant fall in PRA.

In this context it is perhaps also interesting to note that Hyman et al. (1975b) found chronic unilateral renal artery constriction in fetal lambs to be associated with a marked elevation in both fetal arterial blood pressure and plasma renin activity.

It has been suggested (for references, see Boddy, 1976) that the sympathetic nervous system in fetal lambs, as in other immature animals, may not be fully developed at birth. $\alpha$-Sympathetic blockade results in small falls in blood pressure from ~85 days (0.58) gestation, but $\beta$-sympathetic blockade does not appear to affect basal fetal blood pressure until at least 120 days (0.82) gestation. Similarly, fetal baroreflex activity is not fully mature, even at term (Shinebourne et al., 1972). It thus seems possible that the renin-angiotensin system may be of relatively greater importance in regulating fetal cardiovascular homeostasis at a time when the main adult control mechanisms are still not fully functional.

### Effect on the Adrenal Cortex

Angiotensin II has been reported to be a potent stimulus to aldosterone production by fetal lamb adrenals in vitro from as early as 40 days (0.27) gestation (Wintour et al., 1977). Interestingly, the production of corticosterone and cortisol was also significantly increased. The effect on aldosterone production diminished after ~100 days to term, although at term A II was a more potent stimulus than adrenocorticotropin to aldosterone production. Plasma aldosterone has been measured in the anesthetized fetal lamb from 60 days (0.41) gestation and in the chronically cannulated fetal lamb from 90 days (0.61) gestation (Wintour et al., 1975; Brown et al., 1978), after which it rises progressively to term, when it is about 10-fold higher than in the maternal circulation. It can be calculated that more than 80% of the aldosterone in fetal blood is of fetal origin (Wintour et al., 1980).

At 0.86 term the adrenal in vitro is unresponsive to either low sodium or high potassium concentrations, both of which are, in the adult, potent stimuli for aldosterone release. Similarly, increasing plasma potassium concentrations by up to 1 mmol/liter in the chronically catheterized fetal lamb from 109 days (0.74) gestation only evoked very small increments in plasma aldosterone (Wintour et al., 1979). On the other hand, basal concentrations of plasma renin activity and aldosterone have recently been found to be significantly correlated with each other in fetal lambs. Interestingly, the slope of the regression line between aldosterone and renin activity was steeper in utero than after birth (Robillard et al., 1980). The response to an acute stimulus may differ, since Siegel and Fisher (1980) found only a small increase in plasma aldosterone in fetal lambs near term following the administration of furosemide, even though PRA increased considerably. Thus in fetal life, when the ionic environment remains relatively stable, humoral control of secretion may be relatively more important.

The fetal kidney can respond to increased plasma aldosterone concentrations with a marked fall in the urinary $[Na^+]/[K^+]$ ratio, although neither urinary flow rate nor osmotic pressure alter (Lingwood et al., 1978; Siegel et al., 1981). A progressive increase in sodium and chloride reabsorption has been observed in the chronically cannulated fetal lamb from 0.62 gestation (Robillard et al., 1977).

After ~105 days (0.71) gestation, there is a progressive fall in fetal plasma [$Na^+$] and a rise in [$K^+$] which is temporally coincident with the rise in plasma renin to term (Carver and Mott, 1975). Activity of the fetal renin-angiotensin system has recently been shown to be closely linked to renal factors influencing sodium excretion (Stevens and Lumbers, 1981). There is a progressive rise in plasma aldosterone from ~105 days gestation, and it seems likely that the increasing activity of the renin-angiotensin system, possibly in response to the changing [$Na^+$]/[$K^+$] ratio, is exerting a direct effect on the adrenal cortex in vivo, as it has been shown to do in vitro.

### Effect of Volume Changes

*The Response to Hemorrhage or Volume Expansion* Three of five anesthetized fetal piglets studied at 85 days gestation (0.75 term) showed a brisk increase in plasma renin concentration (PRC) in response to the removal of 12 ml of blood. By 111 days (0.97) all of eight piglets studied responded with an increase in PRC (Broughton Pipkin et al., 1981).

In both anesthetized (Broughton Pipkin et al., 1974b; Smith et al., 1974) and unanesthetized (Broughton Pipkin et al., 1974a) fetal lambs the removal of as little as 3% of the calculated fetal blood volume also resulted in highly significant increases in PRA and/or angiotensin II from 0.63 term. Iwamoto and Rudolph (1981b) also showed that the previous administration of saralasin blocked the fetal lamb's ability to restore its blood pressure and heart rate following hemorrhage, and that under these circumstances the umbilicoplacental blood flow fell significantly. Hemorrhage is known to be a potent stimulus to the renin-angiotensin system in adult animals (Davis and Freeman, 1976), a response thought to be mediated via the intrarenal baroreceptors at the macula densa.

Conversely, the administration of 30-75 ml/kg of maternal blood to the fetus from 103 to 118 days (0.70-0.80) resulted in a marked fall in PRA which was maintained for at least 2 hr (Mott, 1978). This fall may, however, have been in part due to the concomitant rise in fetal blood pressure evoked by the rapid infusion. It is, however, also consistent with a functional renal baroreceptor control of fetal renin secretion.

*The Response to Osmotic Changes* In both acutely (Trimper and Lumbers, 1972) and chronically (Fleischman et al., 1975; Siegel and Fisher, 1980) catheterized fetal lambs the administration of furosemide resulted in increased PRA from 110 days (0.75). If basal fetal PRA was initially greater than 8.0 ng/ml per hour, then no further rise was noted. The magnitude of the response increased progressively to term (Siegel and Fisher, 1980). It has also been found (Leake et al., 1979) that the maternal administration of a bolus of hypertonic (225 mEq) sodium chloride at 124 days (0.84) of gestation resulted in a fetal-maternal water flux and an increase in fetal plasma sodium concentration. There was a 3-fold increase in PRA in these animals, together with an 11-fold increase in arginine vasopressin. Both hormones reached plasma levels much in excess of maternal levels. Frederiksen et al. (1975) proposed that the juxtaglomerular cell, at least in vitro, functions as a sensitive osmometer. The observed changes in PRA with altered osmolality reported above suggest that this sensor mechanism is functional in utero.

In conclusion, experiments on the chronically cannulated fetal sheep suggest that from about 90-100 days (0.6) gestation the stimuli affecting the renin-angiotensin system are qualitatively the same as those in the adult. It appears that baroreceptor/volume receptor and osmoreceptor activities are developed and sensitive. Lumbers and Lee Lewes (1979) found propranolol to block isoprenaline-induced PRA increase in the

fetal lamb. This suggests that the β-adrenergic receptor implicated in the control of renin release in the adult (Davis and Freeman, 1976) may also be functional in late fetal life.

## THE RENIN-ANGIOTENSIN SYSTEM IN NEONATAL LIFE

### The Enzyme: PRC and PRA

Brown et al. (1964) were the first to observe that the total renin concentration in human umbilical venous plasma usually exceeded that taken simultaneously from a maternal peripheral vein. Symonds and Furler (1973) found PRC to be essentially identical in four paired maternal venous-cord venous samples. However, Godard, et al. (1976) calculated PRC to be consistently higher in cord venous blood in 21 paired samples; cord arterial PRC was also somewhat higher in 9 samples. Similarly, in 22 paired samples both cord venous and cord arterial PRC were found to be significantly higher than maternal concentrations (J. J. N. Oats, G. D. Lamming, and F. Broughton Pipkin, unpublsihed data). When the same three groups considered paired PRA measurements, the differences were much less marked, although cord venous levels still tended to be higher. Renin substrate is lower in the newborn (see below) so that the relatively low PRA observed in the newborn in the face of high PRC presumably indicates that at this age renin substrate concentration is rate limiting in the production of angiotensin I.

Numerous other investigators have measured PRA at delivery without simultaneously reporting PRC. With the exception of Katz et al. (1974), PRA in umbilical venous or arterial blood following vaginal delivery has been found to be the same or somewhat higher than that in maternal venous blood. Oparil et al. (1976) and Erkkola et al. (1979) have measured PRA in maternal and fetal blood following delivery by cesarean section and found fetal PRA under these circumstances to be lower than maternal levels. Since general anesthesia is known to influence the renin-angiotensin system, it is a little difficult to assess these data, but it seems probable that labor itself normally stimulates the renin-angiotensin system, possibly more in the fetus than in the mother (see below).

Since the chorion is a potential source of renin production (see above), it is theoretically possible that the placenta may be capable of modifying fetal renin concentrations. Godard et al. (1976) found umbilical venous PRC to be higher than arterial PRC in six of nine paired measurements. We have found a similar trend, in that cord venous PRC was higher than cord arterial PRC in 15 of 21 paired samples (J. J. N. Oats, G. D. Lamming, and F. Broughton Pipkin, unpublished data). Values for PRA in paired umbilical arterial-venous samples tend to be much closer, with very little difference between means, whether measured at elective cesarean section (Erkkola et al., 1979) or at vaginal delivery (J. J. N. Oats, G. D. Lamming, and F. Broughton Pipkin, unpublished data).

Hayduk et al. (1972) reported PRC in pooled cord plasma to be twice as high as that in the corresponding maternal plasma. When blood was obtained from the same infants 3-48 hr after delivery, extremely high levels of PRC were observed, to a mean value approximately twice that found at delivery. The infants were fasted for 24 hr and were therefore presumably in a state of some salt and water depletion. Renin levels fell progressively thereafter. Kotchen et al. (1972) also found a somewhat higher mean PRA 3-6 days after birth, although since, as frequently happens with data concerning the renin-angiotensin system, the data distribution was skewed, this difference is difficult to interpret. Dillon et al. (1976) found neonatal venous PRA to have fallen

significantly in 21 babies by 6 days post partum, to a mean not significantly different from that in infants less than 1 year of age.

The observed falls in PRC and PRA with increasing postnatal age may be not only a function of postnatal, but also one of gestational age. Richer et al. (1977) made serial measurements of PRA in four preterm infants born at 30 weeks gestation and found in all a very pronounced fall in PRA to 36-37 weeks. By 40 weeks gestational age (i.e., 10 weeks postnatal) their PRAs were indistinguishable from those of normal term infants. Spot measurements of PRA made on a group of week-old infants of gestational ages 30-40 weeks also showed a fall in PRA over this time (Sulyok et al., 1979), with the greatest fall occurring over the range 30-33 weeks. Both PRA and PRC have been found to fall steadily throughout infancy and childhood, although they may remain slightly elevated until puberty (Krause et al., 1972; Pohlova et al., 1973; Sassard et al., 1975).

### The Substrate

Wernze and Seki (1972) reported renin substrate (RS) concentrations in 18 samples of human mixed cord blood to be only some 40% of those in maternal blood, although still substantially higher than those in nonpregnant adults. Skinner et al. (1972), and Goddard et al. (1976) also found RS to be consistently lower in cord venous blood in maternal-fetal pairs. Skinner et al. (1972) reported two paired umbilical arterial and venous measurements of RS at vaginal delivery in which the levels were effectively identical. Oparil et al. (1976) also reported mean RS concentrations to be effectively the same in cord arterial and venous blood at cesarean section. Plasma renin substrate is still significantly higher 3 days after birth than in children up to 15 years old (Immonen et al., 1981).

### The Hormone

Until relatively recently, the large volumes of plasma required for the radioimmunoassay of A II precluded its measurement in the newborn. The development of increasingly sensitive assay techniques has now allowed its measurement, although such data are still relatively scanty. Godard et al. (1976) reported higher umbilical venous than simultaneously measured maternal venous A II in 8 of 10 patients at vaginal delivery. They also reported higher mean and range values for umbilical arterial A II but, unfortunately, did not make the direct paired comparison. Broughton Pipkin and Symonds (1977) also found both cord arterial and venous A II concentrations to be significantly higher than maternal levels following delivery. Arterial and venous A II concentrations were highly correlated in the 51 pairs obtained; the venous A II was significantly higher than the arterial A II in these samples. As with the renin estimations, cord venous and arterial A II concentrations were lower following cesarean section than following vaginal delivery, and were very similar to maternal values, showing no consistent umbilical arteriovenous difference.

Lumbers and Reid (1977) reported similarly lower levels at cesarean section, without giving maternal values for comparison. Oparil et al. (1976) found fetal A II levels to be lower than maternal levels at cesarean section, with mean cord venous and arterial concentrations again being very similar. Possible factors affecting the renin-angiotensin system during labor are discussed below.

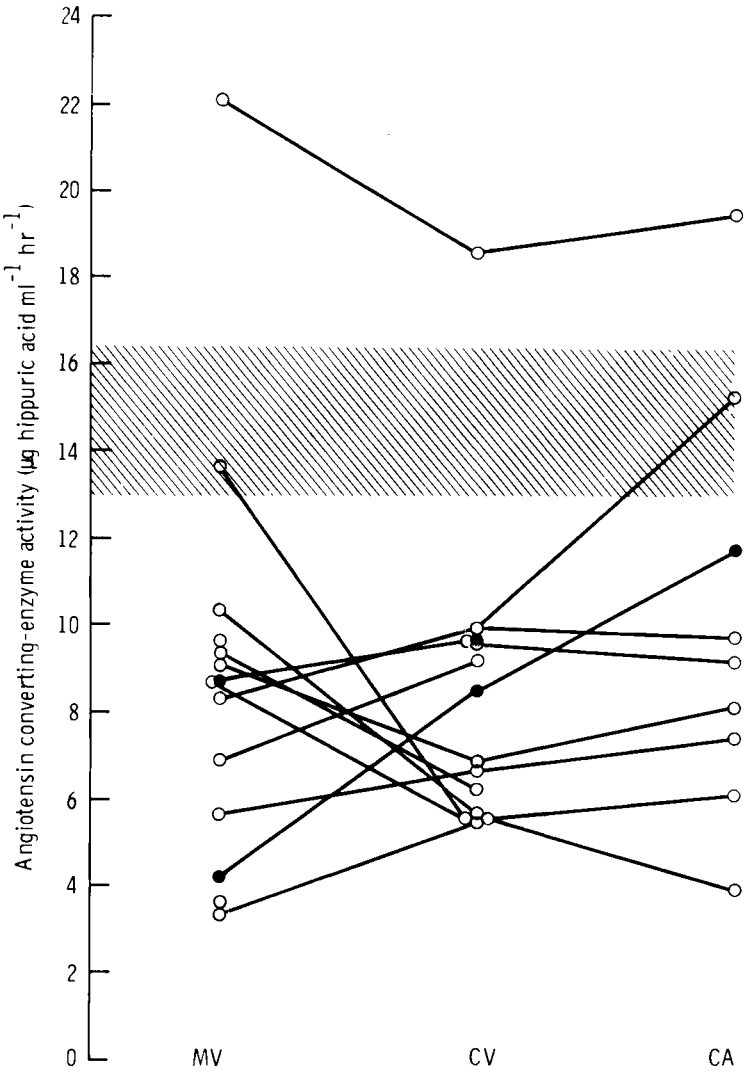


**Figure 2** Angiotensin converting-enzyme activity at term delivery in maternal venous (MV), cord venous (CV), and cord arterial (CA) blood. The hatched area is the mean ± SEM angiotensin-converting enzyme activity in a group of 16 nonpregnant women (○, normal vaginal delivery; ●, cesarean section). (Dr. J. J. N. Oats, personal communication.)

## Angiotensin-Converting Enzyme

Angiotensin-converting enzyme activity (ACE) has been assessed indirectly. Oparil et al. (1976) found it to be very similar in the seven sets of maternal and umbilical samples examined, although umbilical venous levels tended to be slightly lower than arterial or maternal venous levels. A similar pattern was found by Oats et al. (1981) in eight maternal venous, umbilical venous, and arterial sets. By comparison with

nonpregnant levels, those found by Oats et al. at delivery were low in both mother and baby (Figure 2). The available animal work (see above) suggests that somewhat lower values might indeed be expected at delivery. Mattioli et al. (1975) have also reported ACE levels in human infants within 48 hr of birth to be only some 80% of those in normal adults. Interestingly, the value for 11 healthy premature babies of comparable postnatal age was somewhat higher, which parallels the raised PRA in such infants (see above). Johnson and Erdös (1977) cultured human umbilical venous endothelial cells and found them to contain ACE in measureable amounts and to be capable of synthesizing ACE through successive subcultures. Since the placenta acts as the fetal lung, it is tempting to postulate a role similar to the lung in the conversion of angiotensin I to angiotensin II.

A recent case report (Guignard et al., 1981) concerning effects on the human fetus and neonate associated with the maternal administration of Captopril underlines the potential importance of the renin-angiotensin system in cardiovascular homeostasis at this time. Captopril was given to the mother between weeks 25 and 29 for severe maternal hypertension. Amniotic fluid volume fell sharply soon after the start of treatment. The baby was delivered at 29 weeks and was found to have marked systemic hypotension, peripheral vasodilatation, and anuria; it died on the eighth postnatal day. The parallels with the hypotension seen in the chronically catheterized fetal lamb following the maternal administration of Captopril are evident (Broughton Pipkin et al., 1982).

## Factors Influencing the System in the Perinatal Period

### Labor

The data reported above indicate clearly that some stimulus or stimuli associated with labor and delivery are associated with increased activity of the renin-angiotensin system. Soveri et al. (1975) found that the babies of patients with "uterine inertia," who required oxytocin supplementation of labor, had markedly higher PRAs than did those of mothers delivering spontaneously. They also found that the babies of mothers who had had "short" total labors, arbitrarily defined as being above or below the mean, again had considerably higher PRAs. These data are, however, difficult to interpret, both because of the difficulty of assessing the actual time of onset of labor and because the patients with "uterine inertia" were included, whose babies were known to have high PRAs. The second stage of labor can be quantified with slightly more accuracy, and Broughton Pipkin and Symonds (1977) found a statistically significant positive correlation between the duration of the second stage of labor and umbilical venous A II concentrations.

The actual stimuli evoking the increase during labor remain unclear. In lambs, a similar increase in renin and A II concentration is found following vaginal, by comparison with operative, delivery (Broughton Pipkin et al., 1974b). The increase seems unlikely to be related to hypoxia, since experiments on chronically cannulated fetal lambs showed this to be largely without effect on the fetal renin-angiotensin system (Broughton Pipkin et al., 1974a). Extrapolation from the animal experiments previously described suggests that sympathetic stimulation, fluctuations in fetal blood pressure, or possibly hypercapnia occurring during labor might be involved. Babies who had suffered severe birth asphyxia have been found to have significantly raised A II concentrations during the first week of life (Broughton Pipkin and Smales, 1977).

Lumbers and Reid (1977) also found delivery by cesarean section to be associated with lower PRA and A II in cord venous blood. They suggested that the relatively

greater increases in A II seen following vaginal delivery might be due to an increase in the ability of endogenous renin to form A I from substrate, although they did not test this hypothesis.

### Serum Electrolytes and Osmolality

Serum sodium levels are relatively low in the human newborn, and potassium relatively high. Both these factors are potent stimuli for renin synthesis and release (Davis and Freeman, 1976). Similarly, colloid osmotic pressure and total protein, which are low at birth, are even lower in premature infants and show a progressive increase that is highly correlated with gestational age (Baum et al., 1971). Plasma renin activity has been shown to be inversely correlated with sodium intake, and directly correlated with urinary osmolality over the first 9 postnatal days in the human infant (Godard et al., 1979). Fredericksen et al. (1975) showed that juxtaglomerular cells in vitro act as highly sensitive osmometers, and that a 7% reduction in osmolarity resulted in a doubling of the renin release rate. The high renin and A II concentrations of the first few days of life, especially in premature infants, may well therefore be at least partly a response to the altered serum electrolyte and osmolar balance seen at this time.

### Systemic Hypotension

There is a well-documented linear increase in systemic blood pressure in the days immediately following birth, which is partly related to gestational age (e.g., Broughton Pipkin and Smales, 1977; Fawer et al., 1979). A statistically significant inverse relationship between circulating venous A II and blood pressure has also been demonstrated at this time (Broughton Pipkin and Smales, 1977) similar to that seen in the chronically cannulated fetal lamb (Broughton Pipkin and O'Brien, 1978). It seems likely therefore that the high activity of the renin-angiotensin system in the neonate has been in part stimulated by the relative systemic hypotension.

### Maternal Hypertension

There is still considerable controversy concerning the role of the renin-angiotensin system in pregnancy-induced hypertension (PIH). Plasma renin concentration and activity are frequently suppressed, especially in PIH occurring before 34 weeks gestation. In these patients A II concentrations have also been reported to be low by comparison with normal pregnant values (Weir et al., 1973). In PIH of later onset, A II concentrations are raised in ~85% of patients, whether measured before the onset of labor (Symonds et al., 1975) or at delivery (Symonds and Broughton Pipkin, 1980).

When fetal A II concentrations are measured in these patients, cord venous A II levels are also found to be significantly higher than those in babies born to normotensive mothers, whether obtained at elective cesarean section or following vaginal delivery (Broughton Pipkin, 1976). Cord arterial levels are similar to cord venous levels following cesarean section, but only slightly higher than those in babies of normotensive mothers following vaginal delivery. It thus appears that in hypertensive pregnancy the placenta is capable of supplementing fetal A II levels. Further indirect evidence on this point is that in normal pregnancy, following vaginal delivery, the ratio of cord arterial to venous A II tends to unity, with a mean cord arterial of ~180 pg/ml (Figure 3). In hypertensive pregnancy, this mean appears to be reset at a considerably higher value, ~480 pg/ml (Broughton Pipkin and Symonds, 1976).

It is tempting to postulate that, at least in late-onset PIH, the fetoplacental unit is manipulating maternal circulatory control to its own benefit. Uteroplacental blood

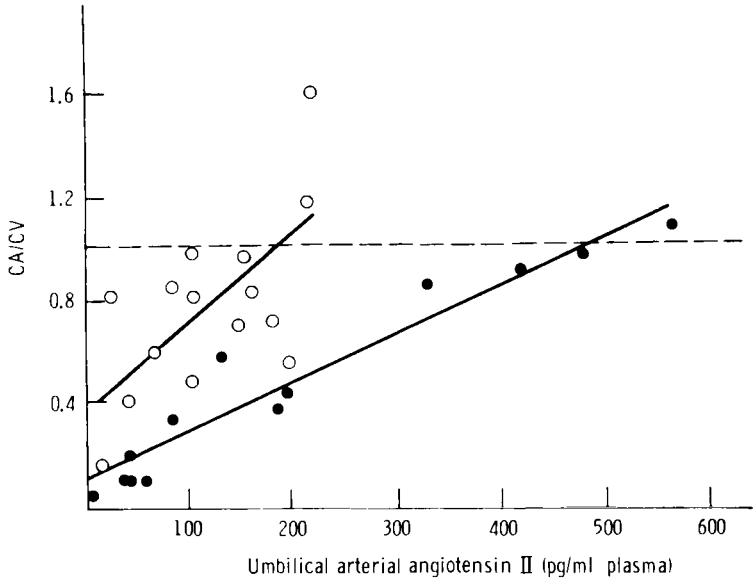


Figure 3 Relationship of cord arterial and venous A II concentrations. The ratio between CA and CV A II concentrations is proportional to the CA concentration. This relationship is significantly altered in hypertensive pregnancy (○, normotensive pregnancy; ●, hypertensive pregnancy). (From Broughton Pipkin and Symonds, 1976, reproduced with the kind permission of the Editors of the *Journal of Physiology*.)

flow is diminished in PIH, and it does not seem impossible that under these circumstances the relatively ischemic fetoplacental unit may be likened to the ischemic kidney. The kidney under such circumstances releases increasing amounts of renin into the circulation until the generated A II evokes a sufficient increase in systemic blood pressure to return renal perfusion pressure to an acceptable level. This mechanism overrides the normal nervous control of the cardiovascular system. If the uterus and/or the fetoplacental unit is capable of similarly modifying circulating A II levels, then a similar increase in systemic blood pressure might result. Animal experiments have shown that increasing A II in the uterine bed results in increased production of vasodilator prostaglandins and consequent increased uterine blood flow (Terragno et al., 1976; Speroff et al., 1977). If a similar effect were exerted in the human, then the increased systemic pressure would be expected to exert an even greater effect on uteroplacental blood flow, to the consequent benefit of the fetus.

### Idiopathic Respiratory Distress Syndrome

Premature infants with severe idiopathic respiratory distress syndrome (IRDS) may develop hyponatremia and hyperkalemia with increased sodium excretion and a decreased glomerular filtration rate (GFR) by comparison with normal premature babies. These symptoms resemble those of hypoaldosteronism. Siegel et al. (1973) reported serum aldosterone levels to be slightly but not significantly lower in babies with stable IRDS than in normal premature babies. However, Mattioli et al. (1975) found angiotensin-converting enzyme activity to be significantly higher in infants with IRDS than in comparable normal premature infants, and Broughton Pipkin and Smales (1977) found

A II concentrations also to be very significantly raised in 11 babies with severe IRDS, the concentration falling as the disease regressed. This suggests the possibility that the adrenal cortex in these infants may not be responding adequately to the stimuli of hyponatremia and high A II concentrations. The pulmonary pressure stays at or near the systemic level for much longer in babies with IRDS than in healthy babies, presumably because of pulmonary vasoconstriction. Angiotensin II is a powerful pulmonary vasoconstrictor in the adult (de Bono et al., 1963). The high observed AII concentrations may therefore, by their action on the pulmonary vasculature, contribute to the vicious circle of IRDS.

## Physiological Actions

### Vasoconstrictor

The GFR in the newborn, measured per unit surface area, is ~30% that of the adult, which may be related to the lower blood pressure at this time. The delivery of sodium to the macula densa also appears to be lower in the immature kidney and PRA has been found to show a significant negative correlation with sodium balance in a group of healthy infants studied at 30-41 weeks gestational age (Sulyok et al., 1979). This combination of hypoperfusion and response to a decreased sodium load might itself be the stimulus for the high renin and A II concentrations of infancy. On the other hand, the very marked increase in GFR seen following delivery (Fawer et al., 1979) might result from the fall in A II with increasing age. Angiotensin II given to adult humans results in a marked fall in GFR and renal plasma flow (de Bono et al., 1963); if it has a similar effect in utero, then the low renal blood flow and GFR observed might be a consequence of, rather than a stimulus to, the high A II levels. Following birth, when the placenta is no longer available as an excretory organ, the fall in A II concentration would permit the sharp increase in renal function.

### Aldosterone

It is not the purpose of this chapter to discuss fully the physiology of aldosterone in the newborn; to do so would require a chapter to itself. However, since in the adult the renin-angiotensin system appears to be a major determinant of aldosterone secretion, a brief summary is given here.

Aldosterone concentrations have been found to be consistently higher in mixed umbilical or umbilical venous blood at delivery, whether by elective cesarean section (Beitins et al., 1972) or vaginally (Beitins et al., 1972; Katz et al., 1974) although Godard et al. (1976) measured aldosterone in a group of infants at delivery and 6 days later and found concentrations to have halved in this time, although they were still significantly higher than those in infants 1 year of age. Sulyok et al. (1979) measured both plasma and urinary aldosterone in a group of infants of gestational age 30-41 weeks studied 1 week postnatally. Interestingly, they found that while plasma aldosterone concentration did not alter significantly with increasing gestational age, the urinary aldosterone excretion per day rose significantly over this period. This suggests the possibility of both an increasing metabolic clearance and increasing synthesis of aldosterone with increasing maturity. Although the authors could find no correlation between plasma aldosterone and either serum or urinary electrolyte profiles, they did find a significant positive correlation between the urinary excretion rate and sodium balance, and inverse correlations with the urinary sodium/potassium ratio and plasma potassium. A similar

inverse relationship has also been described between plasma aldosterone concentration and the urinary sodium/potassium ratio in newborn infants (Raux-Eurin et al., 1977).

Infants born prematurely have also been reported to have higher serum aldosterone concentrations than term infants (Siegel et al., 1973) at a time when renin and angiotensin II levels are also high. Honour et al. (1974) measured urinary tetrahydroaldosterone in two infants at 31 weeks of gestation who ingested a milk formula containing 7 mEq/liter of sodium and compared the values with those in comparable premature infants given a formula with 13 mEq/liter of sodium. The infants given the low-sodium formula showed a marked increase in urinary tetrahydroaldosterone, presumably indicating an increased synthesis of aldosterone. However, inappropriately high salt loss occurs in premature infants, and a relative insensitivity of the distal tubule to aldosterone at this age has been suggested (Greenberg et al., 1967; Honour et al., 1974; Raux-Eurin et al., 1977). Further evidence that the newborn infant can respond to salt deprivation with increasing aldosterone concentration comes from the work of Beitins et al. (1972), who also demonstrated that plasma aldosterone was high in the cord blood of infants born to mothers who had been given a low-sodium diet or diuretics in the latter stages of pregnancy, when maternal aldosterone concentrations were themselves raised.

In none of the studies reported could correlations be demonstrated between plasma renin and aldosterone levels, although Siegler et al. (1977) were able to find such a correlation by 3-12 months. Furthermore, Dillon et al. (1978) could find no correlation between PRA and plasma aldosterone in a group of infants undergoing exchange transfusion in which some large alterations in PRA were observed (see below). Similarly, when maternal and fetal aldosterone concentrations were compared at delivery by elective cesarean section and vaginally, the effect of vaginal delivery appeared to be considerably greater on maternal aldosterone levels (Beitins et al., 1972) although both maternal and fetal renin levels are markedly increased at this time. It remains to be determined whether this dissociation between renin and aldosterone concentrations in the newborn relates to an insensitivity of the adrenal cortex or to the overriding nature of the profound changes in plasma volume and electrolyte balance which are occurring at this time.

### Response to Acute Changes in Blood Volume

Hypovolemia caused by controlled hemorrhage results in marked increases in the activity of the renin-angiotensin system in anesthetized newborn rabbits (Broughton Pipkin et al., 1971) and lambs (Broughton Pipkin et al., 1974b). Conversely, Mott (1978) showed that expansion of fetal blood volume resulted in a fall of renin and A II concentrations. Dillon et al. (1978) have demonstrated a similar response in human newborn undergoing exchange transfusion at gestational ages ranging from 31 to 40 weeks. Nine infants underwent 11 exchanges during which they were maintained in a hypovolemic state (−8% of the calculated blood volume) for 30 min. Five estimates of PRA were made during this time. In 9 of the 11 transfusions, PRA rose by up to about threefold. Conversely, PRA was suppressed in 9 of 10 infants maintained for a comparable period with a volume overload of 8%. Although these infants were not normal, in that they required exchange transfusion, it nevertheless appears that the renin-angiotensin system can respond to acute volume changes from at least 31 weeks of gestation.

### Interrelationship with Arginine Vasopressin

Joppich and Weber (1976) found that 20 μg of arginine vasopressin given intranasally resulted in a highly significant fall in PRA in 12 children 1-25 weeks of age. Urinary sodium excretion rose in 10 of these infants, while urinary aldosterone excretion fell in 4 of the 6 infants in which it was measured. Since antidiuretic hormone is present in only extremely low concentrations in the neonatal period (see Challis and Thorburn, 1976), it seems possible that the high activity of the renin-angiotensin system and aldosterone at this time may be required to offset the effect of low antidiuretic hormone concentrations. No correlation was found between urinary antidiuretic hormone and PRA or urinary osmolality in infants 1-9 days of age (Godard et al., 1979).

In conclusion, it appears that the renin-angiotensin system is, in utero and in the perinatal period, in a state of hyperactivity relative to that of the adult. It seems possible that prior to birth the system may be of especial importance in the maintenance of an adequate systemic blood pressure before the development of adult nervous control mechanisms. Following delivery and during the first days or weeks of extrauterine life the stimuli to and the effects of the system appear to be qualitatively, although not quantitatively, similar to those in the adult. It may also be of relatively greater importance at this time than in the adult in the regulation of salt and water balance and in the complex changes which occur in cardiovascular homoeostasis.

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# 15

# Mineral and Water Exchange between Mother and Fetus

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## INTRODUCTION

The renal tubule, the choroid plexus, and the intestinal epithelium are examples of epithelial barriers across which selective energy-consuming electrolyte transport can be demonstrated. The placenta and fetal membranes share some properties with these tissues and this leads us to ask certain fundamental questions concerning placental transfer of minerals and water:

1. For which minerals are there specialized transport arrangements, and which, if any, cross by simple or restricted diffusion?
2. Is placental transfer of minerals ever rate limiting for either fetal growth or fetal water acquisition?
3. Can hormones (or drugs) modify transfer to integrate it with fetal requirements, and if so, do they act from the fetal or from the maternal circulation and are local or distant sources of hormone production involved?
4. Is the placenta the only important route for mineral and water transfer into and out of the fetus?

While none of these questions can be fully answered at present, an increasing number of experimental findings are becoming available.

## ANATOMY

The human conceptus is surrounded entirely by chorion. In the placenta this is convoluted to a total villous surface area of some 110,000 $cm^2$ (Aherne and Dunhill, 1966). The calculated surface area would be even greater if the microvillous surface were included in the calculation. An alternative exchange area is provided by the remaining chorion laeve, but it has a much smaller surface area, about 1200 $cm^2$ (a value calculated on the assumption that the fetus and liquor occupy a sphere of volume 4 liters surrounded by chorion laeve). In a number of smaller species, notably, the rat, mouse, rabbit, and guinea pig, the chorionic covering becomes incomplete. An everted yolk sac placenta develops which offers a third possible path for maternofetal exchange, a pathway which is probably rather unimportant in mineral absorption (Štulc and Svihovec, 1977).

Within the conceptus exchange will occur between the fetus and the fetal fluids contained in the amniotic and, in some species, the allantoic sac.

## MINERAL ACCUMULATION DURING GESTATION

The total quantities of minerals and water accumulated during gestation may be estimated by fetal carcass analysis. Table 1 shows quantities involved in the case of the human, calculated in molar terms. In Table 1 no allowance is made for the mineral and water content of amniotic fluid or the fetal membranes, or for the water produced in situ by fetal metabolism. It is of interest to note the 100-fold difference in the volume of maternal plasma that must be denuded to provide the measured fetal accumulation of the different minerals. It is striking that while potassium and sodium are accumulated in almost equimolar quantities by the fetus, 20 times as much maternal plasma must be cleared of potassium as compared to sodium.

In terms of maternal plasma cleared, phosphate is the most important mineral. Even so, if we assume half the accretion of phosphorus to take place in the last 40 days of gestation and uterine blood flow to be 500 ml/min at this time (Kelly et al., 1951; Blechner et al., 1974), phosphorus uptake by the fetus may be calculated to consume only some 1% of all phosphate passing through the intervillous space in a unit time. Thus the uterine venous phosphate concentration may be expected to be about 99% of the uterine arterial concentration. (The proportion of delivered phosphorus cleared by the placenta will be still less if phosphorus is accessible to the fetus in any form other than phosphate.) For other minerals the ratio between uterine venous and arterial blood concentrations will be even higher.

Two consequences follow. Firstly, it will be hopeless to attempt to measure the net fetal uptake of any mineral from arteriovenous differences by applying Fick's principle: (uptake = uterine blood flow × venoarterial concentration difference). The available

**Table 1** Mineral Accumulation during Pregnancy

| Substance | Fetal body content (mmol)[a] | Volume of maternal plasma cleared per pregnancy to provide mineral accumulated by fetus (liters) |
|---|---|---|
| Water | 130,000 | 2.5 |
| Na | 243 | 1.8 |
| Cl | 160 | 1.5 |
| K | 150 | 33 |
| P | 520 | 370[b] |
| Ca | 705 | 170 |
| Mg | 32 | 24 |
| Fe | 4 | 225[c] |
| Zn | 0.8 | 9 |
| Cu | 0.2 | 15 |

[a]Composition of 3.5-kg human fetus at term (McCance and Widdowson, 1961).
[b]Phosphorus is taken to be cleared as inorganic phosphate.
[c]Transferrin iron.

chemical methods are insufficiently accurate to measure the venoarterial difference to this degree of accuracy. This problem can be overcome, to some extent, by the use of radioisotopes.

Secondly, the uptake of a mineral by the fetus is not likely to be altered by changes in the rate of uterine blood flow. Mineral uptake is limited, rather, by the area of placental surface available for transfer and by the efficiency of the transplacental transfer mechanisms. It could only be influenced by an alteration in blood flow if the change were sufficiently gross to markedly change the proportion of placenta perfused at all. In general, mineral transfer will be limited by the permeability of the maternofetal exchange barrier ("membrane-limited" transfer) rather than by the flow of minerals to the membrane ("flow-limited" transfer). [This is a very oversimplified introduction to the difference between flow and membrane limitation. For example, the net transfer of a flow-limited solute, such as water, may appear incorrectly to be membrane limited if the above criterion alone is applied. More detailed accounts are available in Faber (1973), Meschia et al. (1967), and Meschia (1976).]

## THEORETICAL ASPECTS OF MINERAL EXCHANGE

### Net Transfer and Unidirectional Transfer

Net uptake across the placenta will occur if there is a quantitative difference between the rate of transfer of molecules of the substance from mother to fetus ($J_{mf}$) and the rate of transfer of identical molecules in the reverse direction ($J_{fm}$). The rate of accumulation is abbreviated $J_{net}$, and

$$J_{net} = J_{mf} - J_{fm}$$

The word *flux* is now usually used to describe a transfer rate. A net flux ($J_{net}$) different from zero results from inequality between the maternofetal and fetomaternal unidirectional fluxes ($J_{mf}$ and $J_{fm}$). The rate of accumulation of a mineral by the fetus may be increased by either an increase in $J_{mf}$ or a decrease in $J_{fm}$.

### Free and Restricted Diffusion

A soluble mineral (which will usually be ionized in solution) may be expected to cross a very permeable barrier by free diffusion in water, in addition to any active transport. The rate of diffusional transfer will depend on the surface area of the barrier available for diffusion and on the degree of obstruction the barrier poses for ion movement, together with the balance, across the barrier, of forces which may influence the movement of ions in solution. The surface area of the barrier and the degree of obstruction it poses depend not only on the anatomy of the maternofetal exchange area, but also on its molecular structure. Transepithelial ionic diffusion probably takes place through water-filled submicroscopic channels which may be small enought to restrict the diffusion of large molecules disproportionately in relation to small ones. Diffusional transfer under these circumstances is described as "restricted diffusion." The degree of restriction will be influenced by the radius of the channels, by the charge on the surface of the channels, which in turn influences the number of water molecules closely associated with the ion in solution while traversing the channels, and by the ease of their access to the barrier across the stagnant "unstirred" layer of plasma up against

the cell membrane (Wright, 1974). These factors may have different influences on the passage of different ions.

The forces which make ions move preferentially across the barrier include temperature differences, water flows, any transbarrier electrical potential difference (pd), and ionic concentration differences. Temperature differences (Conrad and Faber, 1977) and water flows (Weedon et al., 1978) are probably unimportant in mineral movement across the placenta. Concentration differences and any transplacental pd, on the other hand, may be expected to be very important.

### Maternofetal and Transplacental Potential Difference

A pd of approximately 30 mV across a permeable barrier will drive a monovalent ion some three times as fast one way as the other in the absence of other influences. Thus if maternal and fetal concentrations were the same and the transplacental pd were 30 mV, $J_{fm}$ would be either three times larger or smaller than $J_{mf}$, depending on the charge borne by the ion. It is, however, not certain whether there is a transplacental pd of this order in any species. A wide range of maternofetal pds have been recorded between catheters inserted at various sites in the fetal and maternal anatomy, including the large vessels supplying the placenta on either of its surfaces in the bilaterally perfused isolated placenta (Leichtweiss and Schroder, 1981). For example, the mean value in the conscious sheep is 34 mV, fetus negative (Weedon et al., 1978); in the guinea pig it is 18 mV, fetus negative (Mellor, 1969); in the preterm human it is 2.7 mV, fetus negative (Štulc et al., 1978); and in the rat it is 15 mV, fetus positive (Mellor, 1969). In the term human and in the rabbit (Mellor et al., 1969; Mellor, 1969) the recorded maternofetal potential is insignificantly different from zero.

It is not certain whether these recorded maternofetal potential differences are identical to any transplacental potential difference. Faber suggested that if a potential difference were present across the placental exchange area, isotopes of ions which are not pumped across the placenta would achieve a steady-state concentration difference between maternal and fetal plasma after intravenous injection. As none of the ions they studied did so in either the guinea pig or the sheep (Thornburg et al., 1979; Binder et al., 1979), they concluded that there must be only a trivial transplacental pd. They suggest that the measured maternofetal pd, present in most species, is generated elsewhere than in the placenta and that there is no important pd drop across the placental exchange area.

On the other hand, membranes that are homologous with the placenta may generate a potential when mounted in vitro. For example, in the case of the chorioallantoic membrane of the pig, its value can be as high as 80 mV, fetal surface negative (Crawford and McCance, 1960). We have recently provided additional indirect evidence for the existence of a significant pd across the placental exchange area. When negatively charged bromide or iodide ions are injected in labeled form into the fetal circulation of the lamb, their rate of clearance across the placenta into the maternal circulation is significantly greater, the greater the fetomaternal pd (fetus negative) (Canning et al., 1980). This finding is to be expected if the measured maternofetal pd significantly influences transplacental transfer. This controversy is still unresolved; the interested reader should review the symposium edited by Young et al. (1981).

### Maternofetal Concentration Differences

A difference in ion concentration between the maternal and fetal circulations will also influence the rate of transplacental diffusion. The rate of diffusional transfer of an ion from maternal to fetal circulation will vary with the maternal concentration so that

$$J_{mf} = k_1 \times \text{maternal concentration}$$

and, conversely,

$$J_{fm} = k_2 \times \text{fetal concentration}$$

In the absence of electrical and other forces mentioned above and of carrier-mediated or active transfer discussed below,

$$k_1 = k_2 = K$$

so that

$$J_{net} = K \times (\text{maternal concentration} - \text{fetal concentration})$$

K is a "clearance constant" (also called a permeability surface area product) and has the units of volume cubed per unit time, usually milliliters per minute. The clearance constant can be easily measured by estimation of $J_{fm}$ following injection of an isotope into the fetal circulation (Boyd et al., 1976). If the placental surface area could be reliably estimated, the clearance constant per square centimeter could be calculated. This is a permeability constant and has units of length per unit time, usually centimeters per second. For obvious anatomical reasons it is not accurately calculable in the case of placental studies. When $k_1$ does not equal $k_2$, K can still be estimated at a given permeant concentration, but its value is then a coefficient rather than a constant.

For any conclusions about diffusional forces to be valid, the appropriate values for maternal and fetal concentrations must be used. These are the concentration (or, more correctly, activity) of the free ion in plasma water. For an ion like calcium, which is substantially protein bound, the ion concentration in plasma water is very much lower than its concentration in whole plasma. To ignore this difference will lead to a complete misreading of the forces available to influence diffusion. Even for sodium, potassium, and chloride, which are minimally bound to plasma protein, the importance of measuring ion concentration in plasma water, and not in whole plasma, should be borne in mind, since the plasma protein concentration and therefore the water content of maternal and fetal plasmas may be different. When differences in plasma protein concentration exist across the placenta, an ion with the same plasma concentration in each will have different concentrations in maternal and fetal plasma water. In the sheep, for example, maternal plasma is 92.8% water by weight compared to 95.4% for fetal plasma (Weedon et al., 1978), because there is a much lower plasma protein concentration in fetal blood. Allowing for this difference in the amount of water per 100 ml of plasma means increasing the maternofetal concentration ratio for a substance

totally unbound by plasma protein by a factor of 1.03. In other species there is a smaller difference between maternal and fetal plasma protein and the correction is less important. In the guinea pig it may be calculated from the data of Woods et al. (1978) that the correction necessary would be about 1.01. There is no data to allow an accurate value to be calculated for the human, but it is probably about the same as for the guinea pig.

### Facilitated Diffusion and Active Transport

The effect of any specialized carrier mechanism assisting the transfer of a mineral across the placenta will be superimposed on any transplacental diffusion. Active transport involves the consumption of metabolic energy and usually the net accumulation of a substance against its electrochemical gradient. Most active transport systems are stereospecific and can be inhibited and saturated. Passive stereospecific, saturable transfer down an electrochemical gradient is facilitated diffusion, a term first applied interestingly by Widdas when analyzing the kinetics of glucose transfer across the placenta (Widdas, 1952). It may well be that the same biochemical transport mechanisms underlie both active transport and facilitated diffusion, but in the latter energy consumption is absent.

## ACCUMULATION OF MINERALS

### Sodium, Chloride, and Potassium

It can be seen from Table 1 that rather similar molar amounts of these ions are accumulated by the developing fetus.

#### Sodium

Sodium has been the most extensively investigated mineral. Flexner, in the 1940s, organized a beautiful series of studies in which the unidirectional maternofetal flux of radioactive sodium was measured by ashing and counting the conceptus within a relatively short period after injection into the mother. Flexner was careful to make his measurements at a time when the variable measured was the unidirectional maternofetal flux $J_{mf}$ (i.e., the fetal concentration of radioactive sodium was so low that the rate of back-transfer of radioactive sodium from fetus to mother, $J_{fm}$, was still trivial). Results were corrected for sodium-specific activity and given in milligrams of sodium transferred in a unit time. Flexner showed that the unidirectional flux $J_{mf}$ was much higher than the net flux $J_{net}$ estimated from the known growth rates of fetuses. For example, in the human the maximum $J_{mf}$ was 63,000 mg/day of Na when the net flux was 56 mg/day. He coined the term "safety factor" to describe the ratio of the unidirectional maternofetal flux to the net flux (Flexner et al., 1948). He studied a number of different animal species and noted a wide range of different unidirectional fluxes per gram of fetal or placental weight. In general, he noted that species with thicker placentas had lower fluxes. Within a species there was an increase as gestation proceeded, with a decline just before term. In most species a peak value for unidirectional flux was reached after 9/10 of the total duration of gestation had passed (Table 2).

In the goat, for example, the sodium transfer was 30% of its maximum value 5/10 of the way through pregnancy. The maximum rate was achieved 9/10 of the way

Table 2 Reported Values for $J_{mf}^{Na}$ Estimated from Radioactivity of Carcass and Fetal Membranes after Injection of Radioactive Sodium into the Mother

| Species | $J_{mf}^{Na}$ ($\mu$mols/min per gram of placenta) | Unidirectional clearance (ml/min per gram of placenta) | Approximate weight of placenta (g) | Approximate weight of fetus (g) |
|---|---|---|---|---|
| Pig | $2 \times 10^{-5}$ | $1 \times 10^{-7}$ | 225 | 1200 |
| Goat | 0.3 | 0.002 | 200 | 1000 |
| Cat | 0.6 | 0.004 | 14 | 90 |
| Guinea pig | 4.5 | 0.033 | 3 | 60 |
| Human | 4.7 | 0.034 | 400 | 2700 |
| Rabbit | 6.7 | 0.048 | 3 | 30 |
| Rat | 7.5 | 0.050 | 0.4 | 4 |

*Source*: After Flexner and Gellhorn (1943).

through pregnancy, dropping again to 60% of the maximum value at term (Flexner and Gellhorn, 1943).

More recently unidirectional radiosodium fluxes have been estimated by Fick's principle, using either the perfused placenta (Dancis and Money, 1960; Schröder et al., 1972; Štulc and Svihovec, 1977) or the catheterized conscious sheep (Weedon et al., 1978). Armentrout et al. (1977) have published measurements applying the reverse of Flexner's approach, in which they estimated transplacental loss from the fetus following the injection of a known amount of radiosodium into the fetal circulation. With the exception of Schröder's study, in which the bilaterally isolated perfused placenta may have had segments unperfused at low perfusion rates, these reports give values close to those of Flexner, both for ungulates and for guinea pigs. Values of fetomaternal placental clearance of radioions in the sheep are shown in Table 3.

If, for the sake of simplicity, we assume that there is no transplacental pd and that the placenta does not pump sodium actively, then $J_{net} = K \Delta C$, where K is clearance and $\Delta C$ is the maternofetal plasma water concentration difference. Taking the value for sodium clearance of 0.96 ml/min from Table 3, the very approximate $J_{net}$ of 7 mmol/day (Weedon et al., 1978) would be achieved by a $\Delta C$ of 5 mmol/kg of plasma water. This is close to the value of 6 mmol/kg of plasma water found when the whole plasma concentration difference of 1.6 mmol/liter (Table 4) is corrected for the higher plasma water in fetal plasma. Taken

Table 3 Clearance (K) Across Placentas of Conscious Sheep Near Term[a]

| Ion | K (ml/min) |
|---|---|
| $Cl^-$ | $1.6 \pm 0.2$[b] |
| $I^-$ | $10.8 \pm 1.2$[b] |
| $K^+$ | $2.3 \pm 0.4$[b] |
| $Na^+$ | $0.96 \pm 0.10$[c] |

[a]Clearance measured in fetus-mother direction with radioisotopes and Fick's principle. Results (±ISEM) uncorrected for fetal or placental weight or for pd.

[b]Canning et al. (1980).

[c]Weedon et al. (1978).

**Table 4** Plasma Electrolyte Concentrations (In mmol/liter of Plasma)

| | Sheep[a] | | Guinea pig[b] | | Human | |
|---|---|---|---|---|---|---|
| Mineral | Mother | Fetus | Mother | Fetus | Mother | Fetus |
| Na | 145.3 ± 1.2[c] | 143.7 ± 1.9 | 142.2 ± 1.5 | 138.5 ± 0.9 | 138 ± 2[d] | 139 ± 4 |
| Cl | 107.7 ± 1.7[c] | 101.3 ± 2.4 | 102.7 ± 0.9 | 100.6 ± 0.8 | 107 ± 2[d] | 108 ± 2 |
| K | 4.49 ± 0.1[c] | 3.91 ± 0.14 | 4.46 ± 0.21 | 5.08 ± 0.16 | 4.6 ± 5[d] | 6.4 ± 0.2[c] |
| Mg | 0.85 ± 0.08 | 0.81 ± 0.05 | 1.11 ± 0.05 | 1.20 ± 0.03 | 1.31 ± 0.38[e] | 1.39 ± 0.13 |
| Ca | 2.12 ± 0.07[c] | 2.90 ± 0.05 | 2.30 ± 0.06[f] | 2.92 ± 0.04 | 2.23 ± 0.12[e] | 2.81 ± 0.17[c] |
| $PO_4$ | 0.48 ± 0.03[c] | 0.73 ± 0.05 | 0.35 ± 0.02[f] | 0.83 ± 0.02 | 0.46 ± 0.02[e] | 0.62 ± 0.01[c] |

[a]From Armentrout et al. (1977). Chronic sheep.

[b]From Woods et al. (1978). Acute guinea pigs; values corrected for various possible sources of artifact.

[c]Significant maternofetal difference, $P < 0.05$.

[d]From Mellor et al. (1969) at cesarean section; see Faber and Thornburg (1981) for more complete data.

[e]From Shauberger and Pitkin (1979). Cord blood samples.

[f]Significant difference after correction for artifacts of sampling.

alone, this numerical agreement might suggest that sodium ions cross the placenta solely by passive diffusion under a chemical gradient without any active transport and without any influence being exerted by a transplacental pd. This conclusion has been accepted by Conrad and Faber (1977).

If, however, the transplacental pd is indeed the same as the maternofetal pd (mean value 30 mV, mother positive, in the sheep) it is necessary to postulate a more complicated model in which a placental sodium pump is included. This is necessary to adjust for the marked asymmetry in transplacental sodium fluxes that would otherwise occur under the influence of a 30-mV pd and which would rapidly lead to overaccumulation of sodium by the fetus. The sodium pump would have to be active in the direction of the fetus to the mother. Štulc and Svihovec, studying the guinea pig placenta (1977), and Weedon et al. (1978) working on the sheep, have made calculations supporting the concept of such a pump, but on present information it remains quite possible that sodium is accumulated passively by diffusion alone; the status of the pd remains controversial. In the human, recorded maternofetal pds are small, and direct evidence for a transplacental sodium pump is lacking. It is, however, clear that there are specialized sodium transport mechanisms active across the human microvillous membrane when studied in vitro; for example, L-proline is transported by an electrogenic ion-coupled mechanism into syncitiotrophoblast brush border membranes (Boyd and Lund, 1981).

### Potassium

For potassium, information is even more fragmentary. The placenta is more permeable to potassium than to sodium, its unidirectional clearance across the sheep placenta being two and a half times higher than that for sodium (Table 3), and across the perfused guinea pig three times higher measured mother to fetus, and two times higher measured fetus to mother (Bailey et al., 1979). In the chronically catheterized sheep, where results are less likely to be affected by anesthesia or other extraneous factors, potassium, like sodium, has a concentration reportedly higher in maternal plasma than in fetal plasma (Table 4). It might again appear plausible to suggest that this accumulation is solely accounted for by diffusion under the influence of a concentration gradient. With potassium, however, there is evidence to suggest that this simple view is wrong. Firstly, if maternal plasma potassium concentration is lowered by the administration of a potassium-deficient diet, the fetal potassium content (Stewart and Welt, 1961) and plasma concentration (Serrano et al., 1964; Dancis and Springer, 1970) are maintained in the presence of maternal hypokalemia. Secondly, the rate of potassium transfer across the perfused guinea pig is diminished when ouabain, an inhibitor of sodium-potassium ATPase, is added to the perfusate (Bailey et al., 1979). Thirdly, in the human, the plasma potassium concentration in the fetus has been reported to be significantly higher than in the mother (Table 4) and we have also noted a maintained fetal hyperkalemia in certain individual fetal lambs. Fantel (1975) has found that the steady-state concentration difference for potassium in the rat (where the maternal plasma concentration is higher than the fetal plasma concentration) is related by the Nernst equation to the fetomaternal pd (mother negative) found in this species. The maternofetal concentration ratio observed is that predicted if potassium were distributed passively between the maternal and fetal plasmas under the influence of the maternofetal pd alone. However, the quantitative relationship breaks down when the mother is rendered hypokalemic by dietary means, so that other factors must also be involved (Fantel, 1978).

### Chloride

Isotopic chloride permeability has been measured in the fetus-mother direction both by Fick's principle (Table 3) and indirectly from the rate of plasma decline following intrafetal injection (Armentrout et al., 1977). In each case the chloride permeability is low, though not quite as low as sodium. In the primate, chloride permeability is higher when compared to that of urea than it is in the ungulate. Thus in the monkey, chloride clearance is 60% that of urea (Battaglia et al., 1968), whereas in the sheep it is only 3% that of urea (Boyd et al., 1981). There are no published studies on the mechanism of chloride transfer. If there is indeed a transplacental pd, those species in which the fetus is negative with reference to the mother might be expected to have a fetally directed chloride pump. There is no direct evidence for this, but human trophoblast membrane reconstituted into vesicles in vitro carries a chloride pump which can be blocked by certain inhibitors (Boyd et al., 1980).

In conclusion, either electrical forces are unimportant, in which case sodium and chloride cross the placenta passively by diffusion and there is a controlled ion pump for potassium, or there is a transplacental pd, and sodium and chloride transfer across the placenta is governed by a pump or similar mechanism and potassium is either pumped also or drawn into the fetal circulation by fetal electronegativity. There may be species differences.

### Iron

During gestation the fetus accumulates some 300 mg of iron (Widdowson and Spray, 1951). At physiological pH iron is very tightly bound to transferrin, a protein present in both fetal and maternal plasma, and the concentration of free ferric irons may be expected to be as low as $10^{-17}$ M.

Since the first study of fetal uptake using radioactive iron nearly 40 years ago (Pommerenke et al., 1942), placental transfer of iron has been studied more intensively than the transfer of any other mineral. A relatively coherent picture has emerged, though certain interesting questions remain to be answered. The present position has been well reviewed by Morgan (1974).

In primates, including the human, in rodents, and in insectivores, it is likely that the greatest source of iron transferred to the fetus is maternal transferrin, and the mechanisms involved are discussed further below. In ungulates, such as sheep, and carnivores, such as the cat and dog, iron transfer rates to the fetus from maternal plasma are much lower and it is likely that some or most of the iron is transferred directly in the form of placentally ingested maternal red blood cells (Seal et al., 1972). There is both microscopic evidence for such a mechanism in the sheep (Burton et al., 1976) and radioactive evidence in the ferret (Dumartin et al., 1976), where after injection of $^{59}Fe$ to the mother, iron first has to become incorporated in maternal red blood cells before these are ingested by the *poche choriale* of the placenta, allowing fetal iron uptake. With iron we appear to have a definite example of two quite distinct mechanisms for transfer, each applying to a different range of species.

#### Transfer from Maternal Transferrin

In the human at term, transferrin-bound iron is present at a higher concentration in fetal blood than in maternal blood, while the total plasma transferrin concentration is substantially lower in the fetus. When equally saturated, both fetal and maternal

transferrin give up iron equally well to rabbit reticulocytes, suggesting that fetal transferrin does not have a higher avidity for iron; thus the difference in iron concentration cannot be explained by differential protein binding (Fletcher and Suter, 1969). During iron transfer to the fetus the transferrin molecule itself does not traverse the placenta. For example, Gitlin et al. (1964) showed that radioiodinated transferrin crosses the human placenta rather slowly. Similarly, in the rabbit, radioiodinated transferrin is transferred 100 times more slowly to the fetus than radioactive iron from $^{59}$Fe-labeled transferrin (Baker and Morgan, 1970). Different electrophoretic variants of transferrin can be demonstrated on occasion in maternal and fetal blood (Rausen et al., 1961).

Three major questions arise: How does maternal transferrin release its iron to the placenta? In what form is iron while in the placenta? And how does iron leave the placenta to enter the fetal circulation and achieve a higher concentration there than in maternal plasma?

Transferrin receptors can be demonstrated on the maternal surface of the placenta by a number of techniques, including cytochemistry (King, 1976). Within the placenta, iron is found in a number of fractions following maternal injection of $^{59}$Fe. In the rat, for example, it is present bound to transferrin, probably bound to ferritin, and also in a low molecular weight component (Mansour et al., 1972). It is of interest that in placentas which were left in situ after removal of the fetus, these workers found that the placental content of iron was increased. This suggests that the fetus is involved in the removal of iron from the fetal face of the placenta, but it is unclear in what way. Removal does not appear to depend on transferrin, for, using a perfused placental preparation, Baker and Morgan (1970) found that the uptake of iron into perfusion fluid passing the fetal face of the placenta was not altered by the presence or absence of transferrin in the perfusate. Similarly, fetal survival is compatible with the congenital absence of transferrin, though this may have some deleterious effects. Heilmayer et al. (1961) described a case of atransferrinemia presenting at 3 months of age. The sibship (complicated by syphilis) had included two abortions and a neonatal death.

### Control of Iron Uptake

The control of iron uptake by the fetus is an interesting problem, for in the adult gut absorption is the main point of control and iron excretion is very low. Maternal gut absorption does appear to be influenced by the presence of a placenta (Apte and Brown, 1969; Batey and Gallagher, 1977). In the rabbit iron excretion from the fetus to the mother is very low (Bothwell et al., 1958), so that the fetus may need to have some way of switching off placental iron transfer to avoid overaccumulation. There is certainly evidence of a reverse process, for the baby is protected from iron deficiency associated with maternal anemia (Sturgeon, 1959; Parish and Brame, 1954; Murray and Stein, 1971). Various other alterations in maternal iron metabolism also affect placental transfer; for example, a raised maternal iron increases and a very diminished maternal iron decreases iron flux into fetal rats (Matoth and Zaizov, 1977).

## Calcium

The total calcium concentration in fetal plasma is higher than in maternal plasma in humans, sheep, and guinea pigs (Table 4). However, as a large proportion of calcium is protein bound (approximately 50% in humans) and a further small proportion of

the ultrafilterable calcium is complexed to citrate and phosphate, interpretation of this difference as evidence of active placental transport is not straightforward. Nevertheless, the calcium concentration gradient does not result from differences in protein-bound calcium alone, though in the sheep, unlike in the human, the proteins in fetal plasma bind calcium to a higher concentration than those of maternal plasma. The total ultrafilterable fraction and the free calcium ion concentrations are also higher in fetal than in maternal plasma in both species (Delivoria-Papadopoulos et al., 1967; Shauberger and Pitkin, 1979; Care et al., 1980).

The concept of an active transport system is supported by other animal work. The guinea pig placenta, perfused on the fetal side, can transport calcium into the perfusate against its concentration gradient (Twardock and Austin, 1970). This finding, together with the higher concentration of free calcium ions in fetal plasma, must be indicative of active transport of calcium into the fetus, at least in the human, where the materno-fetal electrical potential difference recorded is very small. In the sheep and guinea pig there is the alternative possibility of passive accumulation of positvely charged calcium ions by an electronegative fetus (see below).

Unidirectional calcium fluxes across the placenta have been calculated by analysis of radiocalcium kinetics in anesthetised sheep and monkeys. In the sheep they were calculated to be 215 mg/kg of fetal weight per day, mother to fetus ($J_{mf}$), and 12 mg/kg of fetal weight per day, fetus to mother ($J_{fm}$) (Ramberg et al., 1973); these values are not necessarily diagnostic of active transport, for by applying Ussing's flux equation, it can be seen that the results are compatible with diffusion under the influence of the observed transplacental concentration difference of the free ions and a pd of about 45 mV (fetus negative), a reasonable value. However, the calculated net accumulation of 350-450 mg/kg body weight per day in vivo in late gestation (Braithwaite et al., 1970) is even higher than the asymmetry between $J_{mf}$ and $J_{fm}$ in the acute experiments. In the anesthetised guinea pig, Twardock (1967) calculated from radiocalcium studies the rate of unidirectional transfer of calcium into the fetus. It was about 30 mg/day at 60 days gestation. As with the sheep, this is less than the net accumulation rate of calcium required for fetal skeletal growth, which is about 40 mg/day at this stage of gestation. Either the measured transplacental fluxes of calcium are an underestimate for experimental reasons or, conceivably, the fetus has another source of calcium not apparent in these experiments.

In the primate, kinetic analysis gave a more symmetrical result than in the sheep. The $J_{mf}$ was 391 mg/kg of fetus per day and the $J_{fm}$ was 326 mg/kg of fetus per day (Ramberg et al., 1973), 6-10 times the fetal requirement (McDonald et al., 1965). As with sodium, unidirectional calcium fluxes in the primate are larger in relation to net flux than in the sheep.

In the sheep the control of fetal plasma calcium appears to be governed by the fetus and the fetus probably does this at any rate partly by an effect at the placental level (Care, 1980). Fetal nephrectomy or hypophysectomy abolishes the transplacental calcium gradient which can be restored by intravenous injections of 1,25-dihydroxy-cholecaliferol to the fetus (Care et al., 1980). Conversely, fetal plasma calcium concentrations can be raised by the fetal intravenous injection of parathyroid hormone; calcitonin lowers the fetal concentration (Barlet et al., 1978). Elevation of plasma calcium in maternal plasma fails to increase fetal plasma levels in guinea pigs (Burnette et al., 1968), cows (Barlet et al., 1979a), sheep, or rats (Garel et al., 1972).

The mechanisms of calcium transport are uncertain, but calcium-binding protein has been isolated from the placenta of the rat (Bruns et al., 1978; Marche et al., 1978)

and a calcium-stimulated ATPase is present in guinea pig placenta (Shami and Radde, 1971).

### Phosphate

Like calcium, the concentration of inorganic phosphate in fetal plasma is greater than that in maternal plasma. Only 6-20% of plasma phosphate is protein bound in adult blood (Nordin, 1976). If fetal plasma were the same, it would appear that phosphate is concentrated transplacentally against both the concentration and any electrical gradient; thus inorganic phosphate is actively transported into the fetus.

Wilde et al. (1946), using $^{32}P$ in the guinea pig, first made the observation that the unidirectional flux of phosphate from mother to fetus is almost equal to the phosphate requirement for fetal growth. This was confirmed by Fuchs and Fuchs (1956). The total rate of phosphate transfer rises throughout pregnancy, but if it is prolonged past term artificially, the rise does not continue in the rat (Klem, 1956) or rabbit (Fuchs and Fuchs, 1961). If Wilde and Flexner's (1946) observation in the guinea pig holds true in other species, then it poses several interesting questions. Either the transport system is almost inadequate, is very finely tuned to the requirement of the fetus, or the fetus has another source of phosphate. The mechanism of transplacental phosphate movement is unknown.

### Iodine

In the sheep, ablation of the thyroid gland in mid-gestation results in the birth, slightly post term, of a small hairless lamb that does not survive for more than 24 hr (Hopkins and Thorburn, 1972). This clearly illustrates that the fetal thyroid gland is essential for normal development and that thyroid hormones cannot cross the sheep placenta in sufficient quantity to compensate for an absent thyroid. Although the effects of congenital hypothyroidism may not be as marked in other species, notably, man, they are still apparent; for example, newborn osseous development is delayed. Thus the acquisition of sufficient inorganic iodide by the fetus is important.

The placenta has been shown to concentrate iodide in the rabbit, rat, and guinea pig (Brown-Grant, 1961). Preliminary work in our laboratory suggests that this is also the case in the sheep (Boyd et al., 1980). There are no published measurements of iodide concentrations in fetal plasma, but if radioiodide is injected into the maternal circulation of rabbits or guinea pigs, the fetal plasma radioactivity in inorganic iodide comes to exceed maternal levels, suggesting the probability of active iodide transport (Logothopoulos and Scott, 1956; London et al., 1964). London also showed that if the fetal circulation of the guinea pig placenta is perfused in situ, the achievement of radioiodide concentration in the perfusate that is higher than in maternal plasma is inhibited by injection of thiocyanate into the maternal circulation. Raising the iodide concentration on the fetal side does not inhibit iodide transport into the perfusate (London et al., 1964).

Compartmental analysis of radioiodide disappearance curves in sheep reveals that the rate of transfer of iodide from mother to fetus is high compared to other ions and compared to the rate of transfer from fetus to mother. Unidirectional iodide clearance is calculated to be 45 ml/min in the mother-fetus direction, but only 20 ml/min in the reverse direction (McGuire and Berman, 1978). This information is difficult to interpret without a knowledge of plasma nonradioactive iodide concentrations; nevertheless, there

is strong overall evidence for active transport of iodide across the placenta in the mother-fetus direction. It is very curious that this should be biologically necessary; is it part of a controlling mechanism for fetal growth?

### Magnesium, Lithium, and Fluoride

Most interest in the transplacental transfer of magnesium, lithium, and fluoride has been stimulated by their use as therapeutic agents, rather than by their biological role. Transfer has been investigated by observing fetal plasma responses to acute changes in maternal concentration. For all three ions, in different species it has been reported that raising maternal levels has little effect on fetal plasma levels (Hughes et al., 1973; Ericsson and Malmnas, 1962; Barlet et al., 1979b). However, for fluoride it has also been reported in the sheep that an intravenous dose of fluoride given to the ewe is rapidly transferred to the fetus (Maduska et al., 1980). Gedalia et al. (1964) reported that a close correlation was observed between fetal and maternal plasma fluoride in humans on low fluoride intakes. In mothers with a higher fluoride intake there was no significant change in fetal plasma fluoride. The authors suggested that at low levels fluoride was readily transferred across the placenta, but not at higher levels.

Some evidence suggests that magnesium transfer across the placenta is similar to that of calcium, and thus the maternal to fetal magnesium flux in the sheep is greater than the feto maternal flux (Care et al., 1979), although the asymmetry is not as great as that observed for calcium. Magnesium is concentrated by the placenta in the rabbit (Aikawa and Bruns, 1960; Cittadini et al., 1977). Chronic deprivation of magnesium in guinea pigs does result in a reduction of magnesium in the fetus (Dancis et al., 1971).

### Trace Metals

The relative quantities of the trace elements have been measured in the newborn infant (reviewed by Shaw, 1979). However, little is known about the mechanism of placental transfer of these minerals.

### Water

The sheep fetus each day requires more millimoles of water than it does of oxygen (Barcroft, 1946). While about 20% of this requirement is provided by fetal catabolism (Power et al., 1978), most will cross the placenta or fetal membranes as water.

Study of water transfer, both of its diffusional flux in radiolabeled form and of its bulk flow in response to hydrostatic or osmotic pressure gradients, suggests that the placenta does not represent an important barrier to the transfer of water from mother to fetus or vice versa. In the sheep and primate the steady-state clearance of tritiated water across the placenta is the same as that of antipyrine, even though their permeabilities measured across membranes in vitro are different by severalfold (Meschia et al., 1967). This suggests that the transplacental diffusion of both antipyrine and water is flow limited; if correction is made for shunting, water comes into equilibrium between the two sides of the placenta during a single capillary transit. Using a single circulation technique in the guinea pig, it has been shown that small lipid-soluble molecules such as ethanol and methanol cross the placenta more rapidly than tritiated water, which in turn crosses more rapidly than antipyrine (Bissonette et al., 1979). This is taken as evidence that water and antipyrine transfer are in fact to some small degree diffusion limited and that the steady-state method is not sufficiently sensitive to make this observation; it is not clear whether this is quantitatively important.

Unidirectional flux measurements of labeled water are much higher than the net flux (Hutchinson et al., 1959; Flexner et al., 1948) but, because water transfer is flow limited, it is not possible to calculate the diffusional permeability of the human placenta from these studies. Altering the osmotic pressure on the maternal face of the placenta by infusing hypertonic fluids rapidly leads to net flux of water out of the fetus in both the rabbit (Bruns et al., 1963) and the sheep (Faber and Green, 1972). Similarly, altering the fetomaternal hydrostatic pressure difference induces water transfer across an artificially perfused sheep cotyledon (Power et al., 1978). Power et al. calculated hydraulic conductivity to be 0.059 ml/min per 100 g of placenta per millimeter of mercury, whereas Armentrout et al. (1977) found the coefficient to be $5.6 \times 10^{-8}$ $cm^5$/sec dyne per kilogram of fetal body weight, a value approximately equal to 0.006 ml/min per 100 g of placenta per millimeter of mercury. The 10-fold higher value found by Power may reflect either the leakiness of perfused placentas or unexplained differences between osmotic and hydrostatic forces. In any case, the uptake of water clearly depends on the forces available to drive it and is not limited by a "waterproof" placenta.

Although it is widely agreed that hydrostatic and osmotic forces alone govern the passage of water across the placenta, at first sight a problem remains in that some quantitative observations of these variables are incompatible with water accretion. Measurement of hydrostatic pressure of the maternal circulation by puncturing the intervillous space or spiral arteries of the placenta suggests that the pressure is lower on the maternal side than on the fetal side of the placenta (Seeds, 1965). The same is true in guinea pigs, rats, rabbits, sheep (Moll and Kunzel, 1973), and monkeys (Reynolds et al., 1968). Similarly, both total osmotic pressures (measured by freezing point depression) and colloidal osmotic pressures have been found to be lower in fetal than in maternal plasma. This is despite extensive precautions to prevent false values for total osmotic pressure resulting from fetal hypoxia or from changes in plasma $pCO_2$ on exposure to air (Meschia et al., 1957; Delivoria-Papadopoulos et al., 1969).

However, in considering water flows, a simple balance sheet of hydrostatic and osmotic pressures on the two faces of the placenta is not appropriate. Firstly, the osmotic pressure exerted by a solute across the placenta cannot be derived from freezing point depression measurements alone. Each constituent of plasma will have a different constant by which its concentration difference must be multiplied in calculating the osmotic force it generates; this is known as the reflection coefficient. For a given concentration gradient across the placenta, a relatively impermeable solute will exert a larger osmotic force and have a larger reflection coefficient than a solute which crosses the placenta more rapidly. Differences in reflection coefficient cannot be assessed by chemical estimations or by measurement of osmotic pressure alone. The only published estimates for reflection coefficients across the placenta depend on several assumptions and are therefore not very secure (Thornburg et al., 1979). Secondly, water transfer may be influenced by concentration gradients only present within the capillary bed and dissipated within a single capillary transit (Power, 1981).

Although there is no comprehensive body of experimental data on water transfer, this has not prevented the development of several theoretical models to explain the control of net water transfer in the sheep. Conrad and Faber (1977) proposed that solutes, actively transported across the placenta, together with a hydrostatic pressure difference account for the fetal acquisition of water and of electrolytes, which, the authors argued, follow by passive diffusion. Control of fetal water acquisition will be through

placental electrolyte permeability, which is postulated to be the major constraint on fetal growth and which increases throughout gestation. It is suggested that hormones might reduce the reflection coefficient of the placenta for sodium and chloride and thus also influence the ratio of salt to water acquisition by the fetus (Thornburg et al., 1979). Power et al. (1978), on the other hand, suggested that fetal plasma bicarbonate provides an osmotic driving force for net transfer of water to the fetus and, through its proposed increased accumulation if the fetal circulation is sluggish, a mechanism for an appropriate increase in transplacental water flow. More recently, they suggested that too much emphasis should not be placed on a single metabolite. Instead, they have emphasized the influence that uneven blood flow matching between maternal and fetal capillaries could have on net water gain by the fetus (Power, 1981).

Clearly any solutes maintained at a concentration difference across the placenta will influence the transfer of water. The degree of influence will depend on the molar concentration difference maintained and on the value of the placental reflection coefficient for the substance. Numerically important solutes include amino acids, bicarbonate, fructose, urea, calcium, phosphate, potassium, sodium, lactate, and glucose, among others. If their transport is active, as is almost certainly the case for amino acids, phosphate, and calcium, such solutes are also candidates for the control of fetal water uptake. If sodium transport is indeed active, its transfer, by analogy with other epithelia, may also be very important.

In conclusion, it is clear that hydrostatic and osmotic forces can alter the rate and direction of water transfer across the placenta. However, it is still uncertain how water transfer is regulated. Does the fetus just grow, drop its hydrostatic pressure, and allow more water in, or is solute pumping an important source of control? Any mechanism of water control must be compatible with the observation that the transplacental water and ion flux can be increased several fold by constant drainage of fetal urine without apparent detriment to the fetus or any important change in fetal plasma electrolye concentration (Gresham et al., 1972; Faber and Thornburg, 1977).

## EXTRAFETAL FLUIDS

### Volume and Composition

The amniotic fluid increases in volume to a peak of about 600 ml at about 120 days in the sheep and to a peak of 1000 ml at 30 weeks in the human and then falls (Malan et al., 1937; Gadd, 1977). In the sheep, the allantoic volume increases throughout pregnancy (to a peak of about 750 ml at term), but in the pig the allantois has almost disappeared by 90 days (McCance and Stanier, 1960) and in the human there is no functioning allantois in fetal life.

Table 5 shows the composition of fetal fluids in the human at term and in the sheep 2 weeks before term. In sheep, the osmolarity of amniotic fluid decreases, whereas that of the allantois increases as pregnancy proceeds. During pregnancy sodium concentration falls and potassium concentration rises in both the amnion and the allantois, although potassium changes more markedly in the allantois. Chloride concentration in amnion falls. The concentration of the major electrolytes in both the amniotic and allantoic fluids differ markedly from their concentrations in plasma and urine (Mellor and Slater, 1971) or lung liquid (Adamson et al., 1969). In the human, osmolarity of amniotic fluid and its sodium and chloride concentrations also fall as pregnancy proceeds. No recognized changes occur in potassium concentration (Liley, 1972).

**Table 5** Composition of Fetal Fluids in the Human at Term and the Sheep at 130 Days Gestation[a]

| | Human | | | Sheep | | | |
|---|---|---|---|---|---|---|---|
| | Amniotic fluid[b] | Amniotic fluid[c] | Urine[d] | Amniotic fluid[e] | Allantoic fluid[f] | Urine[f] | Lung liquid[g] |
| Osmolarity (mosm) | 255 ± 3 | | 137.3 ± 11.2 | 270 ± 12 | 283 ± 9 | 200 ± 30 | 294 ± 2 |
| $Na^+$ (mmol/liter) | 128 ± 3 | 133 | 44.1 ± 4.9 | 97 ± 14 | 47 ± 14 | 28 ± 6 | 150 ± 1.3 |
| $K^+$ (mmol/liter) | 3.8 ± 0.01 | 4.5 | 4.7 ± 1.3 | 10 ± 1.5 | 80 ± 16 | 3 ± 1 | 6.3 ± 0.7 |
| $Cl^-$ (mmol/liter) | 101 ± 1 | 111 | 414 ± 3.7 | 105 ± 6 | 20 ± 12 | 26 ± 6 | 157 ± 4.1 |
| Urea (mmol/liter) | 5.2 ± 0.8 | | 10.9 ± 2.8 | 11 ± 2 | 8 ± 1 | 4 ± 2 | 7.9 ± 2.7 |
| $Ca^{2+}$ (mmol/liter) | – | | – | – | – | – | 0.22 ± 0.015[h] |
| $PO_4^{2-}$ (mmol/liter) | – | | – | – | – | – | <0.02 |

[a]Mean values and, where possible, standard errors are quoted.
[b]Gillibrand (1969), values obtained at 40 weeks gestation.
[c]Doran et al. (1970), values obtained after 35 weeks gestation.
[d]McCance and Widdowson (1953) from urines collected at term delivery.
[e]Taken from graphs in Mellor and Slater (1971).
[f]Taken from graphs in Mellor and Slater (1972).
[g]Adamson et al. (1969).
[h]Olver and Strang (1974).

### Pathways for Exchange

There are several sites of possible fluid and mineral exchange between the fetus and amniotic fluid. The main candidates are the kidneys, the gut, the lungs, the surface of the cord and placenta and, early in gestation, the fetal skin. Other sites, such as the lacrimal glands and, later in gestation, the sweat glands, may also contribute to the amniotic fluid but will be quantitatively relatively unimportant. Most work on fetal fluids has been done in the sheep, but even in this species we are nowhere near a quantitative analysis. In all species the degree of direct exchange of water and minerals between maternal circulation, uterine secretions, and amnion through the chorion laeve and amniotic epithelium is uncertain.

### Nonplacental Maternofetal Exchange

The surface area of the extraplacental fetal membranes is tiny in relation to the placental surface area and for this reason it appears improbable that the extraplacental route is of great quantitative importance in fetal mineral and water uptake. Wright et al. (1962) investigated this directly by comparing the fetal uptake of $^{24}Na$ in rats with and without prior placental separation. Rather surprisingly, their results suggest that up to 10% of sodium transfer could still take place in the absence of a functioning placenta. They also demonstrated some direct passage of $^{24}Na$ across the human amnion in vivo.

Thanks to their easy accessibility, the permeability of the amniotic and chorionic membranes have been extensively studied in vitro since the initial work by Garby (1957). Moore et al. (1966) noted that the human chorion is less permeable to small polar molecules than is the amnion; an opposite relationship was found by Seeds (1970). The membranes hydraulic and osmotic permeabilities to water have been thoroughly investigated by Abramovich et al. (1976) and Page et al. (1974). They calculate that a net flux of up to 80 ml of water could take place daily across the amniotic chorionic uterine interface.

Manku et al. (1975), studying the guinea pig amnion, and Leontic et al. (1979), investigating the human, showed an influence of prolactin in in vitro water permeability. France (1976b) suggested that active chloride transport might underlie these hormonal effects, an idea perhaps supported by the work of Battaglia et al. (1969), for they observed a chloride permeability across sheep membranes in vitro that is unexpectedly high in relation to in vivo studies. North and Segal (1976) found that unlike chloride, there was no asymmetry in sodium fluxes across the amnion.

Further study of the overall importance, if any, of the extraplacental route is required and will necessitate in vivo investigation.

### Skin

Seed's (1965) review led him to suggest that amniotic fluid might be found by dialysis of maternal plasma, while, according to Lind et al. (1972), the nature of human fetal skin, which in early gestation (less than 20 weeks) is permeable to sodium and water, means that at this stage of pregnancy the amnion merely represents an extension of the fetal extracellular space. Ultrafiltration or dialysis from either circulation is clearly not the whole explanation for amniotic fluid composition. It and plasma are quite different in later gestation.

Skin specimens taken near mid-gestation from sheep or pigs and investigated by making bidirectional measurements of electrolyte transfer in vitro provide evidence for a pump controlling sodium ion transport (France, 1976a). Such a pump could be expected to lead to fluid shifts, but the importance of skin as a site of fluid exchange must be expected to decrease as pregnancy proceeds and the skin becomes keratinized.

### Surface of the Cord and the Placenta

Tracer studies in humans at cesarean section or hysterectomy have shown that the cord and the surface of the placenta are permeable to water (Abramovich and Page, 1972; Hutchinson et al., 1959). Abramovich and Page (1972) observed an increase in uptake by the cord as uptake by fetal skin decreased.

### Lung Liquid

In the fetal lamb near term the output of lung liquid is 68.5 ml/kg of fetal weight per day (Olver and Strang, 1974). Secretion is switched off with the onset of labor (Walters and Olver, 1978). See also Chapters 10 and 24, by Jobe and Rivers, respectively, in this volume.

### Alimentary Canal

The fetus swallows in utero. According to measurements made using a chronically implanted esophageal flow meter, the fetal sheep swallows 20-800 ml/day (Mistretta and Bradley, 1975); in the case of the human the value may be rather similar, 120-1700 ml/day (Liley, 1972; Abramovich et al., 1979). Mistretta and Bradley reported that the onset and rate of swallowing is so variable that it is difficult to assess the fetal response to a chemical stimulus injected into the amnion. However, Liley quoted De Snoo as reporting an increase in swallowing following injection of saccharin into the amnion, and Liley showed that a marked decrease in swallowing occurred when a radio-opaque solution, Lipiodol, was similarly injected. It has been proposed that a decrease in the rate of swallowing accompanies fetal distress (Bradley and Mistretta, 1973).

The amniotic fluid swallowed by the fetus is absorbed by the alimentary canal. Some exocrine glands secrete fluid into the alimentary canal in late gestation; for example, the liver produces bile (Canning, 1979). However, these fluids are only likely to influence amniotic fluid composition if the fetus defecates in utero.

### Kidney

In the human the urethra becomes patent at 8-9 weeks gestation and from then on the kidneys, by producing urine, can contribute to the composition of the amniotic fluid, itself present from the third week of gestation. In sheep and pigs, urine passes into the allantoic sac via the urachus until the patency of the urethra is established. It is uncertain how much urine passes to the allantois thereafter. Mellor and Slater (1971) noted that in the sheep the urethra is patent at 80 days, while urine is still being passed to the allantois after 100 days gestation. This observation is compatible with the increase in volume of the allantois but is not necessarily true for other species, for example, the pig, in which the allantoic fluid has virtually disappeared by 90 days.

Gresham et al. (1972) observed urine flows of 0.25-0.85 ml/min, about 400-1200 ml/day, in chronically catheterized fetal lambs once they had recovered from surgery. In the sheep, urine osmolarity and sodium and chloride concentrations fall as pregnancy proceeds. The pH and concentration of urea and potassium, on the other hand, remain steady until 10 days before parturition. After this, rapid changes in sodium, potassium, chloride, and urea concentrations are observed. Osmolarity rises markedly, and the urine, always previously hypotonic in relation to plasma, becomes hypertonic (Mellor and Slater, 1972). In the human, however, urine obtained at term delivery is hypotonic (McCance and Widdowson, 1953) (see Table 5).

The gradual changes in urine flow and composition observed in the sheep possibly reflect a maturation of the kidney rather than changes in the control of renal function. Glomerular filtration rate increases in the second half of sheep gestation, but not significantly if correction is made for fetal growth (Alexander and Nixon, 1962; Robillard et al., 1977). The decrease in urine electrolyte concentration found is accompanied by an increasing ability of the kidney to reabsorb a sodium or chloride load, according to Alexander et al. (1958) and Smith's group, who also noted an increase in the absorption of free water as gestation proceeded, especially after 130 days.

As well as the gradual changes observed, it is also possible to induce acute change in fetal urine flow and composition in chronically catheterized lambs. Lingwood et al. (1978) found that antidiuretic hormone administered intravenously before 130 days gestation, caused a drop in urine flow and a rise in urine osmolarity, without a change in blood pressure (see also Lingwood et al., 1980, and Wintour et al., 1981). Changes in fetal urine osmolarity can also be brought about by the ewe drinking (Mellor and Slater, 1973). An acid load to the fetus from either an infusion of acid (Daniel et al., 1975a) or partial occlusion of the cord (Daniel et al., 1975b) results in a decrease in urine pH. Phosphate clearance by the kidney, which is normally very low, can be increased by intravenous parathormone (Smith et al., 1969). It is uncertain how far the alterations that can be achieved have any physiological importance for pregnancy under field conditions, and indeed how important a role the kidneys generally play in fetal fluid and mineral homeostasis or in controlling the composition of amniotic fluid.

## AMNIOTIC FLUID VOLUME

It seems likely that the major source of amniotic fluid is the fetal kidney, a conclusion supported by the observation that renal agenesis in humans or drainage of fetal urine in sheep is commonly associated with a reduced volume of amniotic fluid. Lung liquid, in addition, accounts for perhaps one-fifth of amniotic fluid production in the sheep. The main site of removal of amniotic fluid is probably fetal swallowing. The quantitative role of direct exchange with the mother and of transfer across the cord, placental basal plate, or fetal skin is uncertain.

How the volume and composition of the amniotic fluid is controlled is also unknown. There is some degree of control, for replacement of the amniotic fluid with inappropriate solutions is fairly rapidly corrected. Thus in the sheep, if a volume of amniotic fluid is replaced by an equimolar mannitol solution, the resulting drop in urea and electrolyte concentrations is restored within 6 hr; interestingly, only the change in urea concentration is eliminated if urine is drained throughout the experiment, preventing its addition to the amnion (Wintour et al., 1980). Similarly, in monkeys amniotic electrolyte concentrations are gradually restored to their initial values after replacement of

amniotic fluid by distilled water (Schruefer et al., 1972). The time taken for correction in monkeys, 18-24 hr, is longer than in the sheep.

The mechanism whereby amniotic fluid composition is controlled is unclear. There are various possibilities. Urine flow in the conscious sheep is reduced by antidiuretic hormone and is also influenced by maternal drinking. Lung liquid secretion is diminished near term by catecholamines (Walters and Olver, 1978). Fetal swallowing may be influenced through neural mechanisms by the composition of the amniotic fluid. For example, polyhydramnios occurs in fetuses with abnormalities which preclude fetal swallowing (Liley, 1972). However, ligation of the esophagus does not result in hydramnios in fetal sheep (Wintour et al., 1978) and only in transient hydramnios in monkeys (Minei and Suzuki, 1976). A drop in amniotic fluid volume lasting 24 hr can be induced in monkeys by the intraamniotic injection of 1-10 mg of ovine prolactin (Josimovich et al., 1977). The reason is unknown but may be related to the influence of prolactin on amniotic membranes described above.

Abramovich (1970) and Wallenburg (1977) have made attempts to quantify the bulk turnover of amniotic fluid in the human. The main route of bulk flow out of the amnion is via the alimentary canal as a result of fetal swallowing. This was measured by recovery from the fetus following delivery of a nondiffusable radioactive label injected into the amniotic sac. The volume of urine flow was estimated by ultrasonography or by micturition rates in the newborn. The authors both concluded that in midpregnancy, the volume of liquor swallowed and that of urine voided were approximately of the same order. No estimate was made of the contribution from lung liquid. A similar balance sheet for the sheep suggests a maximum input to the amnion from kidneys and lungs of 1000-1500 ml/day, while the rate of fetal swallowing ranges from 20 to 800 ml/day (Mistretta and Bradley, 1975). The amniotic compartment is obviously an important element in fetal fluid balance, but it is one that cannot yet be adequately quantified.

## CONCLUSION

Returning to the questions posed in the Introduction, it is clear that none of them can be answered with any certainty. There is good evidence for a specialized transport system for some minerals, but even the mechanisms for fetal acquisition of water and sodium are still controversial. Surprisingly little is known as to whether or how the placental transfer of minerals is controlled.

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# 16

# Regulation of Fetal Growth

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## INTRODUCTION

The growth of the fetus is of paramount concern to both the obstetrician and the neonatologist. The manifest consequences of abnormal growth demonstrate the lack of understanding of the mechanisms regulating fetal growth. Data in the human are derived mainly from clinical correlations with birth weight. This chapter is concerned with factors that determine the growth and development of the normal fetus. Because of the numerous gaps in our knowledge of the growth of the human fetus, frequent recourse will be made to experimental data obtained in animals.

The growth and development of the fetus is determined mainly by the fetal genome. Superimposed upon this genetic regulation of fetal growth are two opposing influences. On the one hand, fetal growth is constrained in various ways. For example, the supply of nutrients to the fetus is limited by the capacity of the mother and placenta for supply and transfer, respectively. Other factors constraining fetal growth are poorly defined but are primarily maternal. On the other hand, a stimulus additional to the genetically determined drive to fetal growth and differentiation is provided by hormones and tissue growth factors.

Thus the rate of fetal growth represents the balance between constraining and stimulating forces acting on the genetically programmed drive to growth. In the first half of pregnancy, genetic control is dominant and gives rise to relatively narrow limits of variability of patterns of fetal growth; in the second half of pregnancy, constraints and stimuli become increasingly important and give rise to greater variability of growth and of maturational milestones.

## GENETIC CONTROL OF FETAL GROWTH

The description of growth in biochemical terms is still poorly understood. It remains unclear how the genetic information contained in the fertilized egg guides cell multiplication and differentiation that results in the attainment of the mature human form. A tightly programmed sequence of gene activation and suppression is necessary for development to proceed in an organized manner that allows particular developmental events to occur at precise gestational ages. While this program must be contained within the genome, it is unclear how this is translated into biochemical events on a precise time course.

One of the fundamental controls of growth depends on the ability of cells to "count" the number of divisions it has gone through. The total number of cells in a term fetus lies within narrow limits and is the result of 42 successive divisions of the fertilized ovum. The consequences of inaccurate "counting" will be appreciated from the fact that only five further divisions are required for the fetus to attain adult size. There must be a mechanism by which cell differentiation can be programmed to occur after a specified number of divisions of a cell line.

A hypothesis for a genetic "counting" mechanism has been proposed by Holliday and Pugh (1975). Modification enzymes have been demonstrated in bacterial and viral organisms which act to modify particular bases during the replication of the DNA (for example, the replacement of adenine by guanine). These workers postulated that this modification mechanism could allow the cell to "count" the number of divisions it has experienced at a particular stage of development. The consequent progressive alteration of the sequence of nucleotide bases could lead to the alteration of operon sites, and thus allow a developmental "switch" to become operational. Such a mechanism would provide for synchronous switching in all the progeny of a stem cell, and would also allow for multiple "clocks" to be operative within a single cell line, each being driven by a different operator site.

As a consequence of such a mechanism, the genetic information contained within a cell line would be different from that in other cell lines where other alterations in the genome have occurred. This would provide a basis for the genetic stability of the differentiated state. It is a general observation that differentiated cells do not readily transform either to other types of cells or to the undifferentiated state. As will be discussed in a subsequent section, there may be an interaction between endocrine influences and the genome which provides a further mechanism of regulating genetic switching.

Experiments on the development of the chick wing (Summerball et al., 1973), provide convincing evidence of a developmental clock. The tip of the limb bud, the progress zone, contains dividing cells which form in strict sequence the various structures of the limb from its base to the extremity. If the progress zone from a limb in which the basic structures are nearly fully formed is transplanted to a very young limb from which the progress zone has been removed, none of the structures are formed. On the other hand, if a young progress zone replaces one on the end of a wing that has already laid down all the basic structures, another wing is formed at the end of the first. These results show that there is a temporal order in the laying down of successive structures and this order could well be related to the number of cell divisions that have elapsed in the cells of the progress zone.

Single gene loci can influence fetal birth weight. For example, infants homozygous for cystic fibrosis, an autosomal recessive condition, have reduced birth weight (Boyer, 1955); in contrast, unaffected siblings have enhanced birth weight (Saugstad, 1972). Whether this reflects the fetal genotype or the obligatory maternal heterozygotic state is unclear.

Chromosomal abnormalities are frequently associated with reduced birth weight, for example, trisomy 21, trisomy 18, trisomy 13, 4p syndrome, and 45 XO. In the trisomies, it is believed that the reduction in birth size is a consequence of a reduced rate of cell multiplication (Robson, 1978).

The genetic influence on birth weight in the normal fetus is mediated by multiple gene loci. Correlative studies of birth weight variation between relatives show that both fetal and maternal genotypes influence birth weight (see Tables 1 and 2).

Table 1 Influences on Variation in Birth Weight

| | |
|---|---|
| Genetic | 38% |
| maternal genotype | 20% |
| fetal genotype | 15% |
| fetal sex | 2% |
| Environmental | 62% |
| general maternal environment | 18% |
| immediate maternal environment | 6% |
| maternal age and parity | 8% |
| unknown | 30% |

*Source*: Derived from Polani, 1974.

Mathematical modeling from these and other data suggest that approximately 15% of the total birth weight variation is attributed to the fetal genotype, and 2% to the sex of the fetus. A further 20% of birth weight variation is determined by the maternal genotype and about 30% by the maternal environment (Polani, 1974; Robson, 1955, 1978).

The paternal contribution to birth weight is mediated only through his contribution to the fetus' autosomal genes and sex. The maternal contribution is more profound, being expressed not only through the genes of the fetus, but also through the effect of her own genotype on the environment of the fetus; the latter is as important as the genotype of the fetus itself.

Population studies provide further evidence of the degree of variation in birth weight that may be expected from genetic influences. The Luni tribe of New Guinea have a mean birth weight of 2400 g (Meridith, 1970). At the other extreme, infants born on the islands of Anguila and Nevis have a mean birth weight of 3880 g (Ounsted, 1978). Great variations are also observed within ethnic groups. The mean birth weight of whites of a Neapolitan ancestory is 3030 g, and for those of Norwegian ancestory 3450 g (Ounsted, 1978).

Table 2 Correlation between Birth Weights of Relatives

| Description of sample | Correlation of birth weights r (n) |
|---|---|
| Maternal half-sibs (adjacent birth rank) | 0.581 (30)[a] |
| Full sibs (adjacent in birth rank, nonconsanguineous parents) | 0.523 (367)[a] |
| Full sibs (adjacent in birth rank, parents first cousins) | 0.481 (442)[a] |
| Full sibs (one sib intervening) | 0.425 (654)[a] |
| Full sibs (two sibs intervening) | 0.363 (153)[a] |
| Paternal half-sibs[b] | 0.102 (168)[a] |
| First cousins, maternal sisters | 0.135 (554)[c] |
| First cousins, paternal brothers[b] | 0.015 (288)[c] |

[a]Morton (1955).
[b]Note the lesser effect of paternal genes on birth weight.
[c]Robson (1955).

At the family level, a correlation exists between birth weight and both the height and weight of the parents. There is a marked intrafamily similarity of birth weights (see Table 2; Donald, 1939). In the mother, weight is the more important factor, and when this is corrected for, maternal height has little effect on the size of the fetus.

## MATERNAL FACTORS AFFECTING FETAL GROWTH

### Maternal Constraint

The epidemiological data previously reviewed has demonstrated the importance of the maternal genotype and environment on birth size. It seems likely that the fetus rarely completely expresses its genetically determined potential for growth. Under normal conditions, growth is constrained to a greater or lesser degree by unknown factors in the fetal environment. The phenomenon of growth restraint appears to be a function mainly of maternal influences. While some of these are expressions of the maternal genotype, the major component of growth constraint is independent of a direct genetic component. This phenomenon has been termed "maternal constraint."

The classic demonstration of maternal constraint of fetal growth was made by Walton and Hammond (1938), who crossed Shire horses with Shetland ponies. The birth weights of foals born to Shetland dams were similar to those of pure Shetlands, while foals born to Shire dams were of similar birth weight to purebred Shires. This experiment showed that maternal factors in the horse override that part of the fetal genetic makeup acquired from the sire, but did not clearly distinguish between those maternal influences expressed through the genes of the fetus and those expressed through the fetal environment. In more recent experiments, the genetic component has been eliminated by transplantation of fertilized eggs. Smidt et al. (1967) transferred eggs from normal-sized pigs into dwarf sows and found that the piglets were about half the size of normal piglets. When the experiment was reversed by transferring eggs from dwarf sows into normal-sized sows, the piglets were about twice the size of usual dwarf piglets. Similar results of egg-transfer experiments have been observed in sheep (Hunter, 1956) and rabbits (Venge, 1950).

In man, the evidence for maternal constraint of fetal growth is indirect. For example, whereas there is a high correlation ($r = 0.581$) for the birth weight for half-sibs with a common mother, the correlation for paternal half-sibs is low ($r = 0.102$) (Table 2).

The nature of maternal constraint is unknown. Physical restraint by the uterus is probably not important in monotocous species such as man, in which the potential for additional uterine capacity is readily seen in multiple pregnancy and polyhydramnios. However, the reduced birth weight in twins and further reduction in triplets and quadruplets presumably reflects some of the multiple factors causing maternal constraint. Maternal constraint could operate in many ways. Some of the possible factors, such as maternal placental perfusion, availability of nutrient materials, and placental growth, will be discussed in subsequent sections.

Ounsted and Ounsted (1973) have postulated that there is a predetermined maternal regulator of fetal growth. They postulated that the setting of this hypothetic regulator depends on both the maternal genotype and the extent to which the mother's growth was constrained during her own fetal development. This latter is suggested by the observation that mothers of low birth weight infants were themselves of low birth weight. Similarly, the sibs of low birth weight infants also have reduced birth weight.

Ounsted (1978) has suggested that antigenic differences between the mother and fetus may affect fetal growth. The lesser the antigenic differences, the poorer the growth rate. This hypothesis would explain observations of lower birth weight of monozygotic compared to dizygotic twins and the increase in birth size with increasing parity (consequent upon increased sensitization of the mother by previous pregnancy). Although limited data in experimental animals support such an hypothesis, it has not been generally accepted as a major factor.

Maternal constraint has obvious benefits in easing the mechanical problem of parturition, and there may be more subtle advantages. Constraint limits the demands on the maternal system. Growth of the fetal brain is less affected than other organs in growth retardation, and it is possible that constraints on somatic growth ensure that adequate substrates are available for brain growth.

### Specific Maternal Factors

A large number of specific maternal factors have been shown to reduce birth size. Many of these limit the availability of substrates and oxygen to the fetus by lowering concentrations of substrate in the maternal circulation, by reducing placental perfusion, or by impairing placental transfer.

#### Maternal Nutrition

It is generally accepted that adequate maternal nutrition is a prerequisite for normal fetal growth. However, epidemiological data suggests that there is a wide tolerance for impaired maternal nutritional intake without clinically important fetal growth retardation. In many of these studies, it has not been possible to separate nutritional from other factors that may affect birth size. Maternal nutritional status may be one of the factors determining maternal constraint. Malnutrition prior to and during pregnancy in rats may lead to growth retardation extending into the second generation (Zamenhof et al., 1971). While poor maternal nutrition may generally only have a minor effect on fetal growth, it is possible that substrate limitation may have significant effects on brain growth at critical periods of development.

The relationship between maternal nutrition and fetal growth has been reviewed extensively (Metcoff, 1978). Retrospective studies of the famine in Holland during 1944-1945 in which a previously well-nourished population was exposed to acutely imposed subnutrition demonstrated that reduced birth weight was observed only when the maternal caloric intake was less than 1500 calories during the third trimester (Stein and Susser, 1975; Smith, 1947). Supplementary feeding during pregnancy enhanced birth size in a Guatemalan community of deprived socioeconomic status (Lechtig et al., 1975).

While these studies clearly show that exceptional nundernutrition affects fetal growth, the data do not allow for a clear effect of maternal nutrition on birth size in an industrialized society, separate from other factors such as smoking, alcohol, and drug exposure and maternal health.

The specific nutritional requirements for a normal human pregnancy remain poorly defined (Metcoff, 1978). The fetal lamb requires 72 kcal/day per kilogram in late gestation for normal growth. A total of 60% of the caloric requirement is for energy production and 40% is incorporated into the growing organism (Lemons et al., 1976). Both glucose and amino acids are required to satisfy fetal metabolic requirements. The fetal sheep responds to hypoglycemia by decreasing glucose utilization by fetal tissues.

However, there is no decrease in fetal oxygen consumption and the observed increase in fetal urea production suggests diversion of amino acids from anabolism to catabolism. Insulin and other fetal hormones presumably mediate these responses. Thus decreased availability of glucose will lead to increased catabolism of amino acids and reduced fetal growth (Simmons et al., 1974; Battaglia and Meschia, 1978). In addition, maternal nutrition impairs placental growth in experimental animals by retarding cell division in the proliferative phase of placental growth and reducing placental cell size in the hypertrophic growth phase (Brasel and Winick, 1972).

### Smoking

Smoking is the most significant avoidable maternal factor adversely affecting fetal growth. As well as causing intrauterine growth retardation, the perinatal mortality rate and the incidence of pregnancy complications are increased; the duration of gestation reduced.

The degree of fetal growth retardation increases with the number of cigarettes smoked each day by the mother. The birth weight of infants whose mothers smoke 20 cigarettes per day is reduced by 200 g. The birth length is also reduced (Figure 1). Infants of light cigarette smokers have a slight reduction in birth weight. Although the weight gain of smoking mothers during pregnancy is reduced, several studies have clearly shown that intrauterine growth retardation (IUGR) is not secondary to maternal malnutrition (Persson et al., 1978; Pirani, 1978; Miller and Hassanein, 1964). Placental weight is not decreased, and may be increased in heavy smokers. However, smokers' placentas have an increased frequency of lesions suggestive of placental underperfusion, including obliterative endarteritis, cytotrophoblastic hyperplasia in villi, and necrosis of the decidua basalis at the margins of the placenta (Naeye, 1978).

Both nicotine and carbon monoxide have been implicated as possible causative factors. Nicotine administered orally to pregnant mice or rats induces fetal growth retardation. Nicotine increases uterine vascular resistance and reduces uterine blood flow in rats, but only at plasma concentrations exceeding those achieved during cigarette smoking (Bruce and Parkinson, 1979). Very high doses of nicotine given to pregnant rhesus monkeys cause fetal hypoxia and hypercapnia (Suzuki et al., 1971). Nicotine has been shown to depress placental transfer of amino acids in isolated human placental tissue (Barnwell and Sastry, 1980).

Cigarette smokers are exposed to carbon monoxide leading to the formation of carboxyhemoglobin and consequent tissue hypoxia. The binding of CO to cytochromes of the electron transport pathway is a further factor in the development of tissue hypoxia. In pregnant animals exposed to CO, the fetus has higher carboxyhemoglobin concentrations than the mother, suggesting that the fetus in particular is at risk of tissue hypoxia (Longo, 1976). Carbon monoxide may poison the cytochrome $P^{450}$ system in the placenta and thus reduce placental oxygen transfer (Novy, 1978).

### Environmental Factors

*Altitude* Pregnancy at high altitude slows the growth rate of the fetus. Fetuses born in Peru at 15,000 ft have a mean birth weight 16% less than that of infants born at 500 ft (Kruger and Arias Stella, 1970). Similar observations have been made in a variety of geographical regions. It is likely that the growth retardation is a consequence of decreased oxygen availability to the fetus. Interestingly, the placental weight is significantly increased at higher altitudes, presumably reflecting an attempt at a compensatory growth by the fetal components of the placenta.

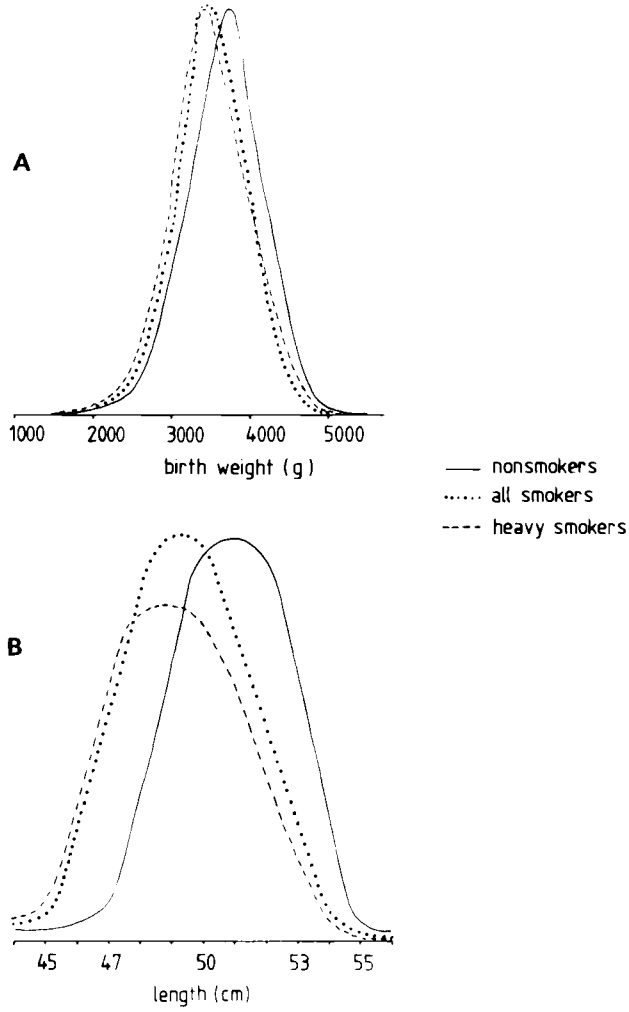


**Figure 1** The effect of smoking on (A) birth weight and (B) birth length. Heavy smokers are defined as those smoking 11 or more cigarettes a day at term. The data are derived from a study of 5772 pregnant Swedish women among whom the prevalence of smoking was 49%, and that of heavy smokers, 20%. (From Persson et al., 1978.)

*Temperature* Exposure to raised environmental temperature is associated with decreased fetal weight in rats, guinea pigs, and sheep. The reduction in fetal size cannot be accounted for solely by a reduction in food intake. Several mechanisms have been suggested, including reduction of uterine blood flow, reduction in placental mass, or heat-induced death of dividing cells. The latter has been demonstrated in the fetal guinea pig brain following very brief elevations of core temperature (Edwards, 1969). The evidence in man is less certain, particularly as elevation of core temperature is generally due to infective illnesses and is of brief duration. The infections themselves may be the primary teratogenic agent. A reduction in birth weight was noted in the infants of mothers who had pyelonephritis with associated fever, compared to those whose mothers had pyelonephritis without fever (Smith et al., 1978).

### Socioeconomic Status

In a number of perinatal surveys social class has been demonstrated to have a significant effect on birth weight. This relationship appears to be due to its correlation with other maternal factors such as maternal age, parity, height, smoking habits, and the incidence of toxemia. Lower social class has no significant effect in its own right (Ounsted and Ounsted, 1973).

### Maternal Diseases

Maternal disorders during pregnancy may have an effect on fetal growth. Impairment of the maternal circulatory status may threaten the oxygen supply to the fetus. Women with severe congenital or acquired heart disease give birth to growth-retarded infants (Novy et al., 1968). Maternal compensations, including increased red cell mass and alterations in the hemoglobin-oxygen dissociation curve, are inadequate in severe cyanotic heart disease. As in the case of fetal growth retardation associated with high altitude, it appears that the supply of oxygen to the fetus is the limiting factor. Isolated deficiency of oxygen has been demonstrated to induce growth retardation in the chick embryo (Metcalfe et al., 1977).

Pregnancy-associated hypertension is commonly associated with IUGR. The uteroplacental blood flow is reduced in this syndrome and there may be occlusive thrombosis of the placental vasculature. Epidemiological studies suggest that growth retardation is most likely when hypertension is accompanied by renal damage, as evidenced by proteinuria or hyperuricemia.

Any chronic dehabilitating disease of the mother may be associated with impairment of fetal growth. For example, growth retardation has been described in mothers with bronchiectasis or other chronic lung disease or with chronic renal failure.

### Infections

Maternal malaria is frequently associated with IUGR and this is linked with placental infestation and extensive histological changes in the placenta (Galbraith et al., 1980).

Several maternal infections may directly affect the fetus and induce fetal growth retardation. It is likely that the fetal infection is the primary cause of the growth retardation, leading to lethal cellular damage of dividing fetal cells. Both rubella and cytomegalovirus have been shown to interfere with fetal cell division. In addition, rubella causes vascular insufficiency in many organs, including the placenta, due to damage to capillary endothelium (Knox, 1978). The effects of the illness on the mother (for example, fever) and on placental growth and function must be considered.

### Drugs

While a wide variety of pharmacological agents have been demonstrated to affect fetal growth in the experimental animal, only a few drugs have been proven to affect human fetal growth, reflecting the practical difficulties of evaluating the effects of drugs during pregnancy. It is likely that many of the agents proven to affect fetal development in the experimental animal also affect that of the human fetus, albeit in a subtle manner. As well as medically prescribed agents, the mother has unlimited access to many other pharmacological agents, including ethanol and aspirin. This discussion is restricted to the effects of drugs on fetal growth.

*Opiates* Infants exposed to intrauterine heroin have a high incidence (50%) of low birth weight. Methadone does not appear to be associated with decreased birth weight in man, although fetal growth retardation has been observed in experimental animals. It is unclear to what extent IUGR is due to an effect of heroin itself or to associated maternal malnutrition (Kandall et al., 1976; Rementeria, 1977). In the pregnant rabbit, morphine affects fetal growth independently of nutritional changes (Raye et al., 1978). Infants born to heroin-addicted mothers have a deficiency of total cell number, suggesting inhibition of cell division in the fetus. In addition, the placenta is hypoplastic (Naeye et al., 1973). Morphine interferes with the placental transfer of amino acids (Barnwell and Sastry, 1980). A further mechanism by which opiates might affect fetal growth is by alteration in maternal or fetal hormone secretion. The endogenous opiates ($\beta$-endorphin and enkephalins) are neurotransmitters with the potential to influence pituitary and pancreatic hormone secretion. The ability of heroin to bind to cytochrome $P^{450}$ and impair placental oxygen transport represents a further potential mechanism by which opiates could affect fetal growth (Novy, 1978).

*Ethanol* A distinct pattern of fetal maldevelopment as a consequence of high levels of alcohol ingestion during pregnancy is now well recognized (Jones et al., 1973). One of the constant features of the syndrome is intrauterine growth retardation; other features are craniofacial, limb, and cardiac anomalies and psychomotor retardation. Preliminary evidence also suggests that infants born to mothers who are moderate or "binge" drinkers also have a higher incidence of growth retardation (Hansen et al., 1978). Fetal growth retardation can be induced in experimental animals by chronic administration of ethanol. These effects appear to be independent of maternal caloric intake.

The growth inhibition and teratogenic effects are probably due to a reduction in fetal cell number, but it remains uncertain whether the toxic agent is ethanol itself or its metabolite acetaldehyde. Other actions of ethanol which may contribute to the IUGR include decreased placental protein and hormone synthesis (Wunderlich et al., 1979), fetal acidosis, and hypotension (Mann et al., 1975).

*Salicylates* An increased stillbirth rate and a reduction in birth weight of about 200 g has been observed in the infants of mothers who took aspirin daily during pregnancy (Turner and Collins, 1975). The cause of the growth retardation remains uncertain, but reduced placental size is observed in rats treated with salicylates (Lubawy and Garrett, 1977). Salicylates freely cross the placenta and inhibit fetal and placental prostaglandin production. Although prostaglandin synthetase inhibitors have various adverse effects on fetal circulation, including pulmonary hypertension, it is uncertain whether retarded growth is attributable to altered prostaglandin synthesis.

*Glucocorticoids* In laboratory rodents, chronic administration of pharmacological doses of synthetic glucocorticoids to the mother induces fetal growth retardation, but similar effects are less certain in primates. Reduced birth weights were reported in the infants of mothers who received prednisone (10 mg/day) prior to and throughout pregnancy (Reinish et al., 1978). In general, glucocorticoids inhibit cell division and promote differentiation of cell function; inhibition of cell replication in the placenta and fetus would lead to growth retardation. Such effects should be more apparent in man in the first half of gestation, the period of rapid cellular division.

*Cytotoxic and Immunosuppressive Agents* There have been a number of reports of IUGR following maternal administration of cytotoxic drugs. These presumably act via inhibition of cellular division. Among the agents implicated are busulfan, methotrexate, and cyclophosphamide.

*Propranolol* Propranolol, a β-adrenergic receptor blocker widely used for treatment of hypertension, crosses the placenta to reach the fetus. Growth retardation of both the fetus and placenta has been described in infants chronically exposed to propranolol in utero (Gladstone et al., 1975; Fiddler, 1974; Pruyn et al., 1979). Several possible mechanisms have been postulated. An increase in uterine muscle tone may occur and lead to a reduction in uterine blood flow. Uterine blood flow may also be reduced secondary to the fall in maternal cardiac output. A reduction in umbilical blood flow has been reported in sheep following propranolol administration (Oakes et al., 1976). Metabolic and endocrine functions of both the mother and fetus might also be affected.

*Other Agents* Many other drugs induce fetal growth retardation in experimental animals. Their relevance to man remains uncertain. These drugs include diazepam, chlorpromazine, haloperidol, barbiturates, and trimethadione. In addition, nonpharmaceutical chemicals such as the heavy metals (arsenic, lead, mercury, cadmium) and industrial chemicals (such as polychlorinated biphenyls or dioxin) have also been associated with fetal growth retardation.

## Uterine Abnormalities and the Site of Implantation

Anatomical abnormalities of the uterus may occasionally be associated with fetal growth retardation. The determinants of the implantation site in the primate uterus are not known. When the site of implantation is in the lower uterine segment, the mean birth weight is reduced by about 200 g (Higginbottom et al., 1975). Placenta previa is associated with decreased birth length and birth weight. The degree of growth retardation relates in part to the number of bleeding episodes; however, it is possible that the lower segment represents a less favorable site for implantation consequent upon the lower regional blood flow to this region of the uterus.

In polytocous species such as guinea pigs, the site of implantation is a more critical factor as a consequence of variations in uterine arterial supply to different portions of the utero-ovarian arterial arcade. This has been used as a basis of experimental growth retardation in polytococous species by ligature of one end of the vasuular arcade (see Dawes, 1968).

## Factors Influencing Uterine Blood Flow

Any factor affecting uterine blood flow may adversely affect fetal growth. Rarely major anatomical variations in the uterine arteries may occur and lead to reduced uterine blood flow. Uterine blood flow rates tend to be less in primagravida than in multigravida (McFadyen, 1979).

*Maternal Cardiovascular Disease* Women with severe congenital or acquired heart disease give birth to growth-retarded infants. In maternal hypertension or preeclampsia, uterine blood flow is reduced. There may be severe arteriopathy of the vascular bed with occlusive thrombosis and placental ischemia or infarction (Novy, 1978).

*Posture and Exercise and Mechanical Obstruction* In the supine posture, the uterus may obstruct the vena cava and reduce uterine blood flow. Mechanical obstruction of the uterine arterial supply has been the main method of producing experimental fetal growth retardation (see below). Recently, chronic maternal exercise has been shown to inhibit fetal growth in the guinea pig. Only a moderate degree of exercise for 15 min twice a day during pregnancy was required to produce a smaller fetus and placenta (Nelson et al., 1980). Conversely, uterine blood flow is probably increased by bed rest (Morris et al., 1956).

*Estrogen* Estrogens increase uterine blood flow in sheep. They are presumably an important factor in the increase in uterine blood flow during pregnancy. Uterine blood flow decreases rapidly after fetal death, possibly due to the decrease in fetoplacental estrogen production (see McFadyen, 1979).

*Temperature* Uterine blood flow is reduced by an increase in ambient temperature (Leduc, 1972), presumably as a compensation for peripheral vascular vasodilation. This is one mechanism by which hyperthermia may lead to growth retardation of the fetus.

*The Chemical Regulation of Uterine Blood Flow* α-Adrenergic agonists reduce uterine blood flow in pregnant sheep, but α-adrenergic antagonists have no effect, suggesting that there is no tonic α-adrenergic activity restricting uterine blood flow (Oakes et al., 1980). The uterus is innervated by sympathetic nerves and the effect of sympathetic stimulation in increasing uterine arterial pressure is α-adrenergic mediated (Fuller et al., 1979). The effects of nicotine on uterine blood flow are mediated by catecholamine release (Resnik et al., 1979). There are conflicting data as to whether the vasodilatory effects of β-adrenergic agonists are significant in the pregnant uterus (Rankin and McLaughlin, 1979), but the growth retardation associated with maternal propranolol administration could be due to reduced uterine blood flow.

Prostaglandins E are potent maternal placental vasocilators, although they cause vasoconstriction of the umbilical arteries. Prostacyclin (prostaglandin $I_2$) reduces the response of the uterus to norepinephrine (Rankin et al., 1979). The pregnant myometrium forms predominantly prostacyclin which may have the function of maintaining vasodilation in the placental bed. Indomethacin, an inhibitor of prostaglandin synthesis, causes vasoconstriction in the sheep placenta (Rankin and McLaughlin, 1979). Aspirin, like indomethacin, is an inhibitor of prostaglandin synthetase and the consequent effects on uterine blood flow could partly explain fetal growth retardation in the infants of chronic aspirin users.

In unanesthetized experimental animals, angiotensin II increases uterine vascular resistance and reduces uterine blood flow, provided that care is taken to avoid elevating systemic blood pressure. Increased renin-angiotensin production may be a factor in the IUGR associated with maternal hypertension and renal disease.

## PLACENTAL FACTORS AFFECTING FETAL GROWTH

### Placental Growth and Its Relationship to Fetal Growth

The factors influencing placental growth have been reviewed recently (Alexander, 1978). The growth of the placenta is not synchronous with that of the fetus, growing more rapidly than the fetus early in gestation so that maximal placental weight is reached at 33 weeks in man. However, placental villous surface area and vascularity continue to increase in late gestation, and it is unlikely that placental weight is an exact determinant of placental function with respect to many of its physiological processes. In general there is an association between the weight of the placenta and the weight of the fetus toward term in all species where it has been studied (Dawes, 1968; Alexander, 1978). This association may or may not represent a causal relationship. The bulk of the placenta is of fetal origin and thus those factors affecting fetal growth could be expected to affect placental growth. On the other hand, placental size might limit fetal growth by limiting the transfer of nutrients or by limitation of hormone production.

Experimental evidence favors the view that placental mass can influence fetal growth in late gestation. Experimental reduction of placental mass in sheep, rats, and monkeys

may lead to a reduction in fetal growth in late gestation (see below). However, in the rhesus monkey growth retardation is only observed if the reduction in placental mass occurs after 110 days (term, 165 days). If the surgical reduction is performed at 80 days, no fetal growth retardation is observed and there is compensatory growth of the remaining placenta (Novy et al., 1977; Hill, 1974). Similarly, in the sheep, minor reduction of placental mass has no effect on fetal growth. However, more extensive reduction in placental mass is associated with fetal growth retardation, although there is partial compensatory growth of the remaining cotyledons, particularly of the fetal component of the placenta (Alexander, 1964; Robinson et al., 1979). The fetal growth retardation is associated with chronic fetal hypoxemia and polycythemia. Fetal plasma glucose, lactate, and pyruvate concentrations are reduced, but plasma alanine concentrations increase (Robinson et al., 1979). These findings suggest that IUGR secondary to a reduction in placental mass is due to restriction of oxygen and carbohydrate to the fetus. In addition, altered placental hormone production may play a role in the genesis of the fetal growth retardation.

The placenta of growth-retarded fetuses is frequently small and demonstrates considerable histological changes. In placentas of preeclamptic women there is frequently placental infarction and degeneration of the syncytiotrophoblast. Intervillous thromboses are reported in the placentas of growth-retarded infants (Rolschau, 1978c).

Limited evidence suggests that fetal hormones may influence placental growth. In anencephaly, the placental weight is decreased (Honnebier and Swaab, 1973), and maternal chorionic somatomammotropin concentrations are reduced (Moshirpur et al., 1978). This reduction in placental mass may explain the slight growth retardation observed in the anencephalic fetus. In the rat, brain extracts, α-melanocyte-stimulating hormone, and growth hormone administered to the encephalectomized fetus increase placental weight (Honnebier and Swaab, 1974). In sheep, histological changes in the placenta are observed following endocrine manipulation of the fetus. Following fetal hypophysectomy there is an increase in the thickness of the epithelial basement membrane, and following fetal pituitary stalk section (Nathanielsz et al., 1978) or adrenalectomy, alterations are observed in the distribution of binucleate cells which are thought to secrete chorionic somatomammotropin (Barnes et al., 1976).

### Abnormalities of the Placenta and Umbilical Cord

Variations in the shape of the human placenta such as an accessory-lobe, bilobate, or reniform placenta appear to have no significant effect on fetal development. However, anomalous cord insertion is frequently associated with low birth weight and may result from the abnormal vascular dynamics of the extreme forms of battledore and velamentous insertion in particular (Rolschau, 1978a; Shanklin, 1978). The cause of IUGR associated with circumvallate placenta may be placental hypoplasia due to reduced cell number (Rolschau, 1978b).

Chorangiomas (hemangiomas) are the most frequent benign tumors of the placenta and are frequently associated with low birth weights infants. It is likely that blood is shunted away from the fetus into the chorangioma (King and Lovrien, 1978).

The presence of a single umbilical artery is a well-recognized association with both fetal growth retardation and other fetal abnormalities. In general, the reduction in fetal weight is only slight and may relate to a reduced gestational age (Rolschau, 1978a).

### Umbilical-Placental Blood Flow

Less data are available regarding the regulation of fetal-placental blood flow than of maternal-placental blood flow because of the technical problems of measuring umbilical blood flow in the experimental animal (Rankin and McLaughlin, 1979).

The umbilical-placental circulation has a low vascular resistance. It receives about half the combined ventricular outputs of the fetus. The umbilical arteries contain a large proportion of muscle compared to elastin and collagen and are probably not innervated distal to the abdomen. It seems likely that the major determinant of umbilical blood flow is fetal cardiac output and the distribution of blood within the fetal systemic circulation. As the fetal heart has little ability to alter stroke volume, umbilical blood flow is highly dependent on the fetal heart rate (Boddy, 1979).

Any interference with fetal-placental blood flow will have major effects on placental function and thus on fetal growth. For example, ligation of one umbilical artery in the sheep causes profound fetal growth retardation (Emmanouildes et al., 1968).

The umbilical blood flow increases with fetal growth but decreases in late gestation relative to fetal weight, reflecting the increased requirement for blood flow to fetal organs (Boddy, 1979).

When the fetus is hypoxemic, there is a redistribution of fetal blood flow to various organs, but relatively little alteration in umbilical vascular resistance. Tachycardia is observed in younger fetuses, resulting in increased umbilical flow. In older fetuses, the arterial pressure increases, but there is bradycardia in response to hypoxemia; however, it is likely that umbilical flow is maintained by the increased perfusion pressure (Boddy, 1979).

A number of peptides and vasoactive substances have been demonstrated to cause umbilical vasoconstriction. These include angiotensin, vasopressin, bradykinins, serotonin, and $\alpha$-adrenergic agonists. Recently, prostaglandins, particularly prostaglandin $E_2$, have been shown to be potent umbilical vasoconstrictors.

There are regional differences in the sensitivity of the umbilical vascular tree to vasoactive substances. Angiotensin II is particularly active on arterioles supplying chorionic villi (Tulenko, 1979). Saralasin, an angiotensin II antagonist, results in a decrease in ovine umbilicoplacental vascular resistance, suggesting that angiotensin II contributes actively to placental vascular tone. Thus, increased renin-angiotensin secretion observed in maternal hypertension might lead to reduced umbilical blood flow and to the genesis of fetal growth retardation (Tulenko and Millard, 1977).

Morphine may produce fetal bradycardia. The resultant decrease in umbilical blood flow is one potential mechanism for the observed growth retardation in the infants of mothers taking opiates or other central nervous system depressants.

### Placental Transfer

Placental transfer of oxygen and nutrients to the fetus and transfer of metabolic wastes from the fetus to the mother is clearly an important and influential factor in determining fetal growth. The mechanisms of placental exchange have been extensively reviewed (Chamberlain and Wilkinson, 1979; Novy, 1978; Suzuki, 1977).

#### Gas Exchange

It is generally accepted that oxygen is transferred across the placenta by passive diffusion and that the placental membrane does not provide significant diffusion resistance. The

transfer of oxygen across the placenta is primarily determined by the maternal and fetal placental blood flow and the higher affinity of fetal hemoglobin for oxygen. However, when the placental diffusing distance is increased or when the surface area for gas exchange is reduced, the diffusing capacity may not be adequate for optimum $O_2$ supply and to maintain normal fetal growth. The diffusion distance may be increased by fibrin deposition in the intervillous spaces and the surface area of exchange reduced by placental infarction. Both these changes may be seen in preeclamptic pregnancy.

### Carbohydrate Transfer

Maternal glucose is the major metabolic fuel of the human fetus, and placental transfer is via a facilitated diffusion mechanism. The major determinants of the amount of glucose reaching the fetus are the maternofetal glucose gradient, the maternal blood flow, and the placental volume. Any factor chronically affecting glucose transfer can be expected to adversely affect fetal growth. In experimental fetal growth retardation due to reduced uterine blood flow, there is a reduction in glucose transfer, but enhanced extraction by the fetal liver of glucose from the fetal circulation (Nitzan et al., 1979).

### Amino Acid Transfer

Most fetal proteins are synthetized from amino acids transported across the placenta via carrier-mediated active transport mechanisms against concentration gradients. The major influences on this transfer are maternal blood flow and adequate maternal nutrition. Placental acetylcholine facilitates placental amino acid uptake, and the adverse effects of both morphine and nicotine on fetal growth could be mediated in part by interference with this cholinergic mechanism (Rowell and Sastry, 1978).

## Chorionic Somatomammotropin

Chorionic somatomammotropin (CS), or placental lactogen, is a polypeptide hormone structurally similar to pituitary growth hormone and prolactin. It is synthetized by the syncytiotrophoblast and in late gestation is secreted primarily into the maternal circulation.

Grumbach et al. (1968) postulated that human CS (HCS) has major metabolic effects on maternal metabolism, ensuring that the nutritional demands of the fetus are met. The potent lipolytic and diabetogenic activity of HCS causes a rise in free fatty acids and decreased sensitivity to insulin when it is administered to nonpregnant women (MacMillan, 1979). These actions mobilize maternal fatty acids for maternal use, thereby sparing maternal glucose for fetal use. Human CS may also increase placental transfer of amino acids by restricting maternal utilization of proteins. There are, however, species differences in the biological activities of CS. For example, ovine CS (OCS) has somatotrophic properties but does not possess lipolytic and diabetogenic activities (Brinsmead et al., 1980a).

Maternal HCS concentrations increase with gestational age in parallel with the growth of the placenta. Consequently, HCS concentrations have been used as an index of placental function. Generally, lower maternal HCS values have been found in pregnancies associated with intrauterine growth retardation. The regulation of HCS secretion is poorly understood. There is limited evidence to suggest that glucose, amino acids, and fetal pituitary hormones may influence HCS release.

The concentrations of HCS in neonatal umbilical cord blood are much lower than in the mother. However, HCS concentrations of up to 200 ng/nl have been measured in

human cord blood, which raises the possibility of a function in the fetus as well as in the mother. In the sheep, plasma OCS concentrations are higher in the fetus than in the mother prior to 100 days gestation (Gluckman et al., 1979a).

While HCS is structurally similar to human growth hormone, it has significantly less somatotrophic activity. Nevertheless, high doses of HCS are reported to stimulate growth in hypopituitary dwarfs. There is a correlation between maternal CS and somatomedin concentrations in both the pregnant human (Furlanetto et al., 1978) and the pregnant ewe (Gluckman et al., 1979b). In the pregnant rat, direct evidence of the role of rat CS in stimulating maternal somatomedin secretion during pregnancy has been reported (Daughaday and Kapadia, 1978; Daughaday et al., 1979). Although CS may stimulate fetal somatomedin generation and thus have a direct effect on fetal growth, there is no evidence to support this. Any role for HCS in fetal growth is indirect and maternally mediated. Recent evidence of normal fetal development in a pregnancy in which no HCS was detectable in the maternal circulation (Nielsen et al., 1979) suggests that HCS does not play an essential role in the regulation of fetal growth.

## FETAL FACTORS AFFECTING FETAL GROWTH

### Hormones

Peptide hormones including insulin and somatomedin do not cross the placental barrier. Thyroxine and cortisol can pass from the maternal to the fetal circulation in the mother, but the passage is impeded to the extent that even elevated maternal levels do not significantly alter fetal levels. The passage of cortisol is impeded by a special mechanism, while other steroid hormones can pass freely. The human placenta is rich in the enzyme 11$\beta$-hydroxydehydrogenase, which converts cortisol to its inactive form, cortisone. The fetus, unlike the adult, has little ability to reconvert cortisone to cortisol.

The elaborate precautions to limit transfer of hormones from mother to fetus are of importance to the orderly development of the fetus, for upon them depends the fetus' endocrine autonomy. If maternal hormones could freely enter the fetus, hormone levels in the fetus would be determined by the vagaries of maternal endocrine behavior rather than by the needs of growth and development. Unfortunately for the fetus, these protective mechanisms cannot withstand the disturbances associated with certain maternal diseases. The fetus of the thyrotoxic mother may be thyrotoxic, not because of excessive passage of thyroxine from the maternal circulation, but because of passage of immunoglobulins such as long-acting thyroid stimulator. Similarly, fetal pancreatic function is altered in maternal diabetes, not by any change in insulin transport, but by fetal hyperglycemia secondary to maternal hyperglycemia.

Similarly, failure of maternal hormones to enter the fetus does not exclude a function for them in fetal growth. Indirectly, by maintaining blood levels of glucose and other nutrients in the maternal circulation, they contribute to the drive to fetal growth. Hormones such as catecholamines, angiotensin II, aldosterone, and prostaglandins present in the maternal circulation of tissues maintain maternal cardiovascular homeostasis, upon which adequate perfusion of the placenta depends.

An understanding of the role of fetal hormones in growth cannot be obtained simply by extrapolating backward from a knowledge of growth in the postnatal period. While it is true that birth represents only a milestone in a continuing process of growth and development, there are certain peculiarities of the intrauterine existence that call for

controlling mechanisms that differ from those of the growing child. The weight of evidence suggests that fetal growth in man is not influenced strongly by growth hormone or thyroxine, two of the major endocrine influences on postnatal growth. Reference has been made already to the likelihood that fetal growth, even in optimal circumstances, is constrained to a considerable degree. The healthy, well-nourished child, on the other hand, probably achieves his full potential for growth. In a subsequent section it will be pointed out that intrauterine existence is a period of life characterized by the beginnings of developmental events, and activation of a variety of "time clocks" which form an important component of ordered growth. By contrast, growth of the child is based on physiological systems already active at birth; with the important exception of puberty, activation of new systems is not needed. The diet before and after birth differs in ways that would be expected to be associated with an altered profile of hormones; apart from a requirement of essential fatty acids for the synthesis of lipids, the fetus obtains little lipid in its diet; calories available to the child from dietary fat are replaced by a greater utilization of glucose in the fetus (Battaglia and Meschia, 1973).

Endocrine influences on fetal growth can be mediated by many potential mechanisms. Hormones may affect the rate of cell division, the transport of glucose into the cell to meet energy requirements, or the transport of amino acids into the cell to meet anabolic requirements. They may affect placental transfer either by affecting transport mechanisms or placental perfusion. In addition, they may be triggers for the differentiation of tissues at specific points in development.

## Insulin

A major fetal hormone exerting an effect on fetal growth is insulin. Insulin does not cross the placenta and is present in the human fetal pancreas and circulation by 10 weeks gestation. The factors regulating fetal insulin secretion have been reviewed in Chapter 4. Evidence for the important role of fetal insulin in the regulation of fetal growth has been obtained from clinical and experimental studies of fetal hyper- and hypoinsulinemia.

*Fetal Hypoinsulinemia* Congenital absence of the pancreas is a rare condition, and is associated with a marked reduction in birth weight and length (the birth weight at term has ranged from 1200-2000 g) (Lemons et al., 1979). Transient diabetes mellitus in the neonate is frequently associated with intrauterine growth retardation. Leprechaunism is a syndrome characterized by severe intrauterine growth retardation, postnatal growth failure, and abnormal facies, calling up the image of a leprechaun. The birth weights and lengths of these infants are comparable to those with pancreatic agenesis. These infants are resistant to exogenous insulin, and recent evidence suggests that the basic defect is a postreceptor intracellular refractoriness to insulin action (D'Ercole et al., 1979; Hill et al., 1980). In a series of intrauterine growth-retarded infants born to mothers who had no cardiovascular or endocrine abnormality, a reduction of fetal endocrine pancreatic tissue and of $\beta$-cell numbers has been reported (Van Assche and Aerts, 1979; Van Assche et al., 1977).

Experimental hypoinsulinism has been induced in rabbit and monkey fetuses by chemical ablation of the pancreas, and these studies support the view that fetal insulin deficiency leads to growth retardation (Hill et al., 1972; Harding et al., 1975).

*Fetal Hyperinsulinemia* Fetal injections of insulin in the rat cause an increase in both birth weight and total lipid content of the carcass (Picon, 1967). Continuous subcutaneous infusion of insulin into fetal rhesus monkeys for 3 weeks commencing at

113-126 days caused fetal hyperinsulinemia, but no change in fetal plasma glucose. Fetal weight was increased with relative organomegaly of the placenta, liver, and heart (Susa et al., 1979).

Many infants of diabetic mothers (IDMs) are large for gestational age. The exceptions are those whose mothers have well-controlled diabetes and no vascular complications. These infants are generally of normal size. The infants of mothers with severe diabetes, vascular disease, and associated hypertension are growth retarded. Uterine blood flow in these mothers is frequently compromised and there is widespread microangiopathy of the spiral arterioles of the placenta.

The fetal obesity and hypersomia of the IDM is generally accepted as a consequence of fetal hyperglycemia leading to fetal hyperinsulinemia (Pederson, 1954; see Chapter 7). These IDMs show increased plasma insulin concentrations as well as an increased insulin content in the pancreatic islets (Van Assche and Aerts, 1979).

Infants with insulinomas or nesidioblastosis ($\beta$-cell hyperplasia) also demonstrate hypersomatism. Wiedemann-Beckwith syndrome is an unusual syndrome of fetal overgrowth characteized by increased birth weight and length, macroglosia, omphalocele, and hypoglycemia. There is generalized organomegaly. Although there is no $\beta$-cell hyperplasia, recent evidence suggests that there are increased numbers of insulin receptors in this syndrome, and it is postulated that the fetal overgrowth is due to end-organ hypersensitivity to circulating insulin (Herzberg et al., 1979).

*The Action of Insulin on Fetal Growth* The concentration of insulin receptors in circulating monocytes and erythrocytes is increased in neonates, particularly premature infants, compared to during later life (Hill et al., 1980). Insulin binding to fetal liver increases during gestation. Recent studies suggest that hyperinsulinemia in the neonate does not down-regulate the number of insulin receptors as normally occurs in adults (Neufield et al., 1979; Greenburg and Howell, 1980). The insulin receptor also binds somatomedin, and insulin also binds to the somatomedin receptor. Recent data suggest that the metabolic effects of both insulin and somatomedins may be mediated by the insulin receptor and that the growth-promoting action of both insulin and somatomedin may be mediated by somatomedin receptors (King et al., 1980b). The action of insulin to promote cell replication may therefore be mediated via the somatomedin receptor. The metabolic actions of insulin in enhancing glucose and amino acid transport into cells, which are mediated by the insulin receptor, may also be important in stimulating fetal growth.

Additional interactions between insulin and somatomedin provide further mechanisms by which insulin could influence fetal growth. Insulin increases somatomedin secretion by the perfused rat liver (Daughaday et al., 1976). Insulin at physiological concentrations increases the affinity of somatomedin-like peptides to the somatomedin receptor. This enhancement is mediated by the action of insulin at the insulin receptor (King et al., 1980a).

Insulin and somatomedin receptors are present in the placenta. Their function is unknown, but they may promote placental growth or the transfer of nutrients across the placenta.

### Somatomedins

The somatomedins (SMs) are a family of peptides (7500 daltons) which are believed to mediate the action of growth hormone on postnatal somatic growth. They are structurally related to proinsulin and have insulin-like metabolic actions. They remain

somewhat poorly defined because of the difficulties of purification and assay. Several different peptides, somatomedin A (SMA), somatomedin C (SMC), insulin-like growth factors I and II (IGF 1 and IGF 2), and multiplication stimulation activity (MSA) are considered members of this family. Recent evidence suggests that SMC and IGF 1 may be identical and that IGF 2 and MSA are analogous.

The liver appears to be the major source of somatomedin production; SMA, SMC, IGF 1, and IGF 2 have been purified from human plasma extracts, and MSA from cultured rat liver cells. The somatomedins circulate tightly bound to a specific binding protein, which itself is growth hormone (GH) dependent. While GH is the major postnatal stimulus to SM production, clinical and experimental evidence suggests that prolactin, chorionic somatomammotropin, and insulin may also stimulate SM secretion under certain conditions. It is becoming clear that the apparent unimportance of GH in fetal growth should not be construed as evidence of similar unimportance of somatomedins. In the fetus, the definition of a somatomedin as a peptide mediating the action of GH will probably have to be amended. Somatomedins stimulate cell mitosis. Recent evidence demonstrates that SM influences the cell reproduction cycle, stimulating passage from the resting phase to the phase of DNA synthesis (Figure 2) (D'Ercole et al., 1980a; Stiles et al., 1979; Rothstein et al., 1980). In addition, somatomedins have insulin-like properties and stimulate cellular glucose and amino acid uptake.

*Somatomedin and Fetal Growth: Clinical Evidence* Measurement of somatomedin concentrations in human umbilical cord blood have generally shown positive correlations between SM concentrations and gestational age, although the concentrations are less than in adults. Several studies have demonstrated correlations between birth size and cord SM. This correlation persists after correction for gestational age. Small-for-dates infants had lower cord SM concentrations than normal-sized or large-for-dates neonates in several studies where different SM assay systems were used (Gluckman and Brinsmead, 1976; D'Ercole et al., 1976; Ashton and Vessey, 1978; Heinrich et al., 1979; Foley et al., 1980).

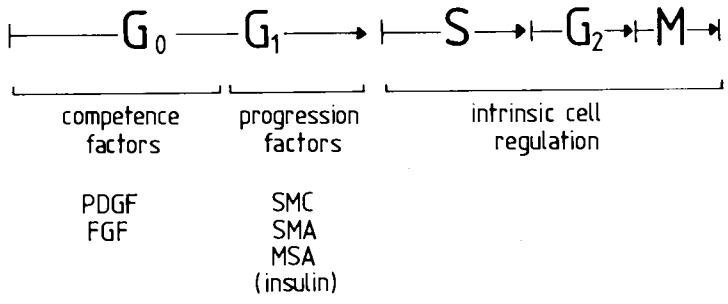


**Figure 2** Model of sequential events in the progression from the resting ($G_0G_1$) phase to the synthesis phase (S) to the mitotic phase (M) of DNA synthesis and cell replication. Competence factors and progression factors are sequentially required to initiate DNA synthesis. Platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) are "competence factors," and the somatomedins SMC, MSA, and SMA are "progression factors." Insulin has weak progression activity and this might be mediated by its affinity for the somatomedin receptor. The subsequent phases of DNA replication appear to be intrinsically regulated by the cell. (Derived from Stiles et al., 1979.)

Laron dwarfism is a form of short stature associated with high circulating GH concentrations but low serum SM concentrations, and is generally accepted as due to a genetic failure of GH to stimulate SM production. The majority of these infants have reduced birth weight and birth length (Brinsmead and Liggins, 1979b). The reduction in birth weight is slight, with a relatively greater reduction in birth length. These small reductions might suggest that SM only has a relatively small influence on fetal growth. However, the degree of intrauterine SM deficiency is unknown, particularly in light of recent evidence of multiple sites of fetal SM production (D'Ercole et al., 1980a) and cord blood SM estimations in Laron dwarfism have not been made.

*The Regulation of SM Secretion in the Fetus* Somatomedin concentrations in the human fetus are lower than those in the adult. The ontogeny of the SM-binding protein is uncertain and this may be a determinant of serum SM values. In postnatal life, the SM-binding protein is itself growth hormone dependent. In one anencephalic fetus, the binding protein was absent at term, suggesting GH dependency of SM-binding protein in utero (D'Ercole et al., 1980b).

Somatomedin concentrations in the cord blood of two anencephalic fetuses were normal (Gluckman and Brinsmead, 1976; Foley et al., 1980), suggesting that the fetal pituitary is not essential for fetal SM secretion. In the experimental animal conflicting results have been obtained. Following decapitation of the fetal rabbit, plasma SM activity did not change (Hill et al., 1979). In the sheep, fetal hypophysectomy caused a reduction in fetal SM concentrations in one study (Falconer et al., 1979), but not in a second (Brinsmead and Liggins, 1979a). Hypophysectomy of the pregnant rat in the mid-gestation does not lead to a fall in maternal plasma SM concentrations until after delivery, when they fall to hypopituitary concentrations (Daughaday et al., 1978, 1979). These data suggest that a placental factor, probably chorionic somatomammotropin (CS) maintains maternal SM generation in the absence of growth hormone. Similarly, in sheep and man, maternal SM concentrations parallel CS concentrations. Although ovine CS stimulates SM production in hypophysectomized adult rats, there is no correlation between fetal OCS and SM concentrations in the sheep (Gluckman et al., 1979a), and no direct evidence of a role for CS in regulating SM in the fetus is available.

Nutrition may have important effects on fetal SM generation. Reduction in placental mass in the sheep is associated with lower fetal SM concentrations (Falconer et al., 1979). Evidence in the neonatal rat suggests the importance of nutritional factors in regulating SM secretion; malnourishment is associated with lower SM concentrations (Sara et al., 1979).

The interactions between insulin and somatomedin have been discussed in a previous section. Insulin stimulates hepatic somatomedin production, increases the affinity of the somatomedin receptor for SM peptides, and may exert its pleotrophic effect via the SM receptor.

*Source of SM in the Fetus*

Somatomedin C does not cross the placenta (D'Ercole et al., 1980a). Fetal rat liver cultures produce MSA (Rechler et al., 1979). Recently, many tissues of the fetal mouse have been found to secrete SMC in organ explants; the liver, lung, kidney, intestine, heart, and brain but not the placenta all appear to produce SMC de novo. The liver is, however, the major site of production and production is maximal in late gestation and decreases after birth (D'Ercole et al., 1980a).

*Somatomedin Receptors in the Fetus* Human fetal chondrocytes are responsive to SM. Somatomedin receptors on circulating monocytes are present in increased numbers in

human cord blood, suggesting that there are increased numbers of SM receptors in the fetus (Rosenfled et al., 1979). Somatomedin receptors are present in many tissues obtained from the fetal pig, but do not change with gestation, except in the lung and placenta, where the affinity is higher in late gestation (D'Ercole et al., 1976). The high concentration of SM receptors in the placenta raises the possibility that SM may affect the transfer of nutrients across the placenta or affect placental growth. Decreased binding of SM to fetal hepatic membranes has been reported in growth retardation secondary to maternal alcoholism (Guyda et al., 1980).

*Evidence for a Distinct Fetal Somatomedin* Limited evidence suggests that the major form of SM in the fetus is distinct from that in postnatal life. This fetal SM, at least in the rodent, appears to be an MSA-like peptide. Multiplication stimulation activity is a group of peptides originally purified from cultured rat liver cells and has similar activities to SMC in its ability to stimulate cells to commence DNA synthesis, the first phase of cell replication (Figure 2) (Stiles et al., 1979). While it is less potent than the other somatomedins in its ability to stimulate sulfate incorporation into cartilage, it has insulin-like activity on fat cells, competes for the SM receptor, and has partial immunoreactive cross-reactivity with SMC/IGF 1, SMA, and IGF 2. It binds to the same SM-binding protein as the other somatomedins (Nissley and Rechler, 1980).

The fetal rat liver in late gestation synthetizes MSA peptides (Rechler et al., 1979), while MSA concentrations as measured by specific radioimmunoassay are high in fetal rat sera and decline to low levels in the 3 weeks after birth (Nissley and Rechler, 1980), whereas SM as measured by SMA radioimmunoassay (RIA) or sulfate bioassay is very low in fetal sera and increases 2 weeks after birth (Sara et al., 1980a; Stuart et al., 1976). These data suggest that MSA-like peptides are the dominant forms of SM in the early growth period, and that other SM peptides influence postnatal growth (see Figure 3). Similarly in the mouse, SM activity measured by a specific SMC radioimmunoassay is lower than that measured by a less specific receptor assay system (D'Ercole and Underwood, 1980).

Indirect evidence in man suggests the presence of a distinct fetal form of SM. Parallel measurements of SM activity by using the traditional sulfate incorporation bioassay and by thymidine incorporation into cartilage segments revealed discrepancies in human cord blood, whereas no discrepancy is generally found in other situations. The sulfate assay measured lower concentrations of SM than in adults, the activity increased with gestational age, and the values were in general agreement with other studies using sulfate bioassays and IGF 1, SMA, and SMC ligand assays. However, the activity as measured by thymidine bioassay was significantly higher in both term and preterm infants, and did not increase with gestational age (Foley et al., 1980). These data suggest that the two SM bioassay systems measure different activities in fetal blood, although in postnatal life they measure comparable activities.

The higher activity measured by the thymidine assay may be due to fetal mitogenic factors having little sulfation activity which are peculiar to the fetus. It is of interest that MSA has marked activity in the thymidine system but relatively little in the sulfation system and the fetal SM may be an MSA-like peptide. In measurements of human cord blood using an MSA receptor assay system, no correlation was found with sulfate bioassay measurements. In contrast to other estimations of fetal SM activity, MSA receptor assay measurements correlated with fetal plasma GH concentrations and decreased in late gestation (Brinsmead and Liggins, 1979c). This suggests that the MSA receptor assay may be primarily detecting a different peptide from the sulfate bioassay in fetal blood.

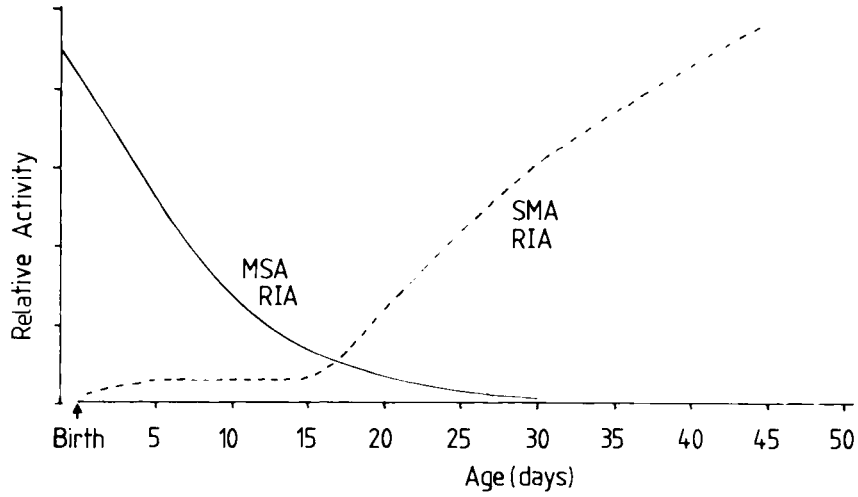


Figure 3 Age dependence pattern of MSA concentrations measured by radioimmunoassay (—) and reactivity in the SMA radioimmunoassay (– – –) in rat serum. The results within each assay are expressed on a relative linear scale. These data suggest the presence of different SM-like peptides in fetal blood to those in the blood of postnatal rats. (Modified from Nissley and Rechler, 1980.)

Using fetal tissues as a target to detect fetal forms of SM, Sara et al. (1980b) found higher concentrations of SM in human and rat fetal serum compared to radioimmunoassayable SMA. This "fetal" SM activity falls during gestation, while "adult" SM activity rises late in gestation.

Recently it has been suggested that the major SM-binding protein in serum in human fetuses prior to 30 weeks is smaller (40000 daltons) than that in older fetuses and adults (150,000 daltons), and similar observations were made in the mouse fetus (D'Ercole et al., 1980b; D'Ercole and Underwood, 1980). Thus the SM-bonding protein also appears to have a distinct fetal form. Its relationship to the adult form remains to be elucidated.

These data suggest that the major form of SM in the fetus is distinct from the forms generally measured in most assay systems. The regulation of its secretion is not known, and the relative importance of "fetal" SM and "adult" SM in influencing fetal growth remains to be investigated.

### Growth Hormone

While the essential role of growth hormone (GH) in promoting somatic growth in childhood is well established, the weight of evidence suggests that GH does not affect fetal somatic growth. Plasma GH concentrations are elevated in mid gestation and decrease in late gestation, but even in cord blood and in the first neonatal week are higher than during later postnatal life. Congenital GH deficiency may occur on a genetic basis or be associated with agenesis of the pituitary gland, or be due to functional abnormality of the hypothalamus. In each of these conditions, birth size is normal and growth retardation is generally not observed until after the sixth postnatal month (Gluckman et al., 1980). In anencephaly the hypothalamus is generally absent and the adenohypophysis is small. Plasma GH concentrations in anencephaly are reduced but not always totally deficient (perhaps reflecting the variable presence of the diencephalic remnant). Growth retardation is not a striking feature of anencephaly, and the small reduction in birth

weight may reflect other associated abnormalities, generalized effects on cell growth of the teratogenic influence, or reduced placental size.

Experimental evidence in a variety of species, including monkeys, sheep, pigs, rabbits, mice, and rats, supports the view that GH does not play a role in regulating fetal somatic growth. In those species such as the monkey or sheep, where fetal hypophysectomy, hypophyseal stalk section, or decapitation to induce fetal GH deficiency is associated with growth retardation, the retardation can be ascribed to the associated hypothyroidism. Where GH deficiency has been induced experimentally without hypothyroidism, fetal growth appears to be normal. Similarly, in mice, rats, and rabbits, where fetal growth is not dependent on the fetal thyroid, these manipulations do not cause fetal growth retardation (Gluckman et al., 1980; Cheek and Hill, 1974; Jost et al., 1974; Jost, 1977; Liggins, 1974).

The reason for the lack of a growth-promoting role for GH in the fetus remains to be elucidated. It may reflect immaturity of GH receptors in fetal tissues. Preliminary evidence of decreased GH binding to fetal hepatic membranes has been observed in the rat, rabbit (Kelly et al., 1974, 1976), and sheep (Gluckman, 1981). Growth hormone may, however, have some actions on fetal carbohydrate metabolism. In fetuses of diabetic mothers, islet $\beta$-cell hyperplasia and hyperinsulin are observed. However, in anencephalic infants of diabetic mothers these changes do not occur, suggesting that GH may have a permissive role of increasing the glucose sensitivity of the pancreatic $\beta$ cell (Hoet, 1969; see Chapter 4).

Glycogen deposition in the fetal liver increases in late gestation and experimental data in the rabbit suggests that this may be GH dependent (Jost et al., 1979). The hypoglycemia observed in the GH-deficient neonate may reflect impaired hepatic glycogen deposition.

### Thyroid Hormones

There are marked species differences in the growth-promoting function of thyroid hormones in the fetus. While fetal hypothyroidism is associated with marked growth retardation in sheep (Thorburn, 1974) and monkeys (Kerr et al., 1972), this is not so in rats or man. Hypothyroid infants are generally of normal birth weight and birth length (Anderson, 1961; Maenpaa, 1972). While thyroid hormones appear to have little effect on birth size, skeletal maturation is delayed.

The reasons for these species differences is not clear. The thyroidectomized fetuses of rats and rabbits fail to show growth retardation, but this can be attributed to the short period of time from thyroidectomy to birth, on one hand, and the long-lived biological effects of thyroid hormones, on the other. The sharp discrepancy between the effects of absence of the fetal thyroid gland in man and in sheep or monkeys is unlikely to reflect fundamental differences in the function of thyroid hormones between species.

It has been suggested that the observed species differences may be due to the extent to which thyroid hormones cross the placenta. It appears that the ovine placenta is almost entirely impermeable to thyroid hormones. Similarly, administration of thyroxine to the pregnant rhesus monkey does not increase fetal plasma thyroxine concentrations (Fisher et al., 1977). It remains uncertain whether clinically significant amounts of thyroxine or triodothyronine cross the human placenta from mother to fetus. While at high levels some transfer may occur, fetal goiter is not necessarily prevented by high maternal doses of thyroxine. However, the amount of

thyroxine needed to permit normal fetal growth may be less than that necessary to suppress TSH secretion and goiter formation.

While thyroxine only has minimal effects on somatic growth, it has a critical role in the growth and functional development of the brain. This will be discussed in a subsequent section.

### α-Melanocyte-Stimulating Hormone

Aspiration of the fetal brain and pituitary in the rat is associated with fetal growth retardation. Growth could be restored by the administration of α-melanocyte-stimulating hormone (α-MSH) to the fetus, but not any other pituitary hormone. The administration of purified anti-α-MSH antiserum to fetal rats inhibits fetal growth. Brain weight was also reduced. These effects on fetal growth could not be reproduced postnatally (Swaab et al., 1978). α-Melanocyte-stimulating hormone is a peptide structurally identical to $ACTH_{1\text{-}13}$, and is present in the intermediate and anterior lobe of the pituitary of the human fetus and pregnant woman (Silman et al., 1976). These intriguing observations have not been investigated in other species and the mechanism of the growth-promoting action of α-MSH in the rat fetus remains unknown.

### Prolactin

Prolactin concentrations in the rat, sheep, monkey, and human fetus rise in late gestation and fall after birth. It is postulated that the high circulating concentration of estradiol in the late-gestation fetus is the major stimulus to fetal prolactin secretion (Gluckman et al., 1980). Prolactin promotes growth in the larval amphibian and neonatal rat (Nicoll, 1978), but there is no evidence to suggest a role for it in regulating mammalian fetal growth. In both humans and rats, suppression of fetal prolactin secretion by the administration of the dopamine agonist bromocriptine to the mother throughout pregnancy has no effect on fetal growth (Bigazzi et al., 1979; Reusens et al., 1979).

## The Kidney and Fetal Growth

The syndrome of renal agenesis associated with abnormal facies and lung hypoplasia (Potter's syndrome) is associated with marked intrauterine growth retardation. While this growth failure is not necessarily due to the agenesis of fetal kidneys, experimental evidence suggests that absence of the fetal kidneys is associated with marked fetal growth retardation. Nephrectomy of the fetal lamb in mid-gestation is associated with marked intrauterine growth retardation associated with some retardation of osseous maturation (Thorburn, 1974). Hepatic glycogen and hematopoiesis is reduced and the volume of amniotic and allantoic fluids is markedly decreased. Fetal nephrectomy later in gestation is associated with reduced birth length, but not decreased birth weight (Brinsmead et al., 1980b). Brain weight is reduced, but placental weight is generally normal. Plasma fatty acid, glucose, growth hormone, thyroid hormone, and insulin concentrations are normal.

The mechanism of fetal growth failure associated with renal agenesis is not known. There are conflicting reports of low and elevated SM concentrations in these fetuses. Similarly, conflicting results have been obtained in postnatal renal failure and may reflect inhibitory substances in the circulation affecting the assay systems or alterations in plasma SM binding protein concentrations (Brinsmead et al., 1980b; Falconer et al., 1979).

The somatomedin concentration in cord blood of a child with Potter's syndrome was normal (Gluckman and Brinsmead, 1976).

Following fetal nephrectomy, fetal plasma renin activity is reduced (Oakes et al., 1975), and the consequent changes in angiotensin production may lead to changes in cardiovascular tone and placental exchange. Vitamin D metabolism may also be disturbed and this may explain the decreased osteoblastic activity and reduced skeletal maturation. The uncertain consequences of reduced amniotic fluid volume must also be considered.

## TISSUE AND ORGAN GROWTH

So far, the emphasis of this chapter has been on the somatic growth of the fetus. We must now consider organ growth, which is more complex because the growth and differentiation of each organ follows an individual time course. For example, endocrine organs are generally well differentiated by mid-gestation in the human fetus; in contrast the lung does not approach maturity until close to birth and the brain is not fully developed until some time after birth. The processes controlling organ development are poorly understood.

The first phase of organogenesis is cellular proliferation, which is followed by differentiation. The latter involves not only the loss of the ability to develop in alternative ways, but also the expression of distinctive morphological and functional characteristics. Other components in organogenesis are change in cell shape, the development of cell adhesion, and, in some tissues, the death of certain cell groups (for example, mammary cell death in the male embryo). The role of the extracellular matrix is critical in certain tissues such as bone and cartilage. Cellular hypertrophy is an important factor in those tissues in which the cells show a finite potential for cellular division, such as neurons and muscle (Goss, 1978; Bernfield, 1978).

The timing and pattern of organ development is primarily genetically determined. However, extrinsic factors, particularly endocrine and mechanical influences, may influence the growth of particular tissues.

### Tissue Growth Factors

Tissue-specific growth factors with important roles in the regulation of fetal organ growth have been described recently. These growth factors are peptides and may be formed either at sites distal to the target organ or in the target organ itself. The peptides have both a mitotic and pleiotrophic (stimulation of the rate of protein synthesis, increased incorporation of nucleic acid precursors into RNA and DNA) effect on their target organ.

#### Epidermal Growth Factor

Epidermal growth factor (EGF) was originally purified from mouse salivary glands and shown to stimulate the precocious opening of the eyelids and the eruption of teeth in newborn mice. It is a mitogen for epidermal cells and certain fibroblastic tissues. It has been found in man, and is also termed urogastrone because of an ability to heal peptic ulcers. The mouse embryo contains both EGF and EGF receptors and the number of receptors increases during gestation (Nexo et al., 1980).

The infusion of EGF into fetal lambs induces marked stimulation of epithelial growth in many tissues, including the pulmonary airways, and also stimulates maturation of

lung function (Sundell et al., 1975). Studies in the fetal rabbit demonstrate that the administration of EGF enhances the differentiation of type II alveolar cells, the source of surfactant. No effects on general somatic growth have been observed (Catterton et al., 1979). These studies suggest that EGF stimulates the proliferation of fetal epithelial tissues, particularly in the lung, and also stimulates alveolar cell differentiation without generalized effects on fetal development.

### Nerve Growth Factor

Nerve growth factor (NGF) was first characterized from extracts of mouse salivary glands, but has been found in many other tissues, at least in tissue cultures. It has been detected in the human placenta. It is a large protein complex (molecular weight, 140,000) consisting of an active $\beta$ subunit and regulatory $\gamma$ and $\alpha$ subunits (Harper and Thoenen, 1980).

The increase in size of sensory and sympathetic ganglia of chick embryos treated with NGF results from the enhanced survival of neurons that would otherwise degenerate. There may also be increased mitosis of glial cells. Neonatal sympathetic ganglia not only contain more NGF than adult ganglia, but they are also more responsive, causing the morphological transformation of sympathetic neuroblasts into differentiated neurons.

Some sympathetically innervated tissues synthesize NGF and it has been postulated that NGF released into surrounding tissue serves as a trophic factor for incoming axons. Nerve growth factor also stimulates the growth or regenerating noradrenergic neurons following brain lesions and hypertrophy of the adrenal medulla (Mobley et al., 1977). Thus NGF appears to be important in the maturation of adrenergic neurons and the sympathetic nervous system.

Nerve growth factor receptors are present in brain tissue. The administration of thyroxine to rats increases the concentration of NGF in the liver, submaxillary glands, cerebellum, cerebral cortex, and brainstem (Walker et al., 1979). The effects of NGF on axonal regeneration in the brain are similar to those of thyroid hormones. These studies suggest a mechanism by which thyroxine might exert its important effects on fetal brain development.

### Other Trophic Peptides

A number of other peptides have been described, but less is known of their potential function in fetal development.

Erythropoietin remains a poorly characterized peptide. The kidney is the main source in postnatal life, but the liver is a major source in the fetus (Zanjani et al., 1977). Erythropoietin stimulates hemoglobin synthesis by human fetal hematopoietic tissues; the sensitivity of this effect is maximum in mid-gestation (Barsch, 1972).

Fibroblast growth factor (FGF), which has been purified both from brain and pituitary tissue, is a mitogen for cells of endothelial and mesothelial origin. It has been shown to stimulate cell replication in vascular endothelium, cartilage, smooth muscle, adrenals, and ovaries. No data are available regarding FGF in the fetus. In some tissues FGF interacts with glucocorticoids and this may be important in the maturational effects of glucocorticoids on some tissues.

A platelet-derived growth factor has been described which stimulates the multiplication of glial and smooth muscle cells. Cells exposed to platelet-derived growth factor become "competent" to replicate their DNA and undergo mitosis. Fibroblast growth factor is also a competence factor. However, the cells will not progress into the "S"

phase unless also exposed to a "progression" factor (Figure 2). The somatomedins are progression factors (Stiles et al., 1979).

A growth hormone-dependent brain trophin has been detected in plasma which stimulates thymidine uptake into fetal rat neurons (Sara et al., 1976). This trophin has not been characterized and its significance to fetal brain growth is not known.

## Organ Growth

The relative importance of intrinsic, endocrine, and mechanical factors regulating growth varies from one organ to another. These principles will be demonstrated by reference to selected organs.

### Kidney

Experiments with renal transplantation suggest that renal growth is primarily regulated by intrinsic, genetically determined factors. When an infant kidney is transplanted into unilaterally or bilaterally nephrectomized adult hosts, the infant kidneys attain full adult size. These results could represent either expression of the intrinsic growth potential of the infant kidney or "compensatory growth" to meet the functional demands of the host. However, when a single infant rat kidney is transplanted into an intact infant host, all three kidneys achieve normal adult size. Similarly, when an adult kidney is transplanted into a unilaterally nephrectomized infant rat, the host's kidney will eventually achieve normal adult size while the transplanted adult kidney remains unchanged (Silber, 1974; 1976). These studies demonstrate that the kidney is programmed to attain adult size irrespective of somatic size or physiological demands (Gross, 1978).

### Lung

The lung seems to have minimal inherent potential for growth for, in the absence of appropriate stimuli, extreme hypoplasia is the rule. This striking contrast with the growth of the kidney is particularly interesting in view of the interaction between kidney and lung–hypoplasia of the kidneys is commonly associated with hypoplasia of the lung. The development of the lung is an excellent example of mechanical and endocrine factors combining in an orderly way to promote a complex pattern of growth and maturation.

The major stimulus to growth of the lung is distension, as is evident from the wide variety of congenital malformations associated with pulmonary hypoplasia. These malformations, which include diaphragmatic hernia, pleural effusion, diaphragmatic paralysis, oligohydramnios, and various neural disorders, have in common a reduction in thoracic volume and thus a limitation of lung distension. The distending force is not from within the respiratory passages, since continuous free drainage of tracheal fluid into a bag in the amniotic sac in fetal sheep does not hamper lung growth (Fewell and Kitterman, 1980). The force appears to be the intermittent negative pressures of fetal breathing, particularly the stronger inspiratory efforts. When the cervical spinal cord of fetal rabbits (Wigglesworth et al., 1977), or fetal sheep (Liggins and Kitterman, 1981) is divided above the outflow of the phrenic nerve, growth of the lungs is impaired, whereas it is unimpaired when the cordotomy is below the phrenic outflow. The difference between high and low cordotomy is that fetal breathing movements are absent and present, respectively. Lung growth is also impaired by merely reducing the amplitude of intrathoracic negative pressures without otherwise interfering with

breathing movements; this can be achieved by increasing the compliance of the chest wall by removing part of some ribs on each side of the chest (Liggins and Kitterman, 1981).

Function, as distinct from growth, of the lung is controlled by hormones. The degree of distensibility and stability of the alveoli necessary to sustain effective air breathing after birth is achieved late in gestation and is attributable largely to the surge of fetal adrenal activity that heralds the approach of term in all species, including man (Fencl et al., 1980). Other hormones, including thyroxine, estrogen, catecholamines, and prostaglandins, contribute to the stimulus to surfactant synthesis and release, to the increased elasticity of the alveolar wall, and to the cessation of alveolar secretion (Liggins and Kitterman, 1981).

### Endocrine Organs

The growth of the thyroid, adrenals, and gonads demonstrate the importance of endocrine stimulation in maintaining growth and functional development of these organs in the fetus. The anencephalic fetus is an experiment of nature in which the hypothalamus is generally absent and the adenohypophysis is hypotrophic. As a result of the lack of hypothalamic stimulation of the pituitary, growth hormone, gonadotropin, and ACTH concentrations in fetal circulation are very low (Gluckman et al., 1980).

In the anencephalic female fetus, the ovaries are small. There is a decreased number of primary follicles which are hypoplastic (Ch'in, 1938). Similar findings are observed in the hypophysectomized rhesus fetus (Gulyas et al., 1977). The male fetus with anencephaly or pituitary aplasia generally has hypoplastic external genitalia and undescended testes. These testes are hypoplastic with few Leydig cells present, and the epididymis is underdeveloped (Ch'in, 1938; Bearn, 1968). Similar observations have been observed in hypopituitary fetal pigs and monkeys (Colenbrander et al., 1979; Tseng et al., 1975). These studies suggest the importance of fetal pituitary gonadotropins in the development of the fetal gonads.

The adrenal gland of anencephalic fetuses is hypoplastic at birth. Prior to 20 weeks gestation, development is normal, but after this age the fetal zone in particular is atrophic, suggesting that fetal ACTH or some other fetal trophin is necessary for continued growth of the fetal zone after 20 weeks gestation (Benirschke, 1956). Similar findings are seen in experimental fetal hypopituitarism.

While the thyroid is of normal size in anencephaly, thyroid hypoplasia is observed in neonates with pituitary agenesis (Gluckman et al., 1980). These conflicting findings suggest that sufficient pituitary thyroid-stimulating hormone (TSH) is secreted in anencephaly to maintain fetal thyroid growth, but in the complete absence of TSH the thyroid cannot develop normally. In the experimental animal, fetal hypophysectomy is associated with thyroid hypoplasia. Further evidence for the role of TSH in stimulating fetal thyroid development is the observation of fetal goiter following maternal ingestion of goitrogens, such as propylthiouracil, which cross the placenta.

### Brain Growth

Despite greatest clinical concern, little is known of the factors regulating the growth and development of the brain. In most syndromes of intrauterine growth retardation, the brain is relatively spared. Brain development is not entirely intrinsically regulated, as hormones may have important effects on brain development.

The effects of thyroid hormones on the developing brain are well recognized from clinical studies of cretinism. Controversy remains as to the degree of the deficit remaining

Table 3 Experimental Models of Intrauterine Growth Retardation

| Basic mechanism of induction of IUGR | Experimental method | Species | Comment | References |
|---|---|---|---|---|
| Maternal mulnutrition | Caloric restriction (50%) | Rat | | Kava et al., 1979 |
| | Vitamin A deficiency | Rat | | Takahashi et al., 1975 |
| | Zinc deficiency | Rat | | Greeley et al., 1980 |
| | Protein/calorie restriction | Rat | | Van Marthens, 1977 |
| | Calorie restriction | Pig | | Anderson and Dunseth, 1978 |
| Environmental | Chronic exposure to hypoxic environment | Rat | Relative sparing of fetal brain and placental weights observed | Van Geijn et al., 1980 |
| | Hypoxemic environment | Guinea pig | | Gilbert et al., 1979 |
| | Maternal hyperthermia | Sheep | | Alexander and Williams, 1971 |
| | | | | Brown et al., 1977 |
| Reduction in uterine-placental blood flow | Ligation of uterine artery to one horn | Rat | Reduction in blood flow to one horn and consequent growth retardation in the fetuses in this horn | Wigglesworth, 1964<br>Brown and Vannucci, 1978<br>Nitzan et al., 1979a<br>Kollee et al., 1979 |
| | Ligation of spiral arterioles | Rabbit | Body, placental, and fetal brain weight reduced | Van Marthens et al., 1975 |
| | Microembolization via the uterine arteries | Sheep | Reduction in fetal $pO_2$ observed; one of the major methods of producing experimental IUGR | Creasy et al., 1972 |

| | | | | |
|---|---|---|---|---|
| Reduction in umbilical-placental blood flow | Ligation of a single umbilical artery | Sheep | Profound growth retardation was induced in this classic experiment | Emmanouilides et al., 1968 |
| Reduction in placental mass | Removal of uterine carunculae prior to pregnancy | Sheep | The carunculae are the site of cotyledonary implantation in sheep and removal leads to reduction in total placental weight | Alexander, 1964<br>Robinson et al., 1979 |
| | Removal of uterine horn prior to pregnancy | Sheep | Placental mass was reduced due to fewer cotyledons although the weight of those remaining was increased | Cefalo et al., 1977 |
| | Ligation of the vessels to secondary placental disk | Rhesus monkey | Ligation of the vessels led to effective destruction of the secondary placenta | Myers et al., 1971 |
| Endocrine manipulation of the fetus | Glucocorticoid administration to mother | Rats | Dexamethasone | Parvez et al., 1976 |
| | | Hamster | Hydrocortisone | Shah and Travill, 1976 |
| | | Mouse | Prednisone | Reinisch et al., 1978 |
| | Anti-α-MSH antiserum to fetus | Rat | | Swaab et al., 1978 |
| | Pancreatic ablation by administration streptozotocin to fetus | Rhesus monkey | Growth retardation observed in fetuses with pancreatic destruction; in those with islet hyperplasia, growth enhancement was seen | Hill et al., 1972 |
| | Pancreatic ablation by administration of alloxan to fetus | Rabbit | | Harding et al., 1975 |

(continued)

**Table 3** (continued)

| Basic mechanism of induction of IUGR | Experimental method | Species | Comment | References |
|---|---|---|---|---|
| Toxic | | | | |
| opiates | Methadone to mother | Rat | | McLaughlin et al., 1978 |
| | | Rat | | Hutchings et al., 1979 |
| | Morphine to mother | Rabbit | Effect not mediated by nutritional changes | Raye et al., 1977 |
| alcohol | Ethanol to mother | Mouse<br>Rat<br>Rabbit | | Schwetz et al., 1978 |
| | | Rat | | Tze and Lee, 1975 |
| | | Sheep | | Rose and Meis, 1979 |
| psychotropic | Chlorpromazine to mother | Rat | | Dailey, 1978 |
| | Diazepam to mother | Hamster | | Ramamurthy and Chaudhry, 1979 |
| smoking | Oral nicotine to mother | Mouse | | Sei and Matsumoto, 1976 |
| | Carbon monoxide plus hypoxia | Rat | | Garvey and Longo, 1978 |
| other | Salicylate to mother | Rat | | Butcher et al., 1972 |
| | | Rat | Placental size reduced | Lubawy and Garret, 1977 |

| | | | | |
|---|---|---|---|---|
| | Methotrexate to mother | Rat<br>Monkey | | Wilson et al., 1979 |
| | Cyclophosphamide to mother | Rat | | Scott, 1977 |
| | S-Fluorouracil to mother | Hamster | | Shah and MacKay, 1978 |
| | Arsenic, cadmium, lithium to mother | Rat | | Matsumoto et al., 1975 |
| | Cadmium to mother | Mouse | | Webster, 1978 |
| | Cadmium to mother | Rat | | Prigge, 1978 |
| | Lead to mother | Rat | Effect both on maternal nutrition and directly on fetus | Dilts and Ahokas, 1979 |
| | Tubocurarine to fetus | Rat | Both fetal and placental weight reduced | Shoro, 1977 |
| Miscellanous | Maternal nephrectomy | Rat | Probably mediated via maternal malnutrition | Nitzan et al., 1979a |
| | Fetal nephrectomy | Sheep | | Thorburn et al., 1970 |
| | Maternal exercise | Guinea pig | Probably mediated via reduced uterine blood flow | Gilbert et al., 1976 |
| | Maternal infection with Coxsackievirus B3 | Mouse | | Lansdown, 1975 |

if thyroxine therapy is commenced immediately at birth, but recent evidence suggests that, even with prompt replacement therapy, functional deficits persist (Wolter et al., 1979). Experimental and clinical data show that fetal hypothyroidism leads to retardation of structural, metabolic, and functional development of the neocortex, the cerebellum and the hippocampus in particular. The binding of thyroxine to brain tissue is enhanced in the fetus. Thyroxine stimulates the synthesis of protein in fetal but not adult brains (Macho et al., 1978), stimulates the synthesis of cerebral gangliosides (Horowitz and Schanberg, 1979), and stimulates neuronal RNA and DNA synthesis (Gomez et al., 1972; Ardeleanu and Sterescu, 1978). Hypothyroidism is associated with abnormalities of catecholamine and dopamine systems in the brain (Rastogi and Singhal, 1979). These effects of thyroxine may be mediated by NGF, as discussed in a previous section.

Glucocorticoids have similar effects to thyroxine on cerebral RNA synthesis (Ardeleanu and Sterescu, 1978) and on ganglioside formation. The effects of sex steroids on hypothalamic development are discussed in a subsequent section. Evidence is accumulating to suggest that biogenic amines including serotonin, histamine, and the catecholamines and acetylcholine are important in regulating brain development, and that drugs that interfere with these neurotransmitters may permanently affect the development of neurotransmitter systems and neurogenesis itself (Baker and Quay, 1969; Lanier et al., 1976).

### Organ Development in Intrauterine Growth Retardation

In the growth-retarded fetus, not all organs are equally affected. In IUGR secondary to interruption of the normal supply line to the human fetus, the organs most growth retarded relative to body weight are the liver, thymus, and lungs. The heart and particularly the brain are larger than would be expected relative to body weight. However, when compared to normal infants of the same gestational age, all organs are relatively small (Gruenwald, 1974). In general, similar observations have been made in experimental growth retardation.

## EXPERIMENTAL FETAL GROWTH RETARDATION

A wide variety of models have been developed which produce experimental growth retardation. Many of these experiments have been referred to in the preceding sections. Many of the experimental models that have been used are summarized in Table 3. It can be seen that generally IUGR has been induced by interruption with the normal supply line of nutrients to the fetus, either by maternal malnutrition, reduction in placental mass, or reduction in uterine blood flow.

## INTERACTIONS BETWEEN HORMONES AND THE GENOME

A distinction can be drawn between those developmental clocks in which the timing mechanism is activated by the genome itself and those in which the genome responds to a stimulus from hormones secreted by organs distant from the cells containing the activated enzymes. The former type of clock has already been considered in the earlier section on genetic control of growth and development; the latter type deserves separate consideration because it is potentially amenable to pharmacological modification, unlike the genetic clock which is relatively immutable. Several examples will be described.

### Sexual Differentiation of the Hypothalamus

Sexual dimorphism of hypothalamic morphology and function with respect to the control of gonadotropin secretion is well recognized as a basic difference between males and females (Toran-Allerand, 1978). Experimental evidence shows that this dimorphism is dependent on exposure of the developing hypothalamus to androgens at a "critical period" of ontogenesis.

The anatomical and functional differences between the male and female hypothalamus vary among species. In the rat there are sex differences in the size of hypothalamic nuclei in the preoptic area, and in the neuronal, synaptic, and dendritic architecture in the hypothalamus and amygdala. Major functional differences include the presence of both tonic and cyclic regulation of luteinizing hormone secretion in the female, but only tonic secretion in the male and the postpubertal development of masculine behavior patterns in the male. The timing of the "critical period" for the effects of androgens on hypothalamic development relates in part to the appearance of sex steroid receptors in the developing hypothalamus. In the rat the critical period has been defined as from the eighteenth fetal day to the fifth postnatal day, and in the sheep from 20 to 60 days gestation (see Chapter 2). The evidence both in humans and subhuman primates is less certain; there is certainly sexual dimorphism of hypothalamic control of luteinizing hormone in man and it is presumed that a "critical period" exists in early or mid-fetal life.

In the absence of exposure to androgens, organization of the hypothalamus will proceed along a female pattern. If exposure to androgens occurs in the "critical period," hypothalamic differentiation will follow a male pattern, irrespective of whether the organism is genetically male or female. Thus hypothalamic differentiation depends on an interaction between the genome and an "endocrine clock." Both the male and female hypothalamus develop in a functionally similar way in the absence of androgen exposure. Both are capable of responding to androgens in a similar manner if exposed to them in this critical period. It is the endocrine environment that therefore determines whether the male hypothalamic form and function develop. This is an example of the second type of developmental clock, in which the genome is responding to a stimulus external to the organ in question.

The internal genitalia provides a further example of hormonally directed differentiation. Studies of various forms of pseudohermaphrodites and the experimental studies of Jost show that the potential for Woolfian duct development exists in both sexes; whether the ducts develop or not is dependent on exposure to androgens at a critical period of development (see Chapter 2).

### Hemoglobin Switching

During the perinatal period there is a change in the form of hemoglobin produced, from fetal (HbF) to adult hemoglobin (HbA). Earlier in fetal life in man and some other species an embryonic hemoglobin is the dominant hemoglobin produced (Nienhuis and Stomatoyannopoulos, 1978). These different forms of hemoglobin differ in the nature of the globin chain and the switching from embryonic to fetal to adult hemoglobin production is a consequence of the activation and suppression of different portions of the genome. While the control of this switching process at different developmental stages is poorly understood, limited evidence suggests that hormonal interactions with the genome may be an important factor. Hypophysectomy of the ovine fetus, while not

affecting the timing of the onset of HbA production, does lead to a slower production of HbA (Wood et al., 1976). In several species, including man, thyroid hormones stimulate HbF synthesis; in the mouse this effect is demonstrable only within a narrow portion of gestation (Fuhr and Dunn, 1978, 1979). Testosterone also stimulates HbF production, and again only during a specific gestational period (Congote and Solomon, 1978). Testosterone increases and estradiol reduces the ratio of HbA to HbF produced by human fetal liver in vitro (Congote et al., 1977).

These studies, and in particular the observations that certain hormones only exert an effect over a specific gestational period, suggest that an interaction between fetal hormones and the fetal genome may control the expression of different portions of the genome coding for globin chains at successive stages of development.

### The Development of Enzymes Within the Fetal Liver

A further example of the regulation of gene expression by an endocrine clock is the timing of the development of enzymes in the fetal liver. Many of the enzymatic functions of the adult liver are not present or present only at very low levels even in the late-gestation fetus. Soon after birth clusters of enzymes either appear or disappear during a series of critical periods. These changes can be correlated with the physiological requirements of the fetus and neonate. The enzyme uridine diphosphate glucuronyltransferase, necessary for the detoxification of bilirubin, appears soon after birth. On the other hand, thymidine kinase, an enzyme necessary for DNA synthesis and thus for cell replication, is present in higher activity in mid-gestation than at term (Greengard, 1978).

Experimental evidence largely derived from the rat demonstrates that these changes in fetal liver enzyme activity can be influenced by endocrine manipulation. For example, cortisol injected into the rat fetus in late gestation will induce the enzymes necessary for hepatic glycogen synthesis and suppress enzymes involved in growth such as thymidine kinase (Greengard, 1978). Other hormones, including glucagon and thyroxine, have also been shown to affect the development of certain liver enzymes.

### Glucocorticoid-Induced Events in Late Gestation

Reference has already been made to the sharp increase in adrenocortical activity that precedes birth in all species so far investigated, and to the relationship of this surge of corticosteroids to maturation of the lung. It remains to be pointed out that the fetal adrenal has a key role in the successful transition from intrauterine to extrauterine life (Liggins et al., 1979). Not only the lung but also numerous other organs undergo accelerated maturation in response to cortisol shortly before birth, a phenomenon collectively termed "preparation for birth." In many species (not including primates), the placenta is included in the organs responding to cortisol. The consequent activation of placental enzymes (particularly those converting progesterone to estrogen) sets in motion the mechanism of uterine activation (see Chapter 19). Thus in these species the fetal adrenal not only prepares the fetus for birth, but also, when it is prepared, determines the time of birth.

With the probable exception of the gonads, there is not an organ or system in the fetus that fails to respond to a greater or lesser extent to cortisol. Some, like brain enzymes, respond sluggishly and show no lasting effects from the prepartum stimulus. Others, like the lung, respond vigorously and are transformed from marked immaturity

to full maturity in the space of days. Even such unlikely systems as the cardiovascular system respond to cortisol; premature infants are more likely to successfully close the ductus arteriosus if they have been treated with corticosteroids before birth.

The endocrine system shows a number of responses to cortisol. Conversion of thyroxine to triiodothyronine is accelerated in anticipation of the need for postpartum surge of triiodothyronine (Thomas et al., 1978), the insulin response to glucose increases (in anticipation of the need for independent homeostasis of glucose), and the conversion of norepinephrine to epinephrine is stimulated (in anticipation of the need for epinephrine to release lung surfactant and dry out the alveoli as well as mobilize glucose).

It is little wonder that premature infants delivered without a prior surge of cortisol suffer a variety of illnesses resulting from immaturity of organs or that prepartum treatment with corticosteroids improves viability.

## CONCLUSION

Disorders of fetal growth and development form an important segment of the problems of obstetrics and neonatology. The diagnosis and rational treatment of the growth-retarded fetus must depend on a detailed understanding of fetal growth. Knowledge of the mechanisms regulating the normal growth of the fetus may lead to therapies that will prevent the development of fetal growth retardation. This chapter demonstrates that our knowledge is superficial and fragmentary. Genetic, maternal, placental, and fetal factors all play an essential role in regulating the development of the fetus. Considerable research effort will be required to define and detail these factors and determine interventions that will enable the disadvantaged fetus to achieve its full potential for growth.

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# 17

# Fetal, Placental, and Maternal Hormones

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## INTRODUCTION

Obstetrical complications frequently occur as varying combinations of abnormal fetal growth, aberrations in the sequencing of fetal maturational endocrine events, or disordered timing of parturition. The placenta, and the fetus and placenta as a cooperative, secretes a diverse profile of polypeptide and steroid hormones into the intervillous space. Measurements of circulating maternal concentrations of these hormones as they change with advancing gestational age can define the passage of normal maturational events or signal the presence of developing fetal complications. Such measurements have thus become increasingly important in the assessment of the at-risk pregnancy.

This chapter reviews the origins, regulatory mechanisms, biosynthetic processes, release, and clinical relevance of polypeptide and steroid hormones secreted by the fetus, placenta, or fetus and placenta from conception until term.

## FETAL PLACENTAL HORMONES

### Protein and Glycoprotein Hormones

#### Origins and Mechanisms of Production

Circulating patterns of pregnancy-related glycoprotein and protein hormones measureable in maternal blood from implantation to parturition are determined largely by placental syncytiotrophoblast secretion into the intervillous space (Midgley and Pierce, 1962; Catt et al., 1975; Marshall et al., 1968; Thiede and Choate, 1963; Sciarra et al., 1963; Van Leusden, 1976; Josimovich, 1973). Production continues without immediate regard to fetal viability. Relatively small but significant amounts of these hormones are also secreted into the fetal circulation; however, little is known about their effects (Lauritzen and Lehmann, 1967; Johannisson, 1968). The list of known secreted placental proteins and glycoproteins continues to lengthen and includes human chorionic gonadotrophin (HCG), human placental lactogen (HPL), human chorionic thyrotropin (HCTSH), human chorionic corticotropin (HCC), beta-endorphin, pregnancy-specific beta-1-glycoprotein, and many others (Diczfalusy, 1974; Genazzani et al., 1975; Liotta et al., 1979). Only HCG and HPL, both readily measurable in peripheral blood by specific radioimmunoassays (Saxena and Landesman, 1975; Yen et al., 1968b; Letchworth, 1976; Saxena et al., 1968b) have been investigated extensively for clinical purposes.

Human chorionic gonadotrophin is a double-chain glycoprotein with a molecular weight of approximately 36,000-40,000 (Bahl et al., 1972). The alpha chain in

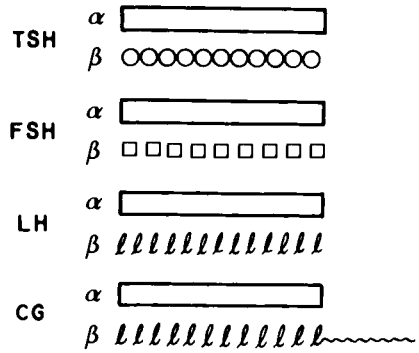


Figure 1 Structural homology of HCG (CG) and the three pituitary glycoprotein hormones LH, FSH, and TSH. The alpha chain of HCG is biochemically and immunologically similar to the alpha chains of the three pituitary glycoproteins; HCG and LH are very similar to one another, except for a "tail piece" at the terminus of the beta chain of HCG, which makes it structurally distinctive. (Courtesy of Dr. Glenn Braunstein, Cedars-Sinai Medical Center, Los Angeles, California).

biochemically and immunologically similar to the alpha chain of the three pituitary glycoprotein hormones luteinizing hormone (LH), follicle-stimulating hormone (FSH), and thyrotropin (TSH) (Figure 1). The beta chain, however, is biochemically and immunologically unique, a feature which has been utilized to develop relatively specific radioimmunoassays for HCG and its beta subunit (Bahl et al., 1972; Morgan et al., 1972). Human CG and LH are structurally similar except for a distinctive "tail piece" at the terminus of the HCG beta chain. The major physiological role of HCG appears to be support of corpus luteum progesterone (P) production, a function essential for pregnancy maintenance through the seventh week as calculated from the last menstrual period of a 28-day menstrual cycle (Csapo et al., 1973a,b). Its functions beyond the seventh week are not known (Van Leusden, 1976), and the mechanisms regulating placental production and secretion of HCG are poorly understood. The circulating half-life ($t_{½}$) is a two-component disappearance curve: The $t_{½}$ of the first component is about 11 hr and the $t_{½}$ of the second about 23 hr (Yen et al., 1968a).

Human placental lactogen is a 190-amino acid, straight-chained polypeptide with a molecular weight of approximately 21,500 (Friesen, 1965; Li et al., 1973). Its molecular structure is similar to that of human prolactin and human growth hormone (Friesen, 1965; Li et al., 1973). The structural similarities between these molecules are summarized in Figure 2. The major functions of HPL probably involve mobilization and metabolism of maternal fat stores (Yen, 1973). Functioning as an insulin antagonist, HPL appears to be involved with the regulation of maternal blood sugar levels so as to provide for fetal caloric requirements (Yen, 1973). This action is undoubtedly a major factor in the diabetogenic effect of pregnancy. The mechanisms regulating HPL production and secretion by the placenta are poorly understood (Boime et al., 1977). The circulating $t_{½}$ of HPL is 12-25 min (Pavlou et al., 1972).

## Circulating Maternal Polypeptide Concentrations in Normal and Diseased Pregnancies

*HCG* Human CG is first detectable in peripheral serum 7-12 days following a conceptual ovulation, that is, days 21-26 of a 28-day cycle (see Figure 3) (Catt et al., 1975; Wide,

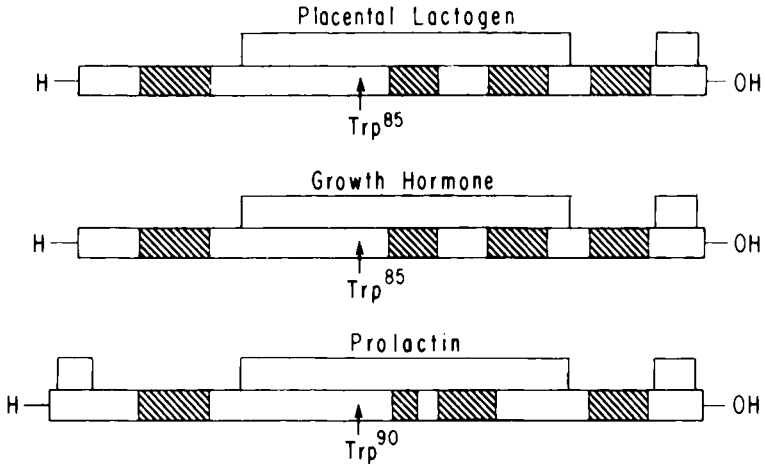


**Figure 2** Structural homology of somatomammotropins. The known portions of replicating sequences of amino acids are represented by the hatched areas. The open areas represent the peptide chains, with the amino terminal indicated by the H and the carboxyl terminal by the OH. Human placental lactogen thus has a strong resemblance to its pituitary somatomammotrophic counterparts. (From H. D. Niall et al., *Proc. Nat. Sci. 68*:866, 1971.)

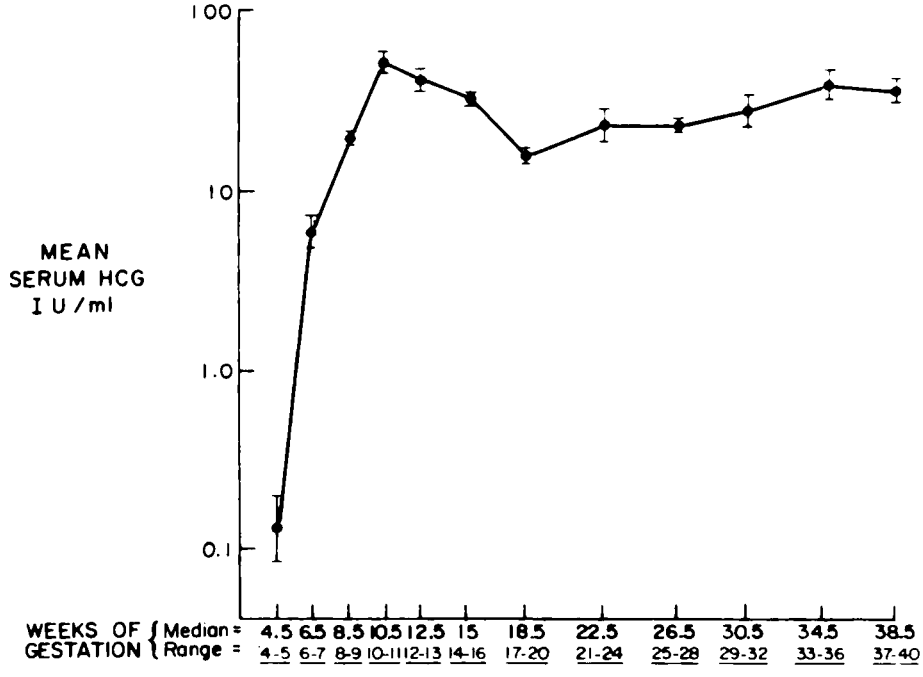


**Figure 3** Serum HCG concentrations from conception to term (mean ± SEM). (From Goldstein et al., *Am. J. Obstet. Gynecol. 102*:110, 1968.)

1969; Mishell et al., 1974). This corresponds closely to the penetration of the trophoblast-covered blastocyst into the endometrial stroma on the seventh postovulatory day and its subsequent direct apposition to the maternal circulation (Boving and Larson, 1972). Human CG levels rise logarithmically over the 10 days following implantation, the concentration doubling every 1.7 days (Marshall et al., 1968). Peak circulating concentrations are found at 9-12 weeks and vary around a mean of about 60,000 mIU/ml (Saxena et al., 1968a). This is followed by a fall between 16 and 30 weeks to a plateau ranging from 12,000 to 28,000 mIU/ml. There is then a small gradual second rise to about 45,000 mIU/ml at term (Figure 3) (Saxena et al., 1968a).

In women presenting with threatened abortion, serum HCG concentrations are well correlated with the ultimate outcome. In one investigation, HCG concentrations exceeding 18,600 mIU/ml, measured at the time of initial examination, were associated with continued maintenance of the pregnancy (Nygren et al., 1973). Values of less than 10,600 mIU/ml were, with unusual exceptions, associated ultimately with abortion of the pregnancy (Nygren et al., 1973).

In women afflicted with untreated gestational trophoblastic neoplasms, HCG concentrations are generally greater than 320,000 mIU/ml. Human CG levels in this range strongly support this diagnosis, provided that the sample is obtained after the fourteenth week of amenorrhea (Teoh et al., 1972). Because with normal pregnancy the highest HCG concentrations are observed between 9 and 12 weeks, extremely high concentrations measured prior to 14 weeks are not diagnostic. Human CG measurements are frequently utilized as a tumor marker to indicate presence of residual tumor following surgical or chemotherapeutic treatment of gestational trophoblastic disease. Specific beta subunit HCG radioimmunoassays, which show negligible cross-reactivity with LH, are particularly suited for this purpose when HCG concentrations fall into the pituitary LH range, where persistent HCG activity might otherwise be masked by endogenous LH. Remission is considered achieved when HCG is undetectable by a beta subunit assay over 3 consecutive weeks. The remission is considered permanent if HCG remains undetectable after 5 years (Pastorfide et al., 1974).

*HPL* Human PL is first detectable during the fifth to sixth week of pregnancy (Figure 4) (Saxena et al., 1968b). Human PL concentrations rise gradually from approximately 0.2 μg/ml between 7 and 10 weeks to a plateau ranging from approximately 6 to 10 μg/ml beginning at 34 weeks. This plateau persists until term (Letchworth, 1976; Saxena et al., 1968a; Spellacy, 1973). For lack of an international reference standard prior to 1977, ranges as wide as 3.3-25 μg/ml had been described in various publications at term (Saxena et al., 1968a; Beck et al., 1965; Samaan et al., 1966; Varma et al., 1971). As a rule, however, from 30 weeks to term there are few normal values under 4 μg/ml (Spellacy, 1973).

In women presenting with threatened abortion in whom abortion does not subsequently occur, HPL concentrations characteristic of the first trimester continue with little deviation. In cases in which abortion does occur, HPL concentrations are very low and are well correlated with outcome if the sample is obtained at 9 weeks or later (Gartside and Tindall, 1973; Niven et al., 1972).

In women afflicted with gestational trophoblastic disease, HPL concentrations consistently fall within a range of less than 10% of the normal level for the estimated gestational age of the pregnancy (Saxena et al., 1968a). The reasons for low HPL concentrations are unknown but may be related to trophoblastic immaturity and to a deficiency of the more sophisticated enzyme systems necessary to produce this hormone. Unusually high

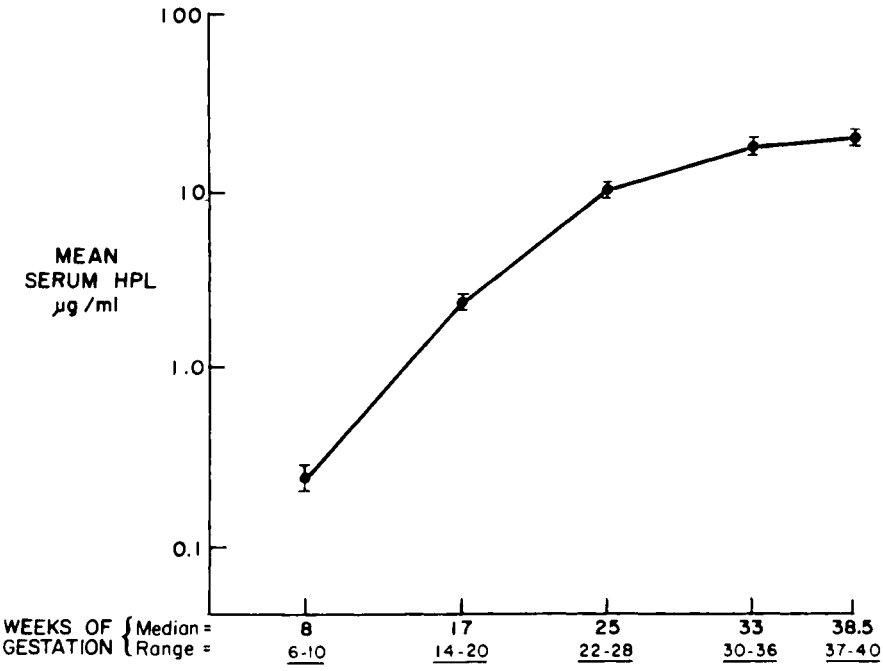


Figure 4 Serum HPL concentrations in normal pregnancy from the first trimester to term. (mean ± SEM). (Modified from Saxena et al., 1968a).

HCG levels associated with very low HPL levels after 8 weeks gestational age strongly support a diagnosis of gestational trophoblastic disease (Saxena et al., 1968a).

In women pregnant in the third trimester in whom there is need to assess fetal well-being, HPL concentrations have been widely suggested as predictive instruments in the assessment of hypertensive toxemia and dysmaturity/postmaturity syndromes (Spellacy, 1973). The utility of this test, however, remains controversial, in that fetal deaths have been reported in the presence of very high levels of HPL. Conversely, term pregnancies apparently normal with entirely normal outcomes have been associated with very low levels of HPL (Trolle et al., 1978; Josimovich, 1977). It is generally agreed that the measurement is not of value in the management of diabetic or rhesus-sensitized pregnancies (Spellacy, 1973). As a general guide, after 30 weeks gestational age, HPL concentrations of less than 4 μg/ml ("fetal danger zone") indicate major fetal compromise (Spellacy, 1973).

## Steroid Hormones

### Origins and Mechanisms of Production

Steroid hormones measurable in maternal blood from implantation to parturition arise from multiple maternal and fetoplacental sources. During the first trimester, relative contributions of circulating sex steroids derived from maternal adrenals, ovaries, liver, and fetoplacental sources shift from maternal to predominantly fetoplacental origins.

*The First Trimester* Immediately following conception, the major source of circulating sex steroids is the corpus luteum, which, supported by increasing HCG production,

continues to function well past its normal 14-day life-span. The corpus luteum remains a major source of circulating maternal sex steroids through approximately 12-13 weeks gestational age (Tulchinsky, 1972a; Blandau, 1973). After the seventh week, the placenta assumes an increasingly important quantitative role in the production and secretion of steroid hormones (the luteoplacental shift), a trend which increases until the time of parturition. This is substantiated by reports that surgical lutectomy prior to the seventh week almost invariably results in abortion, whereas after the seventh week the same surgery does not disturb the pregnancy (Csapo et al., 1973a,b). These findings illustrate the important functional autonomy acquired by the conceptus following the seventh week as it becomes increasingly efficient in steroid hormone production.

*The Second and Third Trimesters* During the second and third trimesters of pregnancy the profile of circulating maternal steroids is the result of a series of complex exchanges centered on the placenta, with precursors originating from the fetal adrenals, fetal peripheral steroid interconversions, fetal liver, and maternal liver.

*The placenta* becomes the single most important source of $C_{18}$ and $C_{21}$ steroids in both fetal and maternal circulations; at term it secretes them in enormous amounts. The placenta is an incomplete steroidogenic organ, in that many of the enzyme systems needed to produce steroid hormones from simple $C_2$ acetate are not present. However, the placenta can extract necessary precursors and intermediates from either fetal or maternal circulations.

*The fetal adrenal cortex* is the central steroid modulator in the fetal placental complex and the chief source of circulating fetal steroid precursors. The fetal cortex is extremely large in contrast to its adult counterpart. The large mass of the fetal adrenal is attributable anatomically to the presence of a hyperplastic internal zone ("fetal zone" or FZ) that rapidly regresses after birth. The outer zone ("definitive zone" or DZ) ultimately makes up the bulk of the postnatal and adult cortex. In utero, the FZ makes

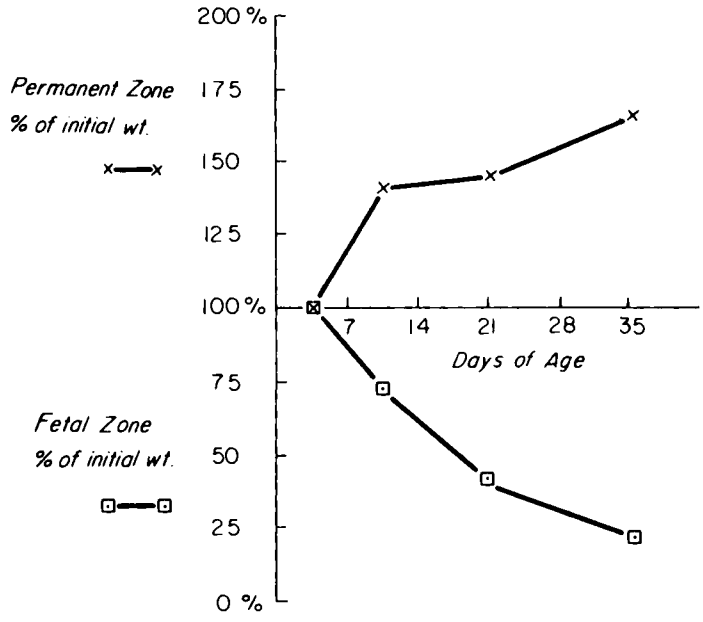


Figure 5 Relative changes in size of permanent and fetal zones of the early postnatal adrenal cortex. (From Reynolds, 1975).

up approximately 80% of the mass of the fetal adrenal from mid-trimester to term. Figure 5 shows the relative contributions of the FZ and DZ to the percentage of newborn adrenal mass during postnatal FZ involution and DZ enlargement. The FZ involutional process is well on its way to completion by the fifth week of newborn life (Reynolds, 1975). Because the FZ persists only during gestation, it appears that its cells are supported by a factor(s) unique to their fetal status. The extreme development of the FZ is believed to be linked with the intense steroidogenic activities of intrauterine life mutually involving virtually all fetal organs, placenta, membranes, and decidua.

The original elements of the FZ first appear at about 35 days (days numbered from the last menstrual flow) and are steroidogenically active by 50 days (Jirasek, 1969). The DZ first appears at about 50 days (Jirasek, 1969), but it is not substantially involved in steroidogenesis until well into the second trimester (Jirasek, 1969), in rough temporal association with development of the hypothalamus and hypophyseal portal vascular systems (Fisher, 1975). Since there is a functioning fetoplacental circulation by 4 weeks (Boving and Larson, 1972), the steroids produced by the FZ are presented to the placenta as soon as the FZ is steroidogenically active. The FZ is the major source of fetal $C_{19}$ steroids until birth.

The FZ produces its full fetal steroid complement by a cooperative interaction with the placenta. The placenta contains steroidogenic enzymes that are complementary to those in the FZ, allowing it to produce its complete steroid profile only when the two are working by exchange of circulating precursor intermediates. Table 1 summarizes the anatomical distribution of the key steroidogenic enzymes required to produce the major known circulating intrauterine steroids. It appears that a functional or actual deficiency of FZ 3$\beta$-hydroxysteroid dehydrogenase (3$\beta$-HSD) is largely responsible for the situation depicted in Figure 6. It is the 3$\beta$-HSD system which allows for the conversion of the $\Delta^5$-3$\beta$-hydroxysteroids to the corresponding $\Delta^4$-3-ketosteroids: $\Delta^5$-pregnenolone ($\Delta^5$P) to P; 17$\alpha$-hydroxy-$\Delta^5$-pregnenolone (17$\Delta^5$P) to 17$\alpha$-hydroxyprogesterone (17P); and dehydroepiandrosterone (DHEA) to androstenedione ($\Delta^4$A) (Figure 6). The placenta, however, provides an abundance of 3$\beta$-HSD activity. This anatomical arrangement allows the FZ, working in a mutual cooperative with the placenta, to produce the full complement of circulating fetal steroids. The available evidence as a whole supports the following scheme. The fetal adrenal extracts low-density

**Table 1** Anatomic Distribution of FZ and Placental Enzymes[a]

| Steroidogenic enzymes | Fetal zone | Placenta |
|---|---|---|
| 3$\beta$-HSD | | X |
| Sulfatase | | X |
| Sulfokinase | X | |
| Hydroxylases | X | |
| $C_{17\text{-}20}$ desmolase | X | |
| Aromatization enzymes | | X |

[a]The FZ 3$\beta$-hydroxysteroid dehydrogenase (HSD) deficiency blocks conversion of $\Delta^5$ to $\Delta^4$ compounds, a function provided by the placenta. Placental sulfatase activity facilitates hydrolysis of $\Delta^5$ FZ sulfoconjugates. Fetal zone hydroxylases facilitate corticoid production and lead to cleavage of the 17-$\Delta^5$P-S side chain by $C_{17\text{-}20}$ desmolase to form DHEA-S. Placental aromatization enzymes facilitate estrogen production from androgen precursors. This is a partial list.

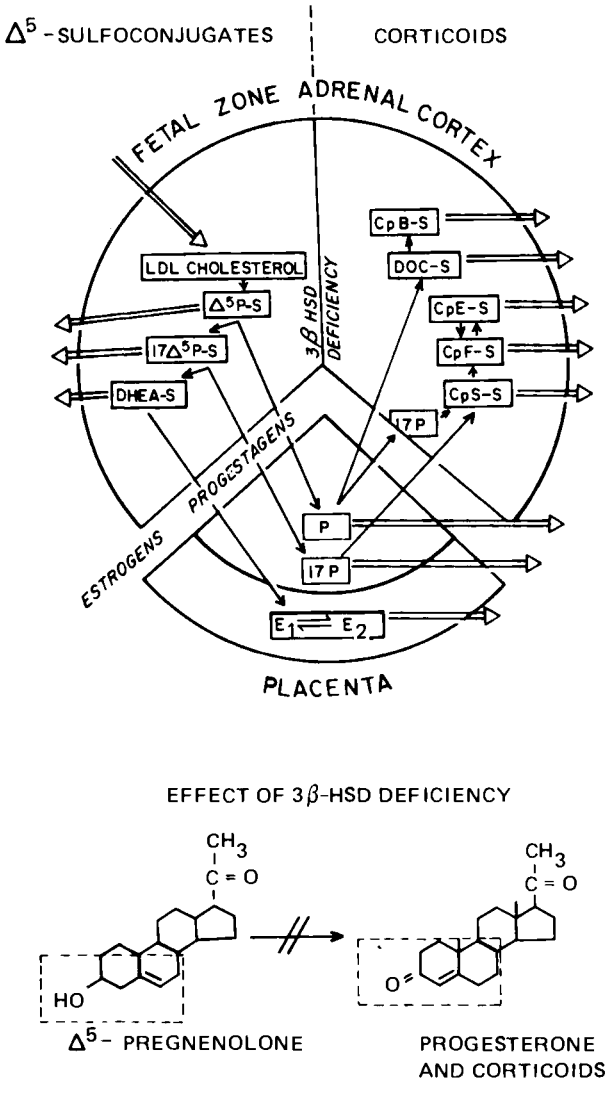


**Figure 6** Exchange of circulating steroid intermediates between the fetal zone and the placenta. Enzyme deficiencies of the fetal zone are offset by enzyme activities of the placenta. The two organs work as a mutual cooperative to produce the extensive profile of circulating fetal steroids that would not otherwise be possible. Although the bulk of fetal zone steroids are secreted as sulfoconjugates, the unconjugated counterparts are secreted in abundance as well ($\Delta^5$P-S, $\Delta^5$-pregnenolone sulfate; 17$\Delta^5$P-S, 17$\alpha$-hydroxy-$\Delta^5$-pregnenolone sulfate; DHEA-S, dehydroepiandrosterone sulfate; P, progesterone; 17P, 17$\alpha$-hydroxyprogesterone; $E_1$, estrone; $E_2$, estradiol-17; CpS-S, 11-desoxycortisol-21-sulfate; CpF-S, cortisol-21-sulfate; CpE-S, cortisone-21-sulfate; DOC-S, 11-desoxycorticosterone sulfate; and CpB-S, corticosterone-21-sulfate). (From Buster and Marshall, 1980.)

lipoprotein cholesterol (LDL-cholesterol) from the fetal circulation and converts it to $\Delta^5$P and its sulfate ($\Delta^5$P-S), which is either secreted or converted intraadrenally to 17$\alpha$-hydroxy-$\Delta^5$-pregnenolone sulfate (17$\Delta^5$P-S) and then to dehydroepiandrosterone sulfate (DHEA-S) (Simpson et al., 1979). Because the FZ is deficient in the 3$\beta$-HSD enzyme, it is unable to metabolize these three $\Delta^5$-sulfoconjugated steroids to the corresponding $\Delta^4$-steroids and therefore secretes them into the fetal circulation in enormous amounts (Table 1). For this reason, the umbilical artery contains very high concentrations of $\Delta^5$P-S, 17$\Delta^5$P-S, and DHEA-S (Reynolds, 1975; Chang et al., 1976). Because the placenta contains an abundance of 3$\beta$-HSD (and sulfatase) activity, the three aforementioned $\Delta^5$-sulfoconjugated steroids are transformed by the placenta as follows: $\Delta^5$P-S to P, 17$\Delta^5$P-S to 17P, and DHEA-S to estrogens. The placenta is therefore a massive producer of P, hydroxylated P, and estrogens and an efficient extractor and processor of circulating sulfoconjugated precursors. Although the placenta produces $\Delta^5$P and P from circulating maternal LDL-cholesterol, the quantitative significance of P produced from maternal precursors is not clear insofar as fetal metabolism is concerned. The progestogens produced by the placenta are returned to the fetal circulation, where they are extracted and converted to glucocorticoids by the FZ. The great bulk of these glucocorticoids are sulfoconjugated at the 21 position; however, large amounts of the unconjugated corticoids are secreted as well. Estrogens made from the placental extraction and conversion of DHEA-S are secreted into the maternal circulation or back into the fetal circulation (Diczfalusy, 1969).

In summary, the FZ is a major $\Delta^5$-sulfoconjugated steroid secretor, synthesizing its steroids probably from circulating fetal LDL-cholesterol. The placenta is a progestogen and estrogen producer secreting these steroids from extracted preformed adrenal $\Delta^5$-sulfoconjugated precursors. Finally, the FZ is a corticoid and corticoid sulfate producer, having made these compounds by the extraction and conversion of placental P and hydroxylated P precursors. Aspects of fetal adrenal function are also considered in Chapter 19.

*Fetal peripheral steroid interconversions* contribute significantly to the profile of circulating fetal steroids. Peripheral fetal steroid interconversions may have as much or more influence on the concentrations of physiologically active steroids and their biological expression as the fetal adrenal cortex and placenta. Although peripheral interconversions involve a wide variety of steroidogenic enzymes, the best understood of these functions relates to the interconversion of cortisol to cortisone through 11-ketoreductase or 11-dehydrogenase enzymes. This interconversion is a major regulator in the fetal maturational cascade and is reviewed in more detail below.

*The fetal liver* is the major source of circulating fetal LDL-cholesterol, since transfer from the maternal circulation is probably minimal (Spellacy et al., 1974). Fetal liver LDL-cholesterol is an important substrate in fetal adrenal steroidogenesis, as was described above. Under conditions of fetal malnutrition, depleted fetal LDL-cholesterol stores may be a rate-limiting factor in the adrenal production of $\Delta^5$-steroid sulfoconjugate (Liggins, 1972). In addition, the fetal liver has a very active 16$\alpha$-hydroxylation of many $C_{18}$, $C_{19}$, and $C_{21}$ steroids (Diczfaulusy, 1974, 1969). The measurement of maternal levels of 16$\alpha$-hydroxylated placental steroids, such as estriol ($E_3$) and estetrol ($E_4$), have been investigated clinically as indicators of fetal well-being, since they are derived largely from 16$\alpha$-hydroxylated precursors of fetal origin.

*The maternal adrenal cortex* is the major maternal source of precursors for placental steroidogenesis, particularly DHEA-S. Approximately 30% of daily maternal production of DHEA-S is extracted by the placenta and converted to estradiol ($E_2$) (Gant et al., 1972). In addition, the maternal adrenal cortex secretes a considerable quantity of cortisol, which after oxidation of the 11-hydroxyl group by placental 11α-hydroxysteroid dehydrogenase enters the fetal circulation as predominantly cortisone (Murphy et al., 1974). The physiological importance of this exchange is not well understood. Since the rate of reconversion of cortisone to cortisol is relatively low, it has been suggested that this conversion protects the fetal tissues from the effects of changes in maternal secretion of cortisol.

*The maternal liver* is the major source of circulating maternal LDL-cholesterol, an important precursor in placental P production (Diczfalusy, 1974, 1969; Hellig et al., 1969); it is also a minor source of 16α-hydroxylated steroid conjugates (Gant et al., 1972; Baulieu et al., 1965). Finally, the maternal liver clears and conjugates placental steroids, which then become water soluble and are efficiently excreted through the maternal kidneys (Kirdani et al., 1972).

## Circulating Maternal Steroid Concentrations in Normal and Diseased Pregnancies

*$C_{21}$ Steroids. Progesterone* concentrations are less than 1 ng/ml during the follicular phase of a normal menstrual cycle (Figure 7). In a conceptual cycle, P concentrations rise to 1-2 ng/ml on the day of the LH peak, rise again sharply to a plateau of 10-35 ng/ml over the subsequent 7 days, fluctuate generally within limits of this plateau through the tenth week (dated from last menstrual flow), and then show a sustained rise which continues until term. At term P concentrations range from 100 to 300 ng/ml (Abraham et al., 1972; Tulchinsky et al., 1972b). As mentioned previously, P originates almost entirely from the corpus luteum prior to 5-6 weeks gestational age. The luteal placental shift occurs soon after the seventh week. After 12 weeks, the placenta is the major source of P (Figure 7). The placenta contains all of the enzyme systems necessary to produce P from circulating maternal LDL-cholesterol and is minimally dependent upon fetal steroidogenesis insofar as P production and secretion to the maternal circulation are concerned (Figure 8). For these reasons circulating maternal P concentrations reflect upon corpus luteum steroidogenesis during the first 5-6 weeks, a mixture of corpus luteum and placental steroidogenesis through the twelfth week, and then primarily placental steroidogenesis from the twelfth week until term.

In women presenting with threatened spontaneous abortion in the first trimester, P concentrations measured at the time of initial evaluation are roughly correlated with ultimate prognosis. Approximately 80% of those with P concentrations under 10 ng/ml will abort (Nygren et al., 1973).

In women pregnant with hydatidiform moles, P concentrations are significantly elevated above the normal range. This disparity is particularly pronounced between the tenth and twentieth weeks of gestation. Blood HCG concentrations exceeding 320,000 mlU/ml after 14 weeks of amenorrhea combined with elevated P concentrations are indicative of hydatidiform mole (Teoh et al., 1972).

In women whose pregnancies are complicated by rhesus isoimmunization, P concentrations are elevated approximately twofold above values for normal pregnancies of comparable gestational ages. This elevation may be related to a two- to threefold increase in placental mass associated with the erythroblastosis (Tulchinsky et al., 1972b). Higher P concentrations are associated with a less favorable prognosis (Tulchinsky et al., 1972a).

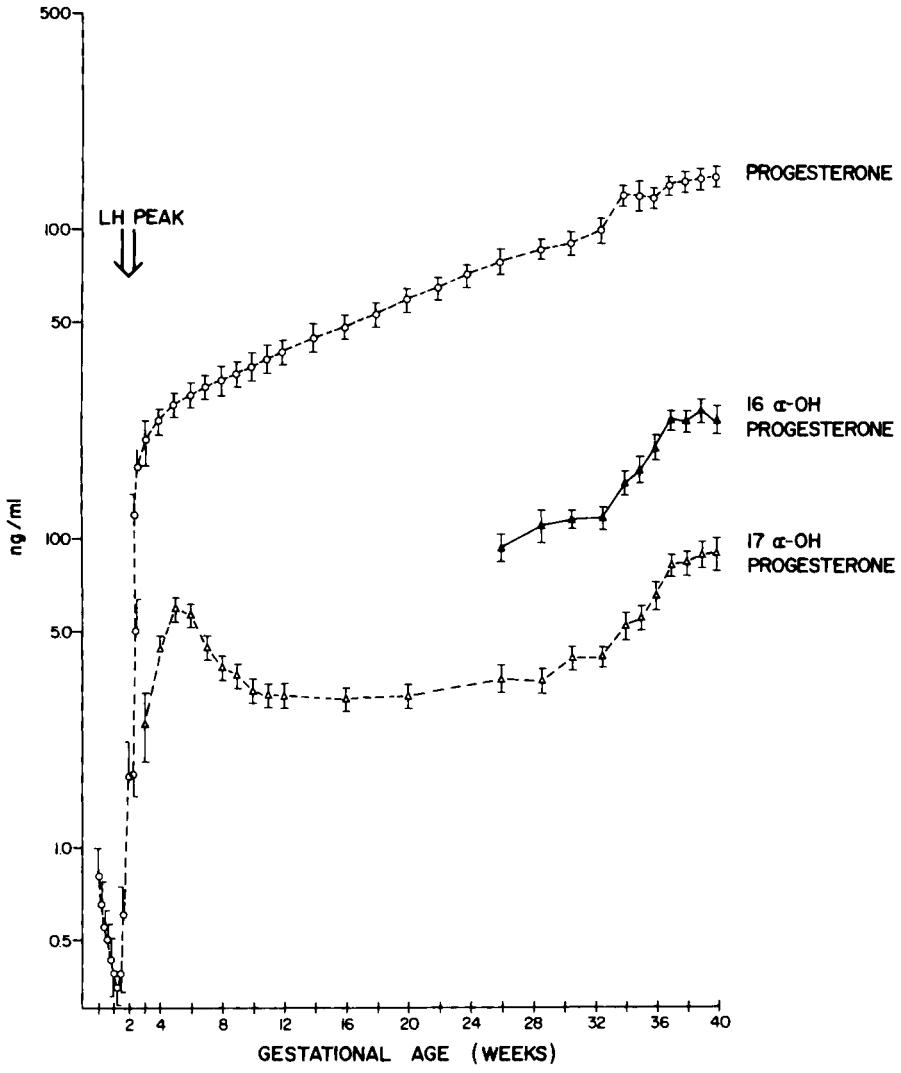


**Figure 7** Mean concentrations (± SEM) of progesterone, 16α-hydroxyprogesterone, and 17α-hydroxyprogesterone from the first trimester until term. Data were compiled from several reports. Gestational ages are calculated from the last menstrual flow. (From Buster and Marshall, 1980.)

*17α-Hydroxyprogesterone* concentrations are less than 0.5 ng/ml during the follicular phase of normal menstrual cycles. In conceptual cycles, 17P concentrations rise to about 1 ng/ml on the day of the LH peak, fall slightly for about 1 day, rise again over the subsequent 4-5 days to a level of 1-2 ng/ml, and then increase gradually to a mean of approximately 2 ng/ml (luteal phase levels) at the end of 12 weeks. This level remains relatively stable until about the thirty-second week, when there begins an abrupt sustained rise to a mean at 37 weeks of approximately 7 ng/ml, a level which persists until term (Figure 7) (Tulchinsky and Hobel, 1973a; Abraham et al., 1972; Tulchinsky et al., 1972a). The rise beginning at 32 weeks is strikingly correlated with the activity of fetal maturational processes known to begin at this time.

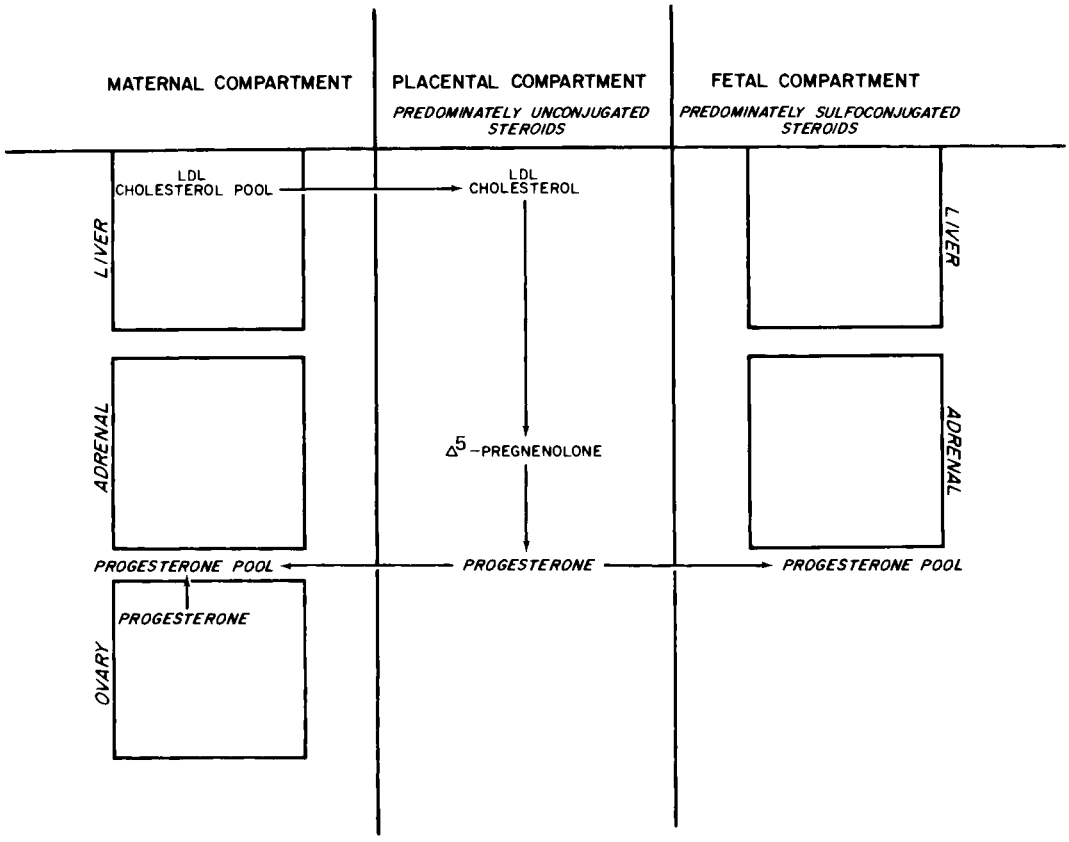


Figure 8 Anatomic compartmentalization of progesterone production. Although several pathways other than the ones depicted are known to exist, the extraction and conversion of cholesterol from the maternal pool is believed to be a major source of fetal placental progesterone production. (Modified from Buster and Marshall, 1980.)

17α-Hydroxyprogesterone originates predominantly from the corpus luteum during the first trimester of pregnancy (Figure 9) (Tulchinsky and Hobel, 1973a; Yoshimi et al., 1969). The ovaries continue to be a significant source of 17P throughout pregnancy. During the third trimester, however, the placenta utilizing fetal $\Delta^5$-sulfoconjugated precursors secretes increasing amounts of 17P and is probably the major source of this hormone at term (Tulchinsky and Simmer, 1972).

In women undergoing spontaneous abortion, falling concentrations of 17P parallel falling concentrations of P (Tulchinsky et al., 1973b). Although still speculative, it is possible that 17P levels could provide useful information in assessing the mechanism of abortion due to corpus luteum dysfunction. Through the time of the luteal placental shift at about 7 weeks, 17P concentrations reflect primarily on corpus luteum steroidogesis (Tulchinsky and Hobel, 1973a; Yoshimi et al., 1969).

*16α-Hydroxyprogesterone* (16P) concentrations fluctuate around a mean of 0.5 ng/ml during the follicular phase of normal menstrual cycles and rise significantly to a mean of 1.2 ng/ml during the luteal phase (Abraham and Samojlik, 1974). 16α-Hydroxyprogesterone rises gradually with increasing gestational age until about the thirty-second week. At

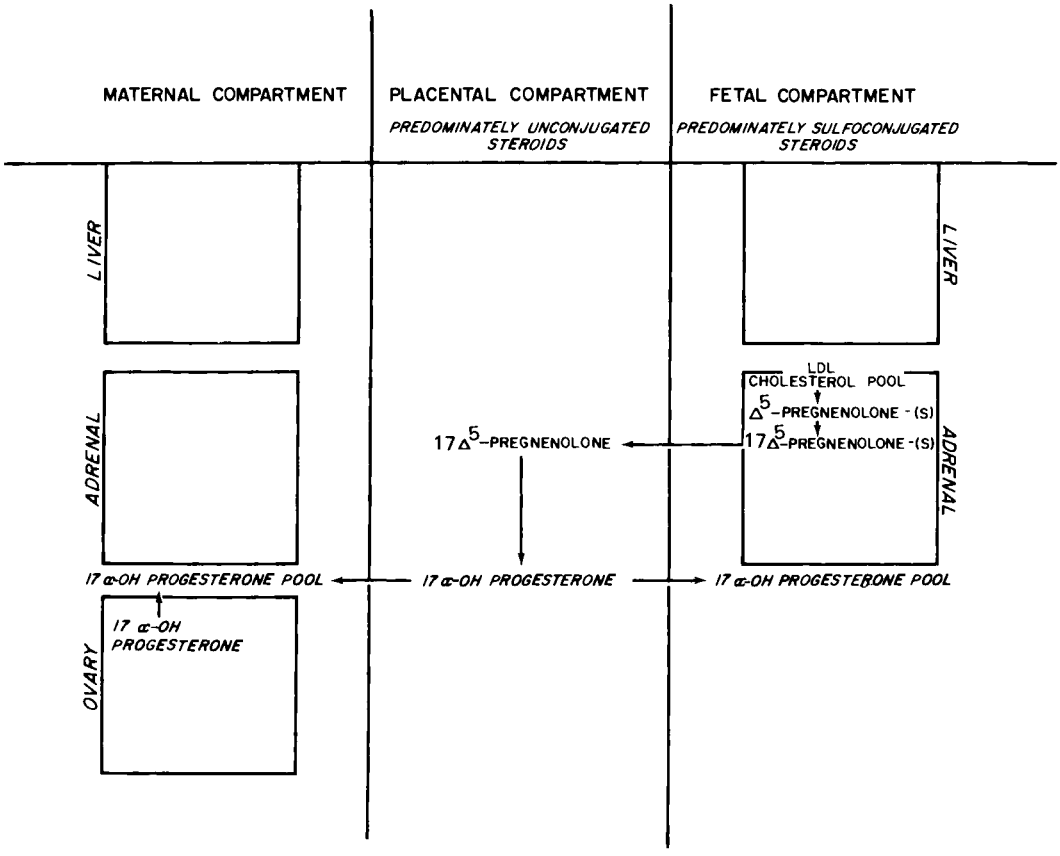


**Figure 9** Anatomic compartmentalization of 17α-hydroxyprogesterone production. The corpus luteum is the major source of 17α-hydroxyprogesterone during the first trimester. The fetus and placenta in cooperation are believed to secrete the great bulk of this steroid during the third trimester. Although several known alternate pathways are not depicted, the suppression of 17α-hydroxyprogesterone concentrations by intravenous maternal cortisol infusion during the third trimester indicates that a major pathway is from fetal adrenal LDL-cholesterol through the $\Delta^5$-$C_{21}$ sulfoconjugates to 17α-hydroxy-$\Delta^5$-pregnenolone sulfate. 17α-Hydroxy-$\Delta^5$-pregnenolone sulfate is converted to 17α-hydroxyprogesterone by placental 3β-hydroxysteroid dehydrogenase and $\Delta^4$ and $\Delta^5$ isomerase systems. (Modified from Buster and Marshall, 1980.)

that time, an abrupt sustained rise begins from a mean at 32 weeks of about 12 ng/ml to a mean at 37 weeks of about 25 ng/ml, which persists until term (Figure 7). The overall pattern of 16P from 32 weeks to term is nearly identical to 17P. Therefore 16P and 17P are similarly associated over time with the initiation of fetal maturational processes known to be activated just prior to term (Figure 7).

Sources of 16P have not been studied extensively but are probably predominantly fetoplacental through later pregnancy (Figure 10) (Abraham and Samojlik, 1974; Buster et al., 1979a). While there is a highly significant third trimester association between rising levels of 16P and 17P, it is possible that the measurement of these two hormones provides data about fetal steroidogenic activities that are related but reflect on very

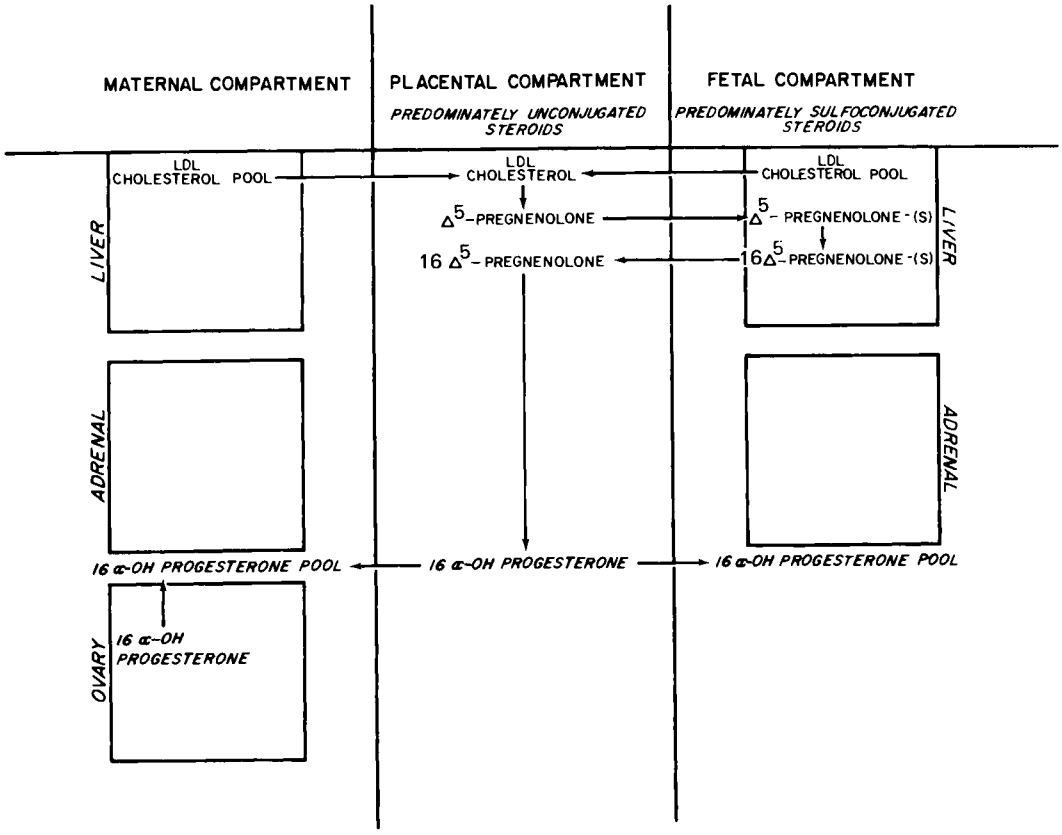


**Figure 10** Anatomic compartmentalization of 16α-hydroxyprogesterone production. The bulk of circulating maternal 16α-hydroxyprogesterone probably originates from the maternal ovary during early first trimester, but shifts progressively toward fetoplacental production during later pregnancy. Although many pathways are probably involved with the production of 16α-hydroxyprogesterone and are not depicted here, the non-suppressibility of circulating maternal 16α-hydroxyprogesterone after a maternal intravenous infusion of cortisol implies that the production of this steroid by the fetus and placenta is not dependent upon fetal adrenocorticotropin hormone (ACTH) regulation. Available data as a whole indicate that the predominant pathways involve fetal liver 16α-hydroxylation of placental $\Delta^5$-pregnenolone, completely bypassing fetal adrenal coversion of cholesterol to $\Delta^5$-pregnenolone, a major site of ACTH steroidogenic regulation. (Modified from Buster and Marshall, 1980.)

different regulatory mechanisms (Buster et al., 1979a). In one study, large maternal intravenous doses of cortisol produced an abrupt drop in maternal levels of 17P, but affected 16P very little (Elsner et al., 1979). It is therefore likely that the availability of placental 16P precursors, unlike that of 17P precursors, is not directly regulated by the fetal adrenal cortex or fetal pituitary adrenal feedback loops. Data available from second-trimester isotope kinetic studies and third-trimester concentration gradient measurements indicate that the major pathway in the production of 16P centers around 16α-hydroxylation of placental $\Delta^5$P-S by the fetal liver (Diczfalusy, 1974, 1969). The final steps probably

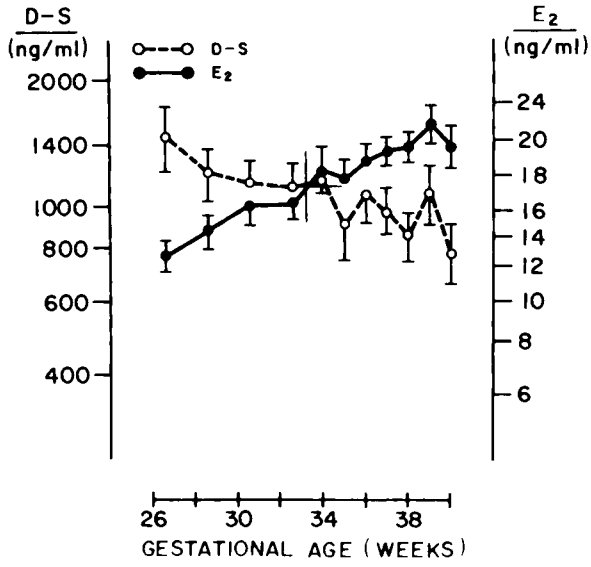


**Figure 11** Maternal DHEA-S and $E_2$ concentrations (ng/ml) from 26 weeks to term. The mean (± SEM) was calculated from 19 normal subjects sampled serially. The increasing metabolic clearance rate of DHEA-S is probably the cause of falling DHEA-S levels with increasing gestational age. (From Buster and Marshall, 1980.)

involve conversion of 16α-hydroxy-$\Delta^5$-pregnenolone sulfate (16$\Delta^5$P-S) of fetal liver origin to 16P by the placenta, which then secretes 16P into the maternal and fetal circulations (Figure 10) (Diczfalusy, 1974, 1969).

*$C_{19}$ Steroids. Dehydroepiandrosterone sulfate* concentrations fluctuate around a mean of 1600 ng/ml throughout the menstrual cycle. In pregnancy, with advancing gestational age, DHEA-S levels decrease, where they fluctuate around a mean of 800 ng/ml at term (Figure 11) (Buster and Abraham, 1972; Buster et al., 1979b).

Dehydroepiandrosterone sulfate originates almost entirely from the maternal adrenal cortex, where its secretion pattern is regulated by maternal adrenocorticotropin (ACTH) (Baulieu et al., 1965). In nongravid women, DHEA-S is cleared by the liver and kidney and excreted into the urine along with the other classical 17-ketosteroids (Baulieu et al., 1965). In pregnancy there is a progressive increase in the metabolic clearance rate (MCR) of DHEA-S ($MCR_{DS}$) which rises 6- to 10-fold above the nonpregnant level, which is about 7 liters/24 hr (Gant et al., 1971). This increase in $MCR_{DS}$ is related in part to irreversible placental extraction of maternal DHEA-S with conversion through intermediates to placental estrogens. The increase in $MCR_{DS}$ is probably the cause of lowered DHEA-S concentrations during pregnancy. In this regard, it is interesting that falling maternal DHEA-S levels plot almost identically to the reciprocal log of rising maternal $E_2$ levels (Figure 11) (Buster et al., 1979b). Thus the rate of this conversion appears to rise in association with increasing placental mass (MacDonald and Siiteri, 1965).

There is evidence to indicate that the efficiency with which the placenta extracts circulating maternal DHEA-S and converts it to estrogens becomes impeded early in the course of disorders associated with impaired placental perfusion (Gant et al., 1975,

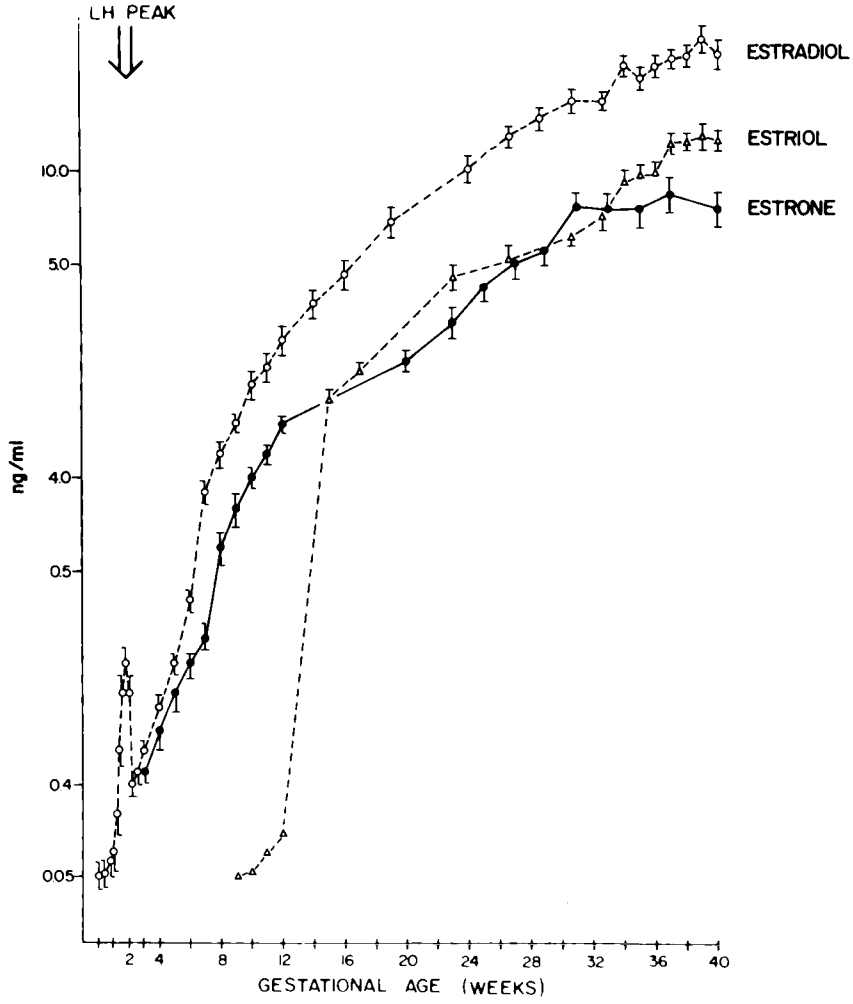


**Figure 12** Mean concentrations (± SEM) of estrone, estradiol, and estriol from early first trimester until term. Gestational ages are calculated from the last menstrual flow. (Modified from Buster and Marshall, 1980.)

1976). Because the $MCR_{DS}$ increases at a subnormal rate long before the onset of hypertension and proteinuria in patients with pregnancy-induced hypertension (Gant et al., 1971, 1975, 1976), a defect in placental perfusion regulation may be a major factor leading to this disease.

*$C_{18}$ Steroids. Estrone* concentrations are less than 0.1 ng/ml during the follicular phase and may reach 0.3 ng/ml during the luteal phase of a normal menstrual cycle. Following a conceptual cycle, $E_1$ concentrations remain within the luteal phase range through 6-10 weeks. Subsequently there is a gradual increase to a range of approximately 2-30 ng/ml at term (Figure 12) (Tulchinsky and Hobel, 1973a; Tulchinsky et al., 1972a; Lindberg et al., 1974a).

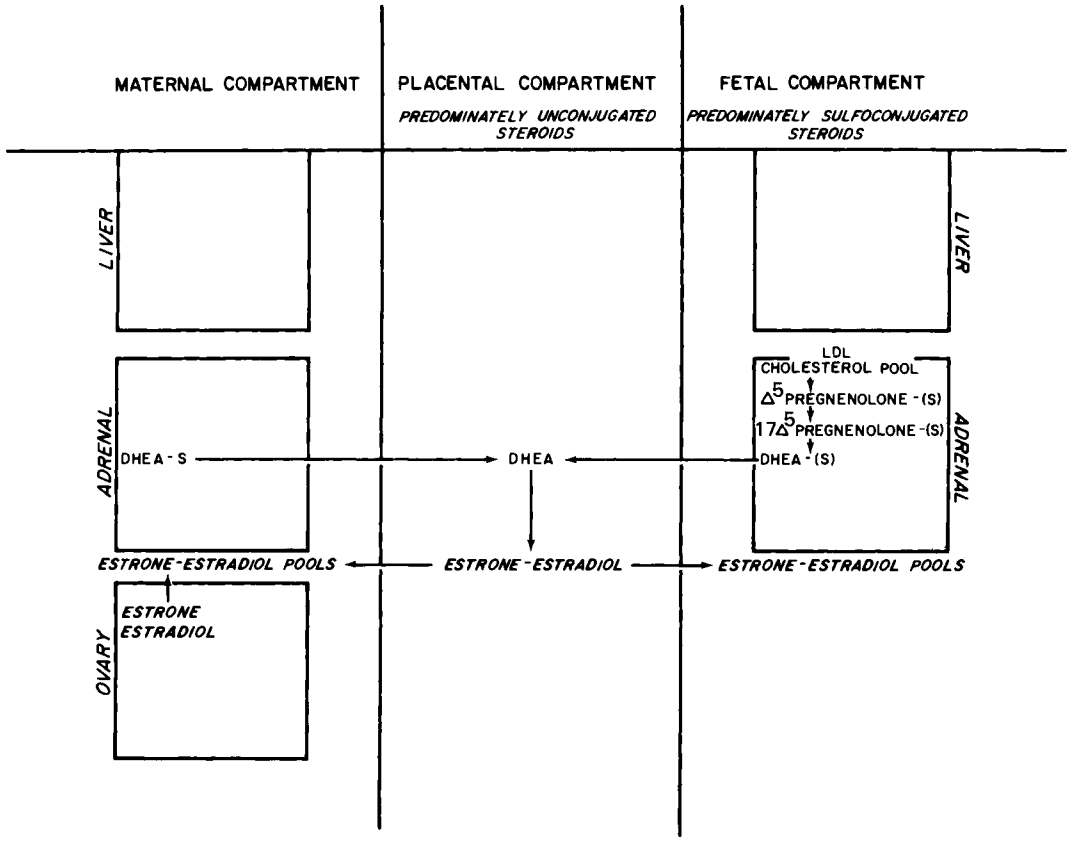


**Figure 13** Anatomical compartmentalization of estrone and estradiol production. During the first trimester the corpus luteum is the major source of circulating maternal estrone and estradiol. Progressing through the second into the third trimester, the placenta becomes the major source of these two steroids. (Modified from Buster and Marshall, 1980.)

Estrone originates primarily from maternal sources (ovaries, adrenals, peripheral conversion) for the first 4-6 weeks. After this time, the placenta secretes increasing quantities of $E_1$, which it synthesizes from conversion of circulating maternal and fetal DHEA-S. After the first trimester the placenta is the major source of circulating $E_1$ (Figure 13) (Tulchinsky and Hobel, 1973a).

Estrone concentrations probably reflect upon the same metabolic processes involved with the production of $E_2$. Individual variations and the range of values for $E_1$ are so wide, however, that concentrations have not been studied extensively for clinical applications (Lindberg et al., 1974a).

*Estradiol* concentrations are less than 0.1 ng/ml during the follicular phase and may reach 0.2 ng/ml during the luteal phase of normal menstrual cycles. Following a conceptual cycle, $E_2$ closely parallels the pattern described for $E_1$, with a gradual increase to a range of 6-30 ng/ml (Abraham et al., 1972; Lindberg et al., 1974a) at term (Figure 12).

Estradiol originates almost exclusively from the maternal ovaries for the first 5-6 weeks. After this time, the placenta secretes increasing quantities of $E_2$, which it synthesizes from conversion of circulating maternal and fetal DHEA-S. After the first trimester, the placenta is the major source of circulating $E_2$ (Tulchinsky and Hobel, 1973a). At term approximately equal amounts of placental $E_2$ are converted from circulating maternal DHEA-S and fetal DHEA-S (Figure 13) (Siiteri and MacDonald, 1966; Tulchinsky and Korenman, 1971).

In women presenting with threatened first trimester abortion, $E_2$ concentrations measured at the time of initial evaluation are roughly correlated with ultimate prognosis. Approximately 90% of those in whom $E_2$ is less than 0.4 ng/ml at the time of presentation will abort (Nygren et al., 1973).

During the third trimester, $E_2$ concentrations show a rough correlation with clinical outcome. Although original reports were promising (Tulchinsky and Korenman, 1971b), more recent studies indicate a considerable overlap between normal and abnormal values (Lindberg et al., 1974b). In addition, $E_2$ concentrations frequently fluctuate to misleadingly low concentrations (Townsley et al., 1973). Since nearly half of $E_2$ secreted at term is converted by the placenta from maternal DHEA-S, it is possible that the wide undulations in maternal adrenal DHEA-S production may explain, in part, this lack of correlation.

Estriol is undetectable at a sensitivity of 0.01 ng/ml in nonpregnant women. It is first detectable at an assay sensitivity of 0.05 ng/ml at 9 weeks (Tulchinsky and Hobel, 1973a) and then increases gradually to a range of approximately 10-30 ng/ml at term (Figure 12) (Tulchinsky et al., 1971a). Between 30 and 40 weeks gestational age, $E_3$ concentrations describe a characteristic bimodal curve, with a first rise beginning at 30-32 weeks, reaching a peak at 32-34 weeks followed by a fall at the thirty-fifth week, and then a second sharp rise reaching highest concentrations at 37-39 weeks gestational age (Buster et al., 1980b). A preparturitional drop frequently occurs during the week or two prior to onset of labor (Buster et al., 1980b). This pattern is closely correlated with gestational age and has been used as a noninvasive marker of fetal maturity. This technique is described in detail later.

Estriol originates almost exclusively from the placenta (Klopper et al., 1973). It is produced principally from placental conversion of fetal 16$\alpha$-hydroxydehydroepiandrosterone sulfate (16-DHEA-S) (Diczfalusy, 1974, 1969; Liggins, 1972). The appearance of $E_3$ in maternal serum at 9 weeks closely corresponds to increasing steroidogenic evolution of the fetal adrenal cortex (Johannisson, 1968; Tulchinsky et al., 1971a). Its continued production is therefore dependent upon the presence of a living fetus (Figure 14). Concentrations of $E_3$ reflect upon fetal viability, fetal anomalies, hydatidiform mole, and fetoplacental well-being.

Fetal death at any time during the second or third trimester produces a striking drop in $E_3$ concentrations within 1-2 hr (Tulchinsky et al., 1971a). Within 4-6 hr following death, concentrations are consistently less than 1 ng/ml in the second trimester and less than 2.5 ng/ml in the third trimester (Tulchinsky et al., 1971a).

Fetal anomalies associated with adrenal atrophy, such as anencephaly, are associated with low concentrations of $E_3$. For this reason, evaluation of unexplained low $E_3$ concentrations should include ultrasonography or x-ray.

Hydatidiform moles are associated with low concentrations of $E_3$ (Tulchinsky et al., 1971a). Presumably this occurs because of the absence of a fetal adrenal and liver. The

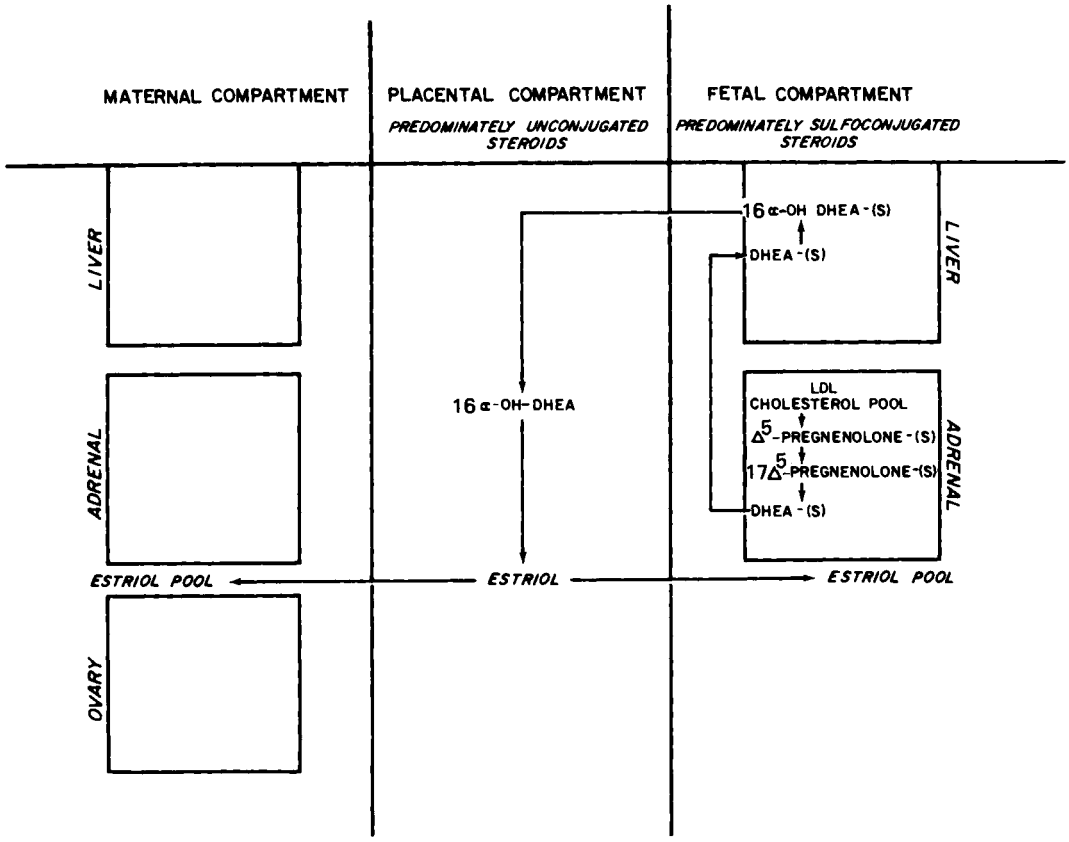


Figure 14 Anatomical compartmentalization of estriol production. The major precursor to estriol production by the placenta is believed to be fetal 16α-hydroxydehydroepiandrosterone sulfate. (Modified from Buster and Marshall, 1980.)

consequent deficiency of fetal 16α-hydroxylated sulfoconjugated precursors would account for the very low $E_3$ values.

Deteriorating fetoplacental health during the third trimester has long been associated with either falling or chronically low $E_3$ concentrations. The weight of available data indicates that placental secretion of $E_3$ is related to such factors as fetal size and fluctuations in fetoplacental oxygen tension. The former has been documented (Loriaux et al., 1972). The latter is strongly suggested by the known rate-limiting effects of fetal precursor availability on placental $E_3$ biosynthesis (Crystle et al., 1973), the decreased availability of fetal precursors in growth-retarded newborn (Reynolds and Mirkin, 1973), and the established observation that steroidogenic pathways involved in the fetal biosynthesis of these precursors are dependent upon the availability of molecular oxygen (McKerns, 1969). Furthermore, published clinical experience with $E_3$ measurements show the best correlations with various hypoxia and malnutritive "placental insufficiency" disorders such as pregnancy-induced hypertension and intrauterine growth retardation (Buster and Ostergard, 1973). The major clinical value of $E_3$ determinations has been in the assurance of fetal well-being and prevention of unnecessary obstetrical intervention

(Buster and Ostergard, 1973). Each patient, however, requires serial sampling in order to establish a trend. Attempts to construct a statistical "fetal danger zone" applicable to all pregnancies have not been successful (Lindberg et al., 1974b).

## HORMONAL REGULATION OF FETAL MATURATION

### Endocrine Regulation of Intrauterine Maturation

In the normally progressing human pregnancy, the preterm acceleration of maturational enzyme kinetics begins at approximately the thirty-second week and is completed by approximately the thirty-seventh week. Delivery at any time from 37 weeks to term is generally productive of a newborn free of the hazards of prematurity. Although a variety of interdependent hormonal events are involved, the weight of available evidence indicates that fetal cortisol is a major effector in the induction to these terminal maturational steps. The ultimate expression of cortisol's actions, however, takes place on the background of changes involving other fetal hormones. Increased circulating fetal prolactin, estrogens, progestogens, and DHEA-S occur and may have their individual or complementary tissue effector activities that augment or otherwise modulate the effects of cortisol. Although cortisol's action is emphasized in the discussion below, the background impact of multiple other hormons is no doubt of substantial but poorly understood significance.

#### Fetal Lung Maturation

The necessary presence of increasing cortisol bioavailability in the development of pulmonary surfactant activity has been documented repeatedly. Beginning at approximately 32-34 weeks gestational age, the choline phosphotransferase pathway becomes increasingly active in the production of dipalmitoyl lecithin, the principal surface-active phospholipid. The exact mechanism by which cortisol performs this function has not yet been clarified; however, it does appear to be acting as an enzyme inductor in close association with thyroxine, prolactin, and estrogens (Liggins, 1972).

#### Regulation of Tissue Glycogen Storage

Cortisol plays a major role in the hormonal control of liver glycogen disposition. Newborn glycogen availability is of major importance in the adaptation to extrauterine life. Liver glycogen content falls rapidly after birth and is almost depleted within 24 hr. During this period of time, maintenance of blood glucose and the supply of glucose to the brain is largely dependent upon liver storage. In addition, the duration of asphyxia that can be withstood by a newborn is directly related to the amount of glycogen stored in fetal cardiac muscle (Liggins, 1972).

#### Fetal Adrenal Medullary Maturation

Cortisol induces the adrenal medullary enzyme phenylethanolamine-N-methyltransferase, the enzymatic complex that facilitates the conversion of norepinephrine to epinephrine. Cortisol is thought to arrive in the medullary region by diffusion and centripetal blood flow from the surrounding cortex. The evolution of this catecholamine function is believed important in the maintenance of fetal circulation during asphyxia. Catecholamines are also important in newborn thermogenesis. In the immature fetal adrenal medulla, norepinephrine comprises the major catecholamine content and product of that organ. With advancing gestational age, there is an increase in the production of

epinephrine to norepinephrine occurring with evolution of this cortisol-induced enzyme (Liggins, 1972).

### Miscellaneous Cortisol Inductions

Cortisol is believed to induce duodenal alkaline phosphatase activity, an event which coincides with a sharp decrease in the ability of the gut to absorb antibodies. Cortisol also induced the multiple hepatic enzymes necessary for carbohydrate, protein, and fat metabolism. Cortisol is related to maturation of the hypothalamopituitary-adrenal axis, to central nervous system growth, and to hypothalamic rhythmicity in experimental animals (Liggins, 1972). This latter activity has been of particular concern because of the widespread clinical practice of maternal corticosteroid administration to accelerate fetal lung maturation during premature labor. Glucocorticoids have been shown to retard the growth of the brain in newborn rats and might have a similar side effect in the human.

## Regulation of Fetal Adrenal Corticoid Production and Peripheral Corticoid Metabolism

The quantities of corticosteroids secreted into the fetal circulation are dependent upon regulatory interrelationships within the hypothalamopituitary-adrenal axis, the placenta and adrenal, the adrenal itself, and peripheral fetal corticoid metabolic interconversions.

### Hypothalamopituitary-Adrenal Trophic Regulatory Functions: Adrenocorticotropin, Prolactin, α-Melanocyte-Stimulating Hormone, and Corticotropin-Like Intermediate Lobe Polypeptide

Adrenocorticotropin hormone ($ACTH_{1\text{-}39}$), prolactin, α-melanocyte-stimulating hormone (α-MSH) (the 1-13 polypeptide sequence of $ACTH_{1\text{-}39}$), and corticotropin-like intermediate lobe polypeptide (CLIP) (the 18-39 polypeptide sequence of $ACTH_{1\text{-}39}$) have been studied extensively as to their roles in the maintenance of the fetal adrenal cortex. Though the role of these trophic hormones has been the center of widespread scientific interest over the past decade, their exact activities and interrelationships are not yet well understood. The possible roles for each of these hormones is reviewed.

*$ACTH_{1\text{-}39}$* Adrenocorticotropin hormone (ACTH)* is detectable in the human fetal pituitary by about the seventh week of gestational age. It is first detectable in human fetal cord plasma at approximately 12 weeks, increasing steadily until about 20 weeks. Fetal plasma levels remain relatively unchanged until approximately 34 weeks gestational age, when a decrement appears which persists until term. Concentrations of ACTH over time therefore do not demonstrate a positive correlation with the increased fetal adrenal mass or increasing steroidogenesis that characterizes the third trimester (Winters et al., 1974b).

Adrenocorticotropin is clearly a major trophin in the regulation of both FZ and DZ steroidogenesis. ACTH cell receptor activity is present in both the FZ and DZ, although FZ activity is diminished somewhat during early second trimester, when HCG is of more importance in its maintenance. The key role of ACTH in FZ ontogeny has long been documented by studies in the anencephalic, in which fetal plasma-immunoreactive ACTH concentrations are low or undetectable and the FZ undergoes atrophy after the twentieth week. In addition, experimental deprivation of ACTH in ovine pregnancies by hypophysectomy, in rhesus pregnancies by surgical anencephaly,

*The abbreviation ACTH refers to the $ACTH_{1\text{-}39}$ polypeptide unless otherwise specified.

and in human pregnancy by long-term suppression with massive maternal corticoid administration have the same common effect, that is, FZ atrophy with low or undetectable circulating fetal ACTH. Administration of pharmacological doses of cortisol to the mother, because cortisol crosses the placenta intact in pharmacological doses, produces marked suppression of fetal ACTH with suppression of fetal $\Delta^5$P-S, 17$\Delta^5$P-S, and DHEA-S (Simmer et al., 1974). There is also suppression of circulating maternal 17P, $E_2$, and $E_3$, the circulating maternal end products of fetal $\Delta^5$-sulfoconjugated secretion, and placental $\Delta^5$ to $\Delta^4$ conversion (Elsner et al., 1979). Recent studies in human fetal adrenal tissues in an in vitro superfusion system show that ACTH stimulates the release of $\Delta^5$P-S and DHEA-S, while isolated definitive zones secrete only cortisol when stimulated by ACTH (Seron-Ferré et al., 1978b; Simpson et al., 1979; Walsh et al., 1979). Adrenocorticotropin hormone acts on the adrenal cell membrane receptor subunit, subsequently expressing its biological effect through adenylate cyclase. Recent in vitro studies indicate that one major focus of ACTH activity within the human fetal adrenal cell is in the uptake of the steroid precursor LDL-cholesterol. Low-density lipoprotein cholesterol becomes available through the metabolism of fetal lipoproteins probably originating in the fetal liver. The presence of ACTH and LDL-cholesterol are both essential in the production of $\Delta^5$P-S and DHEA-S by isolated FZ, and cortisol by isolated DZ (Simpson et al., 1979).

Recent studies in the chronically catheterized fetal rhesus monkey, a model closely analogous to the human, are highly revealing as to the physiologic impact of fetal intravascular ACTH (Walsh et al., 1979). Short-term administration of ACTH to the rhesus fetus, even in large doses, produces little or no change in circulating fetal cortisol concentrations. It thus appears that the FZ is operating at maximum stimulable activity and will therefore not respond to additional ACTH. To demonstrate an in vivo corticotrophic effect of ACTH, it is necessary to administer to the rhesus mother suppressive doses of dexamethasone, which crosses the placenta and suppresses fetal ACTH, thus leaving the receptor sites on the fetal adrenal available to the ACTH administered to the fetus. When this procedure is performed, ACTH administration produces a marked rise in fetal cortisol, DHEA-S, $E_1$, and P (Figure 15). Significant rises in maternal cortisol, DHEA-S, $E_1$, and $E_2$ are also observed (Figure 15). Assuming that the fetal rhesus is analogous to human pregnancy, the following explanation applies: Following dexamethasone suppression of fetal ACTH, with increasing availability of fetal adrenal ACTH receptor sites, fetal administration of ACTH increases the uptake of fetal adrenal LDL-cholesterol, resulting in the formation of large amounts of $\Delta^5$P-S, 17$\Delta^5$P-S, and DHEA-S; $\Delta^5$P-S and 17$\Delta^5$P-S are transported to the placenta and converted to their corresponding $\Delta^4$-3-keto-compounds, P and 17P, which are then recirculated to the FZ and transformed to corticoids. The cortisol rise observed should therefore be the result of the mechanisms shown in Figure 6, responding to the combination of ACTH and LDL-cholesterol. Then DHEA-S is circulated to the placenta, where it is aromatized to $E_1$ and $E_2$. Estrone is selectively released into the fetal circulation, whereas $E_1$ and $E_2$ are released into the maternal circulation.* The rise in maternal cortisol is presumably related to the transplacental transport of fetal cortisol into the maternal circulation, an occurrence well documented in the fetal rhesus (Walsh et al., 1979).

Fetal ACTH is clearly a major adrenal corticotropin. It is likely, however, that its activity is modified by the influence of other trophic modulators.

*In human pregnancy, $E_1$ and $E_2$ are thought to be released into both circulations.

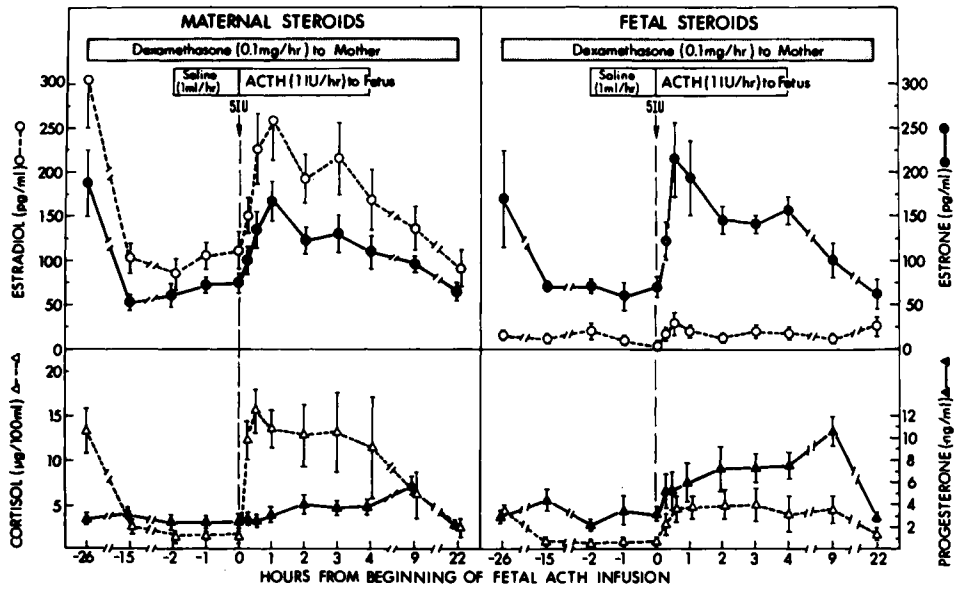


Figure 15 Effects of dexamethasone administration to mothers and ACTH to fetuses on fetal and maternal concentrations of $E_1$, $E_2$, P, and F in chronically catheterized rhesus monkeys. The 26-hr samples were obtained before the start of dexamethasone infusion. Data represent the mean (± SEM) in seven maternal-fetal pairs. Adrenocorticotrophic hormone did not cross from fetal to maternal circulation. (From Walsh et al., 1979.)

*Prolactin* Prolactin is detectable in the fetal pituitary from approximately 10 weeks gestational age. Fetal cord plasma prolactin concentrations describe a biphasic curve. Between 11 and 30 weeks gestational age, prolactin levels remain relatively stable; however, between the thirty-second and thirty-fourth week, prolactin begins to rise and continues to do so until the time of parturition. This third-trimester rise in fetal prolactin concentrations is positively correlated with increasing adrenal mass as well as increasing circulating fetal adrenal steroids and placental estrogens. It has therefore been tempting to speculate that prolactin is a major fetal trophic modulator (Winters et al., 1974a). This hypothesis is further supported by the finding of abundant prolactin receptor activity, particularly in the FZ. Finally, because prolactin is a known 3β-HSD inhibitor in the adult adrenal cortex, it has been postulated as the major effector by which the FZ 3β-HSD deficiency is produced and regulated. While it is tempting to speculate that prolactin may have this activity, it has not yet been documented experimentally and alternative explanations are equally plausible (Winters et al., 1974a).

There is similarly convincing data to indicate that prolactin does not have a significant corticotropic role. The introduction of prolactin into human fetal adrenal in vitro systems has failed to demonstrate effects on steroid production. In addition, thyrotropin-releasing hormone (TRH) induced hyperprolactinemia in the chronically catheterized fetal sheep has failed to produce steroid changes analogous to those observed with ACTH (Lowe et al., 1979b). Finally, chronic TRH-induced hyperprolactinemia

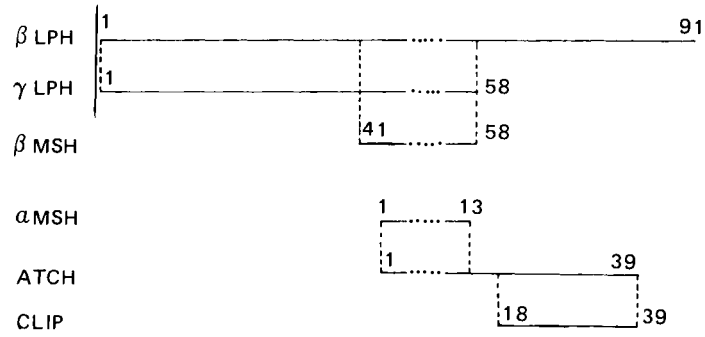


Figure 16 Corticotropin, lipotropin, and melanocyte-stimulating hormones. Beta-lipotropin is a 91-amino acid peptide whose first 58 amino acids are identical in sequence to γ-LPH. Beta-melanocyte-stimulating hormone (β-MSH) shares its 18-amino acid sequence with both β-LPH and γ-LPH. Adrenocorticotrophic hormone (ACTH) is a 39-amino acid peptide whose first 13 amino acids are identical in sequence with α-MSH, and whose 18-39 sequence is identical to corticotropin-like intermediate peptide (CLIP). There is a common heptapeptide core (dashed line) responsible for melanocyte-stimulating activity in β-LPH, γ-LPH, β-MSH, α-MSH, and ACTH. (Courtesy of Professor T. Chard.)

or CB154-induced hypoprolactinemia in the chronically catheterized fetal lamb has no demonstrable effect on fetal adrenal mass, time to parturition, or fetal glucocorticoid production in this species (Lowe et al., 1979a).

It is important to emphasize that the above experiments are only short-term approximations of the totality of gestation. It is therefore plausible that the presence of prolactin over the full duration of pregnancy is necessary for normal fetal adrenal development. Failure to demonstrate prolactin corticotropic activity experimentally may reflect more on the inadequacy of the experimental models involved rather than a lack of this activity.

*α-MSH and CLIP* The fetal pituitary produces a family of polypeptides which are related by molecular configuration to ACTH. Figure 16 summarizes the structural interrelationships of ACTH, beta-lipotropin (β-LPH), gamma lipotropin, (γ-LPH), α-MSH, and CLIP. α-Melanocyte-stimulating hormone and CLIP are thought to be formed in the fetal analog of the intermediate lobe of the adenohypophysis. The ratio of fetal pituitary ACTH activity to the small C-terminal peptides increases sharply with advancing gestational age (Figure 17). A further marked increase is observed in newborn. The increase in the ACTH to small C-terminal peptide ratio is postulated as a central mechanism in the chain of events that leads to atrophy of the FZ and increased activity of the ACTH-modulated DZ of newborn life (Silman et al., 1976).

Other experiments do not support this hypothesis. Infusion of α-MSH into the chronically catheterized fetal rhesus has failed to produce a change in the concentrations of cortisol or DHFA-S (Walsh et al., 1979). Reports of in vivo α-MSH corticotropic activity in the chronically catheterized fetal ovine preparation are conflicting.

## Placental Adrenal Regulatory Functions

The placenta, as the secretor of HCG, is believed to be of importance in FZ maintenance and regulation during the first 20 weeks of gestation. Human CG receptor

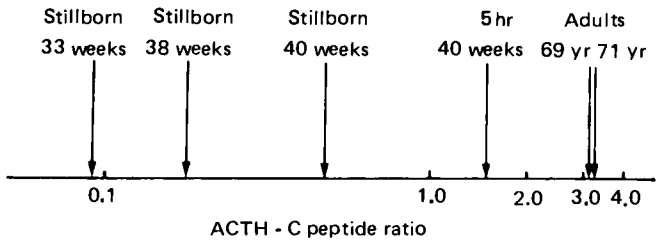


**Figure 17** Ratio of ACTH activity to small C-terminal peptide activity in adult and fetal pituitaries. Fetal pituitaries were obtained from a 33-week stillbirth, a 38-week stillbirth, a 40-week stillbirth, and a term infant who survived 5 hr. Adult pituitaries were obtained at necropsy from patients aged 69-71 years. (From Silman et al., 1976.)

activity is present in the FZ and is known to stimulate fetal adrenal production of DHEA-S both in vivo and in vitro. Human CG appears to be of lesser importance after the twentieth week, when this zone is primarily influenced by ACTH. Fetal zone atrophy following delivery may be due to removal of placental HCG, but loss of other trophic factors may be equally or more important (Seron-Ferré et al., 1978a).

A second influence of the placenta on FZ maintenance may occur as a result of the massive quantities of P secreted into the fetal circulation. One plausible explanation for the FZ 3β-HSD deficiency is mass inhibition of the $\Delta^5$-$\Delta^4$ transformation by the enormous quantities of P presented to the FZ. Removal of the placenta would thus result in FZ atrophy by removal of this functional 3β-HSD inhibitory effect (Bloch, 1968).

### Fetal Adrenal Autoregulation

It has long been known from fetal lamb studies that the fetal adrenal acquires increasing sensitivity to constant amounts of circulating ACTH with advancing gestational age. More recently this has been documented in primates and is supported additionally by in vitro studies in human fetal adrenal tissues. Three different mechanisms have been proposed to explain these events.

*Increasing Fetal Adrenal Mass* The human fetal adrenal increases markedly in mass during the time interval between 32 and 36 weeks gestational age (Figure 18). This increase in cell numbers may be associated with increased ACTH or other trophic receptor availability. As such, the organ as a whole becomes increasingly responsive to unchanging or even decreasing concentrations of trophic hormones (Nathanielsz, 1976).

*ACTH Stimulation of Its Own Membrane Receptor Subunit* Adrenocorticotropin hormone may stimulate an increase in the affinity and binding capacity of its own membrane receptor subunits of the adenylate cyclase receptor enzyme complex (Figure 19) (Nathanielsz, 1976). In addition, it is also possible that cortisol itself may have a positive feedback effect within the adrenal itself, acting by way of the cell membrane ACTH-receptor subunit (Figure 19) (Nathanielsz, 1976).

*Fetal Adrenal Blood Flow* Changing adrenal blood flow, affected by arterial oxygen tension, trophic hormones, or intra-adrenally autoregulated blood flow serve to change the exposure of fetal adrenal receptor subunits to differing numbers of trophic molecules. The relative regulatory importance of fetal adrenal blood flow is poorly understood at the present time (Llanos et al., 1979; Peeters et al., 1979).

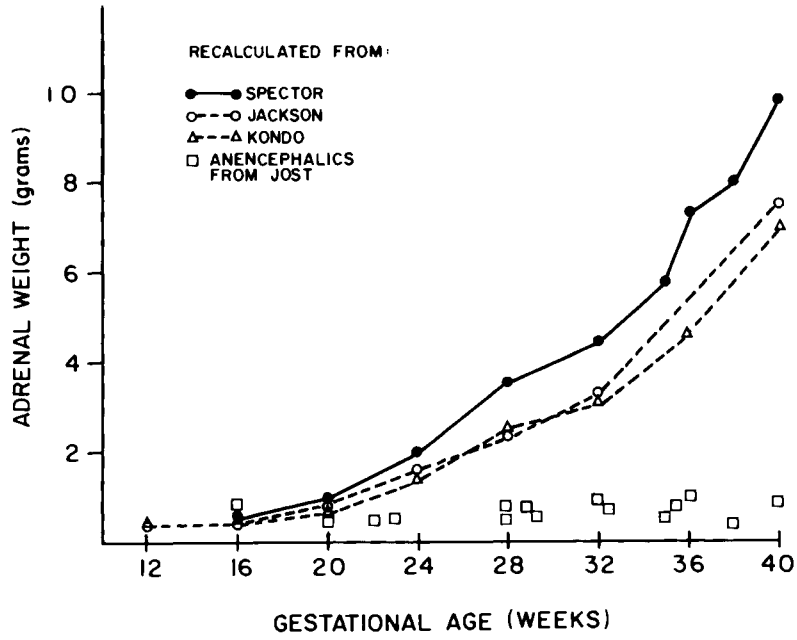


**Figure 18** Total fetal adrenal mass as function of gestational age. Rapid increase in growth velocity begins between 32 and 36 weeks gestational age. In anencephalic pregnancies, adrenal mass does not increase after the second trimester. Weights were obtained and replotted from three separate reports. (Modified from Buster, 1980.)

### Peripheral Fetal Corticoid Metabolic Regulation

Circulating fetal cortisol and amniotic fluid cortisol and cortisol sulfate concentrations are known to increase with advancing gestational age and approaching parturition. Virtually all fetal organs, with the exception of the amniotic membranes and decidua, convert cortisol to cortisone. The intracellular mechanisms of cortisol to cortisone interconversion and its regulatory effect on the biological expression of cortisol is depicted in Figure 20. As parturition approaches, conversion of cortisol to cortisone is suppressed in tissues such as the fetal lung, as is shown in Figure 21. Clearly as the cortisol to cortisone conversions decrease, the net effect is increasing bioavailability of cortisol to maturing fetal tissues.

The tropic control of these peripheral metabolic transformations is not understood, yet the common effects of advancing gestational age and approaching parturition on these functions and on fetal adrenal cortical activities suggest the presence of common trophic modulators (Murphy, 1978, 1979).

### Summary of Fetal Endocrine and Maturational Relationships

Figure 21 is a summary of the endocrine events and corresponding maturational processes discussed earlier. Each panel in Figure 21 contains a vertical bar outlining the interval between 32 and 37 weeks gestational age, the time at which initiation and completion of preterm maturational events are known to occur. Panel A demonstrates the parallel acceleration of the L/S ratio and liver glycogen content beginning at approximately the

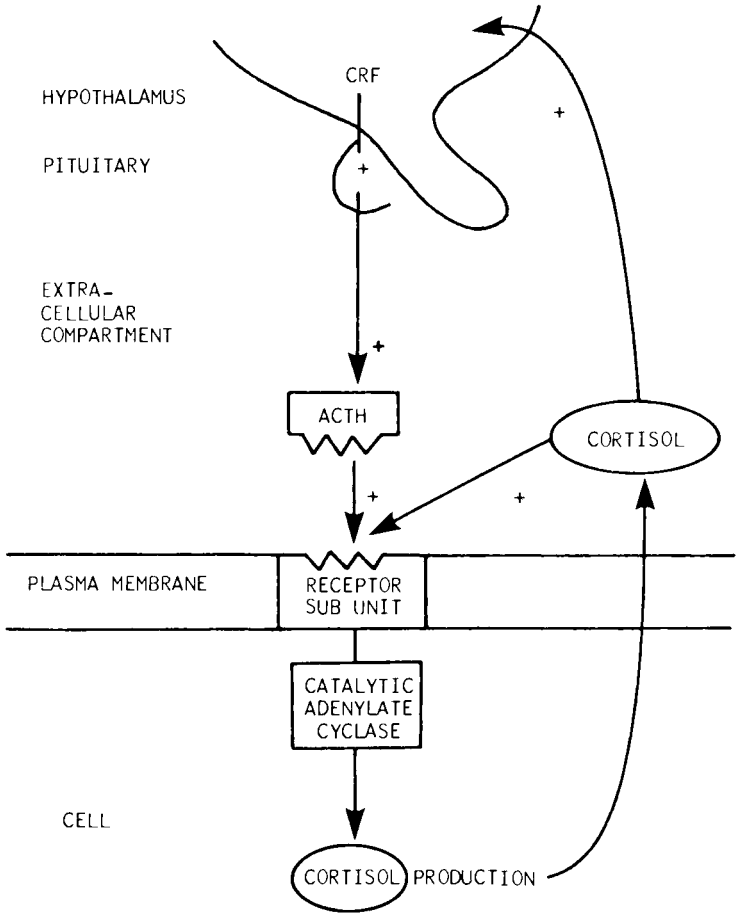


**Figure 19** Diagrammatic representation of possible factors that influence the development of the receptor subunit of ACTH-sensitive adenylate cyclase in the fetal adrenal glucocorticoid-secreting cell. Adrenocorticotrophic hormone may have both short and long positive feedback loops affecting receptor subunit activity (+, stimulatory factors). (Modified from Nathanielsz, 1976.)

thirty-second week and continuing through the thirty-seventh week. Panel B shows accelerating adrenal growth velocity and increasing fetal prolactin as they both become particularly pronounced at approximately 35 weeks gestational age. Panel C demonstrates the rapidly incrementing concentrations of amniotic fluid corticosteroid sulfates, this reflecting on the net impact of increased adrenal secretion and increased peripheral cortisone-to-cortisol conversion. The similarities over time between liver glycogen deposition and the L/S ratio (panel A) and corticosteroid sulfate concentrations (panel C) should be emphasized. Fetal ACTH shows a significant decrement in the 35-40 week interval as opposed to the 26-35 week interval, an event probably reflecting on negative feedback suppression of ACTH from increasing production of fetal cortisol. Amniotic fluid DHEA-S and circulating fetal $E_3$ show significant increments at approximately 35 weeks, as demonstrated in panels E and F, respectively.

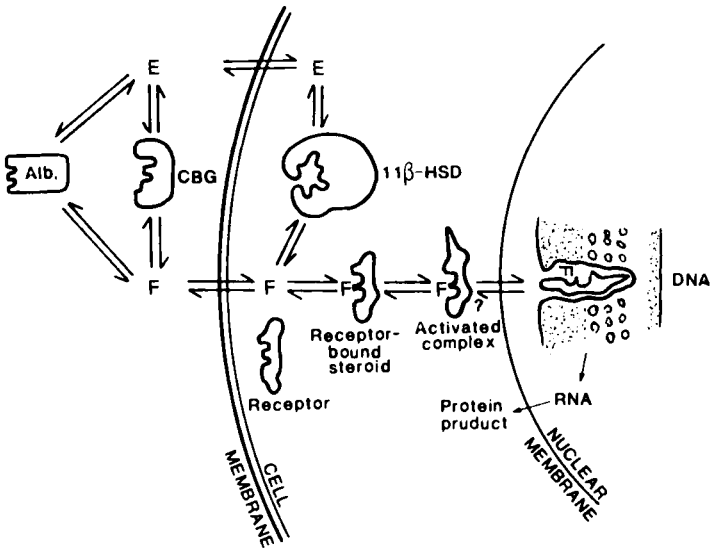


**Figure 20** Mechanism of cortisol (F) action. Target organ cells may autoregulate F interaction with corticoid receptors by diverting F to E through enzyme conversion. (From Murphy, 1978.)

Estriol concentrations, shown in Figure 21, describe a complex curve between 30 and 40 weeks gestational age. The $E_3$ curve is a bimodal structure with a rise beginning at approximately 30-32 weeks gestational age, reaching a maximum at approximately 34 weeks followed by a transient fall or nadir at the thirty-fifth week, and a second rise reaching its peak at between 37 and 39 weeks. The physiologic basis for this complex bimodal structure is not currently understood, but a reasonable explanation for this event is as follows. A sharp elevation of circulating fetal prolactin occurs between 34 and 36 weeks gestational age, as seen in Panel B (Winters et al., 1974a). Because prolactin is known to inhibit adrenal 3β-HSD activity, increased fetal prolactin production may augment adrenal secretion of the $E_3$ precursor, DHEA-S. That this actually occurs is further supported by the abrupt rise in amniotic fluid DHEA-S (panel E) and cord $E_3$ (panel F) after the thirty-fourth week. Thus the abrupt rise observed from the 35-week nadir point over the subsequent 2-3 weeks may in part be explained by a fetal prolactin-modulated mechanism (Buster et al., 1980b).

Recently, the time trend effects of circulating $E_3$ concentrations have been utilized as a noninvasive marker of gestational age and intrauterine maturation (Buster et al., 1980a). By utilizing a motorized withdrawal pump which corrects for the short-term pulsatile variability in $E_3$ concentrations, the characteristic bimodal curve can be reproduced within individual subjects with striking regularity. Recent studies indicate that the juncture point between the two modes, or nadir, corresponds closely with the thirty-fifth gestational week.

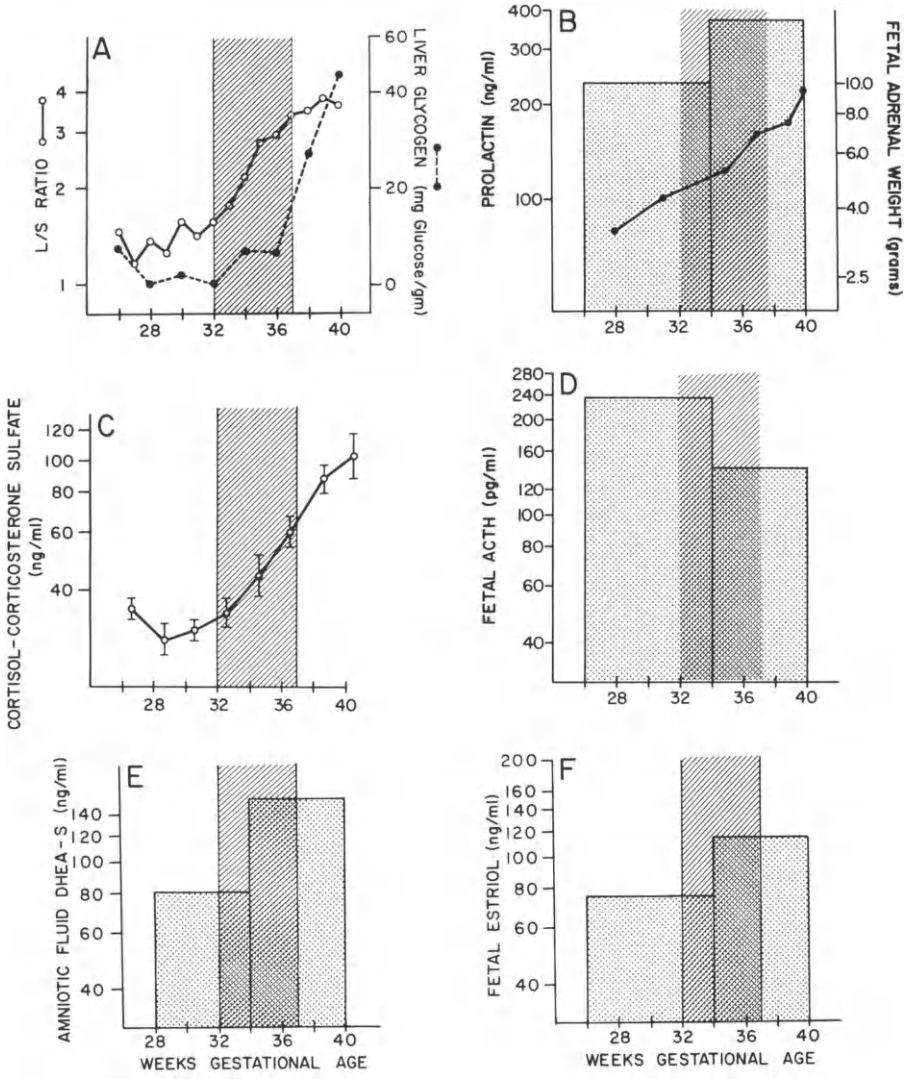


**Figure 21** Composite fetal maturational relationships and endocrine correlates with advancing gestational age. There is a concomitant acceleration of fetal maturational processes, for example, lecithin/sphingomyelin (L/S) ratio and liver glycogen content (A), beginning at approximately the thirty-second week and continuing through the thirty-seventh week, with similar time trend effects. Amniotic fluid cortisol corticosterone sulfate concentrations (C) show a time trend effect similar to the L/S ratio and liver glycogen, as does adrenal growth velocity (B). Fetal adrenocorticotropic hormone (ACTH) drops after 34 weeks (D), presumably as a result of increasing adrenal sensitivity to ACTH. Fetal prolactin increased (B), as does fetal $E_3$ (F) and amniotic fluid dehydroepiandrosterone sulfate (DHEA-S) (E).

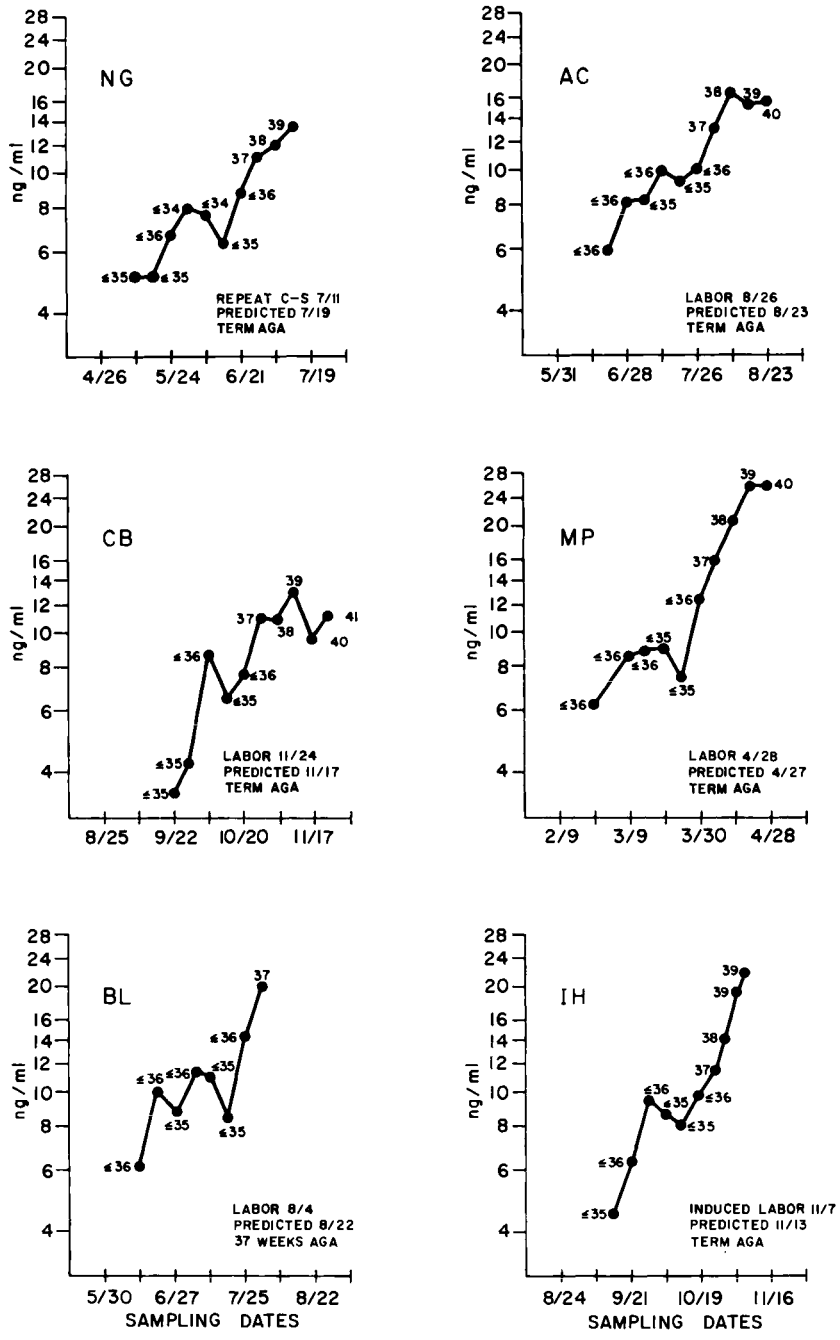


**Figure 22** Estriol time concentration curves in six subjects ultimately dated to the week, demonstrating increasing program precision with increasing numbers of points. Exact predictions can be made as early as the thirty-sixth week but usually are not made until the thirty-seventh week of gestational age (AGA, approximate gestational age). (Reprinted from Buster et al., 1980a).

Utilizing a computerized algorithm which analyzes $E_3$ concentrations relative to level and relative to the development of curve shape, it is possible to make increasingly precise statements relative to gestational age with increasing numbers of points. Figure 22 demonstrates the use of this algorithm in six individual subjects. In the overwhelming majority of both normal and abnormal pregnancies, whenever the algorithm projects a gestational age less than or equal to 36 weeks, an immature fetus with an L/S ratio less than or equal to 2.3 will be found. Whenever the algorithm projects a gestational age less than or equal to 37 weeks, a mature fetus is likely to be found (Buster et al., 1980a,b). The major attribute of this procedure is its noninvasiveness; it is, however, still an investigative instrument and can be utilized only as a guide to optimal timing of amniocentesis for fetal maturity (Buster et al., 1980a,b).

## HORMONAL REGULATION OF PARTURITION

The present level of knowledge does not permit a unified construct that clearly binds the multiplicity of fetal and maternal regulatory mechanisms that trigger the onset of labor in the human. The weight of available data, however, indicates that the final common pathway leading to myometrial irritability, contractility, and actual labor is directed through mechanisms modulating myometrial adenylate cyclase and intracellular calcium sequestration (Korenman and Krall, 1977). Through a series of complex interactions beginning with cell membrane receptor binding by hormones or other regulatory substances, cyclic adenosine 5'-monophosphate (cAMP) levels are either increased or suppressed, eventuating through several steps to increased intracellular sequestration of calcium (increased cAMP), with relaxation or calcium liberation (decreased cAMP) with contraction. Known intermediate details of this process have been reviewed in depth (Figure 23) (Korenman and Krall, 1977). Major effectors interacting with specific cAMP-modulating receptors include the prostaglandins and their intermediates, oxytocin, and catecholamines. Each of these are reviewed below.

### Fetal Factors

Fetal factors include fetal membrane decidual prostaglandin synthesis, fetal steroids, and fetal oxytocin.

#### Prostaglandins

Prostaglandins (PGs) exert both a sensitizing and myometrial stimulating effect through their specific-surface receptors (Korenman and Krall, 1977). The PG sequence beginning with arachidonic acid enriched phospholipids is outlined in Figure 24. Arachidonic acid, the common progenitor to the various PGs and their intermediates, resides in cell membranes of the amnion, chorion, and decidua, primarily in the 2-acyl position of the ubiquitous glycerol phospholipids (Liggins et al., 1977; Ramwell et al., 1977; MacDonald et al., 1974; Schwarz et al., 1976b). Bound as an arachidonate-2-glycerol phosphatide, arachidonic acid is released into the PG sequence by the lysomal enzyme phospholipase-$A_2$ ($PLA_2$) (Liggins et al., 1977; Schultz et al., 1975; Schwarz et al., 1976b). Activity of $PLA_2$ is greatest in the amnion and decidua and is maintained within the stabilized lysosomal membranes of these tissues (Liggins et al., 1977), a stability believed to be

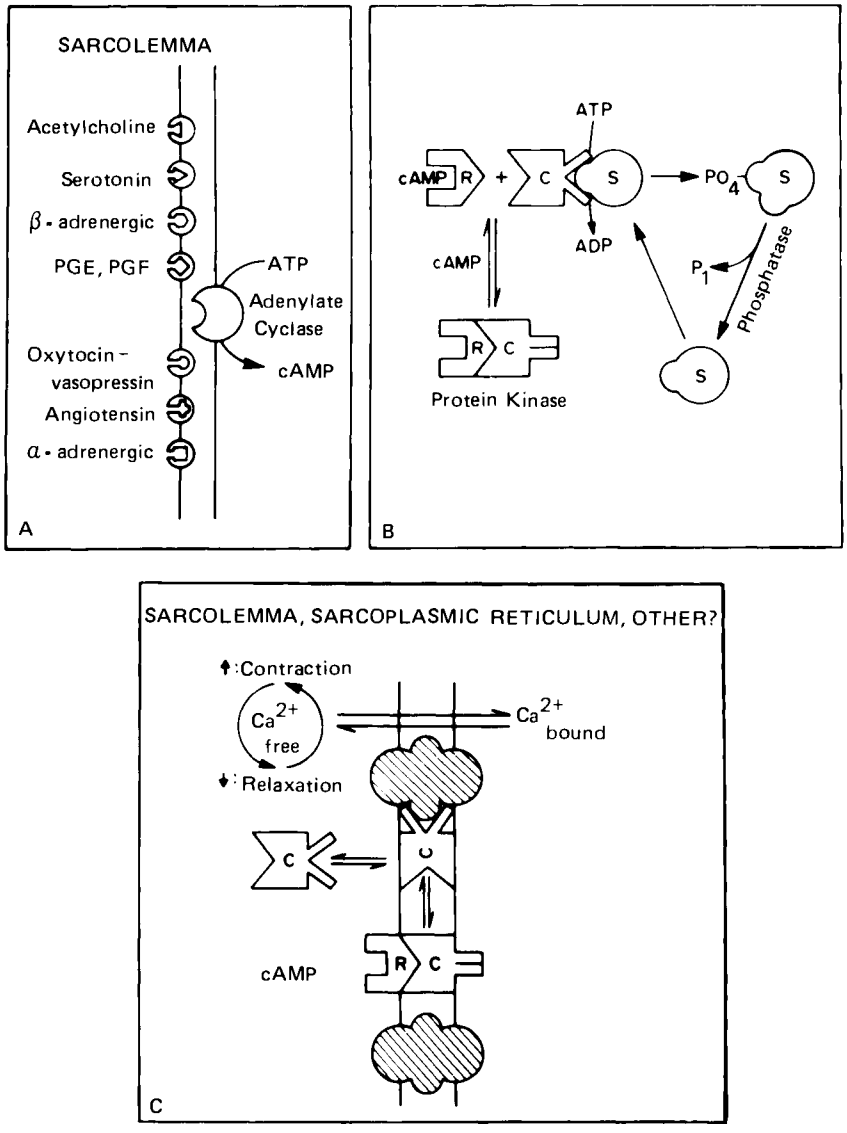


**Figure 23** Possible mechanisms through which the myometrial adenylate cyclase system regulates contractility. (A) Cyclic AMP levels elevated by β-adrenergic stimulation of adenylate cyclase in the sarcolemma are modified by a variety of active agents in a process mediated by their specific membrane receptors. (B) Cyclic AMP activates cytosol protein kinase by binding to the regulatory subunit (R), liberating free catalytic subunit (C), which then phosphorylates specific protein substrates (S), altering their activity or function. (C) In myometrial smooth muscle cells, free catalytic subunits translocate to cell membranes, where they are incorporated through hydrophobic interactions. Increased phosphorylation of specific membrane proteins by the newly acquired protein kinase molecules in some manner increases $Ca^{2+}$ transport activity, lowering the free:bound ratio of intracellular $Ca^{2+}$, causing inactivation of actomyosin $Ca^{2+}$-sensitive ATPase and hence relaxation. (From Korenman and Krall, 1977.)

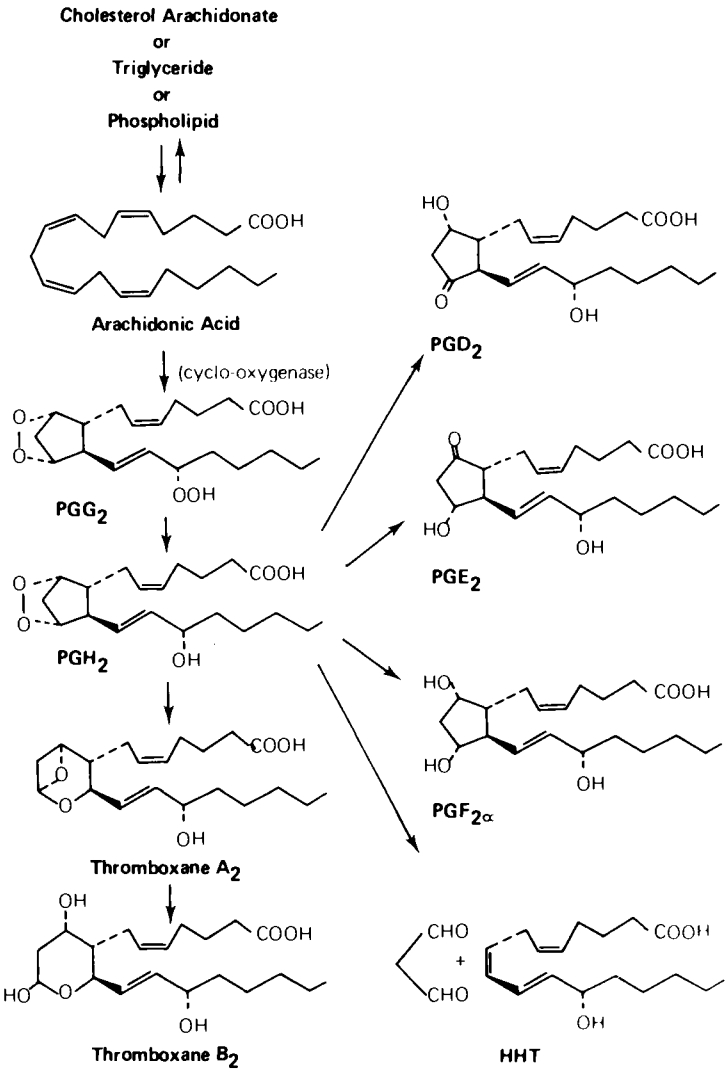


Figure 24 Arachidonic acid cascade. (Modified from Ramwell et al., 1977.)

maintained in part by P and disrupted by estrogens (Liggins et al., 1977; Schwarz et al., 1976a). Activity of $PLA_2$ is increased by any mechanical, toxic, or hormonal factor that disrupts the integrity of lysosomal membranes. Once arachidonic acid is liberated, it is acted upon quickly by cycloxygenase to form $PGG_2$ and $PGH_2$ endoperoxides, which themselves, though short lived, may be biologically active prior to their transformation to the classical E and F series PGs (Ramwell et al., 1977). Phospholipase-$A_2$ activity is believed to be the major rate-limiting factor (Liggins et al., 1977; Ramwell et al., 1977; Schultz et al., 1975; Schwarz et al., 1976b) and may be a major regulatory point in the initiation of parturition. During labor there is a marked elevation of amniotic fluid arachidonic acid, $PGE_2$, and $PGF_2\alpha$, presumably from retrograde movement of these substances from fetal membranes and decidua (MacDonald et al., 1974).

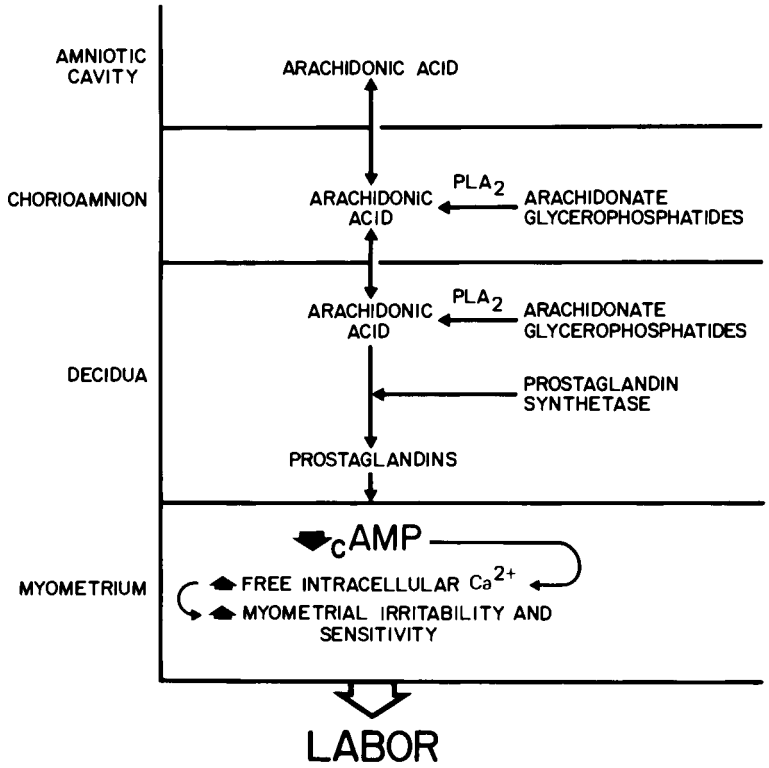


**Figure 25** Anatomic compartmentalization of phospholipids and prostaglandin synthesis in fetal membranes and decidua. The final common pathway for uterine contractility is mediated through cAMP. Prostaglandins are believed to suppress intracellular cAMP levels, which, through intermediate steps, lead to increased intracellular unbound $Ca^{2+}$ and myometrial contraction. (From Buster and Marshall, 1980.)

Prostaglandins secreted into the maternal circulation are cleared by the lungs and therefore are not detected at elevated concentrations in the peripheral circulation (Ramwell et al., 1977). The anatomic localization of these processes is depicted in Figure 25.

### Fetal Steroids

By strong inference from animal data and as suggested by the tendency of anencephalic pregnancies to deliver either too early or too late, it is likely that the fetal hypothalamo-pituitary-adrenal axis is at least a fine-tuning modulator in the timing of human parturition. In human studies, there is no characteristic shift of maternal steroid concentrations just prior to or during labor (Liggins et al., 1977). The demonstration of a P-binding substance (Schwarz et al., 1976a) in the chorioamnion, a protein which competes for P and that may labilize lysosomal membrane, provides a steroid-mediated mechanism that would not be measureable as a change in circulating concentrations of steroids in either the mother or fetus. Although animal models describing fetal steroidal modulation of parturition have been reviewed widely (Nathanielsz, 1976; Thorburn et al., 1977; Lanman, 1977; Liggins, 1977), the extension of these models to man is very uncertain.

### Fetal Oxytocin

Fetal oxytocin concentrations measured after vaginal delivery exceed those measured in newborn delivered by cesarean section (Chard et al., 1971). Oxytocin from the fetal side is transferred into the maternal circulation (Dawood et al., 1977) and could be important in initiating and/or maintaining labor. This is implied from studies in fetal lambs showing that oxytocin infusion on the fetal side stimulates uterine contractions (Nathanielsz et al., 1973).

## Maternal Factors

### Maternal Oxytocin

Hormonal events within the uterus cause it to become increasingly sensitive to maternal influences of oxytocin with advancing gestational age. Oxytocin sensitivity is practically nil until approximately 20 weeks gestational age, at which time it increases progressively to plateau at 36 weeks until just prior to labor, when it increases again (Quilligan, 1973). Maternal oxytocin is released in spurts once labor is started. The further labor has progressed, the greater the spurt frequency (Gibbins et al., 1972; Chard, 1973). Oxytocin acts directly on myometrial receptors to produce its depolarizing effect, possibly by way of adenylate cyclase suppression, and is therefore presumably complementary to the prostaglandins (Korenman and Krall, 1977). Maternal oxytocin may be involved with the maintenance of established labor.

### Maternal Catecholamines

Maternal catecholamines reach myometrial alpha- and beta-receptors both by the circulation and by neuronal transmission. Catecholamine receptors are copious throughout the myometrium, but the exact role of catecholamines in modulating parturition is unknown (Korenman and Krall, 1977).

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# 18

# Energy and Substrate Requirements for Fetal and Placental Growth and Metabolism

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## INTRODUCTION

The purpose of this chapter is to present some of the basic concepts of fetal metabolism which have emerged from studies in several laboratories conducted in a variety of mammalian species. Studies in fetal physiology have been hampered by the difficulties in obtaining access to the fetus in order to collect physiologic and biochemical data. In the smaller mammals this has always limited the data collection to conditions of acute stress, imposed by either the surgical techniques used in obtaining access to the fetus or mother, or the anesthesia used for surgery. There have been several studies which have clearly documented the distortions in normal fetal and placental physiology imposed by stress (Rudolph and Heymann, 1967; Simmons et al., 1974; Battaglia, 1979a). This distortion is particularly relevant to metabolic studies and is compounded, for example, when one attempts to obtain data on fetal metabolism in man, where access to the fetus is virtually confined to the time of delivery. On the other hand, several laboratories have developed techniques for chronic catheterization of the fetal and maternal circulations. These techniques have been applied almost exclusively to the larger mammals and have been confined to the latter 20-25% of gestation.

It is important to point out that most of the comparisons which have been made of species differences in fetal and placental metabolism were not valid comparative physiologic studies, since they compared not only different species, but also species studied under quite different biologic states, (i.e., data collection from one species under conditions of acute stress versus data collected in the second species under chronic steady-state conditions). The definition of a biologic steady state must be viewed in the context of the particular characteristic of metabolism being investigated over a comparatively short period of time, since in a rapidly growing organism such as the fetus, total body pool sizes, total mass of protein, and total quantities of other constituents are increasing, if the time scale of the study period represents a significant fraction of the total gestational length. The value of such steady-state observations carried out in chronic animal preparations is that one can have confidence that the description of metabolic processes which is being obtained is at least compatible with continued growth and survival of the fetus.

## FETAL MASS AND BODY COMPOSITION

The growth rate of the fetus and its body composition are dealt with in detail elsewhere in this volume (see Chapters 6, 7, 8, and 10); however, it is important to emphasize that these factors play a critical role in determining the energy requirements and the requirements for carbon and nitrogen which are imposed upon the mother by the developing conceptus. Mammalian fetal development is characterized by a complete dependence upon a continuous stream of nutrients provided to the conceptus by the uterine blood flow, and to the fetus by the placenta. There is no large store of yolk to sustain the embryo and fetus during development. In marsupials, this period of in utero development and dependence upon placental function is rather brief. The birth weight of the fetus is measured in milligrams, even for marsupial species where adult weight can be as great as 30-40 kg. Thus the fetal mass at delivery is a very small percentage (<0.1%) of maternal body weight, even in species with a litter size as large as 20 (Tyndale-Bisco, 1971). Most of the growth of the fetus occurs postnatally during pouch life, with the energy requirements provided by the marsupial milk. In contrast, eutherian mammals produce a much larger fetal mass, particularly in the small polytocous mammals. For example, in the rat the total fetal mass may equal approximately 20% of the maternal weight at term, and in the guinea pig approximately 50% of the maternal weight. To this fetal weight one must add the combined weights of the placentas. Such a large combined mass of conceptus provides a considerable burden upon maternal metabolism. Primates, including man, are quite different from other mammals, in that they produce a smaller fetal mass in proportion to maternal size, and do so over a much longer gestation (Leitch et al., 1959).

A further factor in determining the caloric accretion rate is body composition. The two components of the body which may vary among fetuses of different species are the water content and the fat content. Both components have a profound impact upon the caloric accretion rate of a fetus, since water contributes nothing to the caloric content and fat contributes 9.5 kcal/g compared to the nonfat wet weight, which has a caloric value of approximately 0.9 kcal/g. In a recent report (Sparks et al., 1980) it was demonstrated that the human fetal caloric accretion rate exceeds that of the fetal lamb, despite a growth in body weight which is only one-third of the lamb, the reason being the much greater fat content of the human fetus at term compared to that of the lamb (16 versus 2%).

The variability among mammals extends to the maternal diet, not only in the composition of the diet, but also in the fast-feed cycles characteristic of a species. Some species such as the vampire bat feed briefly once a day, with a fast cycle which may be as long as 22 hr (Wimsatt, 1969). Others, such as the pregnant guinea pig, feed almost continually. There have been few studies which have looked at alterations in the usual feeding patterns of species induced by pregnancy, although such changes may play an important part in maternal adjustments to pregnancy.

Thus fetal metabolism in mammals goes on against a background of an enormous diversity of maternal diet, gestation length, total fetal mass relative to maternal size, and body composition. This diversity affects those biologic factors, which play a critical role in determining fetal caloric requirements and the metabolic demand placed upon the mother by the conceptus. Such variability among and within species has often been regarded as an obstacle to be overcome in the search for an animal model of human biology. However, this variability provides an elegant entrée for a comparative

physiologic and biochemical approach to identify concepts in maternal and fetal metabolism which bridge species differences, and thus provides a basis for understanding human fetal metabolism (Battaglia, 1978).

## FETAL SUBSTRATE REQUIREMENTS

The substrate requirements of the fetus can be considered in two ways: (1) in terms of caloric requirements and (2) in terms of the total carbon and nitrogen requirements. These requirements, whether expressed calorically or in terms of the elemental building blocks, are defined by the fuel and growth requirements of the organism. Substrates are often spoken of as "fuels," that is, "fetal fuels," "maternal fuels," etc. Often this is an inaccurate description of what is measured in a study, namely, the concentration of a substrate, without delineating whether that particular substrate is used primarily for fuel or for the synthesis and accretion of new tissue. Thus the daily caloric requirements of the fetus will be determined by the growth rate in a particular species, the composition of the new tissue laid down, and the metabolic rate of the fetus. The first two factors, growth rate and body composition, vary quite markedly among mammals. The weight-specific metabolic rate reflected by fetal oxygen consumption is quite similar among fetuses. This was first pointed out by Battaglia and Meschia (1978). Table 1 presents calculations of fetal caloric requirements which have been made for two species, man and sheep. Fetal oxygen consumption has been measured either directly or estimated indirectly for mammalian species which are quite different in size. Since the weight-specific metabolic rate of adults in different species follows the three-fourths power of body weight (Kleiber, 1947), it is clear that the conceptus would represent a tissue mass of lower oxygen consumption than the rest of the mother in small mammals, and an equal or higher oxygen consumption in the larger mammals. This is precisely what has been found in the few species studied thus far (Sandiford and Wheeler, 1924; Behrman et al., 1970; DeMeyer et al., 1971). Table 2 compares several species and illustrates a number of points. First, the two characteristics we have discussed act synchronously in development; that is, as one increases adult size, litter size and the size of individual fetuses as a percentage of maternal size tend to decrease. Thus the percentage of maternal weight represented by fetal weight is only 5-6% in man. This would imply a much higher metabolic demand imposed upon the mother in the smaller mammals compared to the larger mammals. As a counterpoint, however, the weight-specific metabolic rate of the fetus is less than the maternal rate in the smaller mammals. The end result is that the metabolic rate of the total fetal mass, expressed as a percentage of the maternal

**Table 1** Fetal Caloric Requirements (kcal/kg per day)

| | Sheep | Human |
|---|---|---|
| Oxidation | 56 | 55 |
| Growth | 32 | 40 |
| Total | 88 | 95 |

*Source*: From Sparks et al. (1980).

Table 2 Comparison of Metabolic Rates Among Species[a]

| Species | Maternal weight (kg) | Fetal weight (kg) | $M_m$ (kcal/day) | $M_f$ (kcal/day) | Fetal wt./ maternal wt. X 100 | $M_f/M_m$ X 100 | References |
|---|---|---|---|---|---|---|---|
| Man | 56 | 3.2 | 1501 | 180.6 | 5.7 | 12.0 | Battaglia et al. (1968), Bossi and Greenberg, (1972) |
| Sheep | 50 | 4.5 | 1316 | 222 | 9.8 | 16.8 | Battaglia (1979b) |
| Rhesus monkey | 5.3 | 0.43 | 250 | 25.8 | 8.1 | 10.3 | Boyd et al. (1973) |
| Guinea pig | 0.400 | .236 | 48 | 14.1 | 59.0 | 29.7 | Gresham et al. (1971) |
| Rat | 0.160 | .037 | 21 | 3.13 | 23.1 | 15.0 | Battaglia and Meschia (1978); Burd et al. (1975) |
| Lesser horse-shoe bat | 0.006 | .0021 | 1.5 | 0.22 | 35.0 | 14.7 | Battaglia and Meschia (1978), Baur (1977) |

[a] $M_m$, metabolic rate of the mother; $M_f$, metabolic rate of the total fetal mass.

metabolic rate, is quite similar among mammals varying widely in maternal size, fetal size, and litter size.

## PLACENTAL TRANSPORT

The placenta is an organ whose primary function is the transport of nutrients and waste products between the maternal and fetal circulations. All of the following factors may alter the transport of nutrients across the placenta: maternal nutrient concentrations, uterine blood flow, umbilical blood flow, and placental permeability. Maternal nutrient concentrations may be altered by maternal diet, fast-feed cycles, and endocrine changes in the mother. A linear relationship has been described between maternal arterial concentrations and fetal arterial concentrations for a wide variety of nutrients (Figure 1) (Silver et al., 1973; Coltart et al., 1969; Chinard et al., 1956); however, it is important to emphasize that this does not imply a linear relationship between maternal arterial concentration and placental transport of the same nutrients. The implication is often made that an increase in maternal concentration leads to a proportionate increase in placental transport in studies of "maternal fuels," but this is not supported by experimental data. For example, glucose transport has been studied in some detail under chronic steady-state conditions. It has been clearly demonstrated that (1) there is no linear relationship between maternal arterial concentration and placental glucose transport (Simmons et al., 1979) and (2) there is a high rate of placental glucose consumption (Meschia et al., 1980).

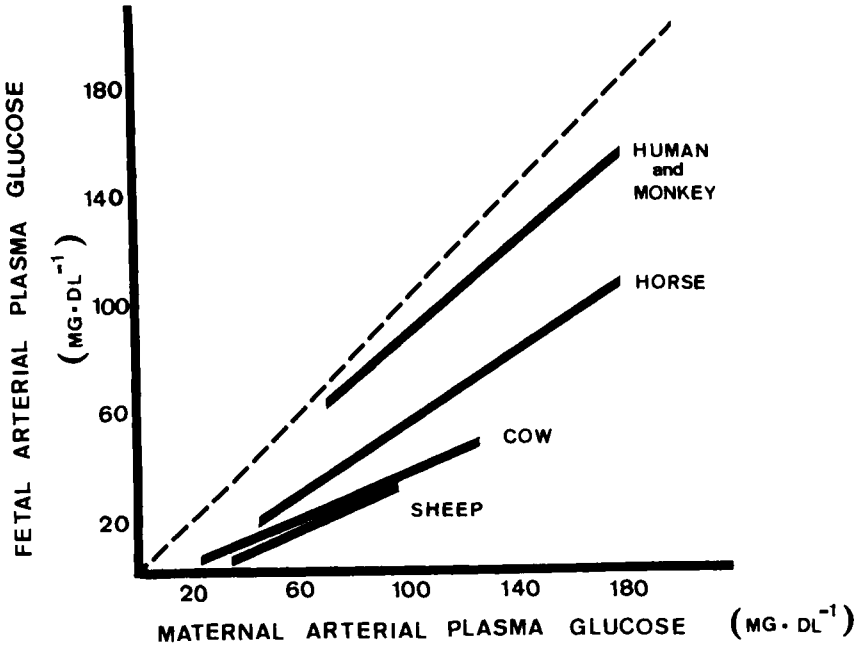


**Figure 1** The relationship of fetal arterial plasma glucose concentration to maternal arterial plasma glucose concentration for the human (Coltart et al., 1969), monkey (Chinard et al., 1956), horse (Silver and Comline, 1976), cow (Comline and Silver, 1976), and sheep (Silver et al., 1973; Simmons et al., 1979).

In 1952 Widdas speculated from data obtained by others that the rate of glucose transport from the mother to the fetus might be described by

$$\text{Placental transport} = K_1 \frac{G}{(G + K_2)} - \frac{g}{(g + K_2)}$$

where G and g are the maternal and fetal arterial glucose concentrations, respectively, and $K_1$ and $K_2$ are constants (Widdas, 1961). In the sheep during the latter 20% of gestation, experimental data have permitted a precise description of the relationship between maternal and fetal concentrations on the one hand, and placental transport on the other. The high rate of placental glucose utilization, discussed elsewhere in this chapter, is reflected by the negative intercept when one plots placental transfer versus arterial concentration differences across the placenta. Thus the Widdas equation becomes modified as follows:

$$\text{Placental transport} = K_1\left[\frac{G}{(G + K_2)} - \frac{g}{(g + K_2)}\right] + \dot{q}_p$$

where $q_p$ is the placental utilization rate of glucose. In the sheep, the only species in which this value has been estimated, $q_p \cong -35$ mg/min. Experimentally, $K_1$ and $K_2$ have been determined and the data used to construct Figure 2, which presents the relationship between maternal arterial glucose concentration and placental glucose transport (Simmons et al., 1979).

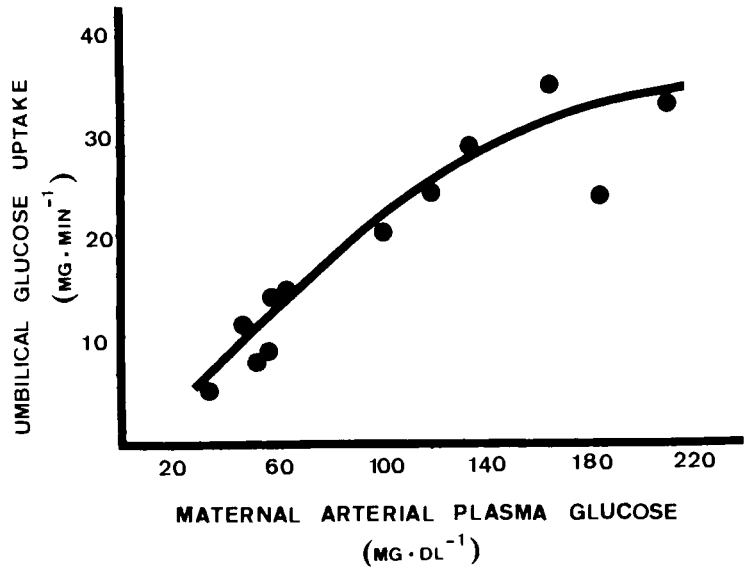


Figure 2 Umbilical glucose uptake versus maternal arterial plasma glucose concentration in the sheep. (From Simmons et al., 1979.)

Some studies have tried to sort out the independent effects of changes in perfusion on the one hand (i.e., umbilical and uterine blood flows) and changes in permeability on the other hand, upon the transport of nutrients across the placenta. It is clear that a simple linear relationship between placental transport and uterine or umbilical blood flow does not exist. This is true even for the placental transport of substances, which are relatively inert and transported by simple diffusion. The clearance of such a substance would be a function of placental permeability and placental perfusion. If the placenta were highly permeable to the compound, its rate of placental transport would be a function of placental perfusion; that is, rates of placental transport would be flow limited. In early studies of sheep and rhesus monkey placentas, antipyrine and tritiated water were shown to fulfill these characteristics; that is, their placental clearances were equal to each other and were a function of uterine and umbilical blood flows (Meschia et al., 1967). More recently, similar evidence has been presented that the placental clearance of ethanol is also flow-limited (Bonds et al., 1979). However, even for these compounds, which are relatively inert and to which the placenta is maximally permeable, the relationship between transport and uterine flow is not linear. As a consequence of this nonlinearity, uterine flow at high flows can vary over wide limits, with relatively small changes in placental clearance. Recently these studies have been extended to placental clearance measurements made under chronic steady-state conditions over a wide range of uterine blood flows (Wilkening et al., 1982). Figure 3 demonstrates the observed relationship between placental clearance and uterine blood flow for ethanol. Thus it is unlikely that the placental transport of nutrients important to fetal growth and metabolism will be linearly related to the rate of uterine blood flow.

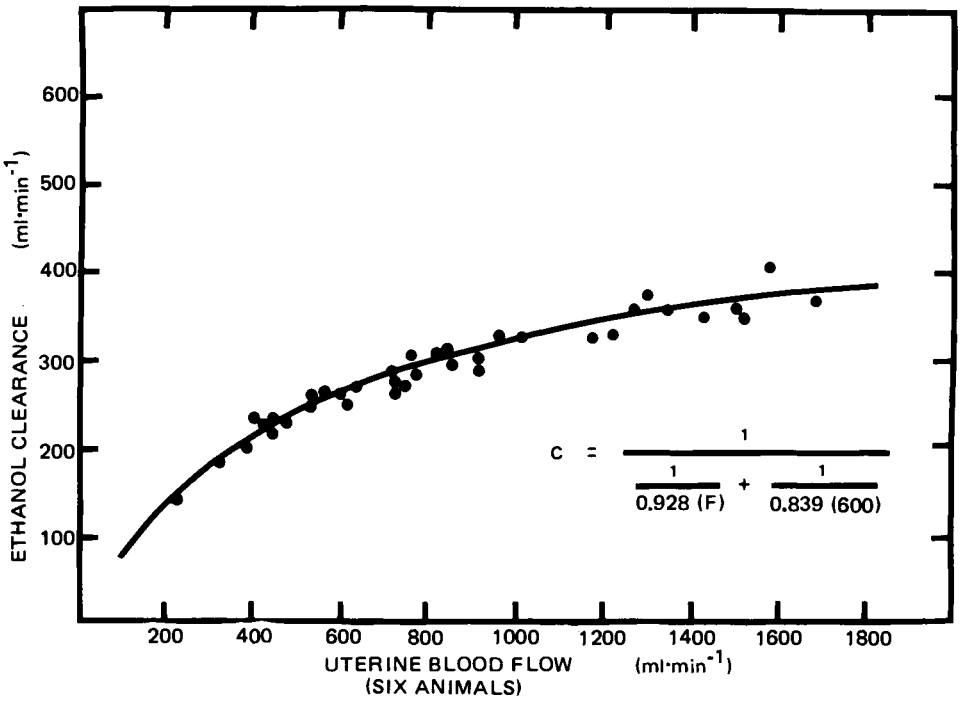


Figure 3 Ovine placental ethanol clearance versus uterine blood flow at constant umbilical blood flow. (From Wilkening et al., 1982.)

## PLACENTAL GROWTH AND MATURATION IN RELATION TO FETAL METABOLIC REQUIREMENTS

We have discussed the variations in fetal growth rate and body composition which occur among different mammalian species. The placental growth curve also varies among species, although in all species the placenta must complete the stages of implantation, growth, and maturation within the gestation. In all mammals placental growth is rapid in early gestation and either continues at a slower rate until term (as in man) or is completed before the end of gestation (as in sheep) (Teasdale, 1976). Figure 4 presents a composite of the placental growth curves of humans adapted from Molteni et al. (1978). Figure 5 presents the estimates of the total placental surface area of several species adapted from the data of Baur (1977). It is clear that the total placental surface area continues to increase up until the end of gestation, despite a relatively constant placental weight and DNA content. There have been few studies which have attempted to measure total placental function under in vivo conditions. It is interesting, however, that a study of the urea-diffusing capacity of the ovine placenta demonstrated the same exponential increase in diffusing capacity with increasing gestational age that is seen with total surface area measurements determined morphometrically (Kulhanek et al., 1974). Similar increases occur in uterine and umbilical blood. flows throughout gestation. Studies of total placental function during gestation should be carried out for the transport of nutrients such as amino acids and carbohydrates. Until such data are available, it cannot be stated with certainty whether placental function shows any decline in late gestation, particularly when expressed in terms of

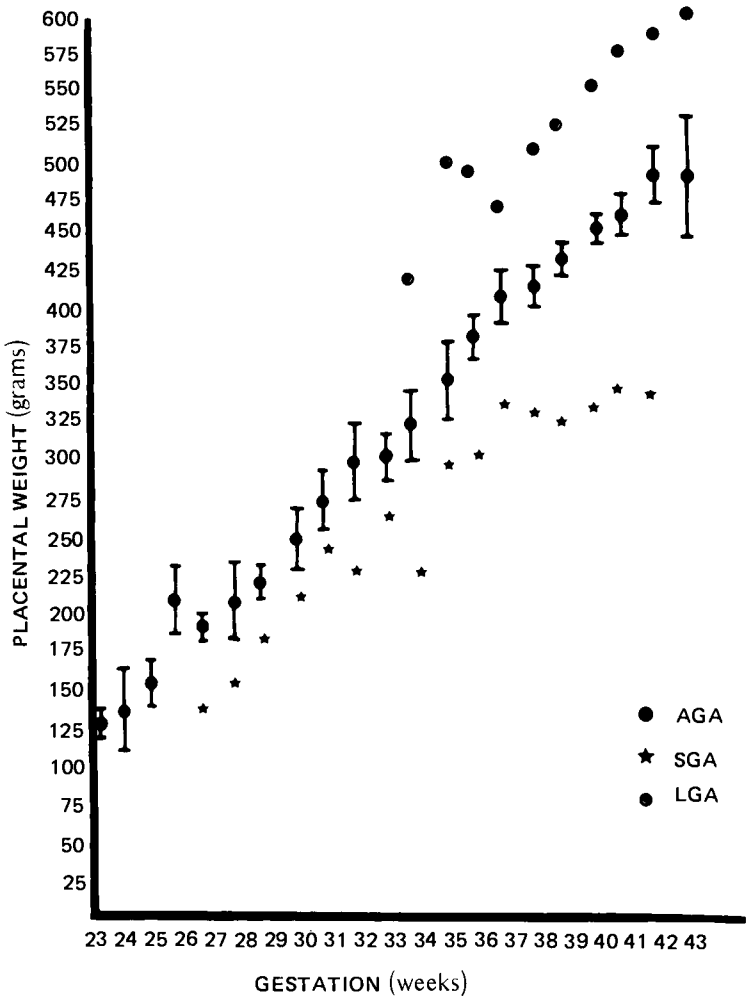


**Figure 4** Mean placental weights for appropriate-for-gestational-age (AGA), small-for-gestational-age (SGA), and large-for-gestational-age (LGA), infants at each gestational age (± SEM given for AGA infants alone). (From Molteni et al., 1978).

units of fetal weight which must be supplied. However, those functions that have been measured in vivo support the concept of continued placental maturation until term, with no evidence of placental "aging." Viewed in this light, the slower rate of fetal growth in late gestation is more likely to be a reflection of endocrine changes occurring in the fetus near the time of parturition than a reflection of a restriction in nutrients supplied by the placenta.

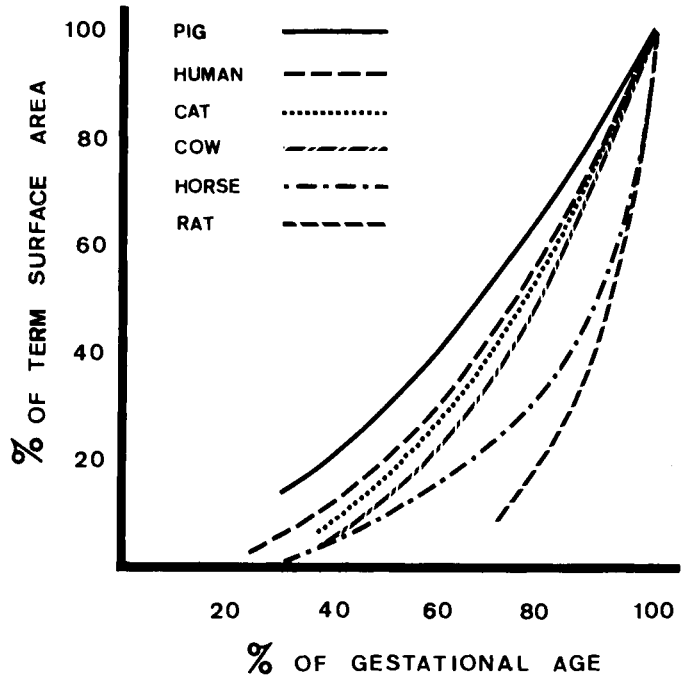


Figure 5 Mammalian placental surface area growth as a percentage of the term surface area versus percentage of gestation. (From Baur, 1977).

## PLACENTAL METABOLISM

There have been extensive studies of placental metabolism conducted under a variety of in vitro conditions, such as placental perfusion systems, incubations of placental slices, and incubations of particulate fractions of placental tissue including microsomal, mitochondrial, and membrane vesicle preparations. Such in vitro studies have proven to be productive and convenient methodologies to delineate placental transport systems, particularly for amino acids, and to describe certain characteristics of placental metabolism in a variety of species. For example, a high rate of lactate production under aerobic conditions has been described under both in vivo and in vitro conditions for at least six mammalian species, including man. Recently, ovine placental metabolism has been studied in vivo under chronic, steady-state conditions (Meschia et al., 1980). These studies have described three rather unexpected features of placental metabolism for the ovine placenta during the latter 20% of gestation: (1) The uteroplacental tissue has a high rate of utilization of both oxygen and glucose, approximately the rate of central nervous system tissue. Fully one-half of the oxygen and two-thirds of the glucose supplied by the maternal circulation to the uterus is used by these tissues rather than being delivered to the fetus. (2) In contrast to the high rate of oxygen and glucose utilization

by the placenta, the sum of amino acid entry into the umbilical circulation is approximately equal to the sum of amino acid exit from the uterine circulation. Hence at this stage of gestation, when placental growth has stopped, the fetus is the predominant site for net amino acid utilization. (3) Two small molecules, lactate and ammonia, are produced by the placenta in relatively large amounts, and both enter the uterine and umbilical circulations. Lactate production by the placenta could account for approximately one-third of the glucose consumption of the placenta, and ammonia for approximately 10-15% of the amino acid nitrogen leaving the maternal circulation (Holzman et al., 1977). The role of these small molecules in fetal and placental metabolism is obscure at this time.

The high rate of placental metabolism raises several interesting questions. First, it is possible that some of the repercussions upon fetal growth from changes in the maternal organism, such as reductions in uterine blood flow and inadequate maternal nutrition, may be mediated indirectly through their effects upon placental metabolism and thus upon placental growth and development. Secondly, the high rates of placental oxygen and glucose consumption are important factors in determining the low oxygen tensions and glucose concentrations of fetal blood. At this time it is impossible to state whether placental metabolism is as high in other mammalian species, including man, until further confirmatory data are obtained under in vivo conditions in other species.

## FETAL OXYGEN CONSUMPTION

In the section on placental metabolism we have pointed out that approximately 50% of the oxygen leaving the uterine circulation is used by the uteroplacental mass and only approximately half of the oxygen is delivered into the umbilical circulation and consumed by the fetus. Fetal development occurs in a relatively low oxygen tension. For many years this was interpreted to mean that anaerobic metabolism was an important component of fetal metabolism. The reason for the low oxygen tension in the fetal circulation of most mammals (including subhuman primates, man, sheep, goats, and cows) is that the placenta tends to simulate a concurrent flow system. Thus the most oxygenated blood of the fetus, the umbilical venous blood, tends to equilibrate not with the arterial blood of the mother but, rather, with the uterine *venous* $pO_2$. Even in those species whose placentas tend to simulate a countercurrent flow system, such as the guinea pig, the $pO_2$ in the fetal circulation is much lower than the maternal arterial $pO_2$ (Moll et al., 1970). However, the low oxygen tension in the fetal circulation does not imply tissue hypoxia. If one considers only those data obtained under chronic steady-state conditions, it is clear that the fetus shows no evidence of a metabolic acidosis or any evidence of excess lactate. Far from being a net producer of lactate, the fetus has been demonstrated to be a net consumer of lactate, obtaining and consuming the lactate produced by the placenta under aerobic conditions. Under normal circumstances, with the mother well oxygenated and uterine flow and umbilical flow within the normal ranges, the administration of oxygen to the mother does not increase fetal oxygen consumption, despite a marked increase in fetal $O_2$ tensions (Battaglia et al., 1968). These observations provide further evidence that under normal conditions the fetus is adequately oxygenated, despite the low oxygen tensions in the fetal circulation. The large umbilical blood flow and the higher oxygen affinity of fetal blood in most mammalian species ensure a high oxygen delivery to fetal tissues.

In a review of fetal energy metabolism, it has been pointed out that fetal $O_2$ consumption expressed per kilogram of body weight is relatively constant among different species, despite marked differences in fetal body size (Battaglia and Meschia, 1978). There have not been enough measurements of $O_2$ consumption rates by individual fetal organs to fractionate total fetal oxygen consumption among the various parts of the body. In the fetal lamb, where cerebral oxygen consumption has been measured, the rate was found to be approximately equal to that of adult brain tissue (approximately 4 ml/100 gm per minute) (Jones, 1979). For a 3-kg fetal lamb with approximately 50 g of brain tissue, brain oxygen consumption would represent roughly 10% of the total fetal oxygen consumption. Similarly, a myocardial oxygen consumption in the fetal lamb of approximately 450 mmol/min per 100 g of left ventricular weight has been reported (Fisher et al., 1980). For a 3-kg fetus this would equal approximately 10% of the total fetal oxygen consumption. Thus the total of the oxygen consumption of the fetal brain and heart in the lamb in late gestation may account for ~20% of the total $O_2$ consumption.

There have been many studies in fetal physiology which have investigated the changes induced in the cardiovascular system and in respiratory gas measurements when fetal $pO_2$ has been reduced; however, there have been no systematic studies of the changes induced in fetal metabolism when fetal oxygen consumption is reduced through hypoxia, that is, when the reduction in fetal oxygenation is sufficient to force relatively large segments of fetal tissues to metabolize anaerobically. On the other hand, there have been studies which have described the changes in the rate of fetal oxygen consumption induced by the increased metabolism of certain substrates. It has been demonstrated that a sustained fetal hyperinsulinemia, for example, with a concomitant fetal hypoglycemia and increased fetal glucose utilization, leads to a significant decrease in fetal arterial oxygenation (Carson et al., 1980).

## FETAL GLUCOSE METABOLISM

Our knowledge of fetal carbohydrate metabolism has undergone substantial changes over the last 15 years. It is clear, for example, that glucose is an important substrate for fetal carbon balance, although it cannot be regarded as the major or sole metabolic fuel of the fetus. Studies from several laboratories have demonstrated that the umbilical glucose/oxygen quotient in all species, including man, is always less than unity (see Table 3). Since the variance of the glucose/oxygen quotients reported in the literature is either not given or often large within a species, the differences between the mean values reported among species in Table 3 are not likely to be significant. It is clear, however, that even if all of the carbon skeleton of glucose were oxidized to $CO_2$, it could not account for the $O_2$ consumption or $CO_2$ production of the fetus. In fact, it is highly likely that much of the carbon skeleton of glucose is used for carbon accretion in various forms within the fetus during its growth. For example, lipogenesis from carbohydrate is likely to occur, since it has been possible to demonstrate the enzyme systems required for lipogenesis from carbohydrate in fetal tissues (Warshaw, 1979).

As was pointed out earlier, in all mammals there is a fairly linear relationship between the arterial concentration of glucose in the fetus and the maternal arterial glucose concentration (see Figure 1). The relationship is somewhat different among those mammals, especially the primates including man, in which the slope approaches

Table 3 Fetal Oxygen and Glucose Uptakes[a]

| Species | Oxygen utilization rate (ml/min per kilogram) | Glucose utilization rate (mg/min per kilogram) | Glucose/ oxygen quotient | Reference |
|---|---|---|---|---|
| Goat (c) | 7.01 | | | Meschia et al. (1967) |
| Sheep (c) | | | | |
| fed, fetus | | | | |
| normoglycemic | 6.0 ± 0.2 | 3.1 ± 0.3 | 0.41 ± 0.2 | James et al. (1972) |
| | | 4.6 | | Crenshaw (1970) |
| | 7.1 ± 0.3 | 4.8 ± 0.4 | 0.52 ± 0.01 | Boyd et al. (1973) |
| fasted, fetus | | | | |
| hypoglycemic | 6.3 ± 0.3 | 2.5 ± 0.5 | 0.31 ± 0.02 | Boyd et al. (1973) |
| | | | 0.17 (0.13-0.23, 95% confidence limits) | Tsoulos et al. (1971) |
| insulin-induced fetal hypoglycemia | 7.7 ± 0.7 | 6.9 ± 0.9 | 0.67 | Simmons et al. (1978) |
| | | | 0.71 | Carson et al. (1980) |
| Cattle (c) | 6.8 ± 0.2 | 5.2 ± 0.3 | 0.57 | Silver and Comline (1976) |
| Horse (c) | 7.4 ± 0.3 | 6.8 ± 0.6 | 0.69 | Silver and Comline (1976) |
| Monkey (rhesus) (a) | 17.0 ± 0.3 | | | Behrman et al. (1976) |
| Human (a) | | | 0.81 | Morriss et al. (1975) |
| Guinea pig (a) | 8.5 | | | Bohr (1900) |
| Rat (a) | 10.4 | | | Data of DeMeyer et al. (1971) as calculated by Battaglia (1979b) |

[a]a, acute; c, chronic.

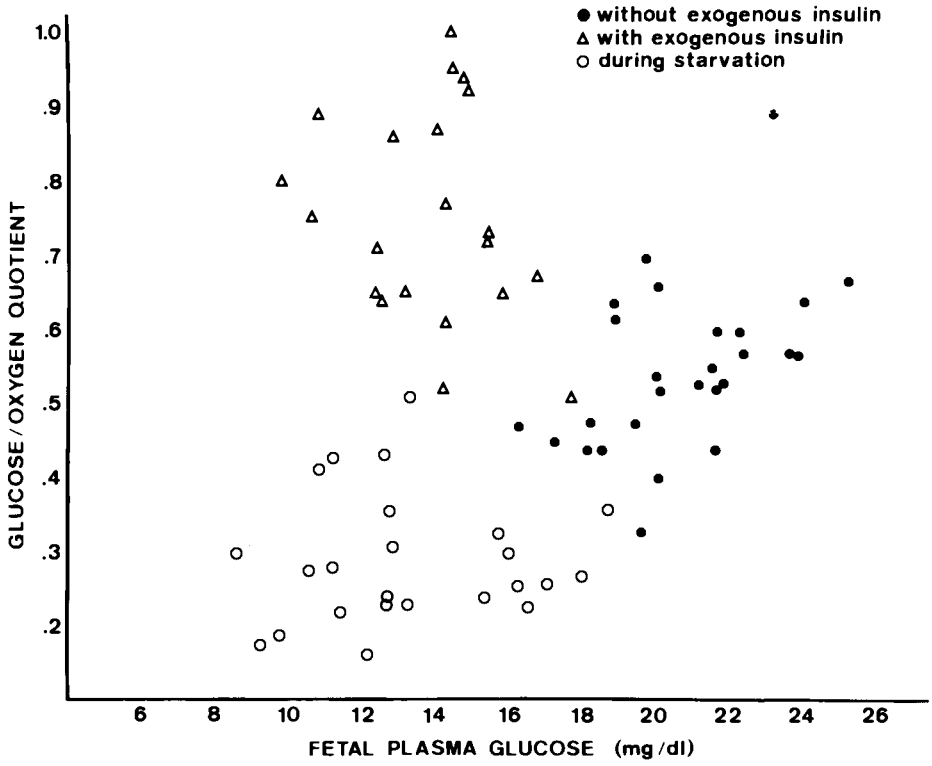


Figure 6 Fetal glucose/oxygen quotient at varying fetal arterial plasma glucose concentrations during the control fed state, with exogenous insulin, and during starvation. (From Carson et al., 1980.)

an identity line. If glucose consumption by the fetus were approximately equal among different mammals, this observation would imply an increasing placental permeability to glucose in primates compared with other mammals. However, glucose uptake by the fetus does not follow a linear relationship to either maternal arterial or umbilical arterial glucose concentration. It is important to emphasize that one cannot automatically assume that fetal glucose consumption changes as umbilical arterial glucose concentration changes. This interpretation is often applied to measurements made in the smaller mammals. Figure 6 compares umbilical glucose/$O_2$ quotients in two groups of fetal lambs, both of which have in common a fetal hypoglycemia (Carson et al., 1980). In one group, umbilical glucose/$O_2$ quotient is increased (that is, a group receiving exogenous fetal insulin infusions) and in another group, it is reduced (that is, in the presence of maternal starvation). In all cases, fetal oxygen consumption either increased or remained constant, implying an increased (insulin infusion) or decreased (starvation) fetal glucose uptake. Thus the fetal glucose concentration in and of itself could not be used to predict whether umbilical glucose uptake were increased, normal, or decreased. The effect of changes in fetal insulin concentrations upon umbilical glucose uptake have been fairly well described. Several laboratories have demonstrated that when fetal insulin concentrations are increased, umbilical glucose uptake is enhanced. This occurs whether or not the hyperinsulinemia is created by an exogenous

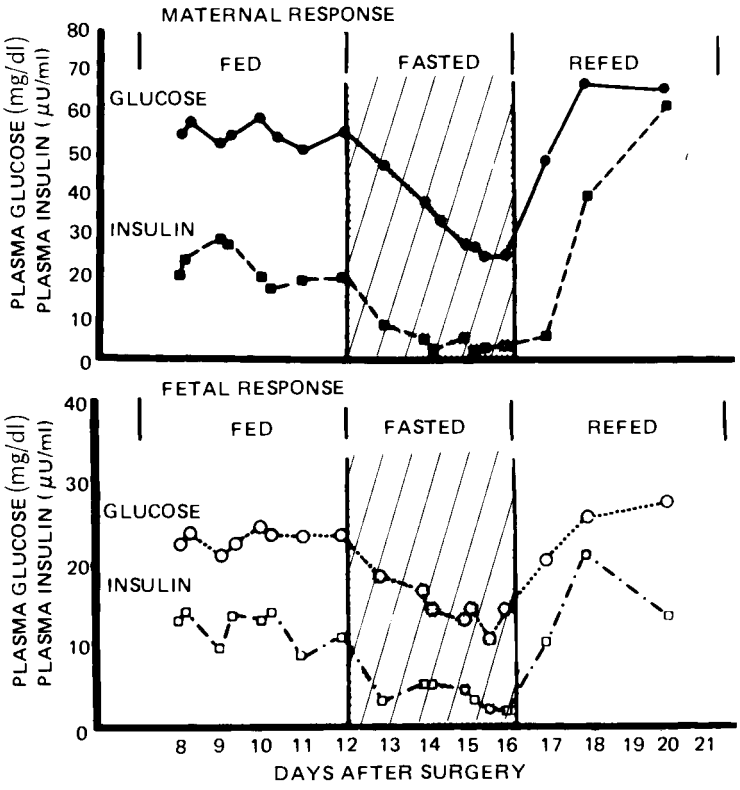


**Figure 7** Maternal and fetal glucose and insulin responses to maternal fasting. (From Philipps et al., 1978.)

fetal insulin infusion (Simmons et al., 1978; Bassett and Madill, 1974) or by the stimulation of endogenous fetal secretion (Philipps et al., 1980). By contrast, hypoinsulinemia induced by maternal starvation is associated with a decreased umbilical glucose uptake (Philipps et al., 1978) (Figure 7).

From measurements of umbilical glucose uptake in a number of species and glucose turnover in newborn mammals, including man, it is clear that the glucose taken up (in milligrams per kilogram per minute) by the fetus or neonate is considerably higher than the rate of glucose consumption by adults. However, an important cautionary note should be introduced here. The measurement of umbilical glucose uptake during fetal life has quite a different physiologic meaning than the measurement of glucose utilization by the organism. Fetal metabolism has just entered an era when we are able, in the same *organism,* to begin to make comparisons of the exogenous supply of nutrients to the fetus with the rate of utilizaton of those same nutrients by the fetus.

In this section we shall discuss in some detail this approach as applied to fetal glucose metabolism, although the same approach can obviously be applied to other carbohydrates, amino acids, and fatty acids. Figure 8 presents in diagrammatic form the entries and exits from the fetal glucose pool. The umbilical uptake, which can be

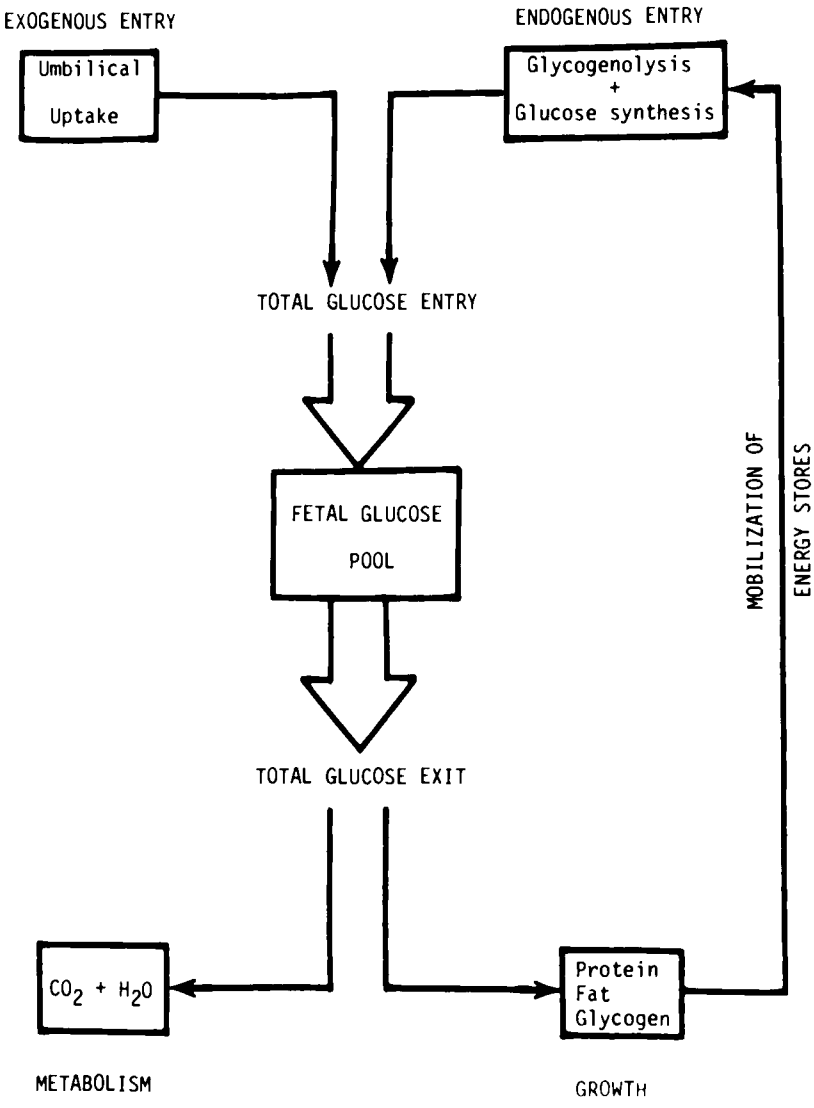


**Figure 8** Fetal glucose balance. (From Battaglia, 1979.)

quantitated during fetal life by an application of the Fick principle, represents the exogenous supply of glucose to the fetus arriving from the placenta. The other entry to the fetal glucose pool is a composite of the total endogenous glucose production, either from glycogen breakdown or from glucose synthesis from carbohydrate or noncarbohydrate sources. The exits from the glucose pool can be through metabolism, that is, used as fuel with the production of $CO_2$ and the consumption of oxygen or as carbon accretion either in protein stores or in energy stores such as fat depots and glycogen depots. The glucose entry or exit from the fetal glucose pool can be determined by a measurement of the glucose utilization rate.

It is not the province of this chapter to discuss the various methodological considerations in the choice of isotopic glucose labels for the determination of the utilization rate or the requirements for the correct application of isotopic dilution techniques to study fetal metabolism. We would point out, however, that most of the data for the interpretation of glucose utilization from glucose labeled at various positions are obtained in adult organisms. Relatively few attempts have been made during fetal or neonatal life to assess the differences in estimates of glucose utilization obtained with glucose labeled at one or another carbon position. Additionally, one must also distinguish between tracer-derived glucose utilization within the fetus and total fetal glucose turnover, the latter rate containing an additional measure of unidirectional glucose flux to the placenta and mother. It is clear from Figure 8, however, that the simultaneous measurement within the same organism of the exogenous supply of glucose represented by the umbilical uptake, and of glucose utilization within the fetus, represents a powerful tool in studies of fetal metabolism. Not only do these measurements give considerable information about fetal metabolism, in and of themselves, but the difference in the two values (total glucose utilization minus umbilical uptake) reflects the endogenous rate of production of glucose from other sources.

Another approach to studying endogenous fetal glucose production that has been used recently involves the infusion of tracer glucose into the maternal circulation. In animal experiments, the tracer usually is either $^{14}C$ or tritiated glucose. In human experiments, more recently, the stable isotopic counterparts have been used, namely, [$^{13}C$] glucose or deuterated glucose. The labeled glucose infusion is continued to a steady-state specific activity in the maternal circulation and the ratio of fetal to maternal specific activities is calculated. This approach rests on the premise that if there is no endogenous fetal production of glucose, the specific activity in the fetal glucose pool should be equal to that in the maternal glucose pool, and the ratio of the two should be unity. If the ratio of fetal to maternal specific activities is less than unity, it indicates that there has been some gluconeogenesis or glycogenolysis in the fetus. It does not give any indication, however, of the rate of glycogenolysis or gluconeogenesis. The method has another inherent limitation, namely, that the sensitivity of this approach in detecting fetal gluconeogenesis will depend in part upon the placental permeability to glucose. Thus this method presents definitive information when the ratio is significantly less than unity. However, the demonstration that the ratio is equal to unity does not unequivocally disprove fetal glucogenesis.

Another more direct approach to the question of fetal gluconeogenesis will become more available as techniques are developed to collect information about individual fetal organs and their consumption rates of glucose. This approach would compare the exogenous supply of glucose (represented by the umbilical uptake of glucose) with the summation of the rates of glucose utilization by individual fetal organs. The extent to which the sum of the rates of utilization exceeds the exogenous supply would then define an endogenous rate of glucose production. This approach awaits further determinations of rates of glucose consumption by individual fetal organs and masses of tissue, particularly the fetal carcass.

From the previous discussion, it is apparent, unfortunately, that a great deal of the controversy in the literature regarding the endogenous glucose production rate

during fetal life is generated by methodological differences. It does seem clear that the potential for glucogenesis is present in the fetal liver of many mammalian species quite early in gestation (Sparks, 1979). Even in a species such as the rat, where phosphoenolpyruvate carboxykinase activity is confined to the cytosol, without a mitochrondrial component, and where this activity is negligible during fetal life, one can demonstrate that this enzyme can be induced in the fetal liver by maternal fasting. Furthermore, with infusions of labeled glucose into the maternal circulation, fetal to maternal specific activity ratios significantly less than unity develop during maternal fasting (Goodner and Thompson, 1967; Girard et al., 1977; Bossi and Greenberg, 1972). Thus it seems likely that even if glucogenesis does not occur at an appreciable rate during normal development in most mammalian fetuses, it can be induced under a variety of conditions, including maternal fasting.

## FETAL LACTATE METABOLISM

As mentioned earlier, lactate is produced by the placenta of many species and is released into both the uterine and umbilical circulations. In fetal lambs, the lactate/oxygen quotient is approximately 0.2 (Burd et al., 1975), and fetal umbilical lactate uptake is approximately 2 mg/kg per minute, or roughly half that of glucose. Thus lactate represents a large percentage of the total caloric and carbon supplies to the fetus. The fact that the fetus is a net consumer of lactate is additional information that fetal metabolism is largely aerobic, despite the low oxygen tensions in the fetal circulation. It is somewhat surprising, given the high umbilical lactate uptake in the fetal lamb, that it has not been possible to demonstrate significant labeling of fetal glucose when [$^{14}$C]lactate is infused into the fetal circulation (Warnes et al., 1977; Sparks et al., 1982). Thus the precise role of lactate in fetal metabolism remains an enigma. Preliminary studies have suggested a relatively high rate of consumption of lactate by the fetal myocardium (Fisher et al., 1980), although the relatively small size of this organ in relation to total body mass would seem to rule out this organ as the major site for lactate utilization within the fetus.

## FETAL FRUCTOSE METABOLISM

Fructose is of interest only in a few mammalian species where it is present in large quantities in fetal blood. In these so-called "fructogenic species" fructose concentrations are on the order of 80 mg%, compared with a blood glucose concentration of approximately 25 mg %. However, even in species where it is present in high concentrations, fructose does not appear to provide a ready source of calories for the fetus, or of carbon for growth. Thus its function even in fructogenic species remains unclear. It is not present in significant amounts in mammalian milk, and thus would not be ingested in large amounts following delivery, even in fructogenic species. In those species, fructose concentration falls rapidly following delivery, reflecting rapid renal excretion of this sugar. The only suggestion of a potential role for fructose has come from studies of maternal fasting in pregnant sheep, where it has been demonstrated that fructose concentrations fall as maternal fasting is prolonged, suggesting that it may be regarded somewhat akin to glycogen stores, representing a relatively slowly mobilized substrate for glucogenesis in the fetus (Hay, 1979).

## FETAL GALACTOSE METABOLISM

A discussion of galactose metabolism during fetal life is relevant not so much for its role in fetal metabolism, but for the changes which go on in fetal tissues in preparation for its dominant role in neonatal metabolism. Figure 9 presents the pathways for galactose metabolism. It should be emphasized that the galactose-metabolizing enzymes have been demonstrated in the fetal liver of a number of mammalian species, including the rat (Segal and Bernstein, 1963), rhesus monkey (Sparks et al., 1976), and man (Segal, 1972). In all three species, the enzymatic capacity for galactose metabolism is clearly present prior to delivery. In fact, in the rhesus monkey, which has been most thoroughly investigated, activities of the three key enzymes involved in galactose metabolism are higher prenatally than they are in neonatal liver, despite the fact that the capacity to metabolize galactose increases approximately fivefold during neonatal life. Thus, for these pathways at least, measurements of enzyme activity provide only a reflection of the potential for metabolism of the substrate galactose, but give little indication of the extent to which the pathways are used. During fetal life galactose metabolism is restricted by its supply. Since it is not normally available to the fetus, galactose only becomes important in metabolism during neonatal life. The primary sugar in most mammalian milks is the disaccharide lactose. On hydrolysis by intestinal lactase, equal quantities of galactose and glucose are provided to the newborn. Galactose is rapidly cleared by the neonatal liver and, in fact, has been used as a measurement of hepatic blood flow (Tygstrup and Winkler, 1958).

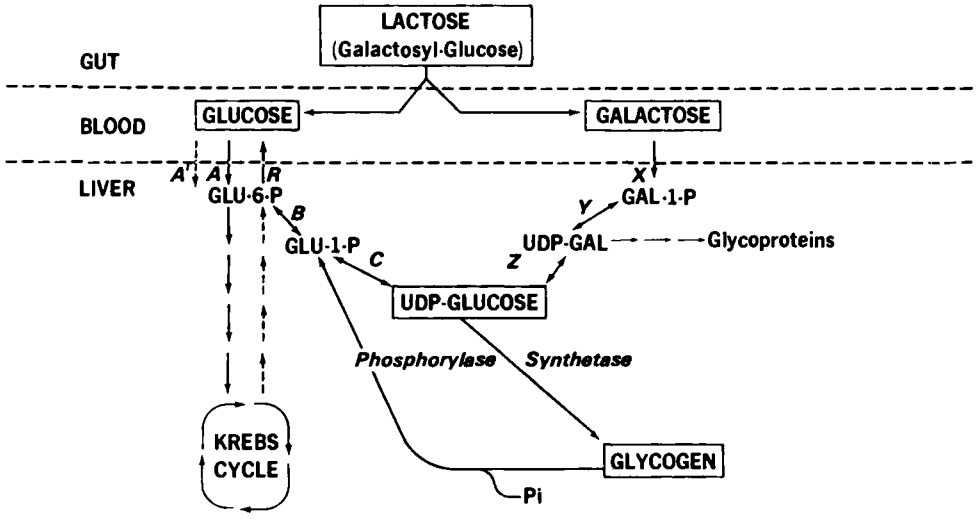


Figure 9 Metabolism of lactose, showing the uptake and metabolism of glucose and galactose (X, galactokinase; Y, galactose 1-phosphate uridyltransferase; Z, epimerase; A, hexokinase; A', glucokinase; B, phosphoglucomutase; C, UDP-glucose phosphorylase; R, glucose-6-phosphatase). (From Sparks et al., 1976.)

## FETAL FAT METABOLISM

In this section we shall consider the placental transfer and fetal metabolism of free fatty acids, including the short-chain fatty acids acetate, proprionate, and butyrate, and the transport and metabolism of glycerol and triglycerides. As with amino acids, one must consider not only the overall transport of free fatty acids across the placenta to the fetus, which may be used both as fuels and as carbon sources for growth, but also the transfer and metabolism of individual fatty acids, particularly the essential fatty acids. These latter fatty acids must be obtained by the fetus from the placenta wither by transfer from the maternal circulation or by synthesis within the placenta (Hull, 1979). There is considerable evidence that placental metabolism plays an important role in providing the essential fatty acid arachidonic acid to the fetus by lengthening the carbon chain of linolenic acid (Crawford et al., 1976). This has been demonstrated for human placental tissue, as well as that of several other mammalian species. In ruminants the metabolism of the short-chain fatty acids is an important feature of metabolism in the adult organism. Thus there have been several studies which have attempted to quantitate the relative contributions of the short-chain fatty acids to fetal metabolism. The data suggest that the transfer of acetate may contribute sufficient carbon to account for approximately 10-20% of fetal $O_2$ consumption and $CO_2$ production (Comline and Silver, 1976; Char and Creasy, 1976). However, these studies of the relative contributions of the short-chain fatty acids need further extension to conditions of maternal fasting and particularly to an investigation of the question of whether the short-chain fatty acids contribute significantly to placental metabolism.

As mentioned earlier, the placental transport of free fatty acids has been well demonstrated in some mammalian species such as the guinea pig, rabbit, and man (Hull and Elphick, 1979); however, there has been no evidence that free fatty acids are transferred to the fetus, even in these species, in amounts which exceed accretion in the tissues as structural lipids or in the considerable adipose tissue stores. This observation suggests that free fatty acids are not used extensively by the fetus as fuels. In addition, attempts to demonstrate the metabolism of free fatty acids to $CO_2$ during fetal life have not been successful. At this time, then, it appears that the differences in placental transport of free fatty acids among species are reflected primarily by differences in the quantities of fetal adipose tissue stores which are built up during gestation, but may not be reflected by differences in the fetal fuels used for $CO_2$ production. A number of studies have suggested that triglyceride fatty acids may cross the placenta in some species. However, the experimental design of these studies does not permit us to arrive at a quantitative estimation of the relative contribution of triglyceride transport as a source of carbon for the developing fetus.

Given the important role of free fatty acids in neonatal metabolism, it is somewhat surprising that their role as fuels during fetal life is relatively small. Warshaw (1979) has suggested that the ativity of carnitine palmityl transferase may be limiting in some mammalian species' fatty acid oxidation, since postnatal changes in the activity of this enzyme tend to parallel the development of fatty acid oxidation. The low activity of this enzyme may be due to a deficiency of the enzyme or to masking of enzyme activity. At any rate, regardless of the reasons for a limited free fatty acid oxidation by the fetus as a whole, this observation in and of itself stresses the fact that the metabolism of certain fetal tissues must be quite different from the metabolism of those same tissues in postnatal life (see the section on metabolism by individual organs).

In both rats and rabbits the conversion of glycerol to glucose and glycogen in the fetal liver has been demonstrated (Gilbert, 1977). The experimental design used in these studies precluded a quantitative estimation of the relative contribution of glycerol to gluconeogenesis during fetal life. However, when [$^{14}$C]glycerol was injected into the maternal circulation, glucose and glycogen in the fetal liver incorporated radioactivity in both the rat and rabbit fetuses. Whether the conversion of glycerol to glucose would become increasingly important to the fetus under conditions of maternal fasting is not known, although it would certainly seem possible, since the pregnant rat develops a marked increase in glycerol concentration with fasting and glycerol readily crosses the placenta from the mother to the fetus. The observation that the plasma glucose specific activity ratio in fetal versus maternal circulations is significantly less than unity when pregnant rats are fasted suggests that substrates such as glycerol may be used for endogenous glucose production in the fetus under these conditions.

## FETAL AMINO ACID METABOLISM

Amino acids must be supplied to the fetus from the placenta and indirectly from the maternal free amino acid pool in quantities sufficient to meet the rates of accretion of individual amino acids in the body. The accretion occurs primarily in the form of protein, with the free amino acid pool of the fetus making a rather minor contribution. For the essential amino acids which cannot be synthesized by the fetus, this requirement is absolute. For the nonessential amino acids this requirement could be met by synthesis of the individual amino acids in the fetus from carbon and nitrogen acquired by the fetus from the placenta in other forms, such as other amino acids. For a mammal such as the hibernating black bear, which carries out its gestation while the mother is fasting, it is clear that the nitrogen required by the fetus is derived from the mobilization of tissue proteins in the mother. On the other hand, for an animal such as the vampire bat, whose diet is essentially protein, the dietary intake of amino acids is in enormous excess of that required for either the fetus or the maternal metabolism. Amino acids provide both carbon and nitrogen to the fetus and thus may not only be used as precursors for the synthesis of protein, but may also provide carbon and nitrogen for $CO_2$ and urea production by the fetus, that is, be used as fuel by various fetal tissues. In addition, the carbon framework of amino acids may be used for accretion in tissues as non-nitrogen-containing compounds.

We are just beginning to obtain information regarding the rates at which amino acids are delivered to the fetus. In part, the slow pace at which a base of knowledge has developed regarding rates of placental transfer of amino acids hinges on methodological difficulties. In general, the Fick principle has been used to quantitate substrate flow to the fetus. This requires a measurement of umbilical flow and of the whole blood arteriovenous differences of the individual amino acids. Unfortunately, the coefficient of utilization of amino acids across both the uterine and umbilical circulations is quite small. The coefficient of utilization is represented by

$$\frac{A - V}{A} \times 100$$

where A is the arterial level of amino acids and V is the venous level. In sheep, it is on the order of 6-8% across the uterine circulation, and in the order of 8-15% across the

umbilical circulation (Holzman et al., 1979). Since the methods for determining the concentration of individual amino acids in whole blood have errors on the order of ±2%, it is clear that a very large number of observations must be made before one can attach significance to the mean arteriovenous difference determined for an amino acid. The problem of applying the Fick principle to quantitate amino acid flow is best exemplified by glycine in studies carried out during ovine pregnancy. The coefficient of utilization for glycine across the uterine circulation is less than 1%, and across the umbilical circulation on the order of 3%. These observations do not imply that glycine is not transported from the maternal circulation to the fetal circulation, because the low coefficients of utilization exist primarily because of the extremely high glycine concentraion in fetal and maternal blood. In fetal blood the glycine concentration is on the order of 850 μM/liter. Thus an appreciable arteriovenous difference of 28 μM/liter represents a very small percentage change in the arterial blood as it perfuses the placenta. It is important to remember, therefore, that the higher the concentration of a substance in the blood, the more difficulty there is in quantitating substrate flow into or out of the placenta.

Despite these difficulties, a number of characteristics of fetal amino acid metabolism have become more firmly established in recent years. It has been known for a long time that the concentrations of most amino acids in fetal blood are considerably higher than the concentrations in maternal blood. This observation, which pertains to both essential and nonessential amino acids, certainly supports the fact that the placental transport of the essential amino acid requires active transport systems; that is, the transport is energy dependent. In the fetal lamb, the quantities of each amino acid entering the umbilical circulation have been determined (Lemons et al., 1976). It is clear from those measurements that amino acids are delivered to the fetus in amounts which far exceed the quantities required for accretion in the body as protein. Secondly, the acidic amino acids aspartic acid and glutamic acid are not delivered to the fetus in appreciable amounts; in fact, glutamic acid is synthesized in the fetus and excreted from the umbilical circulation into the placenta. Thus the fetus must synthesize sufficient glutamic acid, presumably in part from the glutamine it receives from the placenta, to meet its own requirements for accretion and for excretion into the placenta. This characteristic has also been demonstrated for the human placenta during in vitro perfusions, namely, that glutamate is not transferred across the placenta but, rather, is taken up from the fetal side during perfusion (Dancis et al., 1968). Similarly, in vivo acute studies with rhesus monkeys have shown that glutamate is not transported readily across the placenta from the maternal to the fetal circulation (Stegink et al., 1975).

## FETAL CATABOLISM OF AMINO ACIDS

Evidence that amino acids are used not only as substrates for the synthesis of protein but also as fuels by the fetus with the production of $CO_2$ and urea has accumulated from a number of quite different studies of fetal metabolism. The first evidence came from estimates of the urea production rate in the fetal lamb, which were made by utilizing independent measurements of placental clearance for the calculation of the quantity of urea leaving the umbilical circulation (Gresham et al., 1971). Although it is clear that urea, an excretory product of the fetus, must be excreted across the placenta in all mammalian species, the arteriovenous differences of urea across the umbilical circulation are too small to be measured with any precision. Placental clearance for inert compounds is defined as the rate at which the compound crosses the placenta divided by the concentration difference between the maternal and fetal

arterial blood. This can be measured independently by the infusion of radiolabeled urea into the fetal circulation, creating an enormous concentration difference across the placenta for radiolabeled urea. Both umbilical and uterine arteriovenous differences for radiolabeled urea can then be measured and the determination of the clearance of urea obtained. The urea clearance through the fetal kidneys is only approximately 5% of the placental clearance. Thus the placental urea clearance can be safely used to calculate the urea production rate by the fetus. The equation for urea clearance is

$$C_u = \frac{f(U_a^* - U_v^*)_{bl}}{(U_a^* - U_A^*)_{pw}}$$

where $C_u$ is the clearance of urea (ml/min per kilogram of fetus), f is the umbilical blood flow (ml/min per kilogram of fetus); $(U_a^* - U_v^*)_{bl}$ is the arteriovenous difference of [$^{14}$C]urea across the umbilical circulation (dpm/ml of blood), and $(U_a^* - U_A^*)_{pw}$ is the umbilical-maternal arterial concentration difference of [$^{14}$C]urea (dpm/ml of plasma water). Once the clearance has been determined, and the urea concentration between fetal and maternal arterial plasmas measured with precision, the urea production rate can be calculated. In the fetal lamb a urea production rate of approximately 0.54 mg/min per kilogram of fetus was found, that is, a rate of amino acid catabolism sufficient to account for approximately 25% of the oxygen consumption of the fetus. Similar urea concentration differences between maternal and fetal arterial blood have been demonstrated across the placentas of man, horse, and cow (Silver, 1976). Thus in all these species, assuming a placental clearance approximately equal to that of the sheep, the urea production rate during fetal life would be considerably higher than the urea production rate in adults in the same species. Figure 10 compares the estimates of the urea production rate for the human fetus, newborn, and adult, emphasizing the high rate of urea production in fetal life, particularly when compared to the urea production rate in the immediate neonatal period (Jones et al., 1972).

A second study which supports the use of amino acids as fuels has already been alluded to, namely, the quantitation of the umbilical uptake of amino acids (Lemons et al., 1976). This study clearly demonstrates that amino acids are delivered to the fetus in amounts which exceed their rates of accretion in fetal tissue proteins. Since there is no storage form for excess of amino acids as there is glycogen for glucose, or fat depots for free fatty acids, we can infer that amino acids are being used as fuels. More recently, a similar study of the uterine release of amino acids has demonstrated that the quantities of amino acids delivered to the placenta and fetus are in excess of their rates of accretion. In fact, in comparing the uterine release and umbilical uptake of amino acids, it was clear that very little placental retention of amino acids occurred in late gestation, since the estimate of nitrogen released from the uterine circulation was approximately equal to the quantity of nitrogen taken up in the umbilical circulation. We have no knowledge at this stage of the rate of amino acid uptake during early fetal life. However, it would seem likely that amino acids would be delivered to the fetus in excess of their rates of accretion rather than delivered at rates which precisely coincide with their rates of accretion in tissue proteins. Thus it will be important to delineate the excretory forms of nitrogen which exist for the fetus in early embryonic and early fetal life when urea cycle enzymes may not be developed and urea production is not an alternative.

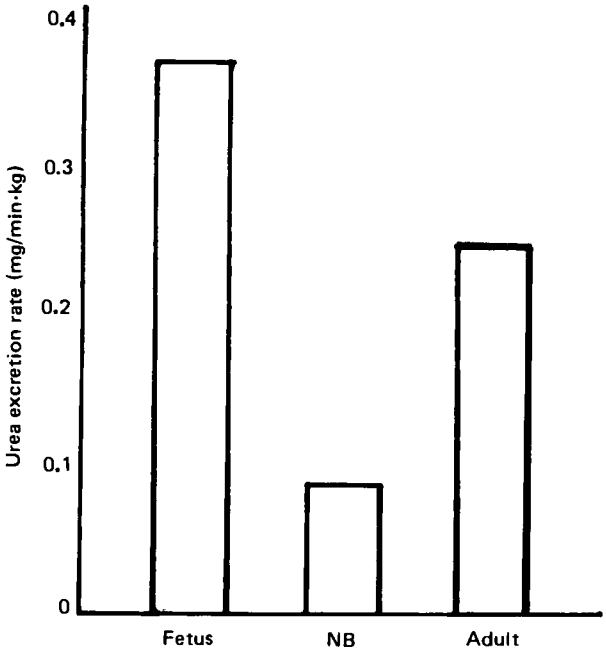


Figure 10 Urea excretion rates for the human fetus (Gresham et al., 1971), newborn (NB) (Jones et al., 1972), and adult (McCance and Widdowson, 1954).

There have been a number of studies which have attempted to measure arteriovenous differences for amino acids across the umbilical circulation in man and in some of the smaller laboratory animals. Unfortunatley, these studies have had a number of problems in design which preclude their providing a description of the quantity of nitrogen or the pattern of amino acid uptake by the umbilical circulation in these species. For the most part, these studies have been carried out acutely at the time of delivery. The concentrations have been measured on plasma rather than whole blood, and flow measurements have either not been carried out or have been estimated from studies in other laboratories. It should be emphasized that, particularly for amino acid uptake, even when a large number of arteriovenous differences are determined under chronic steady-state conditions and flow measurements are made in the same animals, the errors in the estimation of amino acid uptake are considerable. For these reasons it is not yet known whether substantial differences exist in the amino acid profile in the umbilical uptake among different mammalian species.

## METABOLISM BY INDIVIDUAL ORGANS OF THE FETUS

In the last ten years a number of studies have extended our knowledge of the profile of metabolic quotients across several organs of the fetus. For the most part, these studies have been carried out in the fetal lamb, although some data are available in man and other animals. The organs studied have included the heart, the brain, and skeletal muscle. Information about individual organ metabolism is important not only as a final check on our estimates of whole body rates of utilization and production of various

substrates, but also to understand more clearly the effects of maternal or fetal nutrition upon fetal metabolism. It is clear from developmental enzymology that organs mature at different rates within the same organism, and this property is likely to be expressed in quite different alterations of individual organ metabolism to the same common challenge such as maternal starvation or an excessively high dietary intake.

Fetal cerebral metabolism has been studied both in terms of the rates of oxygen and glucose consumption by the brain and in terms of metabolic quotients across the cerebral circulation (Jones, 1979). In fed organisms, whether adult or newborn, glucose/oxygen quotients across the cerebral circulation are approximately equal to unity; that is, sufficient glucose is consumed by the brain to account for all of the $O_2$ consumption and the $CO_2$ production by the organ. In fetal life, cerebral glucose/oxygen quotients have been measured in the fetal lamb; the quotient was equal to 1.02. The rate of cerebral glucose and oxygen consumption by the fetus was approximatley equal to that of the adult ewe. The fact that the quotient is unity, of course, does not establish that glucose is being used as the primary fuel, since the carbon skeleton of glucose may be incorporated into the carbon accretion within the brain and other substrates used as fuels. Suggested alternate substrates have included the ketone bodies beta-hydroxybutyrate and acetoacetate. Their role as alternate substrates for cerebral metabolism has been well established for both neonatal and adult organisms in a number of mammalian species (Warshaw, 1979). Also, it is clear that the carbon framework of the ketone bodies is incorporated into a variety of other compounds, including amino acids and brain lipids. The ketone bodies are unlikely to be significant fuels for the fetal brain under normal circumstances, since ketoacid concentrations are extremely low in the fetus with the mother in the fed state. However, their role could become important in those species that develop a marked ketonemia under a variety of conditions (Freinkel and Metzger, 1979). Ketoacids have been shown to cross the placenta of some mammalian species rapidly, and under those conditions could be important substrates for both carbon accretion and $CO_2$ production by the brain. It is interesting that a similar dependence upon carbohydrate metabolism has been demonstrated for both the fetal heart (Fisher et al., 1980) and fetal skeletal muscle (Morriss et al., 1973). While both observations need further documentation under a variety of conditions, and among different mammalian species, the present studies in the literature suggest that three tissues of the fetus, the brain, the heart, and skeletal muscle, all have carbohydrate/oxygen quotients which are considerably greater than the metabolic quotients for any other groups of substrates. It should soon be possible to account for whole body rates of utilization of individual compounds such as glucose by summing the rates of utilization of the same compound by individual fetal organs. At the present time there are no data on rates of utilization of substrates by the fetal liver or gastrointestinal tract; however, such studies are technically feasible, and it would seem likely that studies of hepatic and gastrointestinal metabolism will soon be forthcoming.

## ACKNOWLEDGMENT

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# 19

# Regulation of Myometrial Function throughout Gestation and Labor: Effect on Fetal Development

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## INTRODUCTION

At term the fully mature mammalian fetus is expelled from its intrauterine environment into one which places very different demands on its physiological systems. During pregnancy, the fetus and its vital organ systems grow and mature in a carefully organized, interrelated sequence. Normal development is dependent on many maternal and fetal factors. The built-in genetic program of the fetus interacts with stimuli mediated through the maternal environment. Stimuli that affect the fetus may have profound influences on the physiological and pathophysiological development of many fetal systems. This is increasingly likely in the later weeks of gestation, when critically important fetal systems such as the brain and lungs are maturing rapidly. As term approaches, pregnancy maintenance mechanisms, cardiovascular, endocrine, and metabolic, in both fetus and mother change. It is necessary to define which of these changes are causes and which are consequences of the processes that bring about labor. Eventually the myometrium is stimulated to undergo coordinated, efficient, expulsive contractions to propel the fetus from the uterus.

There are many reviews of the initiation and control of parturition in the human and experimental animals (Liggins et al., 1973; MacDonald et al., 1978; Nathanielsz, 1978; Thorburn and Challis, 1979). The major aspects of maternofetal endocrine relationships and the initiation of parturition are considered in detail in Chapter 17 by Buster. This chapter will be especially concerned with myometrial function.

It has been known for many years that the uterine muscle is not totally quiescent throughout gestation. However, the physiological significance, if any, of Braxton-Hicks contractions in the human and similar activity in other species has received little attention. This chapter will focus on detailed controlled experimental investigations on the control and effect of myometrial activity throughout gestation in the pregnant sheep and pregnant rhesus monkey. We will comment on work performed with Seron, Taylor, and Martin in the chronically catheterized fetal rhesus monkey preparation

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(Taylor, et al., 1983). This is done in order to demonstrate the presence of myometrial activity in late gestation in the nonhuman primate. Some of our most recent experimental data (referred to as unpublished observations) will also be included where it clarifies the nature of the factors operating in the physiology of contractures. Those increases in intrauterine pressure (IUP) that are more than 3.5 mmHg above the preexisting baseline and last for more than 5 min before returning to baseline, we have designated "contractures." This term has been used to distinguish this type of IUP change from the larger rises in IUP (approximately 20 mmHg) of shorter duration, 0.5-1.5 min, that result in the expulsion of the fetus at term (Jansen et al., 1979). We have reserved the word *contraction* for the expulsive form of myometrial activity at term. We hope that these data from both primate and nonprimate models will prove provocative and will stimulate the study of how the mother may affect the fetus throughout gestation by virtue of changes in myometrial function.

This chapter will consider myometrial activity throughout gestation as well as alteration in this activity that occurs at the time of delivery. It will consider first the characteristics of basal tonic uterine activity, "contractures," that occur throughout gestation (Nathanielsz et al., 1976; Jansen et al., 1979; Nathanielsz et al., 1980; Harding et al., 1982). Next the control of contracures will be discussed. The relationship of contractures to continuous variability in fetal systems and their physiological and pathological significance will be assessed. A detailed analysis of the transition from contractures to the myometrial activity of labor and delivery will then be undertaken.

## METHODOLOGICAL AND EXPERIMENTAL CONSIDERATIONS

This chapter will review data obtained from carefully controlled experimental systems in the nonhuman primate and other species, particularly the sheep. The sources of experimental observations are both in vivo and in vitro systems. In vitro studies of tissue removed from the mother, the placenta, and the fetus at various stages of gestation have yielded much useful information. However, in vitro systems can only suggest the nature of in vivo function and require confirmation by in vivo techniques. In the in vitro systems essential precursors may be unavailable to the tissue, or cellular responsiveness may be lost as a result of the conditions and various agents used to prepare the tissue. Dedifferentiation of developing tissue may also occur. An additional shortcoming of in vitro studies is that neural, endocrine, and cardiovascular influences that play a significant role in the intact animal will not be exerted on isolated tissues in vitro. Thus, while such studies throw light on the intrinsic activity of the tissue, they may lead to misleading conclusions regarding the function of that organ or tissue in vivo. In vitro studies using the highly sophisticated biochemical techniques available throw much light on fetal function, but they can only describe what tissues are capable of doing in utero; whole animal studies will always be required. These general considerations of experimentation with pregnant animal preparations have been discussed in detail elsewhere (Nathanielsz, 1976, pp. 17-29).

Long-term studies in the human with noninvasive techniques have many practical difficulties, and precise information using invasive methods is difficult to obtain for ethical reasons. Chronic instrumentation of fetus and mother over prolonged periods of gestation has been performed in nonhuman primates (Novy et al., 1980) and other large animals, particularly the sheep (Silver, 1980). In the last decade the most successful long-term chronic fetal preparations have been performed in the pregnant sheep, in

which gestation lasts about 150 days. As early as 60 days, vascular catheters can be placed in various fetal and maternal fluid spaces and blood vessels while the ewe is under regional or general anesthesia. Thus after recovery from surgery blood samples can be drawn from sites of specific interest, such as the utero-ovarian vein. At the time of catheter placement, various fetal organs such as the pituitary or kidney can be destroyed or removed (Nathanielsz, 1976; Liggins et al., 1982).

Many fetal physiological variables can also be monitored; for example, stainless steel wires can be placed on the fetal diaphragm to study fetal breathing movements as indicated by the diaphragmatic electromyogram (EMG), on the fetal skull to record the electrocorticogram, to record ocular movements (the electro-oculogram), or to record the fetal electrocardiogram (Harding and Poore, 1982). Fetal and maternal cardiovascular function can be studied using electromagnetic or Doppler flow probes placed on various vessels at the time of surgery, as well as by various indicator dilution and microsphere techniques utilizing vascular catheters implanted at the time of surgery (Rudolph and Heymann, 1980). Continuous recording of intravascular $paO_2$ in the fetal sheep for up to 41 days has led to new insights into the physiological variability of fetal $paO_2$ and the causes and potential consequences of such fluctuations (Jansen et al., 1979). Uterine muscle activity can be monitored throughout gestation by measuring the uterine EMG. It should be remembered that although the uterine EMG gives a clear indication of the input drive to the myometrial cell, coupling to myometrial work may change throughout gestation. Structural changes in the cell-cell gap junction conncections have been shown to relate to functional differences (Garfield et al., 1977). The changes in IUP reflect the actual work performed by the myometrium and can also be monitored, but the EMG gives much useful information regarding changing myometrial function before there are significant changes in IUP (Nathanielsz et al., 1981). These various pieces of instrumentation are exteriorized to the mother's flank, so that following recovery from anesthesia, fetal and maternal function can be studied chronically.

## UTERINE MUSCLE ACTIVITY THROUGHOUT PREGNANCY PRIOR TO THE ONSET OF LABOR

In 1891 Michael Foster, the first professor of physiology at Cambridge University, wrote in his *Textbook of Physiology*, "We may be said to be in the dark as to why the uterus, after remaining for months subject only to futile contractions, is suddenly thrown into powerful and efficient action, and within it may be a few hours or even less, gets rid of the burden it has borne with such tolerance for so long a time." The detailed studies of a large number of investigators over the past decade have demonstrated significant, repetitive, and characteristic myometrial activity at specific periods of the estrous cycle and throughout gestation in several species (Hindson and Ward, 1972; Nathanielsz et al., 1976; Bontekoe et al., 1977; Taverne et al., 1979b,c; Taylor et al., 1983). Evidence will be presented in this section to suggest that this activity is not as "futile" as Foster presumed. In addition, the passive acceptance by the uterus of its contents can now be challenged. When myometrial activity is compared in the nonpregnant and pregnant states, it is clear that the pregnant state influences myometrial activity and in turn it is possible that contractions of the myometrium may exert powerful influences in the developing fetus.

## Physiological Characteristics of Myometrial Contractures

### Studies Using the Chronic Pregnant Sheep Preparation

Studies in the pregnant sheep as early as 100 days gestation (term, 150 days) have shown that regular, tonic increases of IUP lasting between 5 and 15 min can be monitored by either balloon catheters (Hindson and Ward, 1973) or indwelling open-tipped catheters connected to a pressure transducer (Nathanielsz et al., 1976).

A characteristic feature of contractures is their remarkable regularity when recorded as a change in IUP or as the uterine EMG in pregnant sheep from 66 days gestation (Figure 1). The mean duration of the EMG burst was 6.7 min in four animals that did not bear a uterine incision (Harding et al., 1982). Figures in the literature clearly show IUP changes of a tonic nature that have passed without comment as to either their existence or physiological significance. Thus in a study of the changing response of the ovine myometrium to intra-aortic prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) during premature parturition induced by intrafetal dexamethazone infusion, Mitchell et al. (1976) showed contractures before the administration of $PGF_{2\alpha}$ (Figure 2).* In addition, until less than 20 hr before

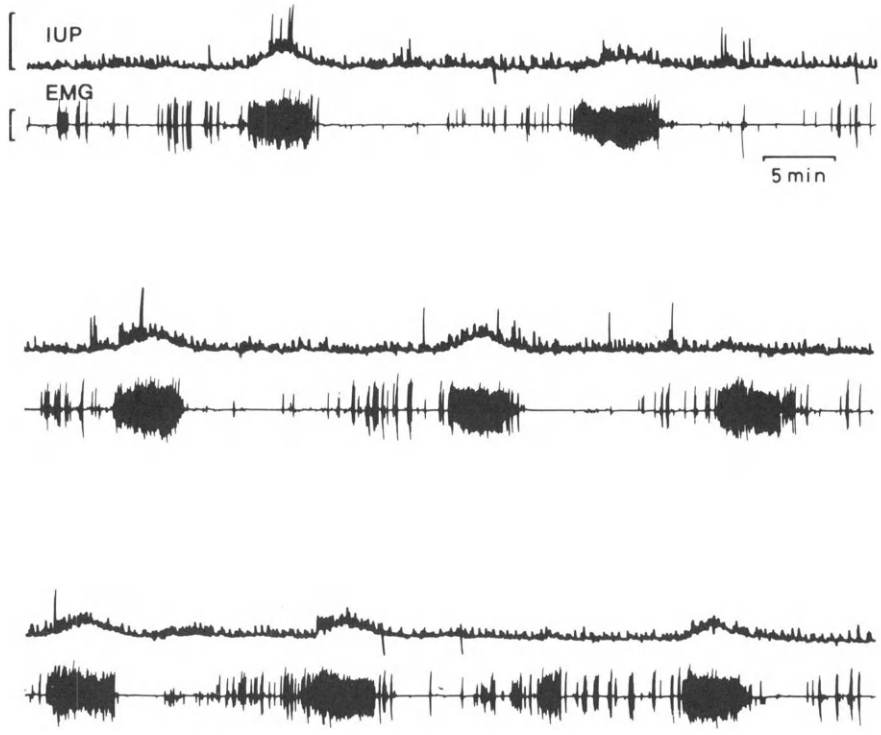


Figure 1 Relationship of IUP and uterine electromyogram at 115 days gestation (calibration bars: IUP, 25 mmHg; uterine EMG, 1 mV). (From Nathanielsz et al., 1980.)

*Where prostaglandins are referred to as a group of compounds the abbreviation PG will be used. Where individual prostaglandins are implicated in a specific function they will be referred to by their particular name.

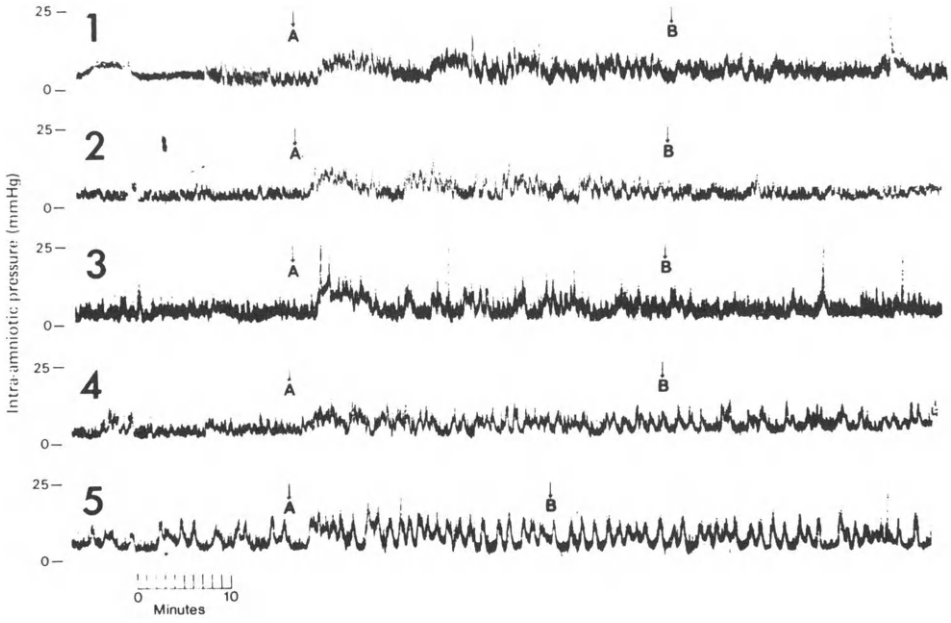


**Figure 2** Intra-amniotic pressure recordings during five experiments in ewe 1, which gave birth to twin lambs 53 hr after the start of intrafetal dexamethasone infusion. Intra-aortic infusion of $PGF_{2\alpha}$ at 94 $\mu$g/min began at A and ended at B. The pattern of contractility shown before starting $PFG_{2\alpha}$ infusion was typical of that observed during the preceding 30 min. (From Mitchell et al., 1976.)

delivery, the response to $PGF_{2\alpha}$ is more of the contracture type than of the contraction type (Figure 2). Similar myometrial activity has been shown in pregnant cows 3 days before delivery. Gillette and Holm (1963) clearly showed a contracture lasting about 10 min. A possible explanation for the absence of comment on contracures in previous studies is that at the fast recording speeds required for stimulus-response-type experiments such as pharmacological studies, the lengthy time scale of contractures determines that they occupy long strips of chart record and the changes are thus too gradual on the recording paper to induce comment. We have verified this possibility by observing the IUP traces of two other groups of workers that were recorded from 6-30 mm/min or more (M. E. Towell and R. Brace, personal communications). The regularity of contractures is best demonstrated at slow paper speeds around 2-5 mm/min (Figure 1).

We have used the pregnant ewe in three separate but related studies of myometrial activity recording IUP and/or uterine EMG from individual animals for several days. The studies were conducted in Cambridge, England (Nathanielsz et al., 1976; and Jansen et al., 1979); Brisbane, Australia (Nathanielsz et al., 1980; Harding et al., 1982); and Los Angeles, (Cabalum and Nathanielsz, 1981, and the unpublished results reported here). In a total of 30 preparations, the mean interval between bursts of myometrial activity was 0.88 hr in England, 0.95 hr in Australia, and 0.78 hr in the USA. In two ewes studied by other workers in Holland at 84-95 and 121-132 days gestation EMG bursts occurred at mean intervals of 0.78 and 0.86 hr, respectively (Scheerboom and Taverne, 1983).

The bursts of EMG activity occurred at approximately the same frequency in preparations in which the uterus has been incised to instrument the fetus and in preparations in which the only surgery performed had been to sew the EMG wires into the myometrium (Harding et al., 1982). The presence of similar myometrial EMG activity in these two states discounts the possibility that the uterine incision acts as an irritative focus for the initiation of contractures. Contracture-like bursts of myometrial EMG activity lasting about 5 min and with a maximal frequency of 3.5/hr have been recorded in the miniature pig late in gestation (Taverne et al., 1979a). Contracture activity appears to be a characteristic of the myometrium when exposed to a particular hormonal environment, since it is present in the nonpregnant ewe at estrous but not at other phases of the estrous cycle (Harding et al., 1982).

### Studies Using the Chronic Pregnant Monkey Preparation

Periodic changes in intra-amniotic pressure have been demonstrated in the chronically prepared rhesus monkey (Harbert, 1972; Novy et al., 1975; Harbert et al., 1979). In a longitudinal study in a large number of pregnancies a diurnal periodicity with higher IUP in the daytime has been demonstrated (Harbert et al., 1979). In contrast, in another study with chronically catheterized pregnant rhesus monkeys, maximum myometrial activity occurred at night (Novy et al., 1980). These differences may be due in part to the different recording techniques, but are also likely to represent the proximity of the period of study to the onset of parturition (see below).

In a recent study we have been able to place EMG wires on the uteri of pregnant monkeys after 130 days gestation (Taylor et al., 1983). Differences in myometrial EMG patterns occurred according to the extent of the surgery and, in particular, depending on whether the fetus was surgically manipulated. When vascular catheters were placed in the fetus in addition to the placement of EMG leads in the myometrium, pronounced EMG activity with associated increases in IUP was seen in the days following surgery (Figure 3). The EMG bursts lasted 45-90 sec and were associated with bell-shaped increases in IUP reaching about 20 mmHg. We have called this type of activity type I contractions. Type I activity showed a marked circadian variation. Maximal activity was present between 8 p.m. and 2 a.m., while only a few type I events per hour were present during the remainder of the day. A second pattern of activity, that we named type II, coexisted with the type I events observed during the daytime. This type II activity had certain similarities to the contractures observed in the sheep. In this pattern, EMG activity lasted several minutes and the IUP increase was less than that observed during a type I event (Figure 3). The amount of type I activity decreased 10 days after surgery, and, although of a much smaller magnitude, still presented a circadian variation,with a maximum during the night. About 10 days before delivery, a progressive increase in the circadian oscillation of type I activity occurred, again the greater frequency observed each evening after the lights went off in the laboratory at 7 p.m., and lasted roughly from 7 p.m. to 2 a.m. As parturition approached, the maximum frequency of type I increased. During the daytime, the uterus showed a limited number of type I events, together with type II events.

In preparations in which the EMG wires have been placed on the uterus but only a small incision had been made into the uterus to insert the intrauterine catheter to measure pressure and no surgery was performed on the fetus, no marked effect of surgery was observed, in contrast to that observed after fetal catheterization. A small circadian oscillation of type I was present throughout the last 40 days of gestation. Type II activity and the period of daily increase in the circadian oscillation of type I

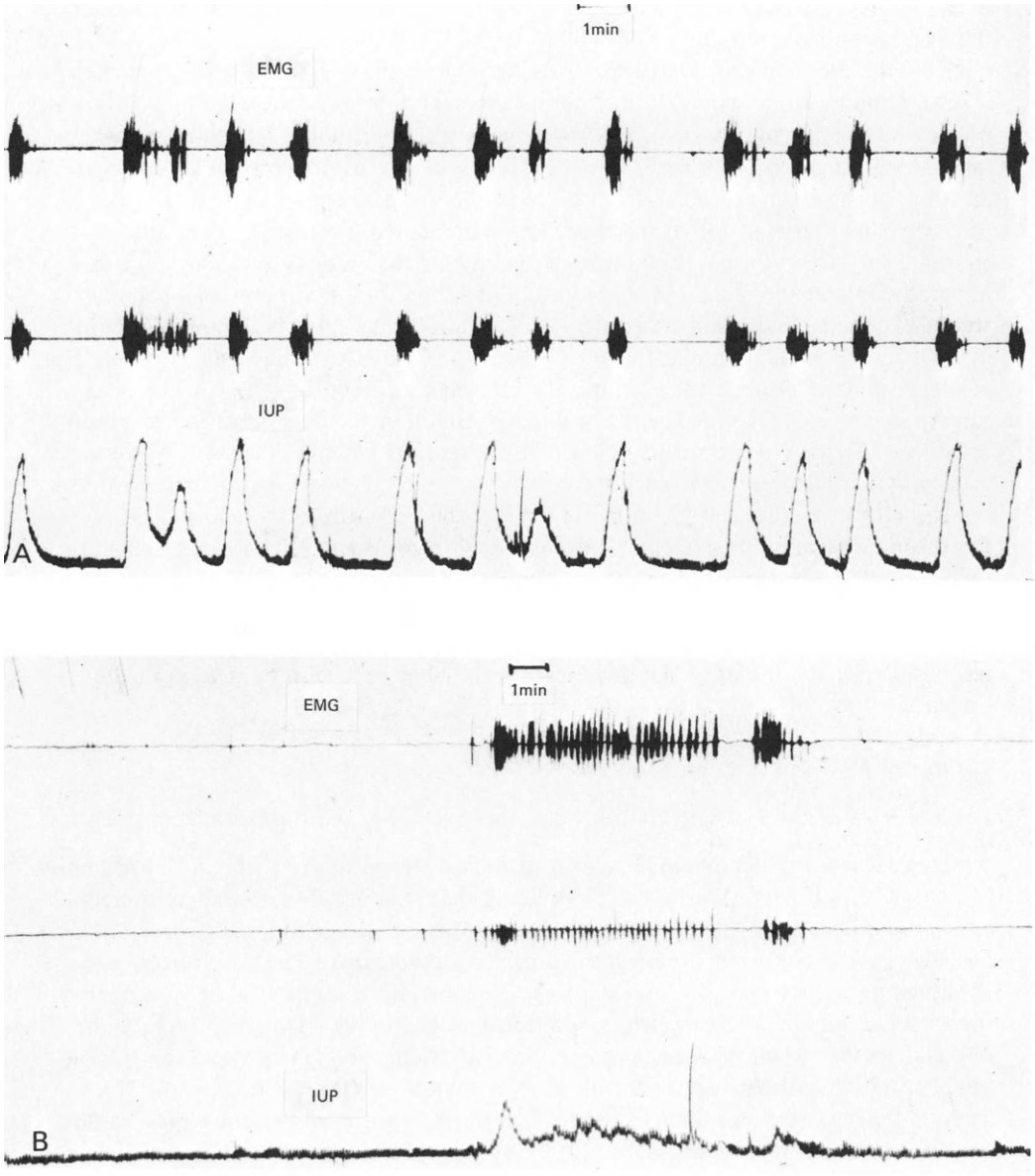


**Figure 3** Uterine EMG and intrauterine pressure recorded in a chronically catheterized monkey preparation in which the fetus was also instrumented. (A) during the period immediately postsurgery in a pregnant monkey on day 127 of gestation and 3 days postoperative. The upper two traces are myometrial EMG records from different sites on the uterus. The bottom trace is intrauterine pressure (maximum amplitude of increase, approximately 20 mmHg). Panel (B) is from the same monkey at 10 a.m., 20 days before delivery. (From Taylor, et al., 1983.)

in the 10 days that preceded delivery were similar to the preparations in which the fetus had been catheterized.

Although these observations in the monkey require more detailed analysis, as well as confirmation by other workers, they permit several important conclusions. Firstly, the nonhuman primate uterus undergoes rhythmic and reproducible EMG activity with associated changes in IUP several weeks before delivery. Although some of the characteristics of this activity differ from those in the sheep, there are certain similarities. Secondly, the uterus of the unanesthetized, instrumented pregnant monkey demonstrated a very clear diurnal rhythmicity in its myometrial activity that may play an important role in the diurnal changes in uterine blood flow that have been demonstrated (Harbert, 1972; Harbert et al., 1979). Thirdly, the fetus is responsible, at least in part, for the oscillation and the amount of type I activity, since when the fetus dies in the immediate postoperative period, the circadian variation of type I is lost after surgery or before delivery. The likely fetal contribution to the modulation of type I is by way of estrogen precursors released from the fetal adrenal as a result of the stress of surgery. In addition, PG generated either as a result of tissue damage and/or under the stimulus of estrogen production may play a role. Fourthly, as reported below in the sheep, availability of continuous uterine EMG records enabled accurate prediction of the onset of delivery at term. Continuous observation and analysis of myometrial EMG and uterine IUP changes and their relationship to endocrine changes in both fetus and mother should prove a sensitive method of investigation of the subtle changes responsible for the initiation and maintenance of labor and delivery at term in the primate.

## Control of Myometrial Contractures

### Possible Role of the Maternal Sympathoadrenal System in the Control of Contractures

A potential role for the maternal sympathoadrenal system in the sheep in the regulation of contractures is suggested by the observation that stressful stimuli such as noise and epinephrine injections will stimulate uterine motility in the gestational period 120 days to 1 day before birth. In the pregnant sheep and rabbit this stimulatory effect was abolished by agents that block $\alpha$-receptors. In contrast, in the last 24 hr of gestation these stimuli inhibit uterine motility (Bontekoe et al., 1980). This accords with the observation that when maternal estrogen concentrations are high at term, epinephrine inhibits uterine activity; a response blocked by $\beta$-blockers (Bontekoe et al., 1977). These differing catecholaminergic effects at different periods of gestation may be due to steroid-induced myometrial catecholamine receptor changes or to differential changes in the rate of myometrial metabolism of the various catecholamines, particularly epinephrine and norepinephrine.

In the anesthetized pregnant monkey both epinephrine and norepinephrine produced a rise in IUP when infused intravascularly. The extent of the IUP increase was related to the preexisting basal uterine pressure and was accompanied by fetal asphyxia. Asphyxia may have been caused by a decrease in uterine blood flow due to vasoconstriction caused directly by the catecholamines and/or secondarily as a result of catecholamine-induced myometrial activity causing compression of the uterine vessels (Adamsons et al., 1971).

In a previous study we demonstrated that an isolated uterine sac that was connected to the rest of the uterus only via its blood supply showed EMG activity similar to the

rest of the uterus. This experiment demonstrated that the fetus need not be present in the sac for EMG bursts to be initiated. However, it did not distinguish between passage of stimulatory humoral agents transported between the different parts of the uterus or the existence of sympathetic nerve fibers coursing along the blood vessels (Harding et al., 1982). The widespread activity of the myometrium as shown by the recording of EMG activity from many sites and the virtually simultaneous appearance of activity over large areas at the uterus is in keeping with the possibility of stimulation of the myometrium by a diffuse nerve net or by electrotonic spread of activity between muscle cells in the myometrium.

Several species have been shown to possess adrenergic uterine innervation that serves the bundles of myometrial cells in addition to the sympathetic vasomotor fibers (Marshall, 1973). Species differences do occur; in the rat for example, there are relatively few fluorescent adrenergic nerves in the uterus. Those fibers that can be seen are vasomotor and have their cell bodies in the sympathetic chain. These fibers are called long adrenergic nerves. In contrast, both the guinea pig and rabbit have short adrenergic nerves originating from ganglia in the cervical region. In parts of the uterus that contain fetuses the catecholamine content of these nerves decreases as pregnancy proceeds. No decrease occurs in portions without fetuses. Electrical field stimulation of these short adrenergic nerves in vitro shows little effect in portions of myometrium surrounding fetuses. This finding is in keeping with the histological sympathetic degeneration in these areas. In addition, these muscle strips with nerves depleted of catecholamine are hypersensitive to the relaxant effect of exogenous noradrenaline. This observation suggests the development of denervation hypersensitivity following the degeneration of the nerves. In contrast, myometrial strips from nonpregnant uterine horns demonstrate a normal relaxant response to nerve stimulation and there is no hypersensitivity to noradrenaline. The fibers stimulated have an $\alpha$-adrenergic action but $\beta$-adrenergic effects that stimulate muscle activity may also occur. These observations support the existence of sympathetic fibers that affect myometrial activity and demonstrate that changes occur in the nerve nets as pregnancy progresses (Elmer et al., 1980). In nonpregnant guinea pigs, oophorectomy reduces uterine norepinephrine content over a period of 3 weeks and this loss can be reversed by the administration of estrogen. In the rat neither oophorectomy or estrogens changed the uterine norepinephrine content (Falck et al., 1974). Thus differences exist between those species with short uterine sympathetic nerve fibers and those that do not possess them. Further work is required at both the in vivo and in vitro levels to determine the nature and extent of catecholaminergic effects on the uterus and the relative roles of direct innervation and blood-borne agents. In addition, studies on the receptor population for different catecholamines or myometrial fibers as well as changes in metabolism of the various amines should be conducted. In addition, peptidergic nerve fibers that contain substance P and vasoactive intestinal polypeptide have recently been demonstrated in the uterus. The functions of these fibers have not been defined (Elmer et al., 1980).

Recently evidence has been obtained in the human that significant changes occur in both red blood cell and myometrial but not endometrial phosphoethanolamine-N-methyl transferase (PNMT) during gestation in the human (Hobel et al., 1982). As pregnancy progresses, PNMT activity in muscle from the lower segment increases. These findings would be compatible with the hypothesis that there is increased methylation of norepinephrine to epinephrine as pregnancy progresses. Such a conversion would

favor increased contractile activity of the myometrium. No changes were observed in the tissue concentrations of the enzymes that catabolize catecholamines, catecholamine-O-methyl transferase or monoamine oxidase. The potential involvement of the fetus in the differences in catecholamine function in the myometrium is suggested by a greater PNMT activity in the pregnant horn at 140 days compared with 100 days and a higher ratio of epinephrine to norepinephrine in the pregnant than in the nonpregnant horn (Hobel et al., 1981). Tissue catecholamine concentration changes have been demonstrated in pregnant and nonpregnant human uteri, some of which may be vascular as well as related to the myometrial cell (Zuspan et al., 1981).

Further work is required at both the in vivo and in vitro levels to determine the nature and extent of catecholaminergic effects on the uterus and the relative roles of direct innervation and blood-borne agents. In addition, studies on the receptor population for different catecholamines on myometrial fibers as well as changes in metabolism of the various amines should be conducted. The recent demonstration of peptidergic nerve fibers that contain substance P and vasoactive intestinal polypeptide in the uterus suggests that other as yet unconsidered molecules with actions on smooth muscle may be of significance in the control of myometrial function. The function of these fibers have not been defined (Elmer et al., 1980).

### The Role of Fetal and Maternal Hormones in the Control of Contractures

Estrogens are stimulatory to the myometrium and very high concentrations of estrone sulfate have been demonstrated in the fetal circulation in the last third of gestation in the sheep (Nathanielsz et al., 1981). Prostaglandins may also play an important role (Challis et al., 1976). Regional differences in various PG molecules have been demonstrated in the myometrium of the pregnant sheep in late gestation (Evans et al., 1981). In control saline-infused animals PGF and PGE concentrations were higher at the tubal and cervical ends of the uterus. This difference was maintained after 70 hr of adrenocorticotropin infusion to the fetus to induce premature delivery at 127 days gestation. These interesting findings require further study to relate them to the possibility of there being pacemaker activity within the myometrium as well as to the endocrine control of contractures. In the only detailed study to date in the sheep with several electrodes placed at several different sites in the uterus, no clear evidence could be obtained for propagation of EMG activity in an orderly and repetitive fashion throughout the uterus (Harding et al., 1982). However, although it was not very pronounced and not commented on in this article, the amount of EMG activity gave the impression of being greater at the tubal and cervical ends than in the body of the uterus. Such a conclusion cannot be drawn too easily with any great degree of certainty, since the extent of EMG activity recorded depends on several factors such as the number of fibers in the immediate locality of the electrodes. In the chronically catheterized pregnant sheep and monkey, myometrial activity is increased in the immediate postoperative period and although it remains to be demonstrated experimentally, the likely causes are increased estrogen production and/or the release of PG from damaged tissue. There is need for a systematic study of the regulation of contractures by hormones produced by the fetus and mother. In the monkey contractures, or type II activity, continue after fetal death, while type I activity is abolished. Thus estrogens produced as a result of secretion of $C_{19}$ estrogen precursors may play a role in the generation of type I activity but may be less important in the regulation of type II contracture activity.

Table 1 Fetal Jugular Vein $pO_2$ Before (−) and After (+) the Administration of 5 mg of Pancuronium to the Fetal Carotid Artery[a]

| Time (hr) | $pO_2$ (mmHg) | Time (hr) | $pO_2$ (mmHg) | Time (hr) | $pO_2$ (mmHg) |
|---|---|---|---|---|---|
| Resting $PO_2$ | | | | | |
| −2 | 100 ± 3.1 | −1 | 99.3 ± 3.5 | −0.5 | 100.8 ± 3.6 |
| Hours after pancuronium administration | | | | | |
| +1 | 108.3 ± 5.5 | +1.5 | 109.9 ± 8.6 | +2 | 113.4 ± 13 |
| +3 | 117.0 ± 8.9 | +3.5 | 113.6 ± 10.5 | +4 | 110.3 ± 5.3 |
| +5 | 108.2 ± 5.5 | +10 | 107.6 ± 6.5 | +11 | 103.2 ± 5.0 |
| +18 | 108.9 ± 3.0 | +21 | 115.4 ± 14.4 | +23 | 104.4 ± 6.6 |

[a]Mean ± SD resting fetal $pO_2$ for each animal was taken as 100% (n = 8). Fetal jugular vein $pO_2$ had risen significantly by the end of 1 hr ($P < 0.01$).

### The Possible Role of Fetal Movement in the Control of Contractures

The possibility that fetal movement in some way initiates the contracures has been investigated by paralyzing the fetus for 36-48 hr with the curare derivative pancuronium (Pavulon). Four chronically catheterized fetuses were observed for 24 hr as a basal period between 117 and 120 days. The time intervals between the contractures and the IUP change generated by each contracture was unchanged in the 24 hr after the administration of pancuronium (Nathanielsz et al., 1982). In addition, succinyl choline paralysis of three fetuses lasting for shorter periods of time did not alter the frequency of contractures (P. W. Nathanielsz, H. K. Yu, and T. C. Cabalum, unpublished observations). Following fetal paralysis, fetal $pO_2$ rose a maximum of 17% at 3 hr after the administration of pancuronium (Table 1). The mechanism of this rise in fetal $pO_2$ remains to be investigated, but it probably indicates the significance of fetal oxygen consumption by activity of skeletal muscle. A similar rise in fetal $pO_2$ has been obtained 90 min after administration of gallamine to the fetus (D. W. Rurak, unpublished observations).

As mentioned above, contractures are still present following fetal death in both the monkey and the sheep. This observation supports the view that fetal movement is not essential to the initiation of contractures. The fetus may, however, modulate the frequency or amplitude of contractures in subtle ways. The relatively infrequent occurrence of contractures, approximately 1/hr, means that animals must be studied for several days to obtain good, statistically sound information. Further work is required to assess whether fetal movement may play a role in the fine tuning of the amplitude or extent and exact location of contracture activity over the uterus.

### Relationship of Contractures to Continuous Variability in Fetal Systems

#### Temporal Relationships

In 1976 we reported a temporal relationship in the sheep fetus between contractures and a switch in the fetal electrocorticogram (ECoG) from low-voltage high-frequency activity (characterized as rapid eye movement, REM, activity) to high-voltage low-frequency activity (non-REM). We proposed that contractures affected the fetus either by impairing placental blood flow and thereby fetal oxygenation, or by producing stimulation of the fetus by constricting it (Nathanielsz et al., 1976). In further studies using an indwelling, continuously recording $pO_2$ electrode designed by Dawood Parker

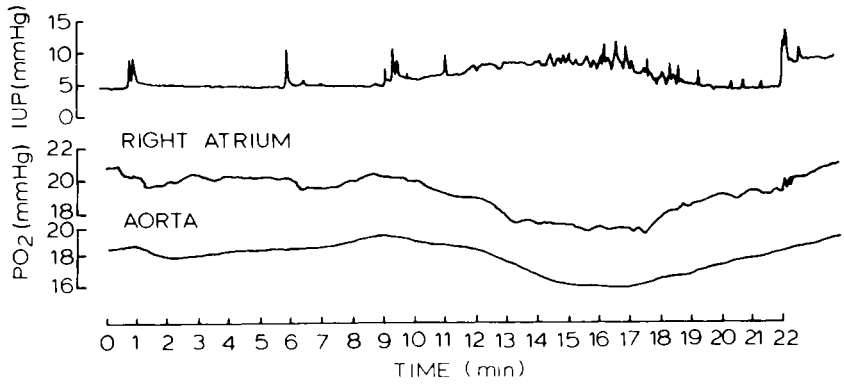


**Figure 4** Simultaneous measurement of IUP and right atrial and aortic arch $pO_2$ in a sheep fetus, gestational age 113 days, 5 days postoperatively. The fetus died during delivery following induction of labor with adrenocorticotropic hormone at 123 days gestation. (From Jansen et al., 1979.)

which we placed in various fetal vessels, we demonstrated that contractures were accompanied by falls in fetal intravascular $pO_2$ (Jansen et al., 1979) (Figure 4).

In a subsequent series of experiments it was shown that contractures are temporally related to the cessation of fetal breathing movements (Figure 5). In addition, gross deformation of the fetal thorax was demonstrated using transit-time ultrasonography. There was a 10-20% decrease in the anteroposterior diameter of the fetal chest and a similar increase in the lateral diameter during contractures (Figure 5) (Nathanielsz et al., 1980). Since increases in intracranial pressure may have important physiological consequences for the fetus, it would be of interest to know the effect of contractures on the dimensions of the fetal skull.* Our current hypothesis as to how contractures affect the fetus is shown in Figure 6.

In the sheep two important observations can be made from viewing the uterus and its contents directly at surgery. First, myometrial activity of a tonic contracture appearance can be generated by stroking the uterus with a finger. These contractures, while widespread throughout the uterus, are absent from some parts of the uterus. Second, the uterine wall is in direct contact with the fetus in several areas. In addition, when myometrial EMG activity is recorded from a larger number of sites throughout the uterus, although activity is widespread during a contracture, EMG bursts at one site are not always accompanied by an IUP rise (see EMG burst number 5 in Figure 1). Similarly, IUP rises are not always accompanied by EMG activity at the site of every pair of recording electrodes in use. It is likely that some parts of the uterus are subjected to less wall tension than others during a contracture and may bulge outward, permitting the uterine wall to come into contact with the fetus over even greater areas and to exert increasing direct pressure on the fetus. In addition, in the sheep, as the volume of the amniotic and allantoic fluids decrease throughout the last half of pregnancy, increasingly larger areas of the uterus may come into direct contact with the fetus as gestation progresses. Similar localized myometrial activity has been observed in the pregnant rhesus

*Note added in proof: Since this manuscript was written it has been demonstrated that contractures produce a rise of 5-15 mmHg in intracranial pressure in the fetal sheep (D. Walker, personal communication).

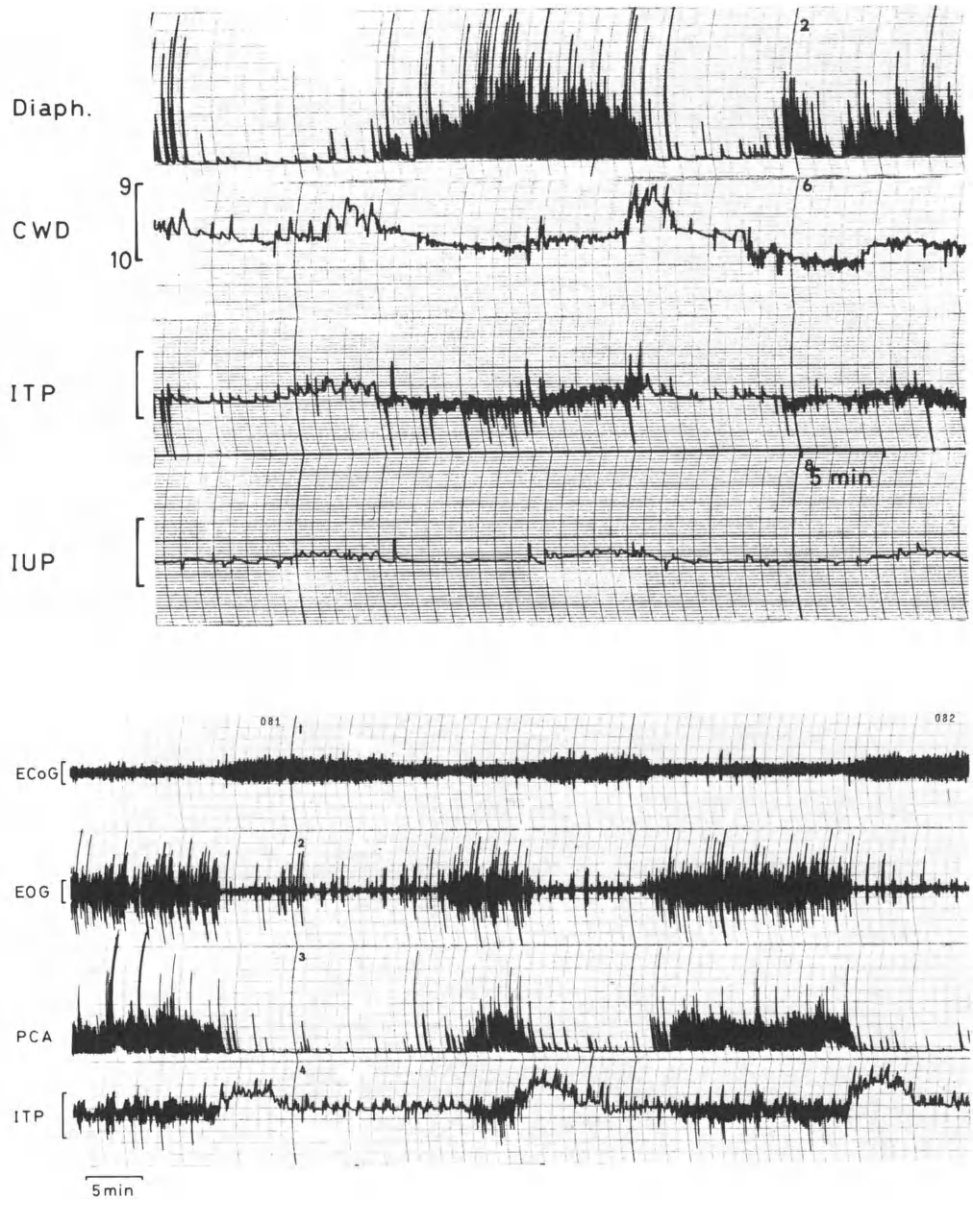


**Figure 5** (Upper) Relationship of diaphragmatic EMG (Diaph.), chest wall diameter (CWD), intratracheal pressure (ITP), and IUP at 132 days gestation (calibration bars: IUP, 25 mmHg; diaphragmatic EMG, in millivolts; CWD = distance between dorsal and ventrally placed transducers, in centimeters). (From Nathanielsz et al., 1980). (Lower) Relationship of ECoG, electro-oculogram (EOG), posterior cricoarytenoid muscle (PCA), and ITP at 134 days gestation (calibration bars: ECOG, 100 $\mu$V; EOG, 100 $\mu$V; IUP, 25 mmHg). (From Nathanielsz et al., 1980.)

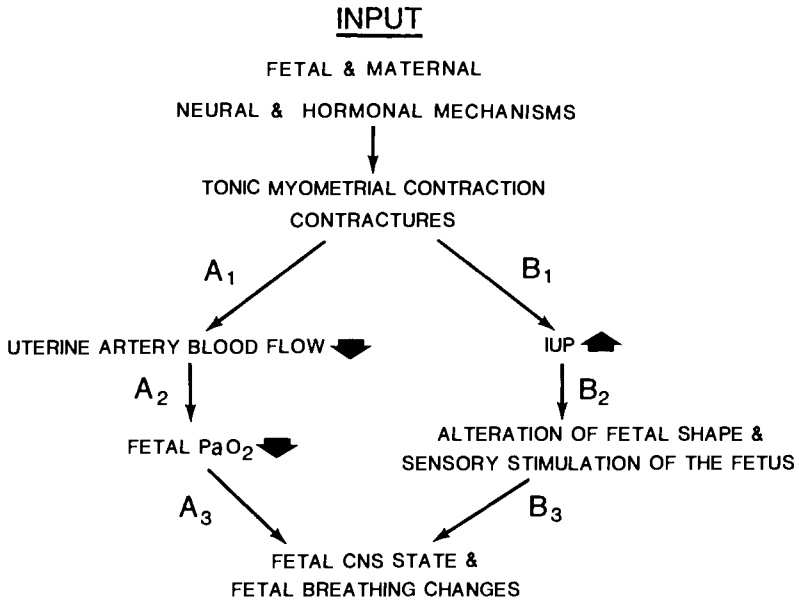


Figure 6 Two hypotheses of the mechanism of production of changes in fetal central nervous system (CNS) state and breathing changes induced by contractures.

monkey both at surgery and from records obtained from two pairs of EMG electrodes placed at different sites on the uterus (Taylor et al. 1983). In the humans, it is a common feature of ultrasonography during amniocenteses for antenatal diagnosis at 16-20 weeks that the passage of the needle through the myometrium stimulates contraction, which can be observed as a thickening of the uterine wall. This myometrial activity is probably localized, since amniotic fluid that was previously present in this area between the fetus and the uterine wall is moved to another site in the uterus, with the result that the fetal parts come into closer approximation with the uterine wall at the site of the needle.

## Pathways by Which Contractures May Affect Fetal Function

*Effects on Uterine Arterial Blood Flow* Contractures may influence the fetus via one or both of at least two routes (Figure 6). At the present time it is not possible to quantify their relative importance. Uterine blood flow is controlled by several factors, including the existing perfusion pressure and the resistance to flow of the vessels in the uterine circulation. Some of the continuous variability in uterine blood flow can be shown to be due to changes in maternal arterial blood pressure (Figure 7). Since the placental, endometrial, and myometrial vessels have to pass into and through the myometrium, contraction of the uterine muscle around these vessels will increase the resistance to perfusion. Evidence has recently been provided in the pregnant sheep to show that contractures are accompanied by a fall in blood flow in both the common uterine artery (Cabalum and Nathanielsz, 1981) and the middle uterine artery (Figure 8) blood flow. There are few long-term studies with continuous recordings of uterine blood flow in the literature. Our data bear a close resemblance to the data and figures shown in an investigation of middle uterine

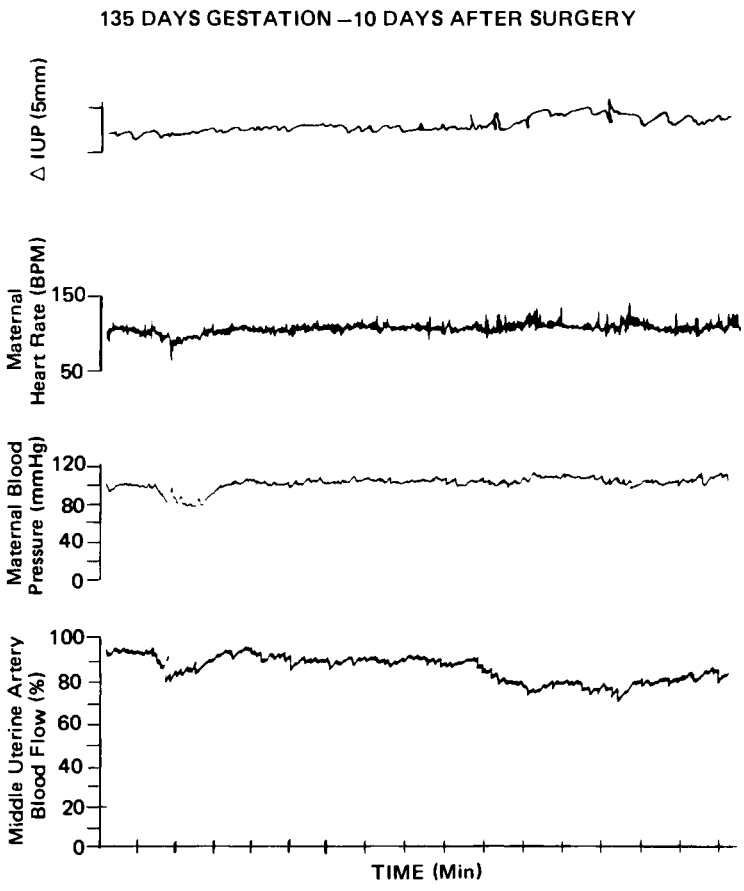


Figure 7 Maternal middle uterine artery blood flow, mean aortic blood pressure, and heart rate in relation to myometrial EMG.

artery blood flow by Lanz et al. (1977). These authors are among the few to consider the existence of variability in uterine arterial blood flow in the undisturbed animal in a stable condition at least 2 days postinstrumentation. They demonstrated that middle uterine artery blood flow has a coefficient of variation [(SD/mean) X 100] greater than 10% in its variation within each hour and the mean value taken every minute for each hour could vary by as much as 20% from hour to hour (from 252 to 300 ml/min). Although these authors did not record IUP, they showed falls in the uterine blood flow of up to 10% lasting 10 min. Such a trace can be seen in the resting period before administration of the test substance (Lanz et al., 1977). This fall is very similar to the fall in uterine blood flow shown in Figure 8. The authors also reported an increase in uterine blood flow when the ewe lies down.

Analysis of postural effects on uterine blood flow is complicated by the fact that the ewe often changes position when a contracture occurs (Figure 9). Thus the effects of postural change must be dissociated from the effect of the contracture. When allowance is made for any contracture effect occurring simultaneously with the ewe lying down, this postural change is accompanied by an increase in uterine blood flow

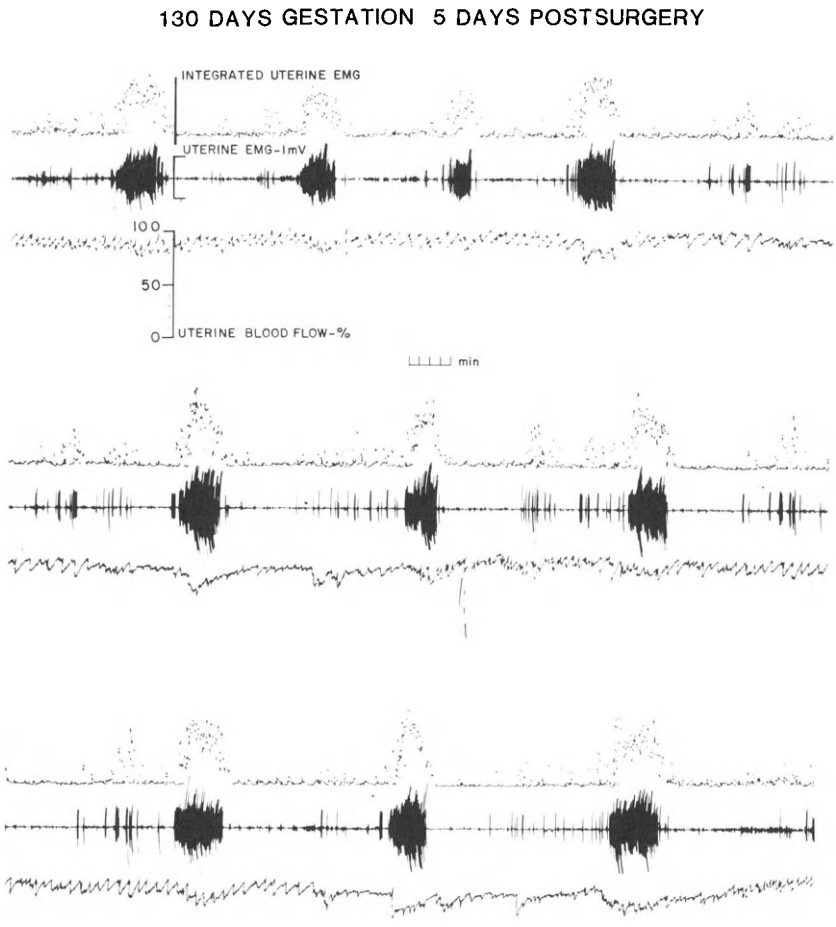


**Figure 8** Maternal middle uterine artery blood flow: electronic summation of flow in both middle uterine arteries in a ewe at 130 days gestation, 5 days postsurgery. Integrated uterine EMG and uterine EMG are also shown.

in the sheep (R. Harding and E. R. Poore, personal communication; T. Cabalum, H. K. Yu, and P. W. Nathanielsz, unpublished observations).

Lanz et al. (1977) demonstrated a prolonged fall for several minutes when the sheep was unduly disturbed. This fall in utereine blood flow occurs even though the maternal arterial blood pressure may rise. There are several possible explanations for this observation: for example, (1) there is direct, neurally mediated sympathetic vasoconstriction in the uterine circulation, (2) circulating catecholamines may act on the uterine circulation, or (3) the stress stimulates a uterine muscle contracture, as we have observed on several occasions.

In the published discussion following a paper by Makowski et al. (1973), Prystowsky reported experiments conducted by himself and his colleagues and stated, "It was concluded that uterine blood flow is probably not regulated by low $O_2$ or high $CO_2$ concentrations, but that vascular resistance is not fixed and does vary spontaneously through

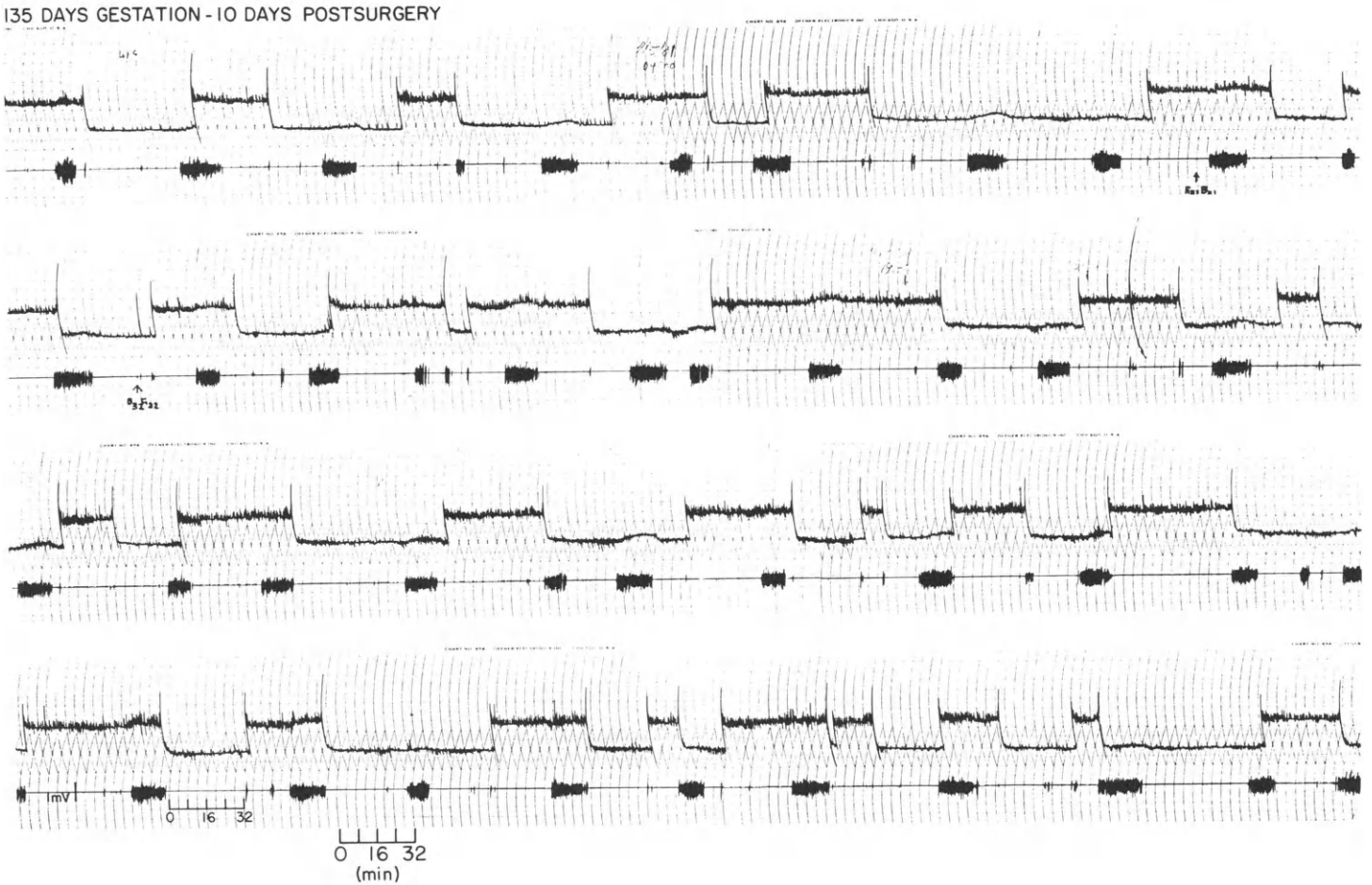


**Figure 9** Intrauterine pressure (IUP) (top trace) recorded from a transducer mounted on the side of the cage and uterine EMG (bottom trace of each pair). The whole recording is continuous. The IUP trace shows the times when the ewe stands and lies down, as well as contractures.

unknown mechanisms, probably not neural." We would propose that among the "unknown mechanisms" producing spontaneous variation is the episodic change in myometrial tone. Further data are required before the exact significance of these relationships is understood. However, contractures represent a route whereby the maternal environment and maternal physiological systems may "talk" to the fetus. As discussed above, stress in the maternal sympathoadrenal system and sympathomimetic agents have been shown to modify myometrial activity in vivo and in vitro in several species. Also, in the rhesus monkey a pronounced diurnal rhythmicity has been shown in both the myometrial EMG and IUP changes (Harbert, 1972; Harbert et al., 1979; Taylor et al., 1983). In addition to the temporal relationship between contractures and decreases in uterine arterial blood flow, several investigators have demonstrated the probable existence of direct effects of the maternal sympathoadrenal system on the uterine vasculature. Future studies should attempt to define the relative roles of direct effects on the uterine vessels themselves and those that are secondary to myometrial contraction. In addition, it is important to identify possible alterations of regional perfusion within the uterus, since redistribution of regional blood flow could constitute an important compensatory mechanism.

*Direct Stimulation of the Fetus by the Contracture: Modification of Sensory Input to the Fetus* With one or two exceptions, such as the studies of taste stimulation in utero (Mistretta and Bradley, 1975), there have been very few studies on fetal sensory development in the sheep or any other species. The linkage between the fetus and the outside world via the mother has been little studied. Using sensitive transit-time ultrasonography, we have demonstrated that contractures are accompanied by pronounced deformation of the fetal chest (Nathanielsz et al., 1980), and with real-time ultrasound it appeared that qualitative changes occurred in the type of fetal movement, since characteristic writhing movements occurred following the onset of a contracture (G. R. DeVore, J. C. Hobbins, C. A. M. Jansen, and P. W. Nathanielsz, unpublished observations). However, the total amount of fetal limb movement may decrease during a contracture (Sheerboom and Taverne, 1983). In addition, stimulation of other parts of the fetus, particularly the fetal skull, may occur.

In preliminary studies, we have noticed that slow removal of 16 ml of fetal tracheal fluid over 90 min modifies fetal breathing movements. This observation suggests that afferent pathways stimulated by fetal lung volume changes can affect fetal neurological activity. Experimental perturbation studies are required to investigate the nature, pathways, and extents of various fetal sensory cues and their maturation throughout gestation. The fetus does demonstrate several circadian rhythms, including circadian periodicity in fetal breathing movements (Dalton et al., 1977). The mechanism(s) whereby the fetus receives cues from the external environment are poorly understood and merit further study.

## Physiological and Pathophysiological Significance of Contractures

Understanding the physiological pathways controlling contractures and those by which contractures influence fetal development is a first step to an understanding of their role in normal and abnormal fetal maturation. Several characteristic features of contractures and the accompanying changes in fetal function that have been described above may play significant roles in fetal development. The rhythmicity of contractures in each pregnant ewe constitutes a remarkably regular feature of the fetal environment. Either through the fall in intravascular $pO_2$ or by sensory stimulation, contractures may

modulate the various fetal rhythms. Since fetal hypoxemia can stimulate fetal adrenocorticotropic hormone (ACTH) release (Jones et al., 1977) it has been suggested that periodic uterine contractures could lead to premature activation of fetal adrenal function and premature labor (Challis et al., 1981).

Contractures may also play a role in a variety of pathological mechanisms. There are a number of conditions such as the effects of cigarette smoking, the effects of pharmacological agents such as caffeine that are known to affect smooth muscle function, the effects of other therapeutic agents whose influence on the myometrium has not been studied, various drugs of addiction, and alcohol, whose harmful effects may be due to an increased incidence of contractures. We have demonstrated that administration of Rompun, a commonly used premedication in surgery on the pregnant sheep, stimulates myometrial EMG activity (Jansen et al., 1983). A variety of maternal stresses of a more psychological nature may also be involved. Clinical impression suggests that maternal stress does affect myometrial activity and epidemiological studies do show an increased incidence of premature labor associated with a variety of maternal stresses (Kaminski and Papiernik, 1974; Papiernik and Kaminski, 1974). Some of these stresses are multifactorial and hence difficult to quantify in an exact, scientific fashion.

The possibility exists that abnormalities in the quantitative amount or qualitative pattern of contractures significantly modify fetal development. In one experimental study, a 50% reduction in the time spent by newborn rats in REM sleep for the limited period of the seventh to the twenty-first day of neonatal life markedly decreased learning capacity and increased the incidence of impotence in male rats in later life (Mirmiran et al., 1981). Contractures have been shown to be associated with a change of fetal sleep state from REM to non-REM. Thus the effect of contractures is to decrease the amount of time the fetus spends in REM sleep. Factors regulating the frequency and intensity of contractures may considerably influence fetal imprinting, particularly if abnormalities in the contracture pattern occur at a critical period of fetal neurological maturation. The siblings of infants who have been victims of sudden infant death syndrome have a greater risk of themselves being affected by sudden infant death syndrome. It has been shown that the siblings of such infants show disturbed sleep state patterning as early as the first week of neonatal life (Harper et al., 1981). We would hypothesize that this difference in sleep-wakefulness patterns in the early postnatal period may reflect abnormal sleep-wakefulness patterns in utero that may themselves result from stress-induced alterations in myometrial activity.

Current views on fetal and neonatal sensory systems suggest that the pattern of sensory input markedly affects development in ways that can be demonstrated by structural studies (Blakemore and Vital-Durand, 1981). Information regarding myometrial activity needs to be obtained in the antenatal clinic and in the antenatal ward. If such information could be integrated with follow-up data postnatally, it would provide a better understanding of the pathophysiology of fetal growth, brain development, and premature labor, as well as complicated, incoordinate term labor.

### Transition of Contractures to Efficient Expulsive Contractions at Term

A better understanding of the mechanisms responsible for the transition from the late pregnant state in which the myometrium is only undergoing "contracture"-type activity to well-coordinated expulsive contractions would greatly aid the clinical management of premature labor, discoordinate uterine activity, uterine atonia, and post-term pregnancies.

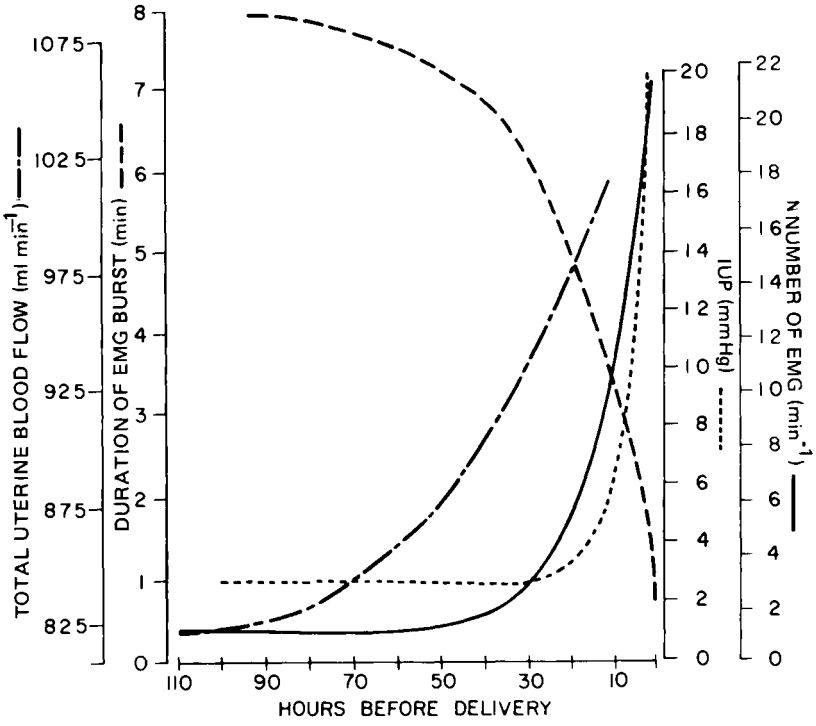


**Figure 10** Total common uterine artery blood flow (measured every 5 min when no contracture was present), duration of individual myometrial EMG bursts, number of myometrial EMG bursts per hour, and increment in intrauterine pressure (IUP) accompanying myometrial EMG activity during induction of premature delivery following the intravascular infusion of 1 $\mu$g of $ACTH_{1-24}$ per hour to a sheep fetus commencing at 120 days gestation. Delivery occurred after 113.24 hr of infusion.

Several early reports stated that observation of IUP changes in the pregnant sheep at term did not demonstrate any significant pressure increase until less than 24 hr before delivery. Continuous recording of myometrial EMG permits clear anticipation of both normal and prematurely induced delivery in the sheep from the changing EMG pattern. Term labor can also be anticipated in the chronic pregnant monkey preparation. The earliest change is a decrease in the mean duration of individual EMG bursts (Figure 10). Whether this is due to the appearance of a qualitatively new type of input to the uterus that results in a different pattern of muscle stimulation or to a quantitative change in the preexisting stimulus or to a difference in the response of the muscle itself remains to be determined. The next change in the EMG bursts is that they become more frequent. The final event is an increase in the IUP change produced by the EMG burst. Since the amplitude of EMG activity is unchanged, there appears to be an improvement in the coupling of electrical and contractile activities. In the sheep gap junctions between the individual myometrial cells appear during parturition and disappear after delivery (Garfield et al., 1977). These junctions could be the ultrastructural mechanism responsible for better-coordinated muscle contraction. Several other changes may contribute to the alteration in the amplitude and duration of the IUP wave form; there are likely to be biochemical and ionic changes in the muscle cells as a result of

the changing endocrine milieu. In addition, recruitment and activation of myometrial cells may be faster. Finally, connective tissue changes have great importance in the cervix at the time of delivery (Fitzpatrick, 1976). The connective tissue matrix of the myometrium and the rest of the uterine wall may greatly influence the efficiency with which activation of myometrial cells is converted to IUP change. Little attention has been paid to such changes in the consideration of normal and abnormal labor.

## SUMMARY

Using electromyographic as well as pressure recordings from the uterus, it has been demonstrated that tonic, long-lasting episodes of myometrial activity (contractures) throughout the major part of gestation in several experimental species, including the pregnant rhesus monkey. In the sheep, contractures have a temporal relationship to a fall in uterine blood flow and a 25-30% fall in fetal intravascular $pO_2$. If the fetus is brathing when a contracture occurs, there is also a temporal association with cessation of fetal breathing movements and a switch from REM to non-REM sleep.

The regularity of contractures in a given pregnant animals, their response to various stresses, and the marked harmful effects shown in the newborn rat deprived of REM sleep indicate that contractures may have important physiological and pathophysiological effects on the fetus.

## ACKNOWLEDGMENTS

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The data relating to myometrial function in the long-term monkey studies were obtained in collaboration with Drs. Maria Seron, Mary Martin, and Norman Taylor. Many others have participated in some of the studies, in particular Drs. Alan Thomas, Kenneth Lowe, and Patricia Jack in Cambridge, England, and Drs. Richard Harding and Robin Poore in Brisbane, Australia.

The investigations reported would not have been possible without the skilled and dedicated technical assistance of numerous people, especially Ms. Fiona Bass and Ms. Kathryn Fontwit. Finally, we would like to thank Ms. Chrysanthi Arseculeratne for her expert attention to the manuscript.

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# 20

# Antepartum Monitoring of Fetal Heart Rate

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## INTRODUCTION

In 1962 Hammacher described a fetal heart rate monitor operating through the maternal abdominal wall and displaying true fetal beat-to-beat heart rate. Four years later the same author, in order to demonstrate the value of this instrument, published a series of examples (Hammacher, 1966), one of which is shown in Figure 1. This figure demonstrates a clearly abnormal antepartum fetal heart rate (FHR) with reduced variability and marked late decelerations with spontaneous contractions at 38 weeks of pregnancy. On the basis of the pathological antepartum FHR, a prelabor cesarean section was performed and a growth-retarded, depressed fetus was delivered who had an uneventful neonatal course.

Since that time, not too much has changed in the interpretation of ominous FHR tracings or in the management of antenatal problems. However, considerable additional knowledge—albeit incomplete—on the physiological background has been accumulated, as well as extensive experience in the clinical application and potential of Hammacher's technique.

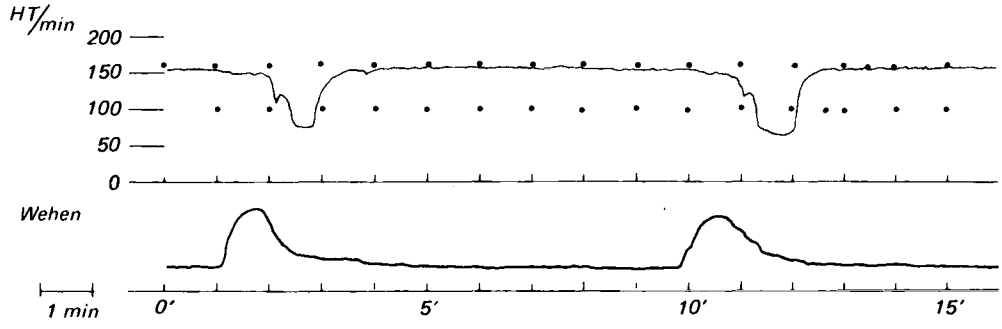


Figure 1 Antepartum cardiotocogram with reduced variability and late decelerations with spontaneous contractions at 38 weeks of pregnancy. The fetus was delivered by cesarean section, depressed and growth retarded. (From Hammacher, 1966.)

## PHYSIOLOGICAL BACKGROUND

The *regulation* of the fetal heart rate is complex, involving cerebral cortical and subcortical influences; the vasomotor center in the brainstem; peripheral reflex activity, mainly through the aortic chemoreceptors and the baroreceptors; humoral factors such as catecholamines; and finally cardiac autoregulation. The main mediators of cardiac control are the parasympathetic and sympathetic autonomic nervous systems.

Intrinsic rhythms, exogenous stimuli operating through the fetal cerebral cortex, and fluctuations in fetal blood gases (Jansen et al., 1979) are present throughout normal pregnancy in the animal and probably also in the human. They all contribute to a highly dynamic state of fetal cardiovascular performance throughout normal pregnancy, on which might be superimposed pathological events and influences.

### FHR Variability

Under physiological conditions the fetal beat-to-beat intervals are constantly subject to small changes from one heart period to the other, known as short-term variability. Due to a certain periodicity in the direction and size of these changes, they result in oscillations of the fetal heart rate around its mean level; these are called long-term variability. Long-term variability is defined by the amplitude and frequency of the oscillations, 10-25 beats per minute (bpm) and 2-6 cycles/min in the normal fetus. In the FHR tracing short-term variability is superimposed on long-term variability in the form of minimal deflections, but it cannot be reliably interpreted with the naked eye with conventional monitoring display techniques; thus, unless otherwise mentioned, FHR variability in this chapter means long-term variability.

#### Physiological Influences

The understanding that a reduction in FHR variability may be due to a physiological fetal low-activity phase known as "quiet sleep" is crucial for the interpretation of an FHR trace in clinical monitoring. Semiquantitative studies in 1972 showed the relatively frequent occurrence of phases of low-amplitude variability in 180 cardiotocograms (CTGs) from 40 normal fetuses. The low-variability periods never exceeded 50% of the recording time of 30 min (Rüttgers et al., 1972), in contrast to the situation with chronic antepartum hypoxia, where the low-variability periods were clearly of longer duration (Table 1).

Sleep phases may be interrupted and wakeful states induced by various external stimuli, of which manual stimulation by palpation and acoustic stimulation (Luz, 1979) are the most important in practice. Under physiological conditions, FHR variability reflects the balance of the autonomic nervous control of the fetal cardiovascular system. A rise in parasympathetic tone causes an increase in FHR variability, while enhancement of sympathetic tone results in a decrease. With advancing gestational age, parasympathetic drive increases and so does the variability of the fetal heart (Wheeler et al., 1979). However, in the last 10 weeks of pregnancy this change is small (Wheeler et al., 1979) and at present may be disregarded for practical purposes.

Dawes et al. (1981a) have shown that short-term variability decreases with FHR acceleration and increases with deceleration, as it does with fetal breathing. According to deHaan et al. (1971) and Roemer et al. (1979), there is a trend toward an inverse relationship of the FHR baseline level and variability. However, this does not seem to be an absolutely constant phenomenon (Dawes et al., 1981a) and, according to Wheeler et al. (1979), is true only within a given fetal activity state.

Table 1 Relationship Between FHR Variability (Amplitude) and the Simultaneous Fetal Breathing Pattern[a]

| Amplitude of FHR variability | Less than 10 bpm | | Greater than 10 bpm | | Not interpretable | |
|---|---|---|---|---|---|---|
| | Time (min) | Percentage of time (%) | Time (min) | Percentage of time (%) | Time (min) | Percentage of time (%) |
| Total fetal breathing | 482 | 26.9 | 1176 | 65.7 | 132 | 7.4 |
| Continuous, regular | 149 | 30.9 | 177 | 15.1 | * | |
| Discontinuous, irregular | 73 | 15.1 | 488 | 41.5 | * | |
| Absence of breathing | 260 | 53.9 | 511 | 43.5 | * | |

*$P < 0.001$.
[a]Data obtained by transabdominal electrocardiogram and B-scan real-time ultrasound from 57 patients (1790 min of recording).
*Source*: From Garoff et al. (1981).

## Pathological and Pharmacological Influences

The association of a loss or reduction of FHR variability with conditions suggestive of chronic fetal hypoxia is one of the findings reported by clinical investigators over several years (Hammacher, 1966; Kubli et al., 1972; Emmen et al., 1975) and is supported by data presented in Table 2. The relationship of FHR variability and hypoxia, however, is complex. With milder degrees of acute hypoxia, an increase in FHR variability is observed in experimental animals (Dalton et al., 1977; Stange et al., 1977). This is in line with observations made by Willcourt et al. (1981) in the human fetus, where falls in fetal trancutaneous $pO_2$ ($tcpO_2$) in labor were associated with enhanced FHR variability. With severe fetal hypoxia and marked acidemia, loss of variability is seen in the experimental animal (Dalton et al., 1977), as well as in the human fetus. What might be specific to the human species and so far has not been reproduced in the chronically instrumented animal is a reduction of FHR variability in the early stages of chronic hypoxia when substantial measurable hypoxia and acidemia are not yet present. This is common—over 90% of the growth-retarded fetuses

Table 2 Incidence of Reduced Variability and Its Relative Duration in 90 CTGs of 30 min from 12 Cases of Antepartum Fetal Death

| | | Bandwidth and relative duration of irregularity | | |
|---|---|---|---|---|
| | | ⩾ bpm ⩾ 25% | 5-10 bpm ⩾ 50% | ⩽5 bpm ⩾25% and/or 5-10 bpm ⩾ 50% |
| Records (90 = 100%) | N | 58 | 25 | 75 |
| | % | 64.0 | 27.0 | 83.3 |
| Fetuses (12 = 100%) | N | 12 | 9 | 12 |
| | | 100 | 75 | 100 |

*Source*: From Kubli et al. (1972).

monitored by Flynn et al. (1979) showed some impairment of activity or reactivity. According to Halberstadt (1981), evolution of fetal compromise seems to be accompanied by progressively longer low-activity periods. It seems probable that this reduction of FHR variability early in the course of chronic fetal distress is not a direct effect of hypoxia or acidemia on cardiovascular regulation but, rather, it indicates depression of general, motor, and cardiovascular activity due to depressed brain function on the basis of non-measurable borderline hypoxia or other adverse conditions.

Increased parasympathetic tone is probably responsible for the increased variability with acute hypoxia and during decelerations, whereas enhanced sympathetic tone may in part be responsible for decreased variability with more protracted hypoxia.

*Fetal infection* is a primarily nonhypoxic condition associated with loss of FHR irregularity and generally associated with tachycardia. Rare instances of fetal heart block are also associated with loss of irregularity at a bradycardic level. The same mechanism may be responsible, at least in part, for the "smoothing out" of FHR tracings on the bottom of severe decelerations and with terminal bradycardia.

Pharmacologically, drugs which are known to depress the central nervous system are a well-recognized cause of reduced FHR variability (Petrie et al., 1978). According to Fischer (1976), the amplitude of long-term variability is usually more affected than the frequency. In clinical practice drug effects must be ruled out with a nonreactive antepartum record.

### Sinusoidal Patterns

Regular sinusoidal oscillations of FHR have been described in various conditions of fetal compromise, notably severe hemolytic disease, anemia from other causes, cardiac failure, and severe asphyxia, especially in the preterminal state (Kubli et al., 1972; Baskett and Koh, 1974; Young et al., 1980). They are also seen in the recovery phase of asphyxia, with certain drugs, and when good fetal outcome is reported (Young et al., 1980; Romanini et al., 1980; Sibai et al., 1980).

The pathogenesis of sinusoidal patterns is unclear. They may reflect loss of nervous control over the heart, high-output cardiac failure, or simply the undamped response of a sensitized cardiovascular system (Young et al., 1980). As far as can be judged from the published examples, it would seem that sinusoidal patterns with a regular and smooth appearance with low frequency (<2 cpm) are ominous, particularly if the amplitude is high. Sinusoidal patterns which are less regular and of higher frequency appear to be associated with a more favorable fetal outcome.

Sinusoidal patterns which occur transiently are virtually never an indication for intervention by themselves. Their significance in the individual case has to be evaluated in conjunction with preceding and associated FHR alterations and other indices of fetal compromise if present.

## Baseline FHR

The FHR baseline is the mean level of the fetal heart rate in the absence of accelerations or decelerations. It is determined visually over time periods of 5 or 10 min.

### Physiological Influences

There is a weak inverse relationship between basal FHR and gestational age (Rüttgers et al., 1972). With advancing gestational age the baseline FHR also becomes more

variable (Ruttgers et al., 1972). In general, the baseline rate reflects the balance of sympathetic and parasympathetic influences.

### Abnormal Baseline Heart Rate

*Tachycardia* Fetal hypoxia tends to increase baseline FHR owing to increased fetal catecholamine levels associated with increased sympathetic drive. This is especially true for acute and subacute fetal hypoxia. With chronic hypoxia fetal catecholamine reserves may become depleted and it has been shown by Kubli et al. (1972), and later by other investigators, that baseline FHR levels are usually within the normal range when chronic antepartum hypoxia is present. Thus baseline tachycardia is neither a constant nor a reliable sign of chronic antepartum fetal hypoxia.

Maternal fever often causes fetal tachycardia, but this is generally not very pronounced. In contrast to this, amniotic and fetal infections are associated with marked fetal tachycardia, usually accompanied by loss of variability.

Pharmacological *parasympathetic blockade* by atropine causes fetal tachycardia, but in practice fetal tachycardia induced by maternal administration of beta-mimetic drugs is more important. Baseline tachycardia of this type is generally mild, with normal FHR variability.

Severe baseline tachycardia in the range of 200 bpm or more, irrespective of its origin, carries the risk of fetal cardiac failure (Klein et al., 1979).

*Bradycardia* It is rare to find baseline bradycardia as a symptom of antepartum hypoxia, although with impending death it is generally observed as a terminal event. Occasionally persistent bradycardia may be the only sign of a clinically unrecognized premature placental separation.

Mild baseline bradycardia (100-120 bpm) with normal variability reflects increased parasympathetic tone, mostly of unknown origin, although occasionally this may be associated with chronic cord compression. More marked, persistent bradycardia is suggestive of fetal heart block, which is often associated with congenital heart disease, but this is far from being a constant association. Out of 20 fetuses subsequently shown to have congenital heart anomalies by Garite et al. (1979), only 2 exhibited arrhythmia and 3 bradycardia.

## FHR Deviations

### Accelerations

Accelerations are short-lived, transient episodes of an increase in FHR above the baseline level. If small, they are generally considered as a component of "baseline variability." In practice, different cutoff levels of amplitude are used to define a deviation as an acceleration, most often of 15-20 bpm. Accelerations of this magnitude are associated with fetal movements in nearly 100% of cases.

Accelerations may be provoked in the healthy fetus by external stimuli, but Wood et al. (1979) have suggested that they may also be a response to mild hypoxia. Dawes et al. (1981a) have found a periodicity of spontaneous accelerations of more than one every 2 min; however, the sample included many small accelerations of less than 5 bpm. Irrespective of the cause, accelerations seem to be evidence of increased sympathetic drive and their occurrence represents increased and—of particular importance to the physician—intact fetal reactivity. In recent years, accelerations have become a major element in the evaluation of the nonstress test (Goodlin and Schmidt, 1972; Paul and Miller, 1978; Fischer, 1976).

### Decelerations

Decelerations are transient episodes of slowing of the FHR below the baseline level. The frequency with which they are found in normal antepartum records depends again on definition. If very small downward deflections, which otherwise might be included in the baseline variability, are identified as decelerations, these can be shown to frequently follow accelerations in a rather systematic pattern (Dawes et al., 1981a). In practice, decelerations are those downward deviations which are clearly distinct from the baseline variability. Thus in a study of normal fetuses during late pregnancy, the results of which are shown in Table 3, decelerations related to uterine contractions were found by Rüttgers et al. (1972) in 40% of the fetuses (n = 39), or in 11% of the 182 CTGs and in 28% of those with contractions. They were mostly (in 7% of the CTGs) of the mild, variable type, occurring in a nonrecurrent way with occasional contractions. Nonrecurrent contractions related to sporadic movement and causing mild variable decelerations may be part of a normal antepartum record.

*Late decelerations* have been shown by various investigators, and most recently by Parer et al. (1980), to be hypoxic in origin and thus abnormal. In 10% of fetuses with a normal pregnancy outcome, occasional but nonrecurrent late decelerations were seen. This evidence, presented in Table 3, suggests that occasional late decelerations without other adverse FHR characteristics are not a cause for intervention, although they cannot be regarded as completely harmless.

*Repetitive and persistent late decelerations,* on the other hand, should be regarded as a reliable sign of fetal hypoxia. They constitute, together with loss of variability, the major component of the diagnosis of antepartum fetal hypoxia (Hammacher, 1966; Kubli et al., 1972; Emmen et al., 1975) (Table 4). Marked and atypical contraction-related variable decelerations have the same significance as late decelerations (Kubli et al., 1972; Kubli et al., 1978; Visser et al., 1980) (Figure 2). Acute hypoxia such as occurs commonly with maternal supine hypotensive syndrome results in marked decelerations.

### Fetal Behavioral States

Sterman (1967), Timor-Tritsch et al. (1978b), Junge (1979a,b) and others have demonstrated sleep-wake cycles in the human fetus similar to those found in the experimental animal and in the human neonate (Dalton et al., 1977; Junge, 1979a,b). Active states are related to "rapid eye movement" sleep (REM) or true wakefulness in the experimental animal

Table 3 Incidence of Decelerations in Uncomplicated Late Pregnancy

| | Number of fetuses | Number of records | Number of records with contractions |
|---|---|---|---|
| N | 39 = 100% | 182 = 100% | 70 = 100% |
| Total decelerations | 16 = 41.0% | 20 = 10.9% | 20 = 28.5% |
| Variable decelerations | 11 = 28.0% | 13 = 7.1% | 13 = 18.0% |
| Late decelerations | 4 = 10.0% | 5 = 2.7% | 5 = 7.0% |
| Undefined decelerations | 1 = 2.5% | 2 = 1.1% | 2 = 2.8% |

*Source:* From Rüttgers et al. (1972).

Table 4 Incidence of Decelerations with Antepartum Fetal Death

| | Number of fetuses with contractions | Number of records | Number of records with contractions |
|---|---|---|---|
| N | 10 = 100% | 90 = 100% | 48 = 100% |
| Total decelerations | 9 = 90.0% | 45 = 50.0% | 45 = 93.8% |
| Variable decelerations | 3 = 30.0% | 8 = 8.8% | 8 = 16.7% |
| Late decelerations | 9 = 90.0% | 43 = 47.0% | 43 = 89.6% |
| Undefined decelerations | 6 = 60.0% | 12 = 13.3% | 12 = 25.0% |

*Source*: From Kubli et al. (1972).

(Dalton et al., 1977) and human newborn (Junge, 1979a,b), and probably also in the human fetus, and are characterized by a clustering of increased variability of the fetal heart and an increase in body movements and breathing activity. Quiet sleep, or non-REM sleep, in the animal and human neonate is accompanied by a reduction in heart rate variability, as well as motor and respiratory activity. Species differences, however, are evident (Dawes et al., 1981a). In the fetal sheep breathing movements are closely correlated with marked FHR variability (Dalton et al., 1977).

In the human fetus the relationship between breathing movements and active states in terms of FHR long-term variability and body movements exists but is not close (Manning et al., 1979; Garoff et al., 1981). In their more recent sophisticated study, Dawes et al. (1981a) demonstrated that in the human fetus breathing movements are associated with an increase in short-term (beat-to-beat) variability, but not with increased long-term

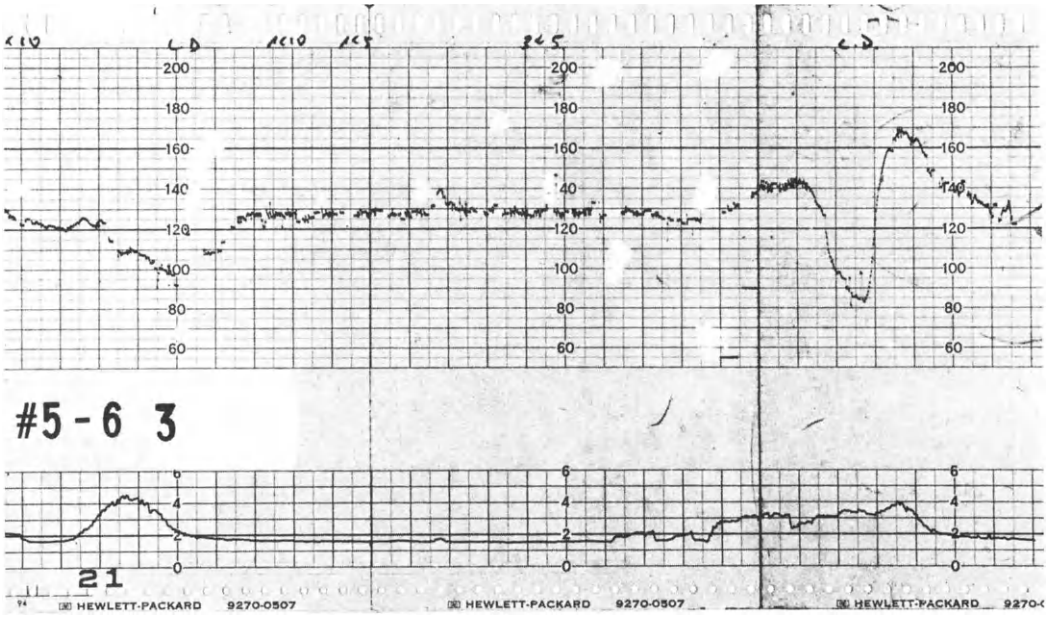


Figure 2 Pathological record with late deceleration and atypical variable deceleration.

variability or body movements. However, the strong association of trunk movements with fetal heart rate accelerations (Timor-Tritsch et al. 1978a; June, 1980) and their clustering during active state with increased FHR variability is firmly established.

Complete sleep-wake cycles in the human fetus range from 60 to 75 min, with quiet phases averaging 20 min but lasting up to 30 min (Sterman, 1967; Timor-Tritsch et al., 1978b; Junge, 1979b). For practical purposes it is important to know that an antepartum FHR record of up to 30 min may occasionally coincide with a physiological fetal sleep phase with low motor activity which will appear on the trace as reduced FHR variation.

Diurnal periodicity, as shown for body and respiratory movements, might also be expected for the human FHR, but so far this has not been studied thoroughly. Similarly, alterations in fetal metabolism, such as those produced by a glucose load or maternal feeding, seem to influence the cardiovascular system (Miller et al., 1979), but this remains incompletely explored (Aladjem et al., 1979).

### Antepartum FHR with Congenital Malformations

In principle, the integrity of the central and peripheral nervous system and cardiovascular system is necessary for a normal FHR. With abnormal antepartum FHR, the incidence of major fetal malformation is increased and was 5% in our own experience (Kubli et al., 1978) and 10% in the population studied by Powell and Towell (1980). Whereas there are no specific FHR abnormalities that typify a fetal malformation (Garite et al., 1979; Powell and Towell, 1980; Hagens et al., 1979), the incidence of abnormal FHRs (antepartum or intrapartum), such as late decelerations, decreased variability, and/or decreased reactivity, was found to be 70% (19 out of 27) in cases with cardiovascular or central nervous system malformations, but only 2 out of 14 with other major malformations (Garite et al., 1979). Out of 30 cases with severe malformations analyzed by Hagens et al. (1979), only 6 had consistently normal FHR records, of whom 4 had a meningocele.

## BASIC DIAGNOSTIC CRITERIA: DEFINITION OF NORMAL AND ABNORMAL FHR AND CLASSIFICATION OF RECORDS

### Basic Diagnostic Criteria

The basic diagnostic criteria underlying antepartum monitoring are as follows:

1. The presence of intact fetal cardiovascular activity as shown by the presence of normal baseline variability and accelerations occurring spontaneously or after stimulation indicate intact fetal reserves and rule out severe fetal compromise with impending fetal death.
2. Reduced fetal cardiovascular activity or reactivity may be, but not necessarily, a sign of fetal hypoxia. Complete loss of fetal reactivity virtually always means very severe fetal compromise.
3. The occurrence of decelerations, except for sporadic, mild variable decelerations, before labor means antepartum hypoxia. However, hypoxia may be mild and transient and may not necessarily lead to fetal compromise. Persistent or repetitive late and marked atypical variable decelerations are always ominous.
4. Evidence of reduced fetal activity or reactivity, on the one hand, or of decelerations as isolated phenomena, on the other hand, indicates potential problems. It

is used to designate an individual record as prepathological or suspicious for which immediate obstetric intervention is rarely necessary. However, further evaluation by additional tests and repeated and frequent monitoring is indicated.

5. A marked loss of baseline variability and/or loss of accelerations combined with decelerations, particularly if they are late, always means fetal compromise requiring further evaluation and often obstetric intervention.
6. The duration of an individual record should be such that the chances of false pathological results due to coincidence with a physiological low-activity state are minimal; external stimulation may be helpful for this purpose. The frequency of recording will depend on the clinician's assessment of the significance of the traces and on the underlying clinical situation, and may be hourly or weekly.

### Criteria Describing Normal Antepartum FHR

The normal antepartum FHR is defined by a baseline level between 120 and 155 bpm, long-term variability with an amplitude of more than 10 bpm and a frequency of 2-6 cpm, the presence of accelerations with a frequency of at least two per 20-min interval and an amplitude of more than 15 bpm, and the absence of decelerations, except for the sporadic mild variable type.

### Criteria Describing Abnormal Antepartum FHR

Fetal heart rate characteristics known to be associated with potential fetal compromise are the occurrence of decelerations (late and atypical variable) with contractions or movements, the absence or diminished frequency of accelerations, a reduction (<10 bpm) or virtually complete loss (<5 bpm) in the amplitude of long-term variability, a reduction in the frequency of long-term variability (<2 cpm), and marked deviations of baseline FHR (over 170 or under 100 bpm).

Fetal heart rate abnormalities of minor or questionable significance consist of minor baseline deviations (155-170 and 120-100 bpm) and an increase in the amplitude (>25 bpm) and frequency of baseline variability.

### Classification of Antepartum FHR Records

For clinical purposes, the large variety of possible antepartum FHR patterns may be categorized into three major groups.

#### Normal Records

The normal antepartum FHR record is characterized by a normal baseline FHR, normal long-term variability, the presence of accelerations (more than two in a 20-min interval, over 15-20 bpm), and the absence of decelerations with contractions.

It is essential to ensure that the record is obtained over a minimum of 20 min.

#### Prepathological or Suspicious Records

The prepathological or suspicious record exhibits signs of either reduced fetal activity/reactivity or fetal hypoxia with intact reactivity, such as the following:

1. Reduction of long-term variability and of accelerations in the major portion of a 30-40 min record, but that is reversible to an active pattern of about 20-min duration by external stimulation (Figure 3); and sporadic, nonrepetitive late decelerations in the presence of intact activity/reactivity

2. Reduction of long-term variability and of accelerations that is not reversible by external stimulation to a persistent active phase about 20 min long, and repetitive late decelerations with intact reactivity
3. Isolated deviations of the baseline FHR and atypical (sinusoidal) variability patterns with no other FHR abnormalities and with no apparent cause

Cases (1) and (2) represent different degrees of abnormalities and thus have different implications in terms of the intervals allowed until repeat testing. Case (3) represents undefined pathology needing further evaluation.

### Pathological Records

A secure diagnosis of fetal compromise necessitating delivery can only be made with evidence of hypoxia on the FHR trace combined with signs of decreased fetal activity/reactivity. If either of these symptoms is very marked (e.g., complete loss of variability without reaction to stimulation or very marked decelerations), the record is also rated as pathological, even if the symptoms occur as isolated phenomena. The following are characteristic of the pathological record:

1. Reduction of long-term variability under 5 and over (10 bpm) and of accelerations, with nonrecurrent late decelerations (decelerative CTG according to Visser et al., 1980)
2. Reduction of long-term variability and of accelerations, with recurrent late decelerations
3. Loss of long-term variability (<5 bpm) and of accelerations, with late decelerations (recurrent or nonrecurrent) (Figure 2)
4. Persistent loss of long-term variability or a smooth sinusoidal pattern without decelerations (Figure 4), and very marked decelerations as isolated phenomena

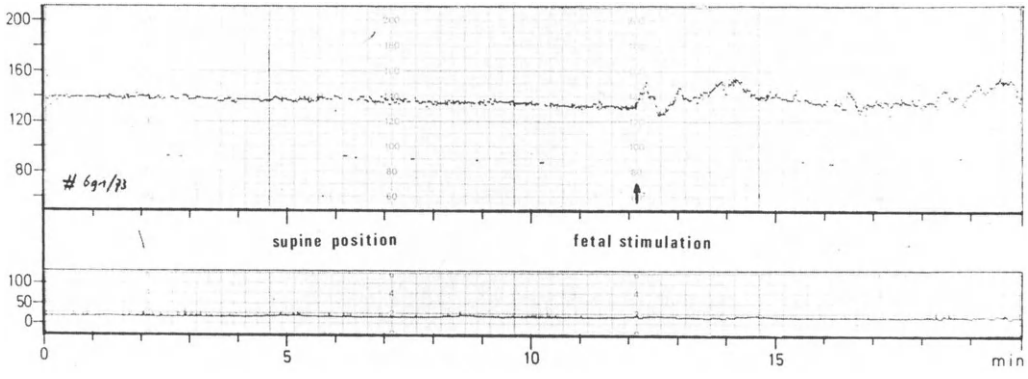


Figure 3 Prepathological antepartum record. There is marked reduction in variability, but good reactivity to stimulation by palpation.

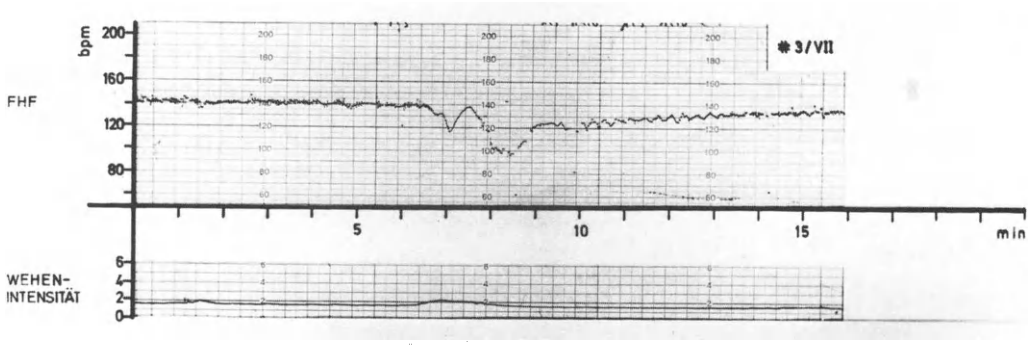


Figure 4 Pathological antepartum record with loss of variability, late deceleration, and sinusoidal pattern following the deceleration.

Figures 1, 3 and 4 represent increasing degrees of pathology; all, however, are signs of serious fetal compromise which will eventually end in fetal death if the fetus is not delivered. Figure 2 represents a rather atypical pathology, where complete loss of reactivity, even in the absence of decelerations, generally indicates severe fetal compromise. The significance of isolated severe decelerations depends on whether a known reversible cause is present or not.

## TECHNICAL PROBLEMS

### Signal Acquisition and Processing

Abdominal wall cardiotocography is possible by phonocardiography, ultrasonocardiography, and electrocardiography.

*Phonocardiography* provides a relatively clean and well-defined signal which makes true beat-to-beat recording possible, however, the method is susceptible to noise and is adversely influenced by a variety of conditions such as maternal obesity and an anterior placenta, so that, for routine monitoring, the method is too time-consuming and has been abandoned in most centers.

*Ultrasonography* is the most widely used method today. Abdominal wall monitoring by this technique has often been criticized because interpretation of FHR variability by this method is less reliable than electrocardiograph signal owing to the variable point on the signal envelope from which the ratemeter may be triggered. Against these theoretical considerations stands our own experience and that of others: Antepartum FHR monitoring by ultrasound does provide valuable clinical information, particularly if one accepts that long-term variability is more important than beat-to-beat variability. Dawes et al. (1981b) have analyzed the technical quality of ultrasound records and found that ultrasound failed in 40% of the recording time to give adequate signals, but that the records as a whole were suitable for further electronic analysis. According to Solum et al. (1981), about 30% of the heart beats are missed by ultrasound, but ultrasound records still allow assessment and evaluation of FHR long-term variability with sufficient reliability, the correlation for this parameter with direct electrocardiograph recordings being acceptable, with a correlation coefficient of 0.7. At present some commercial companies are experimenting with more sophisticated signal processing using autocorrelation techniques, with promising preliminary results. It seems probable that with these

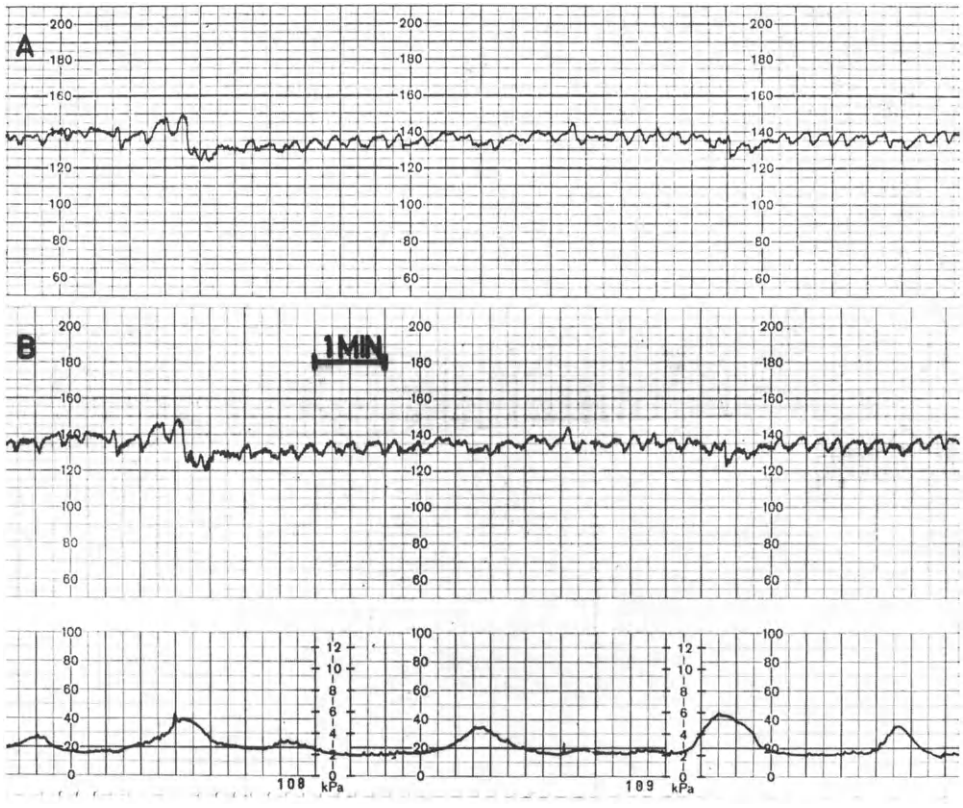


**Figure 5** Comparison between (A) abdominal wall ultrasound with the autocorrelation technique and (B) direct fetal electrocardiography (first stage of labor).

refined signal-processing techniques the quality of ultrasound records will be further improved in the future (Figure 5).

At present prerequisites for the reasonable use of abdominal wall ultrasound techniques are (1) skilled personnel experienced in the handling of the transducer and in the recognition of technically inadequate records; (2) the restriction of signal averaging, as this may create artificial variability; (3) experience in the recognition of technical artifacts; and (4) strict rejection of all technically inadequate records for interpretation.

*Abdominal wall electrocardiography* provides a precise signal (R wave) for true beat-to-beat FHR recording. With modern instrumentation satisfactory records have been considerably enhanced compared to earlier times. Even so, the percentage of positive tracings is small in the critical period between 27 and about 35 weeks (Rüttgers, 1976; Klöck and Haufner, 1977; Carter et al., 1980), so that electrocardiography is unlikely to take the place of ultrasonography for the purposes of routine monitoring.

## Practical Details of Antepartum FHR Testing

In order to avoid the supine hypotensive syndrome, the mother should be in a left lateral or semirecumbent position. The duration of a nonstress test in our experience has been standardized to 30 min, with acceptable results. With low spontaneous fetal

cardiovascular activity, external stimulation is applied after about 15 min. Paul and Miller (1978), Keegan and Paul (1980), and Keegan et al. (1980) have proposed a standardized time schedule for the nonstress test consisting of an initial 20-min monitoring period. If the test is nonreactive, a second trace of 20 min is obtained after external stimulation.

The protocol of the oxytocin stress or challenge test (OCT) has been described in detail by Freeman (1975), Schifrin et al. (1975), and Huddlestone et al. (1979). After a period of baseline recording, oxytocin is infused, starting with low levels (<1 mU/min) and proceeding with increasing dosages until a frequency of three contractions per 10 min is obtained. The time required for a single test is about 50-120 min (Paul and Miller, 1978).

If the nonstress test is nonreactive, external stimulation by palpation and gentle shaking of the fetus is performed. This method is simple but not standardized, and Keegan and Paul, (1980) could not statistically corroborate its effect in the reversal of patterns from nonreactive to reactive. A reasonable alternative might be standardized acoustic stimulation as described by Luz (1979) and others, since this is followed in the normal fetus by a rather well-defined acceleration.

### Long-Term Monitoring

Long-term monitoring over 24 hr was done by Halberstadt (1981) in 29 pregnancies for research purposes using abdominal electrocardiography and telemetry. On a practical level this method might be useful in very critical situations in which an attempt is made to delay artificial delivery for a short time, for example, for induction of fetal pulmonary surfactant. The applicability of this type of monitoring, however, is limited by the low success rate of abdominal electrocardiography from 27 to 35 weeks, the very period of gestation of interest.

## QUANTITATION AND SEMIQUANTITATION: SCORING SYSTEMS

Various attempts at computer-assisted analysis and quantitation of intrapartum FHR have been made, but difficulties still exist, especially in the reliable quantitation of FHR variability (Escarcena et al., 1979). Moreover, until recently, none of the systems have proved to be applicable to antepartum monitoring because of the problems associated with noise and the low quality of the abdominal wall signal. Recently Dawes et al. (1981b) have elaborated a system of signal processing and numerical analysis with antepartum records which they felt might have the potential for future clinical applicability.

At present the interpretation and qualitative classification (normal, prepathological, pathological) of records is done visually and analysis of the different FHR parameters (baseline, variability, deviations) is done by considering their qualitative and quantitative appearance. The number of possible combinations is virtually infinite. Visual examination by an experienced observer is still the best and most sensitive method of interpreting individual FHR records; however, because it is so subjective, its reproducibility and–for research purposes–credibility are limited.

In the absence of applicable methods of true quantitation, semiquantitation by scoring systems improves reproducibility. Scoring systems will not improve the results of the experienced observer, but they are almost indispensable for training purposes, communication, and research, as well as for the establishment of policies within departments.

Table 5 Scoring System According to Meyer-Menk[a]

| Parameter | Score 0 | 1 | 2 |
|---|---|---|---|
| Baseline level (bpm) | <100, >180 | ⩾100, <120; >160; ⩽180 | ⩾120, ⩽160 |
| Amplitude of fluctuation (4 bpm) | ⩽5, sinusoidal | >5, ⩽10 (⩾25) | >10, <25 |
| Frequency of fluctuation (cpm) | <2, sinusoidal | ⩾2, ⩽4 | >4 |
| Deceleration pattern with uterine contractions | Late deceleration pattern frequency ⩾25%, marked variable pattern, severe supine syndrome | Late deceleration pattern frequency <25%, moderate or milk variable deceleration pattern, early deceleration pattern | Lack of deceleration, single mild variable, deceleration, dip 0 |
| Acceleration with arousal test or fetal movements | Absolute lack of acceleration (negative response) | Atypical shape, no spontaneous acceleration | Acceleration with fetal movements (positive response) |

[a]A total of 10 points is optimal; 0 point is the worst result: 8-10 points, normal record; 5-7 points, prepathological or suspicious record; and 0-4 points, pathological record.
*Source*: Meyer-Menk et al. (1976).

Since the description of the first scoring system by Kubli (1971) several further systems have been presented by Hammacher et al. (1974), Fischer et al. (1976), and Pearson and Weaver (1978), among others. The score devised by Kubli (1971) did not include accelerations and as such has a lower diagnostic accuracy compared with the newer scoring systems (Garoff et al., 1978; Wilken et al., 1980). These systems all utilize more or less the same parameters in somewhat different ways. The diagnostic potentials of the different systems are not significantly different (Garoff et al., 1978). It would seem, however, that the 10-point scoring systems devised by Fischer et al. (1976) and by Meyer-Menk et al. (1976) (Table 5), which are similar, combine easy applicability with reasonable diagnostic accuracy and can thus be recommended for clinical use. Keirse and Trimbos (1980) emphasized the fact that with this score, no false identification of poor fetal condition occurred and the risk of unnecessary intervention was minimized.

Adis et al. (1978) have analyzed the results of antepartum FHR nonstress testing in 143 cases of nonrisk pregnancies, 148 cases of risk pregnancies with surviving babies, and 16 cases of antepartum fetal death, the latter from 1968 to 1975. The results are shown in Figures 6, 7, and 8.

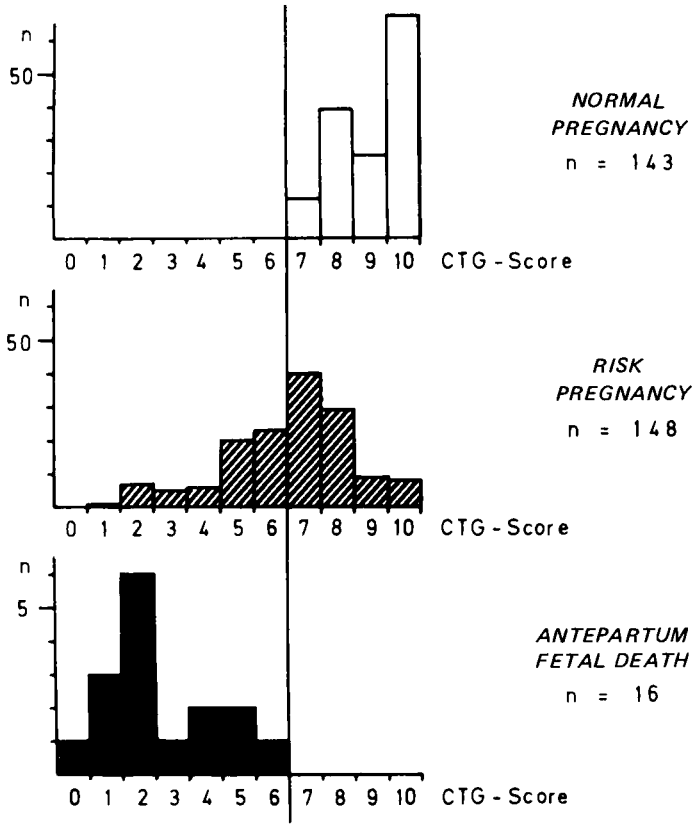


**Figure 6** Frequency distribution of the scores of the last record before delivery in normal pregnancy, risk pregnancy, and antepartum fetal death. Scores of 8-10 were normal; 5-7, prepathological or suspicious; and 0-4 pathological. (From Adis et al., 1978.)

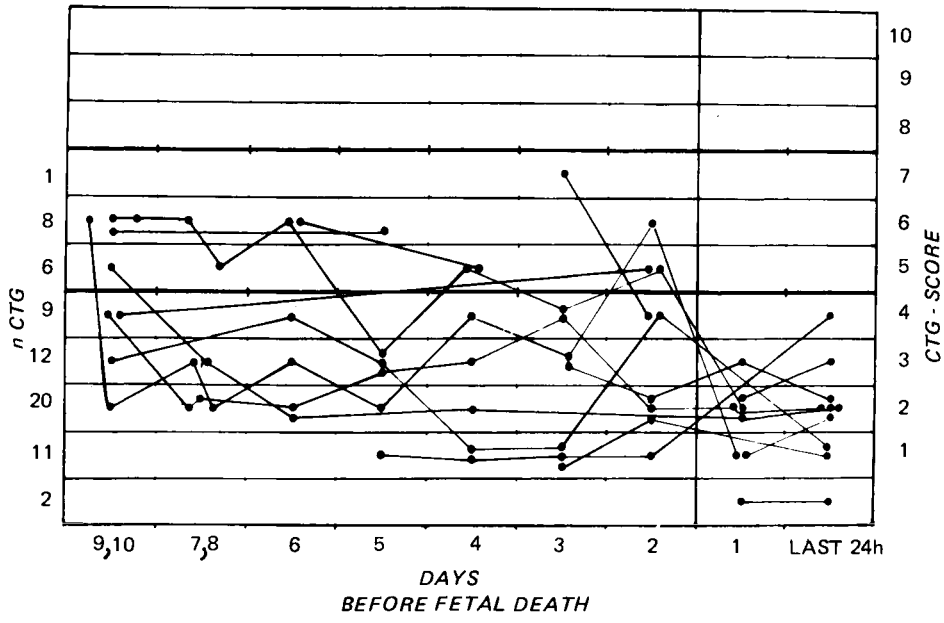


**Figure 7** Results of CTG scores during the last 10 days before fetal death in 16 cases of antepartum fetal death. Scores of 8-10 were normal; 5-7, prepathological or suspicious; and 0-4, pathological. (From Adis et al., 1978.)

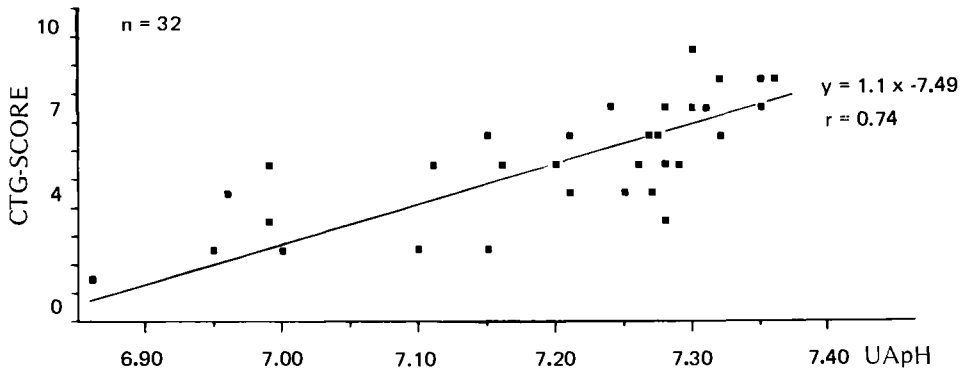


Figure 8 Correlation between the CTG score of the last antepartum record and the umbilical artery pH in 32 risk pregnancies with prelabor cesarean section. (From Adis et al., 1978.)

## CLINICAL EXPERIENCE

After 10 years of experience with clinical antepartum FHR monitoring, the situation is characterized by a widespread acceptance of antepartum FHR monitoring, which has resulted in increasing incorporation of this tool into clinical obstetric practice; a growing trend to abandon the OCT as a primary approach of antepartum monitoring in favor of nonstress monitoring; considerable variation between different groups in the protocols used for the practice, interpretation, and management of the nonstress test; and increasing obstetric intervention with pathological antepartum records, making the end point of antepartum fetal death a rarity. Although this, in itself, proves to some extent the value of antepartum monitoring, it makes evaluation of the practical importance of the procedure as a whole and of different protocols increasingly difficult. Until now there has been a virtually complete lack of controlled prospective studies.

### Oxytocin or Contraction Stress Tests

Antepartum FHR testing with artificially induced contractions has been described in the late 1960s by Pose et al. (1969) and Kubli et al. (1968), among others. It has become the preferred approach to antepartum testing in the United States, whereas it was rapidly abandoned in Europe in favor of nonstress testing. In the meantime a large literature has accumulated on the OCT (Freeman, 1975; Schifrin et al., 1975; Huddlestone et al. 1979) and contraction stress testing has become a well-standardized procedure with strict protocols for distinguishing negative from positive tests. A test is rated positive if late decelerations are recurrent. Hyperstimulation and equivocal and unsatisfactory tests all occur with a frequency of 5-10%.

Failure of the test to predict antepartum fetal death when done at weekly intervals has been reported to be in the range of 2.2 per 1000 (Huddlestone et al., 1979), 5 per 1000, and even 10 per 1000 (Paul and Miller, 1978). Paul and Miller stated that the incidence of false negative results using these criteria is less than 1%. These figures, however, seem high. The recommended frequency of performing the test at weekly intervals has also been challenged by some (Salerno and Kay, 1978). Positive tests, in general, are followed by obstetric intervention, yet 50% or more of vaginal deliveries

occur after positive tests without apparent fetal distress, thus indicating the test results as false positive (Paul and Miller, 1978). The rate of false positives has been markedly reduced by additional consideration of fetal movements (Keegan and Paul, 1980). The major inconvenience of the OCT is that it is time-consuming. In addition, the reliability and predictive abilities of the test do not seem to be particularly good.

At present, it is the view of the author that the OCT as a primary approach to antepartum monitoring will disappear completely in a short time; however, it will keep its place in a small percentage (less than 5%) of cases as a second-line diagnostic tool with suspicious nonstress tests (Kubli et al., 1978; Keegan and Paul, 1980; Keegan et al., 1980).

## Nonstress Testing

### Protocols and Interpretation

A nonstress test generally consists of one 30-min period or two 20-min periods (see above). The test may be interpreted in one of three ways:

1. Visual evaluation of the record, considering all available FHR parameters and classifying the recordings into normal, suspicious, and pathological. Further subgroupings may be used as recommended (Kubli et al., 1978; Visser et al., 1980; Breart et al., 1981).
2. Visual evaluation of the record, considering all available parameters, and semiquantitation by the use of one of the scoring systems. The results are again grouped as normal, suspicious, and pathological, with various degrees of severity (Keirse and Trimbos, 1980; Lyons et al., 1979)
3. Restriction of the analysis to the assessment of the presence or absence of accelerations (Paul and Miller, 1978; Keegan and Paul, 1980; Keegan et al., 1980).

The third approach, that of Paul and Miller (1978), is especially interesting, since it constitutes an extremely simplified way of interpreting antepartum records, relying only on the presence or absence of accelerations and dividing results into either reactive or nonreactive groups. In about 20-30% of cases the test is nonreactive, requiring further evaluation. A contraction stress test is only employed when the pattern remains nonreactive. If the nonstress test is nonreactive and the OCT is negative, a further test is generally done after 1 week. With this protocol applied to high-risk pregnancies, unexpected and unpredicted fetal antepartum deaths occurred at a frequency of 5 per 1000 within 1 week of a reactive nonstress test, and even 17 per 1000 within 1 week of a nonreactive nonstress test combined with a normal (negative) contraction stress test (Druzin et al., 1980).

The protocols with "full visual analysis," with or without semiquantitation of the records, are in our view clearly less rigid and more individualized. Several possible results, depending on the different classes and subclasses of records, may be obtained. Furthermore, the concept of nonstress testing as understood by the author implies frequent testing. Since in the nonstress state the onset of compromise manifests itself by the increasing duration of low fetal activity periods (Halberstadt, 1981), the chance to assess one such period is enhanced with increasing monitoring frequency. It is thus possible to establish guidelines for the intervals of repeat testing and the performance of OCT, but within this framework variation may be considerable (see below). Failure to

prevent antepartum fetal death with this protocol is rare. The overall incidence of antepartum death in our experience is 4 per 1000, and unpredicted antepartum fetal death within 1 week of a negative test occurred with a frequency of 1 per 1000 in an unselected population consisting of high- and low-risk patients (Kubli et al., 1978). Figures of the same order were found in a similar population by Schifrin et al. (1979).

A direct comparison between the approach of considering accelerations only and that of full visual analysis is not possible. Differences in failure rate reflect not only the differences in the protocol and diagnostic approach, but also differences in patient material and other characteristics of the clinical setup. Properly conducted and controlled prospective studies are lacking. Nevertheless, the nonstressed antepartum record contains more information than simply the presence or absence of accelerations. If the interpretability of long-term variability is indeed controversial—especially when averaging monitoring techniques are used—this is certainly not true for the absence or presence of decelerations with spontaneously occurring contractions.

In our study of normal late pregnancy (from 27 weeks gestation) spontaneous contractions were present in 60% of all patients and 40% of all CTGs, the frequency rising from about 20% before 30 weeks to almost 100% near term (Rüttgers et al., 1972). With frequently repeated monitoring, the chance to record one or several spontaneous contractions in an individual patient will increase, especially in pathological pregnancies. It is our feeling that spontaneous contractions are more adequate to detect true fetal compromise than artificially induced contractions with the ever-present risk of hyperstimulation. More recently Keegan and Paul (1980) proposed spontaneously occurring contractions as an alternative to the OCT.

## Diagnostic Accuracy and Reliability

*Antepartum Fetal Death* Fetal hypoxia which threatens the life of the fetus almost invariably manifests itself in pathological alterations of FHR. Out of 86 cases of fetal death (ante- and intrapartum) published in the literature and recently compiled by Solum (1980), FHR was normal in only 2 cases. The striking differences in antepartum FHR between normal pregnancies and antepartum fetal deaths are demonstrated in Figures 6 and 7. Nevertheless, antepartum fetal death can occur shortly after a normal FHR has been recorded, the major cause probably being acute hypoxia occurring before the next scheduled test. If these cases are classified as failures or "false negatives," the reported rate varies from 1 to 10 per 1000. It is our personal opinion that with proper monitoring practice, this figure should not exceed 2-3 per 1000. This view is supported by recent data of a collaborative study involving 7500 patients where the antepartum rate (corrected for malformations) was 3.2 per 1000 (Freeman, 1981).

*Adverse Intrapartum or Neonatal Events* The rate of "false negative" results of antenatal FHR recording in terms of adverse intrapartum or neonatal events is more difficult to assess, since they are difficult to compare between different authors because of different end points. Keirse and Trimbos (1980) found 5%, Weingold et al. (1980) 2% (corrected to 0.7%), and Rayburn et al. (1978) 3%. In our own material this figure was 10% (Garoff et al., 1978 and Rochard et al. (1976) found 20% related to the total number of negative (normal) tests. There were no "false positive" results in Keirse and Trimbos' material (1980), but 25 and 30% at two different occasions in our own material.

Thus one can say that the reliability of a normal antepartum nonstress test to predict a normal outcome without adverse intrapartum or neonatal event is about 90%.

Similarly, clear-cut pathological records have a high specificity. The problem, as always, is with mildly pathological and suspicious records (Keirse and Trimbos, 1980). Nevertheless, as shown in Figure 8, there is a significant correlation of antepartum FHR to such sensitive and short-lived parameters as cord blood pH, provided that cases of prelabor cesarean section are selected (Adis et al., 1978).

### Clinical Management

*Normal Antepartum Record* Despite the small risk from acute hypoxia in the antepartum period, it is known from earlier studies (Kubli et al., 1972; Schmid and Baertschi, 1976; Adis et al., 1978) that the natural course of the more common chronic fetal hypoxia due to placental dysfunction is slow, and that 2 weeks or more may pass from the first cardiotocographic signs of hypoxia until fetal death. Therefore with a normal antepartum record the standard interval for repeat monitoring is 1 week and it may be longer in low-risk pregnancies. In very high risk in-patients, however, it is our policy to repeat the tests at shorter intervals, depending on how serious the risk is. The rationale for this is the fact that occasionally acute and subacute hypoxia such as is associated with silent abruptio placentae may occur, and that an antepartum fetal death in a hospitalized patient with a viable fetus does not seem to be acceptable any more. It will be important, however, to determine the cost-benefit aspects of increasingly frequent nonstress testing.

*Pathological Records* With pathological records, in general, obstetric action is needed, unless a reversible cause for the fetal hypoxia may be found and normality is restored with subsequent tests. Logically, the likelihood of substantial fetal compromise is high with persistent FHR pathology, and clearly lower with sporadic pathology (Kubli et al., 1978; Visser et al., 1980).

Unfortunately it is rarely possible to predict from a single record the precise interval which is left until fetal death (Kubli et al., 1978). Even with a severe pathology, this interval may range from 1 to 7 days (Visser et al., 1980). In general there is time for the clinician to repeat the test and, if deemed necessary, for additional diagnostic procedures and/or induction of fetal lung maturity to be undertaken. In an ominous situation, frequent monitoring up to several times daily is mandatory, or even continuous monitoring over 24 hr by abdominal electrocardiography if technically feasible.

In view of the relatively high incidence of permanent brain damage with extreme pathology (3 out of 30, according to Visser et al., 1980) management should be aimed at removing the fetus from the adverse environment in utero before the occurrence of this type of pathology, or at least at limiting its duration as strictly as possible.

The question as to whether vaginal delivery may be attempted with a pathological antepartum record depends on the degree of pathology and additional clinical features such as the presence or absence of growth retardation, prematurity, and mechanical factors. Mild or inconsistent FHR pathology in a term fetus is not an indication for cesarean section, but one for attempted vaginal delivery with full monitoring precautions. Severe and consistent pathology, particularly in the presence of growth retardation and/or prematurity, is an indication for prelabor cesarean section.

## CONCLUSIONS AND RECOMMENDATIONS

Antepartum FHR monitoring today is a widely accepted tool in the management of risk pregnancies. It's value as a means of assessing severe antepartum fetal hypoxia is considerable. The failure rate, in terms of antepartum fetal death after normal tests, is

reported to be 1-10 per 1000, depending on the patient material and possibly the monitoring techniques. In general, it should not exceed 2-3 per 1000.

As a primary approach nonstress testing is superior to OCT. The preferable method of interpretation of the nonstress test is "full visual analysis," with or without semi-quantitation by scoring systems. Records are divided into normal, prepathological, and pathological, considering the symptoms of fetal activity/reactivity on the one hand and the presence or absence of decelerations on the other. Further evaluation of prepathological nonstress tests by OCT is necessary in a small percentage of cases only. This approach seems preferable to the alternative of restricting analysis to the assessment of accelerations as signs of fetal reactivity.

Obstetric management of normal and abnormal records is dependent on the degree of eventual pathology and the underlying clinical situation.

Markedly improved signal processing with commercial monitors is to be expected in the future, which will facilitate full visual and possibly even electronic analysis of antepartum FHR records.

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# 21

# Technical Aspects of Fetal and Intrauterine Pressure Monitoring

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## INTRODUCTION

Although Cremer showed as early as 1906 that the fetal electrocardiogram (FECG) could be recorded from the mother's abdomen (Cremer, 1906), the lack of practical technology to exploit this discovery meant that for many years the Pinard stethoscope remained the only clinical method for assessing fetal heart activity. Intermittent recordings and the difficulty of obtaining a reliable count during contractions have led to some serious misunderstandings of fetal physiology. For example, in 1963 Sir Andrew Claye (sometime President of the Royal College of Obstetricians and Gynaecologists) wrote,

> The foetal heart rate, rhythm and intensity should be noted every two hours during the first stage (of labour) provided that the membranes are intact. After the membranes have ruptured, it should be done every half an hour. *Auscultation should be done as long after a contraction as possible, to allow a heart which has slowed to return to its normal rate.* (Claye, 1963.)

The significance of fetal heart rate (FHR) slowing in association with contractions only became apparent with the introduction of continuous FHR monitoring. This involves the detection of fetal heart activity, either electrical (Hon, 1958; Caldeyro-Barcia et al., 1966) or mechanical (Hammacher, 1967). However, the electrical methods involved direct attachment of the electrodes to the fetus, either via the mother's abdomen (Caldeyro-Barcia et al., 1966) or through the cervix, requiring artificial rupture of the membranes (ARM) (Hon, 1958). Consequently, the noninvasive method of microphonic monitoring of fetal heart sounds (derived from fetal heart valve movements) was the first to become established clinically in the form of a "cardiotocograph" (Hammacher, 1967).

## FETAL PHONOCARDIOGRAPHY

The determination of FHR from fetal heart sounds presents a number of formidable technical problems Curran (1975). Each cardiac cycle in the fetus produces two main heart sounds, associated with the closure of the atrioventricular valves (S1) and aortic (pulmonary) valves (S2). Complicated electronics are necessary to compute the differing intervals $S1_1$-S2 and S2-$S1_2$ and thereby derive the interval $S1_1$-$S1_2$, representing a complete

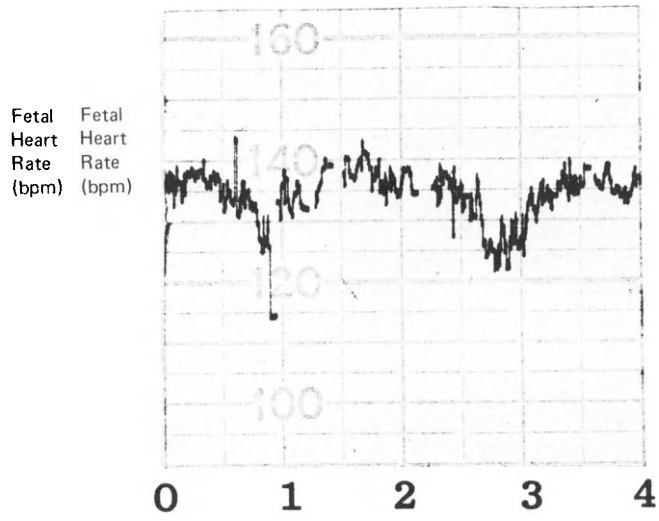


Figure 1 "Continuous" fetal heart rate recording derived from fetal heart sounds.

cardiac cycle. The reciprocal of this interval, $1000 \times 60/(S1_1 - S1_2)$ (msec) is known as the "instantaneous heart rate" in beats per minute (bpm). At a normal fetal heart frequency of 140 bpm, there will therefore be 140 separate estimates of rate per minute. At the usual chart paper recording speeds of 1-3 cm/min, the instantaneous FHR appears to be almost a continuous line, as the "dots" representing individual estimates of rate tend to join together (Figure 1). Because of this, such recordings are often referred to as the "continuous fetal heart rate," although, strictly speaking, this is a misnomer.

Phonocardiography is a truly noninvasive method of monitoring the fetal heart rate, unlike ultrasonic FHR monitoring (see later). It shares this advantage with the abdominal FECG and magnetometer monitoring (Kariniemi and Hukkinen, 1977). Unfortunately, phonocardiography is very susceptible to interference from extraneous noise generated by maternal or fetal movement or even rustling bedclothes, and this severely limits its usefulness during labor. It also often gives poor recordings if the mother is obese or if there is hydramnios or twins (when it may be difficult to position the microphone close enough to the fetal heart).

## FETAL ELECTROCARDIOGRAPHY (DIRECT)

A reliable signal of fetal heart activity during labor can be obtained by placing an electrode directly on the fetus via the cervix. This necessitates ARM, a procedure which has been questioned by some on the grounds that the loss of the cushioning effect of the forewaters increases intracerebral pressure, thereby causing reduced cerebral blood flow, cerebral hypoxia, and permanent central nervous system damage (Schwarcz et al., 1973). Recent work has, however, suggested that these early fears are unfounded (Cibils, 1980). Many clinicians feel that the increased reliability of the FHR recording obtainable with direct electrode application and the possibility of detecting meconium

staining of the liquor and/or oligohydramnios justify routine amniotomy as an aid to fetal monitoring (Steer et al., 1976). Also ARM has the benefit of accelerating induced labor (Steer et al., 1975) and often reducing or even avoiding the need for oxytocin infusion (Steer, 1977b, 1979; Carter and Steer, 1980).

## PRINCIPLE OF DIRECT ELECTRODE FUNCTION

A sharpened piece of wire (usually stainless steel) is inserted under the skin of the presenting part of the fetus. Its electrical potential is then compared with that of a similar electrode in contact with the maternal vagina. Fetal skin is a relatively poor electrical conductor and the most efficient electrical pathway between the two electrodes is through the fetal head and thorax via the blood-filled umbilical cord to the placenta and then back through the maternal tissues to the vagina. This pathway contains the fetal heart and an FECG of between 25 and 250 $\mu$V can nearly always be detected. The maternal ECG signal, although relatively larger, is nearly identical at the two electrodes and can therefore be largely eliminated by "common-mode rejection."

Good contact of the vaginal electrodes with the maternal tissues is essential for a satisfactory recording, and if the presenting part is low down as, for example, in the second stage of labor, the maternal electrode may emerge from the vagina, with consequent loss of signal. It is also important that as much of the fetal electrode as possible is inserted under the fetal skin to minimize direct "leakage" currents between the two electrodes, which otherwise diminish the voltage of the FECG signal obtained (Steer, 1977b). Frequently, failure to obtain an adequate tracing in clinical practice is due to inadequate insertion of the electrode. Use of the digital storage oscilloscope now provided as part of modern cardiotocographs greatly facilitates diagnosis of this particular problem, particularly when it is associated with the so-called "bedding-in process" of the electrodes. Metal electrodes placed in contact with tissues become conductive by generating a "cloud" of charged ions at their surface, through which electron transfer between the metal and the tissues can take place (Geddes, 1972). Some metals (e.g., copper and zinc) ionize so readily that, while they make excellent contact, the metal ions disperse into the tissues, with toxic effects. They are therefore unsuitable for long-term use as electrodes. Metals such as stainless steel ionize less readily and do not seriously contaminate the tissues, but as a result their surface layer takes longer to build up. While the surface layer remains poor, a great deal of noise is generated by even small amounts of electrode movement and this obscures the recognition of the FECG by the cardiotocograph, although the presence of the FECG can usually be confirmed by inspection of the oscilloscope tracing. If an electrode is applied and an FECG can be recognized on the oscilloscope despite failure to obtain a cardiotocographic FHR recording, it is wise to wait at least 10 min for the noise level to reduce as the surface ion layer improves, following which the cardiotocograph will usually begin to produce a satisfactory FHR recording.

## ACCURACY OF FHR DETERMINATION

Derivation of the fetal heart rate from the ECG signal is much more accurate than from the fetal heart sounds, since the R wave of the ECG is much larger than any other and its duration is relatively brief (10-15 msec). This makes it easier to time

than the heart sounds, which produce a more diffuse signal. The accuracy of rate determination from the ECG signal is limited by five factors: (1) the size of the signal, (2) the level of background noise, (3) the shape of the signal, (4) the nature of the signal detection circuits, and (5) the accuracy of the timing circuits.

The first and second factors clearly interact and their relationship is known as the signal-to-noise ratio. With the commercial cardiotocographs currently available the signal must stand out clearly from the background noise if it is to be detected and the FECG must be a minimum of 10-15 $\mu$V above the level of background noise.

However, recent developments using miniatureized charge-coupled devices in series (like a string of small capacitors) have greatly improved the ease with which on-line correlation analysis of wave form can be carried out. A series of FECGs are detected using simple threshold logic and then summated in a microprocessor to produce an improved FECG wave template (Greene et al., 1980). The ECG template is then fed onto one side of a 128 N series of microcapacitors (the difference between each capacitor representing about 1 msec). The incoming real-time voltage signal from the scalp electrode is passed down the other side of the series of capacitors, and every millisecond a correlation function is derived by summating the voltage difference across the capacitors. The correlation reaches a maximum (and the voltage difference a minimum) when an incoming FECG signal is directly opposite the FECG template. This improves firstly the number of FECG complexes which can be distinguished from background noise and, secondly, the accuracy of R-wave peak detection (since this is predicted from a 128-point analysis rather than a single-point analysis, which is likely to be perturbed by noise (Wheeler et al., 1979). The use of correlation techniques can result in a 60% improvement in usable data when there is a poor signal-to-noise ratio (Nagel and Schaldach, 1980).

## SIGNAL DETECTION

Factors (3) and (4) also interact. Two main systems of signal detection are commonly used, threshold detection and "zero crossing" detection (equivalent to peak detection of the undifferentiated signal) (Figure 2). Both systems use the differentiated 50-Hz filtered signal, which helps to accentuate the characteristic high frequency of the ECG compared with noise. If a constant threshold trigger is used, changes in R-wave amplitude will cause changes in the timing of the trigger point in relation to the peak of the signal, since this point moves proportionately up and down the leading edge of the R wave. This clearly introduces a small variable error into rate determination. It can be overcome to some extent by using an automatic gain control to standardize the height of a signal, but this introduces further problems if the ECG has multiple peaks (Figure 3), since first one peak and then the other may trigger the ratemeter. As a result sudden repetitive jumps in rate occur, a shortened interval being balanced by a longer one and vice versa.

The characteristic appearance of repeated artifacts of similar rate difference has been called "tramlining". It is important to recognize this artifact, for it may obscure or even be mistaken for true beat-to-beat variation (Figure 4). Tramlining can be overcome by detecting each zero crossing of the differentiated signal (equivalent to the various peaks of the ECG) and triggering from the zero crossing associated with the largest undifferentiated signal amplitude. If this should happen to be the second peak, triggering is locked onto this peak unless another peak subsequently exceeds it in amplitude by 30% or more, whereupon the preferred trigger is altered.

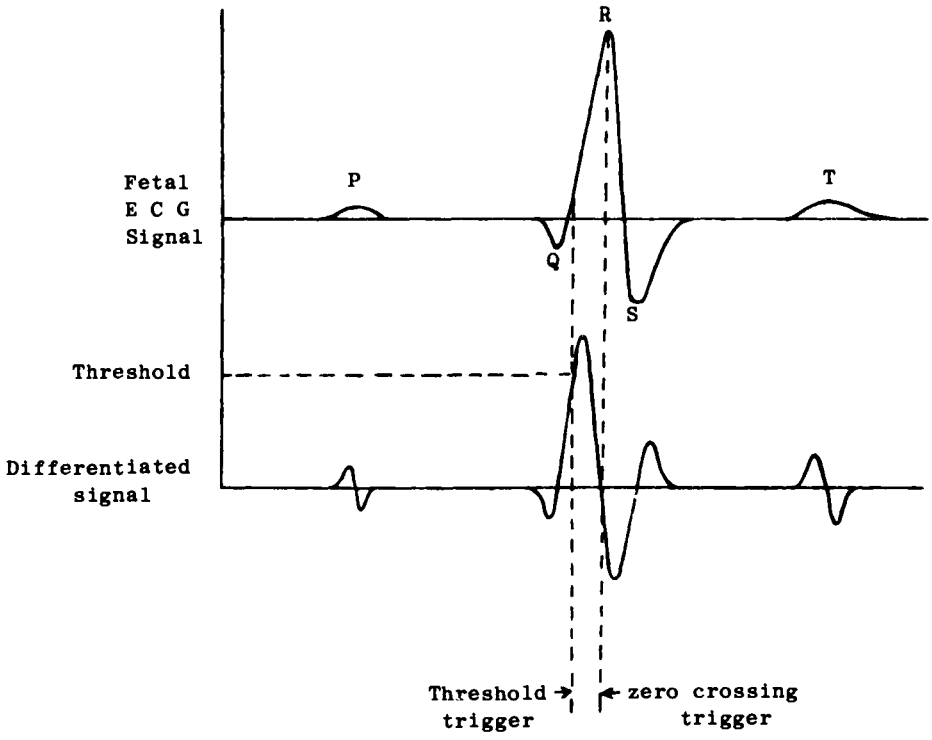


Figure 2 Schematic fetal ECG signal with differentiated signal below. The difference between the trigger pulse produced from threshold detection and that from the zero crossing of the differentiated signal is clearly seen.

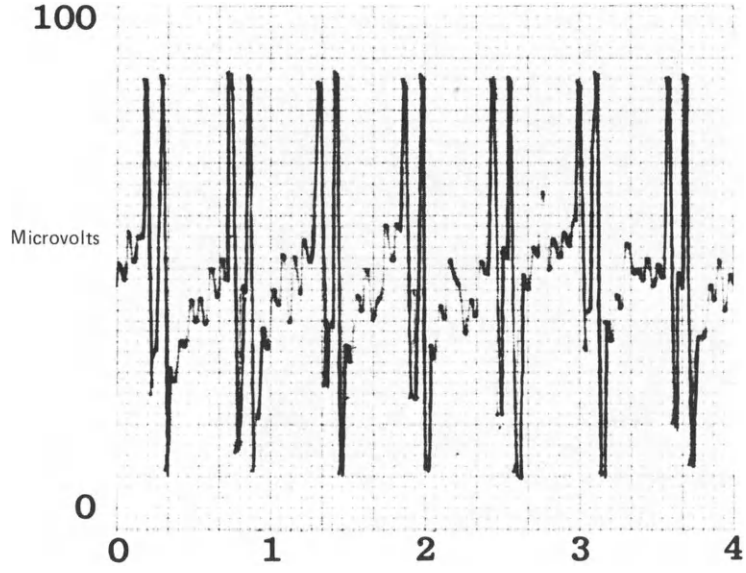


Figure 3 Double-peaked fetal ECG.

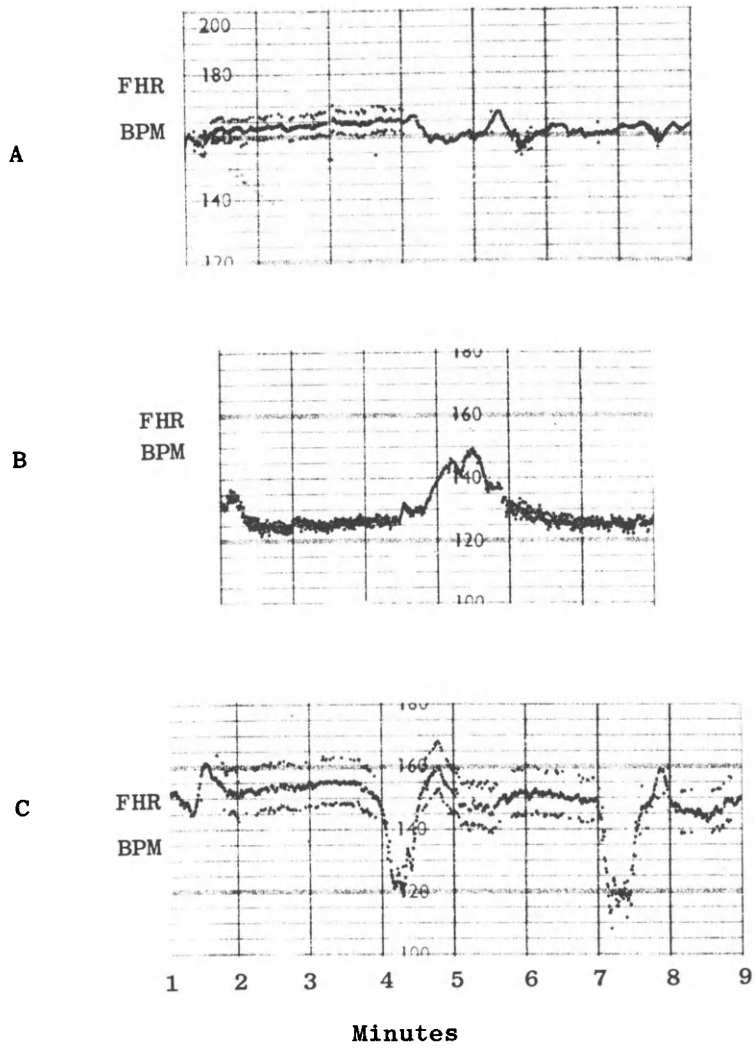


Figure 4 Three traces showing varying degrees of "tramlining". (A) moderate tramlining followed by a normal trace, (B) mild tramlining simulating increased beat-to-beat variation; and (C) marked tramlining.

## "BEAT-TO-BEAT" AND "BASELINE" VARIABILITY

Despite the electronic technology just described, the perturbing effect of noise (e.g., note the baseline noise of Figure 2) means that the timing accuracy of the FECG cannot normally exceed ±1 msec (Wheeler et al., 1979). Since the majority of normal beat-to-beat ("short-term") fetal heart rate variation is less than 4 msec (equivalent to 1.3 bpm at 140 bpm) (Wheeler et al., 1979), it can only be measured accurately using special electronic and statistical techniques. [For further references on the measurement of beat-to-beat fetal heart rate change, see the following: Dalton and Holt (1976), Dalton et al. (1977), De Haan et al. (1971), Detwiler et al. (1980), Henry et al. (1979b), Laros et al. (1977), Lauersen et al. (1976), Modanlou et al. (1977), Yeh et al. (1973), and

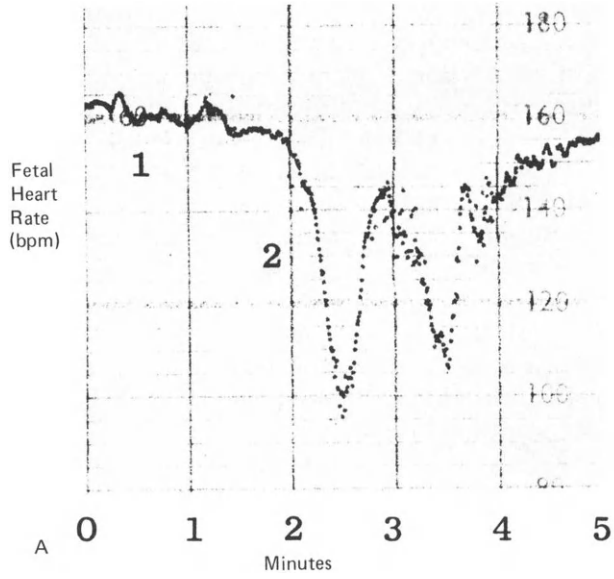


**Figure 5** (A) Normal baseline variability (1) (individual beat-to-beat changes not visible) and rapid change of heart rate (2) during deceleration rendering beat-to-beat changes visible.

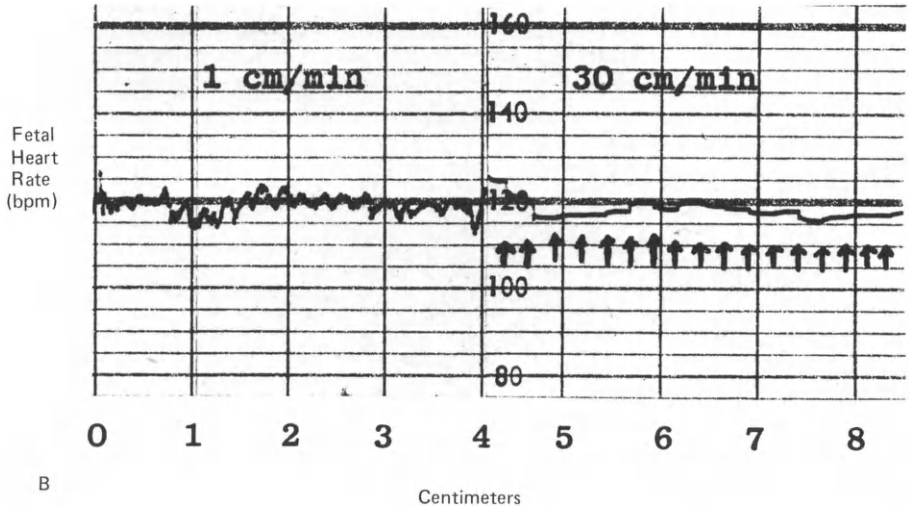


**Figure 5** (B) Fetal heart rate beat-to-beat changes visualized by use of a fast chart speed.

Young et al. (1976)]. It cannot be seen on the cardiotocogram with a paper speed of 1 or 3 cm/min unless the change of rate is very rapid, or the chart speed is increased considerably (Figure 5) (Steer, 1977b). The parameter often incorrectly referred to as "beat-to-beat" variation during clinical interpretation of a cardiotocogram (see Chapter 20) is in fact an oscillation in heart rate of 5-15 bpm, with a cycle length of 10-20 sec (Paul et al., 1975). This should more correctly be referred to as "baseline variability" (Steer, 1976, 1977a). There is no general agreement as to the best way of expressing beat-to-beat variation, although a considerable number of mathematical techniques have been used, such as the standard deviation (or some similarly derived statistic) of the R-R intervals. One such measure, the "varindex," is available on a commercial fetal monitor (Hojaiban, 1976). The simplest is the standard deviation of the R-R intervals over varying epochs; the most complex is a Fourier frequency-power spectrum analysis. However, the clinical value of these measurements in terms of their superiority over visually assessing the trace has yet to be demonstrated.

## ELECTRODE TYPES

The earliest direct fetal electrode which could be applied via the cervix was the clip electrode introduced by Hon in 1963 (Figure 6), but it suffered from problems of reliable attachment to the fetus. These difficulties were overcome by the introduction of the "spiral" electrode (Figure 6), which, with the aid of a special introducer, can be applied by feel alone. This electrode is much less prone to becoming dislodged. It is well suited to the taut skin of the fetal scalp, but tends to tear the skin of the buttock in a breech presentation, and for this application the clip electrode is to be preferred because the loose skin of the buttock actually makes attachment of this type of electrode easier. A third alternative for both sites is the Showell Surgicraft Copeland electrode (Figure 6), in which a single stainless steel electrode is applied by twisting the

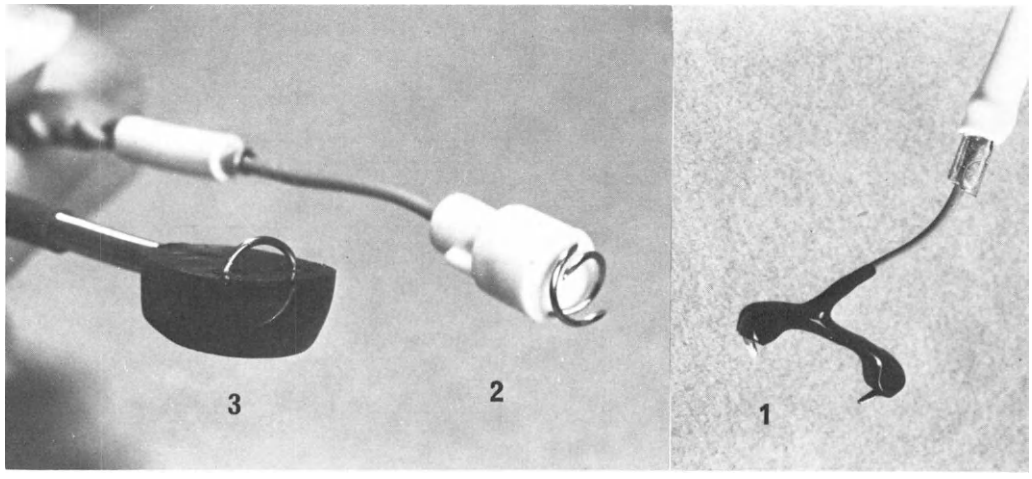


Figure 6 Three types of fetal electrode: (1) the Hon clip electrode, (2) the Hon spiral electrode, and (3) the Showell Surgicraft Copeland electrode.

stem of the device. The "head" of the electrode has to be pressed firmly, flat against the presenting part. This can be impossible if the cervix is long and tubular, only admitting a finger, whereas a spiral can be applied easily in this situation. On the other hand, the Copeland electrode is easier to remove by twisting (in an anticlockwise direction) the stem protruding out of the vagina. Calvert and Newcombe (1980) have also shown that the reusable Copeland electrode is the cheapest to use, provided that it lasts for at least four applications.

## DOPPLER ULTRASONIC CARDIOGRAPHY

This technique relies upon the fact that piezoelectric crystals made of quartz or titanate ceramics vibrate in response to an applied electric current. This produces sound waves at the same frequency as the electrical oscillations; however, the amplitude of the radiated sound waves becomes maximal (for any given applied voltage) at the resonant frequency of the crystal. To locate the fetal heart with sound it is necessary to use very high frequency sound waves and the crystals are constructed to resonate at approximately 2 million to 2.5 million times per second (2-2.5 MHz)—hence the term *ultra-* (high frequency) *sound.* Despite careful manufacture, slight differences in the resonant frequency of the crystals occur, and the frequency of the energizing electrical circuits has to be carefully tuned to produce the optimum output (normally 7 $mW/cm^2$; rated maximum, 12 $mW/cm^2$). This is why ultrasonic probes should be kept with a specific machine; transferring transducers from one fetal monitor to another without retuning inevitably results in some loss of efficiency.

Because of differences in the acoustic impedance of different tissues, impinging ultrasound is partially reflected at tissue interfaces. In pulsed ultrasound short bursts of sound are transmitted. The sound then returns to a receiving crystal in which a small electric current is generated, detected as an "echo" of the transmitted pulse. The transit time from transmitted sound to echo is a measure of the distance of the reflecting interface. This is the method used to measure biparietal diameter.

To detect a movement of tissue interface, a continuous beam of low-intensity ultrasound is transmitted. If reflection occurs at a stationary interface, the reflected and transmitted beam have the same frequency; however, if the interface moves, it changes the frequency of the reflected wave. This is known as the Doppler effect. An electronic analysis of the change of frequency will reveal the rate and duration of the tissue interface movement.

This technique was first applied commercially to fetal heart movement detection by Smith Kline in the United States (Doptone, 1966) and subsequently to continuous fetal heart rate monitoring by Sonicaid in the United Kingdom (1968). At first, probes contained a single transmitting crystal, focused to give a narrow beam, and a single receiving crystal. This gave good tissue penetration, but even a small amount of fetal movement causes loss of signal as the heart moves out of the beam. To overcome this problem, either a plastic lens was used to produce a divergent or "wide" beam or multielement transducers were constructed. These helped to reduce the effects of fetal movement, but, because the beam is divergent, beam intensity drops off rapidly with distance and the amplitude of the echo is reduced. This requires that the transmitter be surrounded by multiple receivers (usually three to six) to increase echo detection sensitivity. Such a system still suffers from the drawback that tissue penetration is reduced because of the falloff of sound intensity with distance. In addition, because of

the larger volume insonated, unwanted echoes degrade the heart signal, reducing the signal-to-noise ratio. A narrow beam system is therefore still useful in situations such as maternal obesity, hydramnios, and twin pregnancy, where good penetration and a high signal-to-noise ratio are important.

The nature of the signal derived from insonating the fetal heart has been well described by Organ et al. (1973) and Lauersen et al. (1976). Various low-frequency Doppler signals (100-300 Hz) are produced by ventricular wall movement. When the signals are processed electronically to make them audible, they sound stethoscopic (i.e., "lub-dup," similar to the sound heard on auscultation) or "sawlike." Valve movements, on the other hand, produce high-frequency (1000 Hz) signals which are of brief duration, comparable to the duration of the QRS complex of the ECG. These signals have a characteristic sharp "slapping" sound. There may be as many as four distinct valve sounds per cycle (atrioventricular valve opening and closing, aorto-pulmonary opening and closing), but their relative amplitude will depend upon the exact direction of the impinging beam. Aortic valve closure normally gives the largest signal of the four.

It must be emphasized that a heart rate derived from detection of ultrasound signals may not be exactly the same on a beat-to-beat basis as the rate derived from the fetal

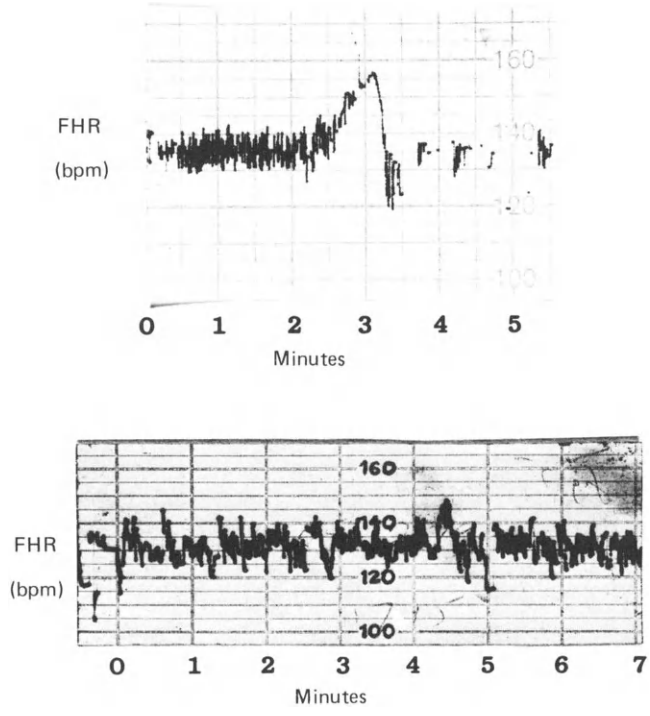


Figure 7 Two examples of baseline variability obscured by spurious beat-to-beat variation produced by a poor ultrasound signal (top trace, Hewlett Packard; bottom trace, Sonicaid).

ECG, since one reflects the mechanical activity of the heart, and the other its electrical activity. While the two are closely related (Organ et al., 1973) extraneous factors such as venous filling pressure, stroke volume, and peripheral resistance will change their temporal relationships (Goodlin et al., 1975; Robinson et al., 1978).

The easiest ultrasonic signal to obtain from the fetal heart is that derived from ventricular wall movement. However, the low-frequency, diffuse nature of this signal make it very difficult to time accurately. Any fetal heart rate derived from this signal will show a great deal of spurious beat-to-beat variation which lacks any recognizable pattern (Figure 7). Apart from the fact that such spurious beat-to-beat variation may be misinterpreted as genuine, it also tends to obscure baseline variability, which is very important for clinical interpretation. Various techniques can be used to overcome this to some extent, although the assessment of variability with ultrasound is inherently less precise than with the ECG (Wheeler et al., 1980).

1. *Clinical techniques.* Care should be taken to ensure that whenever possible, fetal heart valve movement is used as the source of the ultrasonic signal. The beam direction should be adjusted so that a single valve signal predominates and thus provides a clear trigger for the ratemeter. Care should also be taken to ensure the best signal-to-noise ratio. This means (1) using an adequate coupling medium, such as arachis oil, between the transducer and the maternal skin (air is a very poor conductor of ultrasound); (2) finding the location where the transducer is nearest the fetal heart, consistent with obtaining a clear valve signal (auscultation is often useful in this context); and (3) using a narrow beam transducer if tissue penetration is a problem, for example, with obesity.

2. *Electronic techniques.* Most systems now employ some filtering to enhance sensitivity to 1000 Hz and thus accentuate valve signals (Organ et al., 1973). In autocorrelation "depth ranging" a digital code is superimposed upon the transmitted signal, and by selecting the echoes with a given delay in the superimposed code, these echoes (which are from a coherent depth) can be enhanced (Lauersen et al., 1977). The majority of commercial fetal monitors now provide for "three-beat" averaging (or averaging over a similar time scale) (Figure 8). This may be standard (e.g., Sonicaid), an option to be specified on ordering (e.g., Hewlett Packard), or user selected via an average-nonaverage switch on the monitor (Corometrics).

Ultrasound fetal heart rate monitoring is widely regarded as a noninvasive technique because a vaginal examination is not necessary and transducers are not attached directly to the fetus. However, energy in the form of high-frequency sound at a power of 7-12 $mW/cm^2$ is radiated into the maternal and fetal tissues, and the technique is not passive as, for example, with phonocardiography. Recurrent doubts have been voiced about the safety of ultrasound, but intensive studies (e.g., Mannor et al., 1972) have not revealed a significant hazard at such low power inputs. At very high power, a great deal of heat is generated in the tissues, which are "fried" (Hill, 1968), but at lower power no significant sequelae, such as chromosomal damage, have been demonstrable using a variety of techniques. For references on the effects of ultrasonic irradiation on chromosomes, see the following: Coakley et al. (1972), Hill et al. (1972), Looby Watts et al., (1972), and Buckton and Vashon Baker (1972).

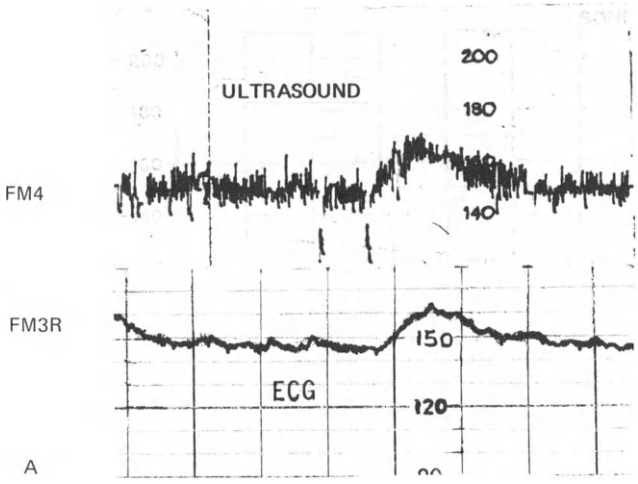


Figure 8 (A) Simultaneous FHR traces obtained by ultrasound (ventricular wall signal) and ECG. The spurious beat-to-beat artifact generated by the combination of a poor ultrasound signal and beat-to-beat processing is clearly seen when compared with the simultaneous unaveraged ECG-derived record.

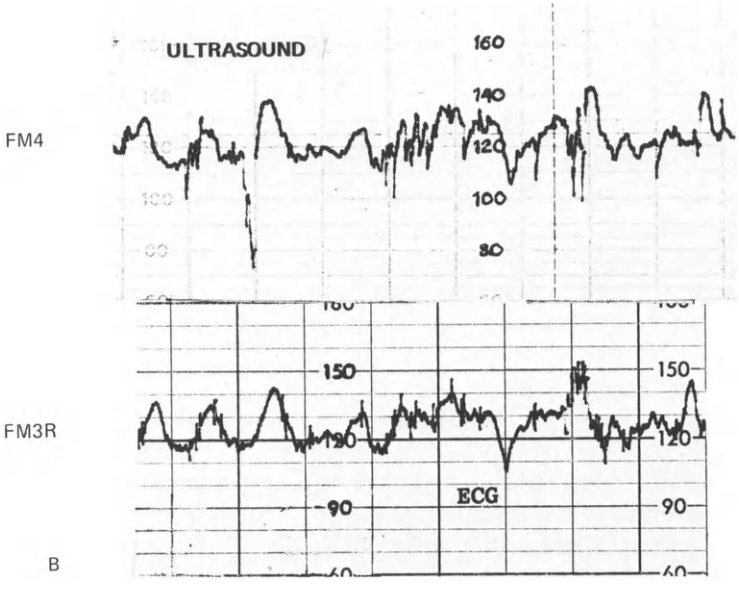


Figure 8 (B) With the same signal source, approximate three-beat averaging (1.2 sec) of the ultrasound-derived rate produces a tracing which is much more similar to that derived from the ECG.

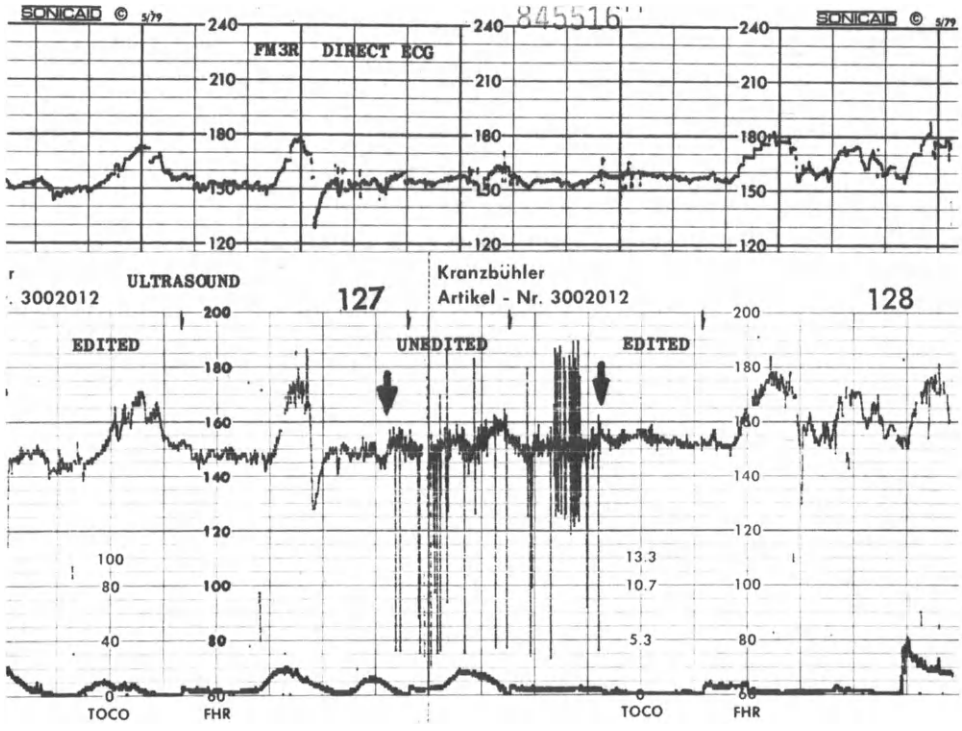


Figure 8 (C) The Kranzbuhler monitor allows a choice between averaged ("edited") ultrasound and unaveraged ("unedited") recordings. The improvement in the "readability" of the tracing in the edited mode is striking. The averaging process enables true baseline variability to be seen (as confirmed by the simultaneous unaveraged ECG recording) by removing spurious beat-to-beat "jitter" from the ultrasound tracing (the amplitude of the variability is apparently greater in the Kranzbühler tracing owing to the different chart paper scale).

## FETAL ECG MORPHOLOGY

Since the first demonstration of the fetal ECG by Cremer in 1906, attempts to analyze FECG morphology have been hampered by a poor signal-to-noise ratio. Satisfactory FECG morphology analysis has required the use of directly applied silver/silver chloride electrodes (Lee and Blackwell, 1974) and/or processing techniques such as digital group averaging, in which the cumulative addition of hundreds of consecutive complexes results in the elimination of random contaminating noise (Rhyne, 1969; Pardi et al., 1971). Asphyxia of the fetus is thought to be associated with changes in the P wave, PQ interval, and ST segment (Pardi et al., 1974), but these changes are so complex and varied that on-line microcomputer trend analysis is the only practical clinical approach (Marvell et al., 1980), and so far this is only at an experimental stage.

## MEASUREMENT OF FETAL CARDIAC ELECTROMECHANICAL INTERVALS

The measurement of cardiac electromechanical intervals has been useful in the management of adults with acute heart disease and it therefore seemed worthwhile investigating

similar parameters in the fetus. The most popular measurement has been the preinjection period, the time between the Q wave of the FECG and the opening of the aortic valve (Organ et al., 1973), but other mechanical intervals such as the phase of isovolumetric contraction between closure of the mitral valve and opening of the aortic valve, and the left ventricular ejection time between aortic valve opening and closure, have also been measured (Robinson et al., 1978).

The detection of the Q wave normally requires an electrode in direct contact with the fetus and therefore the measurements have usually been made in labor. However, the use of the R wave of the abdominally derived FECG allows antepartum studies to be performed. The valvular events are usually detected using high-frequency Doppler shift ultrasound, but phonocardiography can also be used (Goodlin et al., 1975). Computer or statistical averaging is generally necessary to achieve reproducible results and these techniques are as yet in their infancy. So far it has not been possible to show results of reliable clinical significance.

## THE ABDOMINAL FETAL ELECTROCARDIOGRAM

From approximately 20 weeks gestation the antepartum FHR can often be derived from the fetal ECG detected at the surface of the maternal abdomen, although this is often rather difficult between 28 and 34 weeks owing to the low amplitude of the signals at this time (Klöck et al., 1976; Wheeler et al., 1978; Carter et al., 1980). The exact reason for this low amplitude is not known, but it is usually attributed to the vernix caseosa (a poor electrical conductor) which often covers the fetus at this gestation. Figure 9 shows the likelihood of obtaining a satisfactory trace at various gestations. The success rate approaches 100% at term with the mother at rest and not in labor, although even then in 20% of patients a number of different electrode placings have to be tried before a satisfactory trace is obtained. Success of the technique during labor is limited to about 50% of patients by the level of electromyographic interference produced as a result of maternal restlessness, and recording during the second stage of labor is impossible for the same reason.

When the abdominal fetal ECG is used to record the FHR, the maternal ECG which contaminates the signal must be removed. The "blanking" technique usually employed in commercial fetal monitors also removes fetal signals when they coincide with maternal complexes, so that pulses have to be substituted artificially to avoid underestimating the fetal heart rate by 50%. Since this interference affects 15-20% of the fetal signals (Wheeler et al., 1978) a loss of 30-40% of the genuine beat-to-beat information is inevitable. To avoid this problem, a system has been designed which uses an analog store to substract maternal complexes from themselves and also from the combined fetomaternal complexes, thus preserving all the fetal signals (Wheeler et al., 1978). This system is not yet commercially available.

Abdominal ECG recording is easy to perform: It does not require a tight belt to hold the electrodes in place and (unlike ultrasound) is truly noninvasive. The FHR trace is often of a higher quality than that obtained by ultrasound. The major disadvantage already referred to is the uncertainty of obtaining a FHR trace between 28 and 34 weeks gestation and in labor (particularly the second stage).

At present, the improved accuracy of FHR measurements using R-wave peak detection of the abdominal FECG is not of major clinical importance, since most interpretations are based on baseline rate, the presence or absence (and timing) of accelerations

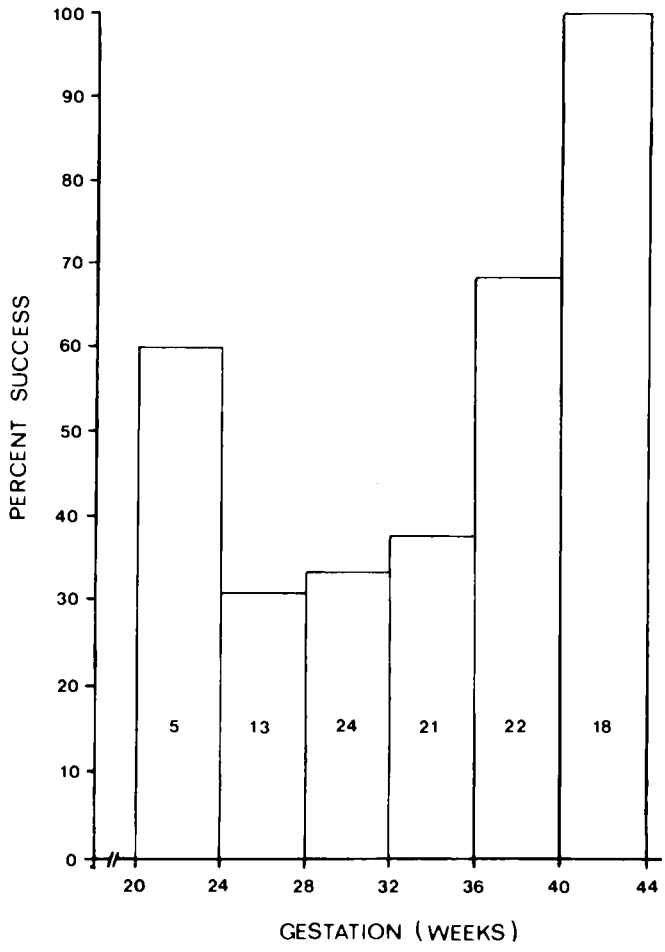


Figure 9 Percentage of successful FHR recordings against fetal maturity. The numbers in each block indicate the total number of recordings made in each group. (Data from Carter et al., 1980.)

or decelerations, and baseline ("long-term") variability. At present most clinical experience is based on traces obtained by ultrasound (which, as pointed out previously, gives information not about fetal heart electrical activity, but about its mechanical performance). Ultrasound, especially when a properly tuned transducer coupled to a three-beat averaging circuit is used, is capable of producing traces of excellent quality for the clinical measurements described above. Therefore, although such a system cannot give any information about "short-term" variability, convincing demonstration of the clinical value of this variable is required before there will be a persuasive argment for the more widespread use of abdominal ECG cardiotocography.

## pH MONITORING

Since its introduction by Saling in 1961, fetal capillary blood sampling and pH estimation has remained the most practicable arbiter of fetal response to hypoxia (Beard et al.,

1971a,b; Beard and Simons, 1971; Edington et al., 1975; Young et al., 1980). In theory, blood pH is an index of the degree to which fetal hypoxia has resulted in anaerobic glycolysis with the consequent development of a metabolic acidosis. However, the technique cannot distinguish between acute failure of placental perfusion and consequent respiratory acidosis due to hypercapnia (for example, the result of maternal hypotension) and chronic interference with fetal oxygenation which leads to more serious metabolic acidosis (Takemura, 1973). Ideally the $pCO_2$, $pO_2$, base excess, and blood lactate concentration should be measured so that the distinction between a respiratory and a metabolic acidosis can be made. In practical terms, however, careful assessment of the FHR trace with reference to the duration and type of abnormality seen, and its rate of deterioration or recovery, can usually suggest the appropriate etiology of a fall in pH. However, a growth-retarded fetus subjected to the mild hypoxia of labor contractions may develop an acidosis more rapidly than FHR changes (reflecting the hypoxia) would suggest (Modanlou et al., 1974; Rooth, 1973). In such a case, repeated fetal blood sampling (for example, every half hour) is the only safe course. Occasionally simultaneous measurement of maternal pH (or, ideally, the maternofetal base deficit difference) will be needed to exclude an infusion acidosis of the fetus of maternal origin (Roversi et al., 1975).

Despite the demonstrated effect of fetal pH measurement in reducing the incidence of false positive diagnosis of asphyxia in populations screened by electronic FHR monitoring, thereby preventing an undue rise in the cesarean section rate (Beard, 1968; Beard et al., 1971b; Young et al., 1980), 60% of all obstetric units in the United Kingdom do not use the technique (Gillmer and Combe, 1979). This is probably due to the following factors: (1) the cumbersome and time-consuming nature of the sampling process, (2) difficulties in obtaining consistent pH readings on the samples obtained, and (3) difficulties in the interpretation of the results, both technical and those resulting from a lack of understanding of the role of pH measurement in the diagnosis of fetal asphyxia.

1. Fetal blood sampling involves a large number of separate pieces of equipment (amnioscope, sampling blade and holder, capillary tube, long-handled forceps, dental swabs, silicone grease, ethyl chloride spray, light source, and fiberoptic connector; sterile towels, gowns, gloves, and masks are also required). These have to be assembled with care, as it is important to maintain an adequate aseptic technique. The operator needs at least one assistant, preferably two, who is properly trained in the layout of the instruments. In addition, it has been customary to place the mother in the lithotomy position, in the belief that it makes sampling easier. This is undignified for the mother, and uncomfortable for both her and the operator, who has to crouch on the floor, or on a low stool. In addition, it may produce supine hypotension in the mother, a tendency accentuated by the use of epidural anesthesia. Supine hypotension produces an acute reduction in placental perfusion, which leads to fetal hypercapnia and an acute fall in fetal blood pH. This can easily be misinterpreted as indicating a significant fetal metabolic acidosis, with consequent performance of an unnecessary cesarean section. Many authorities now recommend the use of the left lateral position for sampling. This not only prevents iatrogenic fetal acidosis, but is more comfortable for the patient and, with experience, for the operator. In cases where factors such as a high fetal head and a cervix only 1-2 cm dilated make the left lateral position unsuitable (the head floats away when pressure is applied with the amnioscope to exclude liquor), the lithotomy position may be used (with an assistant stabilizing the fetal head), provided that lateral tilt with a pillow or wedge is used.

The widespread adoption of the 2-mm guarded blade has now largely eliminated the complication of fetal hemorrhage which has been recorded following scalp stab blood sampling (Beard et al., 1966), although occasional disasters have been recorded associated with fetal coagulopathies (Hull, 1972; Hull and Wilson, 1972).

Contamination of the sample with liquor has to be avoided by judicious pressure on the amnioscope used to expose the fetal skin; excessive pressure, on the other hand, can lead to skin circulatory stasis and failure to obtain sufficient bleeding for satisfactory sampling (not to be confused with the failure of bleeding which occurs with terminal asphyxia and fetal circulatory collapse).

At one time emphasis was placed on the importance of obtaining a continuous unbroken column of blood in the capillary tube used to suck up the fetal blood sample from the skin. In practice, air bubbles can be eliminated when the sample is transferred to the pH meter in which the pH is to be estimated. Some loss of $CO_2$ may result from the exposure of the blood to air, but this will only raise the pH significantly in cases of respiratory acidosis; the lactic acid present in a metabolic acidosis will still produce a low pH reading.

2. The commonly used pH meter is in essence a simple voltaic cell in which two metals of different electrovalent potential, connected by a wire, generate an electric current when placed in an electrolyte (Hill, 1970). The two metals are mercury (with mercurous chloride, the so-called calomel electrode) and silver (with silver chloride). When they are placed in contact with blood and connected by a wire to form a circuit, a potential difference is generated. The metals are usually connected to the blood sample with salt solutions known as bridges (mercury-mercurous chloride-saturated potassium chloride-blood-saturated silver chloride-silver). The potential difference (pd) is measured with a potentiometer and read off in millivolts. In this simple form, the pd is a sum of the effects of the various ions present in the blood sample. Hydrogen ion (or, more properly, the hydroxonium ion, $H_3O^+$) can be measured selectively by placing in the circuit (between the blood and the silver chloride) a very thin membrane of a special glass selectively permeable to $H_3O^+$. The pd developed in this system is then representative of the $H_3O^+$ concentration (the negative logarithm of the $H_3O^+$ activity is pH). The potentiometers used must be of very high impedance, to prevent significant current flow, as this would otherwise affect the ionic constitution of the blood being measured, and progressively alter the result being obtained. The potentiometer can be calibrated in pH units rather than millivolts by the use of standard buffers of known pH.

A number of serious problems exist which interfere with the accuracy of the pH measurement using the standard technique. Probably the most important of these is the generation of liquid junction potentials (Kater et al., 1968). Owing to interactions between constituents of the blood such as proteins and the salt bridging solutions, layers of charged ions build up at the junction interfaces which interfere with the pd developed by the $H_3O^+$ ion. The exact composition of blood varies from one sample to another and this means that although a high degree of repeatability of readings on the same sample may be obtained, readings of different samples can only measure pH with an accuracy of $\pm$ 0.03-0.05 pH units. Clinical judgments therefore have to be made with this inescapable level of error in mind.

Another major problem is blood protein deposition on the $H_3O^+$ permeable glass membrane. This gradually "poisons" the membrane so that equilibrium at a steady

pH takes longer and longer. The process is accelerated if the blood sample is washed out of the electrode with water (which hemolyzes the blood) rather than iso-osmotic saline. Equally, allowing the saline to dry out repeatedly in the electrode can cause crystalline sodium chloride deposits to build up, blocking the electrode; this can be avoided by washing through with distilled water after use. The sequence sample-saline-water should therefore always be used. Buffers used for calibration should be washed out with water before a sample is tested, as even a small amount of contaminating buffer will markedly affect the pH of the sample. Electrodes can be regenerated by "etching" the surface of the glass clean with dilute acid.

3. Whenever possible, action should only be taken on pH values obtained from at least three aliquots of the fetal blood sample. As explained above, these should be repeatable to within ±0.01 pH units, although the absolute accuracy of the results in only within ±0.03 (Kater et al., 1968). If the three aliquots show variation greater than ±0.01, the result should be regarded with great caution, as it may be an error due to (1) technical errors in measurement or (2) contamination of the sample, for example, with liquor.

Results must be evaluated carefully within the clinical context to prevent unnecessary cesarean section for low pHs resulting from potentially reversible respiratory acidosis (hypercarbia). An example already quoted above is when a low pH results from hypercarbia secondary to supine hypotension in the mother. This condition is readily correctable by turning the mother to the left lateral position and, if necessary, infusing intravenous fluids rapidly (for example, Hartmann's solution). A similar situation may occur when there is hypotension following an epidural "top-up." Acute reduction of placental perfusion with a hypercarbic fetal acidosis may also occur when there is uterine hyperstimulation, for example, with oxytocics. Appropriate management is to correct the problem where possible, by infusion of intravenous fluids or reduction of oxytocic infusion, and only to resort to cesarean section where this is not possible (Sutton and Steer, 1979). Equally, normal pH values only exclude acidosis as a cause for fetal distress, which may still occur due to infection or mechanical effects (for example, excessive head compression) in the absence of asphyxia.

## CONTINUOUS TISSUE pH MONITORING

Using the Saling technique of fetal blood sampling, deductions about the acid-base status of the fetus are made from an arterialized capillary sample (arteriolar dilatation is induced using an ethyl chloride spray). As described above, a major disadvantage of this technique is that it is intermittent. While the ideal solution would be a continuous intravascular pH probe, this is unlikely to be technically feasible on the presenting part of the fetus. However, using a miniaturized electrode, it is possible to measure continuously the pH of the subcutaneous tissue. Stamm and co-workers (Stamm et al., 1974) designed such an electrode, identical in its basic concept to the one described above for scalp blood pH estimation, but with the pH-sensitive Li-Ba-Si glass forming the tip of a probe with a liquid KCl junction just below it (Figure 10). The probe is inserted into the subcutaneous tissue of the presenting part of the fetus. The pH of the tissue lying between the tip and KCL junction can then be measured.

There are a number of major problems associated with the use of this electrode to measure the tissue pH of the fetus during labor.

1. *Penetration of the skin and subsequent fixation.* The present technique is to apply a large spiral electrode and use a special instrument to make a central skin

puncture through the middle of the spiral. The pH electrode is then screwed into the center of the spiral electrode, which should hold the tip firmly under the skin surface. Various studies show a 20-40% measurement failure rate due to problems with electrode fixation (Flynn and Kelly, 1980; Huch and Huch, 1977; Lauersen et al., 1979; Sturbois et al., 1977; Weber and Hahn-Pedersen, 1979; Weber et al., 1978; Wood et al., 1978).

2. *Storage and preparation of the electrode for use.* The electrode cannot be left with the reference solution in situ, as KCl crystals form. If the electrode is stored dry, however, a conditioning time of 24 hr is required after filling with reference solution to restore normal function. Recommended sterilization is immersion of the disassembled electrode in Cidex (2% aqueous glutaraldehyde) for 3 hr. Following this, the electrode is rinsed thoroughly with sterile water and then filled with reference solution. Several hours are then required for electrode stabilization.

3. *Calibration.* This must be performed using the pH 7 and 7.4 buffers supplied, heated to 37°C in a heating block.

4. *Breakage.* The electrode is expensive and relatively fragile so that frequent breakages are reported, 1 in 4.2 applications (Sturbois et al., 1977), 1 in 13 (Wood et al., 1978), and 1 in 40 (Lauerson et al., 1979).

5. *Correlation.* A good correlation between tissue pH and capillary blood pH in the fetus has been demonstrated (Sturbois et al., 1977) and the normal range of the two variables is similar (Weber and Hahn-Pedersen, 1979); however, this should not lead us to suppose that they are one and the same (Rithalia et al., 1979). Tissue pH is very susceptible to changes in the extent to which the tissues are perfused and in distressed babies changes in peripheral circulation may well produce differential changes in tissue and capillary pH.

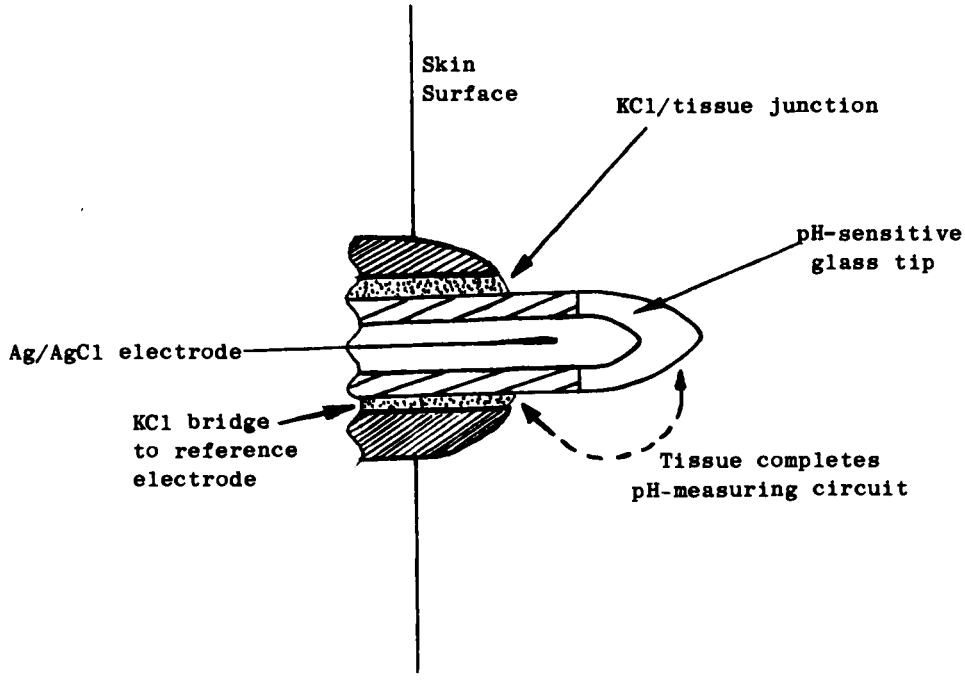


Figure 10 Schematic cross section of the Stamm continuous-reading tissue pH electrode.

## CONTINUOUS INTRAPARTUM $pO_2$ MEASUREMENT

These techniques are based upon the transcutaneous monitoring of gases which diffuse through tissue and are measured on the skin surface. The methods available are based on a number of different techniques. Polarography is the simplest and cheapest to produce and has been most widely used to date (Frankenberger et al., 1979). Its use has been pioneered by the Huchs of Marburg (now Zurich).

### Polarography

A membrane-covered modified Clark $pO_2$ electrode is fixed airtight onto hairless skin after in vitro calibration and heated directly to produce local hyperemia. Oxygen molecules diffuse from the dilated capillaries through the avascular epidermis to the cathode and are there reduced. The current produced at the cathode is a measure of the $O_2$ level. The power required to maintain the temperature is a useful measure of the local blood flow (the greater the blood flow, the more power is required to maintain the temperature, as heat is carried away in the bloodstream).

A number of practical difficulties have limited the routine use of this type of instrument: The cervix must be at least 4 cm dilated for satisfactory application of the electrode, the membranes must be ruptured, the fetal skin has to be shaved with a special long-handled razor, fixation (most satisfactorily achieved with tissue glue) is not always easy or secure, and there are potential hazards associated with overheating of the skin should the local circulation fail.

Knowledge of transcutaneous $pO_2$ levels is a useful aid to the interpretation of unusual FHR patterns (particularly variable decelerations; late decelerations are almost invariably associated with hypoxia), but it cannot reveal the effect on the fetal tissues of reduced $pO_2$ tensions. Modanlou et al. (1974) showed clearly that growth-retarded fetuses can become acidotic despite oxygen tensions which are no different from those tolerated easily by normal fetuses in labor. A $pO_2$ estimation can therefore be a useful adjunct to the management of labor, but it cannot replace pH or possibly lactate measurement as an indicator of fetal response to a hypoxic stress. Clinical aspects of these techniques are dealt with in Chapter 22.

## MEASUREMENT OF INTRAUTERINE PRESSURE

The simplest method of recording intrauterine pressure (IUP) is to introduce a fluid-filled rubber bag into the uterine cavity and to connect it by tubing to a mercury manometer. This technique was first described by Schatz in 1872 and used in the United Kingdom by Bourne and Burn as early as 1927. They found, however, that the introduction of the bag into the uterus required general anesthesia with nitrous oxide or chloroform, and this made the technique unsuitable for routine clinical use. Methods were therefore developed to measure IUP via the abdominal wall. Reynolds et al. (1948) introduced the strain gauge tocodynamometer in which a central piston was mounted on a brass ring placed on the mother's abdomen over the uterus. The piston pushed down, indenting the uterus. As the uterus contracted, the hardening muscle pushed on the piston, and this increased force was registered by a strain gauge. Because the force generated depended upon the initial amount of uterine muscle indentation, only a relative measure of uterine activity (in grams force) could be obtained. The true intrauterine pressure remained unknown. This technique is still widely used in commercial fetal

cardiotocographs today. It is satisfactory if all that is required is an indication of the timing of contractions, to aid in the interpretation of the fetal heart rate trace, either antepartum or in labor. In abnormal labor, however, the measurement of true intrauterine pressure becomes important. For example, the tocodynamometer measures only the activity of the local area of muscle which it indents. Using a single tocodynamometer in incoordinate uterine activity may therefore give a tracing which appears to indicate a good contraction. Intrauterine pressure measurements, however, will reveal that because only a small part of the uterus is active at any one time, there are only small and irregular elevations of pressure shown on the recorder. To overcome this problem, Reynolds et al. (1948) used three tocodynomometers to assess activity at different parts of the uterus. Caldeyro-Barcia et al. (1950) were able to show, using this technique, that a wave of contraction passes down the uterus, with the longest and strongest contraction occurring at the fundus, which they called fundal dominance. However, the use of multiple tocodynamometers is not practical in day-to-day clinical use, and Smyth (1957) made an attempt to produce a tocodynamometer which would give an accurate reading of intrauterine pressure. The principle of his device is as follows: If a small area of the abdominal wall and underlying uterine wall is turned into a flat diaphragm by pressing upon the external surface with a flat plate, the pressures on each side of the body wall will be equal. By measuring the force upon the plate, the internal pressure can be measured, provided that the area of contact is known. To eliminate any pressures arising from bending of the body wall at the edges of the flattened area, the pressure plate is surrounded by a guard plate which flattens an additional "surrounding" of body tissues. In principle, this system works well; however, the pressure required to achieve the appropriate flattening is so great as to be unacceptable to the patient except for short periods. Gas-filled tambours have been designed to exploit the same principle, but Lacroix (1968) has shown that in 75% of patients the contraction pressure is underestimated, sometimes by as much as 40%.

Karlson (1944) attempted to produce direct readings of intrauterine pressure by introducing a pressure transducer through the cervix into the uterine cavity. Unfortunately his transducer was based on the variable resistance of carbon granules subjected to differing pressures (as used for many years in post office telephones) and he was not able to obtain reproducible pressure measurements. More reliable transducers were unfortunately too large to introduce directly into the uterine cavity. In 1952, Williams and Stallworthy published a simplified technique of direct IUP measurement. They passed an open-ended fluid-filled polythene catheter through the cervix into the amniotic cavity and then connected it to an external pressure transducer. This technique is relatively atraumatic and is capable of providing very accurate IUP records. It is still widely used today.

The open-ended fluid-filled catheter attached to an external pressure transducer does, however, have a number of signficiant problems associated with its use.

1. *The "baseline problem."* Baseline tone, or "resting intrauterine pressure," is the pressure within the uterus when it is not contracting. There is a component of pressure due to the elastic recoil of the tissues of the uterus and an additional hydrostatic component which varies with the depth below the upper fluid level of the uterus. The contribution to the overall measured pressure of the hydrostatic component will vary from zero when measured level with the upper fluid level of the uterus, to approximately 35 $cmH_2O$ (25.7 mmHg, 3.43 kPa) if measured at the lowest fluid level of the upright uterus. In practice, baseline tone is therefore not a single value, but varies according to

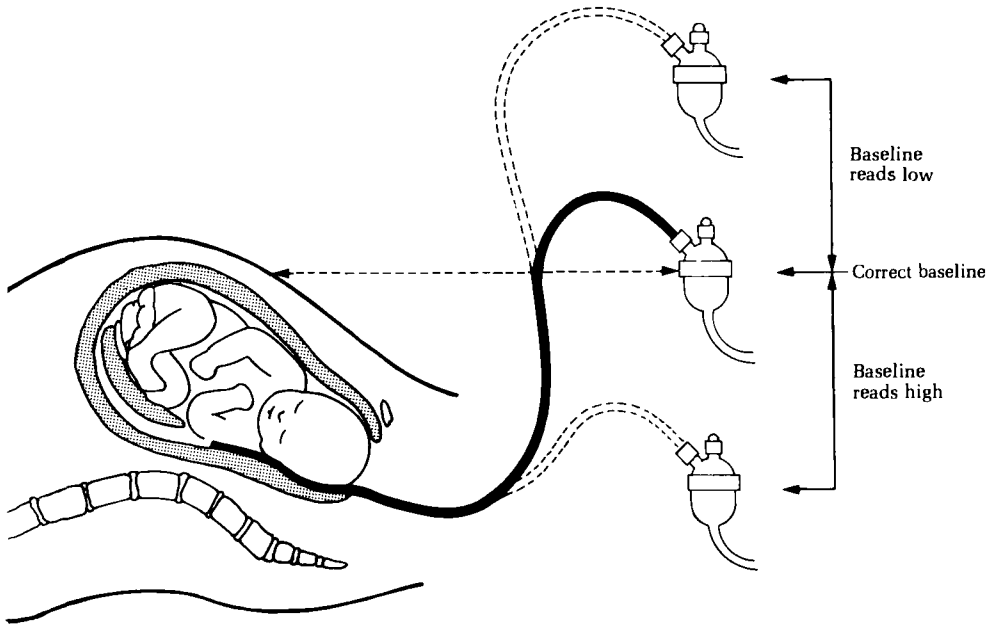


**Figure 11** Changes in registered intrauterine pressure produced by changes in external transducer position.

the position of the measuring transducer. It is traditional to place a measuring external transducer level with the symphysis pubis; using this fixed point for the transducer, the contribution of the hydrostatic pressure to the registered "baseline tone" will vary with the posture of the mother and the consequent degree of "uprightness" of the uterus (Figure 11). The exact value of the hydrostatic component is usually unknown, and therefore the measurement of baseline tone is usually arbitrary to within ±10 mmHg (1.3 kPa). Arroyo and Mendez-Bauer (1975) and Mendez-Bauer et al. (1975) have suggested attaching the transducer to the maternal abdomen over the fundus of the uterus to minimize the effects of changes in posture, but this technique is rather cumbersome and has not yet found general clinical acceptance.

It should also be clear that the fluid within an open-ended catheter is normally in direct continuity with the amniotic fluid, and the hydrostatic pressure measured is determined by the relationship of the upper level of amniotic fluid to the pressure transducer. The position of the catheter tip within the uterine cavity (provided that it is in continuity with the amniotic fluid) therefore has no effect on the measured pressure, as incorrectly claimed by some authors (Odendaal et al., 1976).

2. *Mechanical damage to the uterus, placenta, or fetus.* One fetal death and a number of cases of severe fetal anemia have been recorded following damage to fetal blood vessels in the placenta at insertion of polythene catheters (Trudinger and Pryse-Davies, 1978; Nuttall, 1978). The catheter can also become wound around the cord, interfering with umbilical cord blood flow (Trudinger and Pryse-Davies, 1978; Cave et al., 1979). Perforation of the uterine wall may also occur (Chan et al., 1973; Tutera

and Newman, 1975), although in this event it is usually sufficient simply to withdraw the catheter and reinsert it in another direction.

3. *Infection.* A number of workers have reported an increase in the incidence of endometritis with the use of intrauterine catheters (Amato, 1977; Hagen, 1975), although some say that this is rare (Chan et al., 1973), others find no effect (Tutera and Newman, 1975), and yet others claim that any effect is due to artificial rupture of membranes (Gibbs et al., 1976). Two potential sources of infection exist: (1) Infection may be carried in during placement of the catheter and (2) the pressure transducer may be inadequately sterilized before use. If bacteria are present in the dome or on the diaphragm of the transducer, a direct fluid path exists between the bacteria and the uterine cavity. It has been shown that bacteria are unlikely to ascend a catheter filled with sterile water within 24 hr, but if the catheter is filled with a nutritive medium (such as amniotic fluid), then chemotactic growth down the catheter into the uterus is possible (Roberts and Steer, 1977). Effective sterilization of the transducer (for example, with 2% aqueous activated glutaraldehyde) is therefore important, as is proper aseptic technique for placement of the catheter in the uterus. Catheters should be flushed through with sterile water and not be allowed to fill with amniotic fluid.

4. *Catheter blockage.* Because the tip of the catheter may become blocked with blood or vernix on insertion, it is normal to flush through the catheter with sterile water following insertion. Most commercially supplied catheters also have additional holes in the side of the catheter near the tip. Further blockage is therefore only likely to occur if there is leakage of fluid at the connection of the catheter to the transducer, or from the transducer itself. This causes a flow of liquor into the catheter, carrying with it vernix and blood clot. Complete blockage is easy to recognize because a totally flat tracing is produced, but a partial blockage may simply attenuate the signal, with the result that a variable underestimate of uterine activity is made. This may lead the obstetrician, for example, to administer an excessive dose of oxytocin.

5. *Patient mobility.* Fluid-filled catheters have a limited length, restricting patient mobility.

6. *Catheter movement.* Movement of the catheter causes large artifactual variations in recorded pressure.

7. *Care of equipment.* There is difficulty in clinical use related to the need for calibration, flushing, and assembly of parts.

A new design of catheter tip pressure transducer (Sonicaid-Gaeltec) overcomes some of these problems (Steer et al., 1978). It is a miniaturized bridge strain gauge deposited on a thin metal pressure-sensing surface and mounted on the end of a 900-mm woven Dacron or polyurethane catheter (preferably stiffened with a stainless steel wire). The transducers are stored and sterilized in a perspex tube filled with 2% aqueous Cidex solution. The catheter tip is relatively soft, round, and flexible and it is therefore unlikely to perforate the uterus or traumatize the fetus. The transducer is introduced within the uterine cavity so that blockage or attenuation of the signal cannot occur. The connections are easy to make and calibration straightforward. The electrical cable connecting the transducer to the recording equipment can be as long as required. No increase in intrapartum or postpartum sepsis has been associated with the use of these catheters. Although the exact level of baseline tonus cannot be measured (the position of the catheter tip—and hence the transducer—relative to the fundus is uncertain), the pressure readings are independent of gross patient movement since the transducer moves with the patient.

## ANALYSIS OF INTRAUTERINE PRESSURE READINGS

The classic parameters of uterine activity are frequency, pressure, and the duration of contractions and baseline tonus (Figure 12). The first attempt to derive a single measure to represent all these variables was made by Bourne and Burn in 1927. They used a planimeter to measure the total area below a tracing which represented the intrauterine pressure varying with time. Unfortunately, they did not quantitate their units.

In 1958 Caldeyro-Barcia introduced the Montevideo unit, which is a multiple of the pressure of contractions and their frequency. Finding the level of baseline tone difficult to measure accurately, and of no clinical significance in the majority of labors, he used for his unit the peak contraction pressure minus the baseline tone ("active" pressure) rather than the absolute value of the maximum pressure above atmospheric. The Montevideo unit is therefore the mean active pressure per 10 min multiplied by the frequency of contractions per 10 min. In practice, the Montevideo units in any particular 10-min period can be derived by simply adding together the active pressure of each contraction within that period.

El-Sahwi et al. (1967) included the duration of contractions in a new measurement which they called the Alexandria unit, arguing that the duration of the contraction is an important variable in the relationship between uterine activity and placental blood flow (Towell, 1966). A similar approach was used by Steer and co-workers in 1975 when studying induced labor (Steer et al., 1975).

All the studies described so far analyzed uterine activity retrospectively, using tedious manual assessment of cardiotocograph traces. Not only is the assessment difficult to make if uterine activity is incoordinate, but it is not practical for widespread clinical use "on line" because of its time-consuming nature. In the

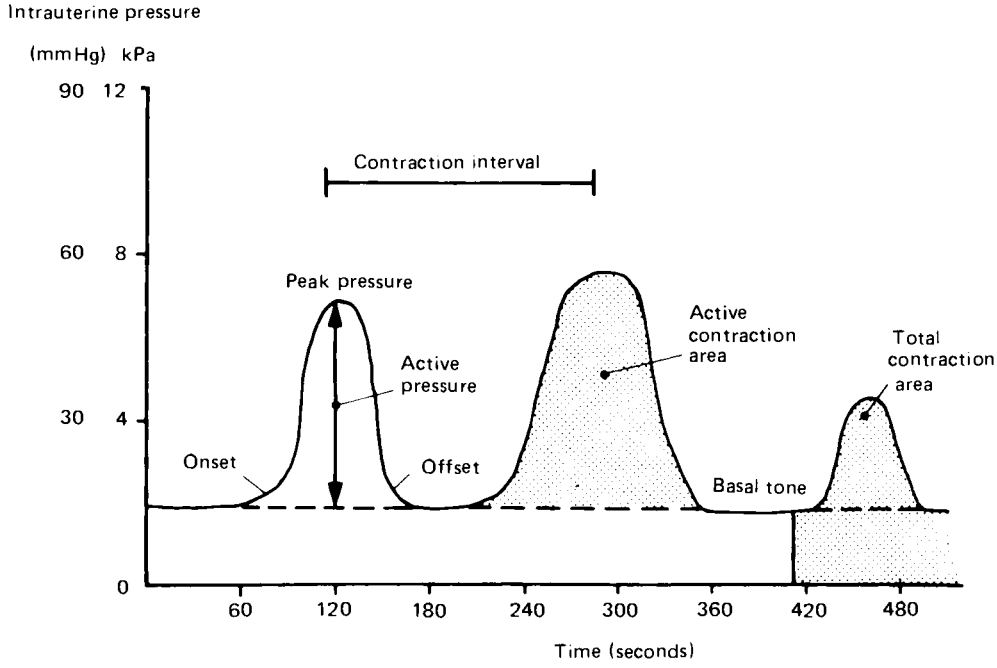


Figure 12 Terminology of uterine contractions.

search for a more practical method of on-line quantitation of uterine activity, Hon and co-workers (Hon and Paul, 1973; Miller et al., 1976) returned to the planimetric technique first described by Bourne in 1927. They used an on-line electronic integration technique described by Jilek and co-workers in 1972 (Jilek et al., 1972) and currently available as a function of the Corometrics fetal monitor. Because of the technical difficulty in electronically determining basal tonus, they decided to integrate the whole of the pressure curve above "zero" pressure as recorded on the cardiotocograph. (This "zero" is in fact atmospheric pressure in absolute terms and reflects the absence of additional hydrostatic pressure above atmospheric pressure.) They maintained that since abnormally elevated basal tonus is known to have an adverse effect on maternal placental perfusion and hence on oxygenation, any measurement of uterine activity should include the effects of basal tonus. In practice, using currently available techniques, the true level of basal tonus is impossible to determine accurately for reasons already explained above.

It appears that this limitation has never been appreciated by Hon and his co-workers. In a recent paper they described a study of the effect of posture on uterine activity during labor (Read et al., 1981). As might be expected from the physics explained above, change in the mothers position from the recumbent to the erect position produced no significant short-term change in the level of uterine activity as measured by Montevideo units (i.e., the contractions themselves did not change), but there was an immediate increase in the total contraction area of 120 UAU (uterine activity units); (Torr min). (The baseline level of UAU was not given, but in a previous article a mean value for UAU in established labor was given as 300 UAU Torr min (Miller et al., 1976). This increase is entirely accounted for by an increase in measured basal tone of 12.25 mmHg (1.63 kPa). The authors conceded that "the increase in intrauterine baseline tonus observed in our patients may have been an artifact," but went on to state that they "attempted to minimize this [artifact] by placement of the transducer relative to the estimated catheter tip." As previously explained, with an open-ended fluid-filled catheter system (which the authors used in previous studies, although their exact technique is not stated in this latest article) the position of the catheter tip is irrelevant in the measurement of baseline tonus, since the amniotic fluid in the uterus and the fluid in the intrauterine catheter behave as a single continuous column. The relevant reference point is therefore the fundus of the uterus.

Simple calculations based on the dimensions of the pregnant uterus show that using the fluid-filled catheter technique (with external transducer), there are uncertainties in the measurement of "true" basal tone (due to postural changes) which amount to 10-15 mmHg (1.3-2 kPa). In the example quoted above, from Read et al., the change was 12.25 mmHg. When total uterine contraction area is measured, this can lead to differences in the measured area of 1800 kPas over 15 min, depending on whether the patient is recumbent or erect. The average value of active contraction area (contraction area above baseline tone) is 1100 kPas/15 min (Steer, 1977b). The total contraction area of an erect patient with no contractions whatsoever can therefore exceed the value obtained in a recumbent patient in active labor! In contrast, the measurement of active contraction area has the following advantages: Firstly, when the patient has no contractions, the value of the measurement is near zero. The normal range of values in established progressive labor (lower 10th centile and upper 90th centile) is 700-1500 kPas/15 min. Within this range the rate of progress in labor is linearly correlated with the active contraction area (Table 1). Secondly, it is relatively easy to design an electronic system which measures and subtracts baseline tone continuously so that the measurement of

Table 1 Results in 20 Cases of Oxytocin-Induced Multigravid Labor (Initial Bishop Score ⩾6)

| Variable measured | Correlation with rate of cervical dilatation in the stable phase | |
|---|---|---|
| | r | *P* |
| Frequency | 0.23 | NS |
| Pressure | 0.36 | NS |
| Montevideo units | 0.51 | <0.05 |
| Active contraction area | 0.60 | <0.01 |

active contraction area is accurate to within 10% (Carter and Steer, 1976). Such a system will adjust automatically to apparent changes in baseline tone produced by postural changes, so that only the effects of the contractions themselves are measured (Steer and Carter, 1977). The fact that the baseline tone is being subtracted continuously from the measurement of active contraction area does not mean that it cannot therefore be monitored continuously as a safeguard against hyperstimulation. Firstly, the actual pressure measurement can be displayed constantly on the chart recorder of a cardiotocograph. An electronic alarm system can be used which will sound automatically if the baseline pressure sits at levels programmed as potentially dangerous (e.g., 4 kPa, 30 mmHg) for excessive lengths of time (e.g., more than 3 min). A further refinement is to use a system which will activate an alarm if there are rises in baseline tone which might, if continued, lead to the baseline tone becoming excessive. For example, the alarm can be set to sound if the baseline tone rises by more than 2 kPa (15 mmHg) in 3 min (Carter and Steer, 1979, 1980). Because such a system reacts only to baseline tone, contractions are ignored, provided that there is a normal baseline established between each contraction. Excessive contractions are recognized because they generate a contraction area which exceeds the upper limit of normal.

## MEASUREMENT OF THE ACTIVE CONTRACTION AREA

The measurement of the active contraction area, produced every 15 min, is known as the "uterine activity integral" or UAL (Figure 12). As described above, the measurement of true active contraction area is very dependent upon the subtraction of the correct level of baseline tone. In the majority of cases, when intrauterine pressure is being recorded accurately via a catheter tip pressure transducer, the measurement of UAI displayed will represent the true level of uterine activity. In some circumstances, however, this accuracy is reduced. Firstly, when the monitor recording the pressure is switched on, if the baseline setting of the IUP is incorrect and is then adjusted, the baseline recognition circuits take a short time (usually 40 sec) to adjust to the new baseline pressure.

This period may be prolonged if there is a great deal of fetal or maternal movement, producing pressure spikes which prevent the clear recognition of the baseline. During this time the baseline is registered at an indeterminate level below the true baseline and a spurious contraction area will be measured. The initial reading produced by the UAI system should therefore always be ignored. Secondly, changes in posture produce changes in baseline tone of up to 15 mmHg (2 kPa). Because of the nature of the electronic circuitry used, downward changes will be recognized within a few seconds, but upward changes can take up to half a minute to be recognized, depending on the degree of change. A spurious additional value of between 60-120 kPas/15 min may therefore be added to the next 15-min reading of UAI (usually the figure will be much less than this). Thirdly, placing the mother on a bedpan may raise the intrauterine pressure by as much as 30 mmHg (4 kPa), although it is usually less than this. The subsequent UAI reading may be increased by a value ranging from 200 kPas/15 min to 700 kPas/15 min, depending on the length of time the mother spends on the bedpan, and this should be borne in mind when interpreting the results. (Note: The measurement of UAI is currently commercially available as a function of the Sonicaid FM3R fetal monitor).

## VARIATIONS ON THE MEASUREMENT OF THE ACTIVE CONTRACTION AREA

Rossavik (1978) described an approximation to the active contraction area by approximating each contraction to a triangle and calculating the "impulse" I of a single contraction by the formula $I = A \times B/2$, where A is the active pressure (kPa) and B is the duration of the contractions (sec). The total uterine impulse in kilopascals for any period is the sum of the impulses of the contractions during that period. Rossavik then went on to categorize labors by the total impulse needed for each centimeter of cervical dilatation. This is a measure of "resistance" to the progress of labor according to the formula: progress is proportional to the uterine impulse divided by the resistance. Progress is the rate of cervical dilatation in centimeters per hour and "resistance" is an ill-defined entity dependent on bony and soft tissue opposition to descent of the fetal head and resistance of the cervix. He then went on to show a close correlation between a high total impulse per centimeter dilatation and the need for instrumental or operative delivery, irrespective of the mean level of uterine activity per unit time, and pointed out that kilopascals per centimeter of dilatation was superior in this respect to the rate of progress alone, calculated from a partogram. For example, a slow labor with poor uterine activity (average or low impulse per centimeter of dilatation) was likely to end in a spontaneous delivery, whereas an equally slow labor associated with a high impulse was likely to end in instrumental or operative delivery.

Henry et al. (1979a) have also used on-line measurement of the active contraction area in the clinical management of their patients. Active contraction area was measured using a mainframe computer system which samples intrauterine pressure (measured from an intrauterine catheter) every 2 sec. They also felt that "since [active contraction area] is measured with respect to a baseline, it is almost independant of errors due to hydrostatic pressure and patient movement, which are inherent in IUP monitoring." Thus they preferred active contraction area measurement to the measurement of total area above atmospheric pressure. They expressed the area measurement in Torr seconds (compare kiloPascal seconds in the SI system). This unit was termed "energy" and the cumulative Torr seconds per half hour are referred to as "power." The drawback to

this measure is that it is not altogether appropriate for uterine contraction area measurement. This view was supported by the majority of those present at the Fourth Annual Joint Meeting of the Biological Engineering Society and the Hospital Physicists Association, as reported by Parsons (1976). He stated that the general feeling was that the terminology was clear and the integration must be referred to as kiloPascal seconds (N sec/$m^2$), and not as "work" (N m).

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# 22

# Maternal and Fetal Acid–Base Balance and Blood Gas Measurement

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## INTRODUCTION

Pregnancy is characterized by significant changes in breathing and in the acid-base balance as a result of the hormonal stimuli beginning at conception, the increasing oxygen requirements, and the mechanical effects of the enlarging uterus in later weeks. Changes in maternal breathing will naturally influence fetal blood gases and acid-base balance, since the mother and her fetus represent a biological unit whose direct gas and substance exchange takes place in the placenta.

The changes in the blood gases and acid-base balance of the mother during labor and delivery are particularly marked. These result from breathing changes due to maternal physical work, pain, and fear and from the work of the uterine muscles. These factors add to the effects of contractions on uterine blood flow to alter the acid-base balance of the fetus.

Our knowledge about maternofetal changes in pregnancy and during labor and delivery is already substantial, particularly regarding maternal values. Significant progress regarding maternal and cord $pO_2$ and $pCO_2$ values was made during the 1960s. However, our knowledge, especially regarding the fetus, is far from complete. For many years the major reason for this was the limitations in technology. The glass pH electrode was not developed until 1912 (Lundsgaard, 1912) and direct $pCO_2$ measurements in blood were first made in 1954 (Stow and Randall, 1954). Finally, $pO_2$ measurements in small amounts of blood have only been clinically practical since 1956 (Clark, 1956). A second reason which still limits our investigative efforts is the lack of access to the human fetus in intact pregnancy. Only since the introduction of the scalp sampling technique of Saling (1966) has access to fetal blood gases and acid-base balance during labor been a practical clinical possibility.

During the last few years, methods have been developed which allow us to measure some blood gas and acid-base variables continuously. While these techniques are not yet ready for routine clinical use, they promise to provide further insight into the fluctuations of blood gas and acid-base balance under varying conditions. With this concept in mind, the current results of continuous blood gas and pH measurements in the mother and in the fetus are reviewed in this chapter. This review is preceded by a summary of the known changes in acid-base balance, blood gases, and blood compartments in the mother during pregnancy and labor and in the fetus during labor and delivery. We refer the interested reader to some

excellent reviews of these topics: Bartels et al. (1972), Bartels and Wulf (1965), Beard (1968), Crawford (1975), Derom (1968), Hytten and Leitch (1964), Kirschbaum and DeHaven (1968), Kubli (1968), Novy and Edwards (1967), Prowse and Gaensler (1965), Rooth (1980), Towell (1976), and Wulf and Manzke (1964).

## CHANGES IN BLOOD GASES, BLOOD COMPARTMENTS, AND ACID-BASE STATUS IN THE MOTHER DURING PREGNANCY

In Table 1 normal values for blood gases and acid-base data for nonpregnant women are given. This data is meant to be the basis of comparison for the most important physiological changes occurring in women during late pregnancy (Table 2).

Some of the major changes in blood gases and acid-base balance result from the alterations in breathing already present in the first weeks of pregnancy (Zuntz 1910; Hasselbalch and Gammeltoft, 1915), attributed by most to the influence of progesterone (Döring and Loeschcke, 1947; Döring et al., 1950). Outstanding is the large increase in the respiratory minute volume. This is almost exclusively due to an increased tidal volume (Bartels et al., 1972, Cugell et al., 1953), since the respiratory rate is relatively constant. The minute volume is about 40-50% higher in pregnant than in nonpregnant women (Bartels et al., 1972; Cugell et al., 1953). Because the dead space does not change significantly, this results in an increase in alveolar ventilation of 60-70%. This increase is in excess of what is required and must be regarded as hyperventilation. The resulting low $pCO_2$ values have long been recognized as typical of the pregnant status (Hasselbalch and Gammeltoft, 1915; Döring and Loeschcke, 1947; Rossier and Hotz, 1953).

An additional characteristic of the respiratory physiology of pregnant women is the decrease in functional residual capacity (FRC) in part due to a decrease in residual volume (RV) caused by elevation of the diaphram. The reduction in functional residual capacity is further explained by the fact that an increase in tidal volume is due almost exclusively to a decrease in expiratory reserve volume. By the end of pregnancy the FRC is reduced by approximately 20% (Bartels et al., 1972; Cugell et al., 1953; Friedberg, 1980; Heidenreich, 1980). This results in a reduction of the "buffer" for breathing irregularities. Therefore rapid changes in blood gases are more common in pregnant than in nonpregnant women. Together with high oxygen consumption during labor, this reduced FRC explains the observation that even skillful tracheal intubation in a parturient woman breathing air frequently causes arterial $pO_2$ to fall to 50-60 mmHg after only 30 sec of apnea (Bonica, 1972).

As is seen in Table 2, decreased $pCO_2$ values during pregnancy are confirmed by all investigators. The $pO_2$ results are less uniform; the range is 83-106 mmHg. However, in most of the studies the values measured are similar to those in the nonpregnant state. Therefore increases in $paO_2$ during pregnancy, if seen, are less than one would expect as the result of the measured decrease in $paCO_2$. This puzzling observation may be explained by an uneven ventilation-perfusion ratio and changing amounts of venous admixture during pregnancy or by diffusion abnormalities. Because of the small arteriovenous difference for $CO_2$ or because of the fact that $CO_2$ diffuses more freely, an impairment in either process might be only measurable for $pO_2$. In fact, a decrease in diffusion capacity has been measured in pregnancy (Lehmann, 1974). There is still speculation whether this diffusion abnormality results from interstitial water retention secondary to progesterone. According to this hypothesis, hyperventilation should be considered as an attempt to maintain a normal $pO_2$.

Table 1 Values for Blood Gases and Acid-Base Data for Nonpregnant Women[a]

| | | | | |
|---|---|---|---|---|
| $pO_2$ (mmHg) | 94.0 | *Respiration and Circulation* (1971) | 96.0-98.0 | Ulmer et al. (1976)[b] |
| | 94.8 | Rooth and Sjöstedt (1962) | | |
| | 86.3 | Stojanov (1972) | | |
| $sO_2$ (%) | 97.0-98.0 | *Respiration and Circulation* (1971) | | |
| | 95.0-97.0 | Rossier and Hotz (1953) | | |
| Hemoglobin (g %) | 12.0-16.0 | Göltner (1980) | | |
| | 13.0-14.5 | Hytten and Leitch (1964) | | |
| | 13.4 | *Respiration and Circulation* (1971) | | |
| Hematocrit (vol %) | 37.0-44.0 | Göltner (1980) | | |
| | 39.8 | Hytten and Leitch (1964) | | |
| | 41.5 | *Respiration and Circulation* (1971) | | |
| $O_2$ capacity (vol %) | 17.5-19.5[c] | *Respiration and Circulation* (1971) | | |
| | 18.0 | | | |
| | 19.5-20.5 | Rossier and Hotz (1953) | | |
| $O_2$ content (vol %) | 17.0-19.0[c] | | | |
| $O_2$ affinity [$p_{50}$ (mmHg)] | 26.1 | Bauer et al. (1969) | 27.1 | Müller and Müller-Plathe (1979) |
| | 27.0 | Humpeler et al. (1973) | 25.0 | Prystowsky et al. (1969) |
| | 26.8 | Meier et al. (1978) | 25.3 | Wulf et al. (1966b) |
| pH[d] | 7.40 | Bonica (1974) | 7.38-7.41 | Rossier (1953) |
| | 7.38 | Cohen et al. (1970) | 7.42 | Sjöstedt (1962) |
| | 7.39 | Documenta Geigy (1969) | 7.40 | Stojanov (1972) |
| | 7.40 | Fadl and Utting (1969a) | | |
| | 7.40 | Friedberg (1980) | | |
| $pCO_2$ (mmHg) | 40.0 | Astrup (1957) | 39.0 | *Respiration and Circulation* (1971) |
| | 37.3[e] | Boutourline-Young and Boutourline-Young (1956) | 40.0 | Rossier and Hotz (1953) |
| | 36.4 | Documenta Geigy (1969) | 37.1 | Sjöstedt (1962) |
| | 39.2 | Fadl and Utting (1969a) | 38.4 | Stojanov (1972) |
| | 39.0 | Friedberg (1980) | | |
| Bicarbonate (mmol/liter) | 24.0 | Bartels et al. (1972) | 26.0 | Gutsche (1979) |
| | 25.0 | Bonica (1974) | 24.1 | Prystowsky et al. (1961) |
| | 22.0-27.0 | Davenport (1973) | 23.9 | Sjöstedt (1962) |
| | 21.3-25.0 | Documenta Geigy (1969) | 23.5 | Stojanov (1972) |
| | 24.0-28.0 | Friedberg (1980) | | |
| Buffer base (mmol/liter) | 47.0 | Bonica (1974) | 46.2 | Sjöstedt (1962) |
| | 48.0 | Documenta Geigy (1969) | 48.1 | Stojanov (1972) |
| | 47.0 | Kirschbaum and DeHaven (1968) | | |

Table 1 (continued)

| | Value | Reference | Value | Reference |
|---|---|---|---|---|
| Base excess (mmol/liter) | +1.0 | Cohen et al. (1970) | −1.0 | Kirschbaum and DeHaven (1968) |
| | +1.0 | Documenta Geigy (1969) | | |
| | +0.1-0.6 | Fadl and Utting (1969a) | +1.1 | Sjöstedt (1962) |
| | | | −0.5 | Stojanov (1972) |
| Lactate (mmol/liter) | 0.85 | Hendricks (1957) | | |
| | 0.80-1.40 | Samueloff et al. (1961) | | |

[a]Blood gases, saturation, $O_2$ capacity, and pH are from arterial or arterialized blood.
[b]For women 20-30 years old.
[c]Calculated from the above data.
[d]Values rounded off to three significant figures.
[e]$paCO_2$.

Table 2 Values for Blood Gases and Acid-Base Data for Women in Late Pregnancy[a]

| | Value | Reference | Value | Reference |
|---|---|---|---|---|
| $pO_2$ (mmHg) | 106.1 | Andersen and Walker (1970) | 97.1 | Rooth and Sjöstedt (1962) |
| | 96.6 | Blechner et al. (1969) | | |
| | 92.0 | Lucius et al. (1970) | 96.8 | Schlick et al. (1977) |
| | 83.0 | Milewski and Schumann (1977) | 93.9 | Stojanov (1972) |
| | | | 96.2 | Vasicka et al. (1960) |
| | 103.0 | Romney et al. (1962) | | |
| $sO_2$ (%) | 97.2 | Blechner et al. (1969) | | |
| | 96.0 | Wulf (1962) | | |
| Hemoglobin (g %) | 11.5-13.5 | Göltner (1980) | | |
| | 11.0-13.0 | Hytten and Leitch (1964) | | |
| | 11.9 | Lucius et al. (1970) | | |
| | 12.3 | *Respiration and Circulation* (1971) | | |
| Hematocrit (vol %) | 34.0-41.0 | Göltner (1980) | | |
| | 35.8 | Hytten and Leitch (1964) | | |
| | 34.3 | Lucius et al. (1970) | | |
| | 37.5 | *Respiration and Circulation* (1971) | | |
| $O_2$ capacity (vol %) | 16.0 | Lucius et al. (1970) | | |
| | 15.0 | Metcalfe et al. (1972) | | |
| | 14.8 | Rossier and Hotz (1953) | | |
| | 14.6 | Wulf (1962) | | |
| $O_2$ content (vol %) | 14.0 | Wulf (1962) | | |
| $O_2$ affinity [$p_{50}$ (mmHg)] | 26.0 | Bartels (1970) | 28.6 | Meier et al. (1978) |
| | 26.2 | Hellegers and Schruefer (1961) | 25.0 | Prystowsky et al. (1969) |
| | 27.4 | Huch et al. (1981) | 26.3-26.5 | Wulf et al. (1966b) |
| | 28.0 | Lucius et al. (1970) | | |

Table 2 (continued)

| | Value | Reference | Value | Reference |
|---|---|---|---|---|
| pH[b] | 7.40 | Andersen and Walker (1970) | 7.47 | Lucius et al. (1970) |
| | 7.42-7.44 | Bartels et al. (1972) | 7.41 | MacRae and Palavradji (1967) |
| | 7.42 | Blechner et al. (1969) | 7.46 | Rooth and Sjöstedt (1962) |
| | 7.43 | Cohen et al. (1970) | | |
| | 7.44 | Derom (1969) | 7.42 | Rossier and Hotz (1953) |
| | 7.44 | Friedberg (1980) | 7.43 | Schlick et al. (1977) |
| | 7.44 | Lim et al. (1976) | 7.44 | Sjöstedt (1962) |
| | | | 7.43 | Stojanov (1972) |
| $pCO_2$ | 31.9 | Andersen and Walker (1970) | 31.3 | MacRae and Palavradji (1967) |
| (mmHg) | 28.7 | Blechner et al. (1969) | | |
| | 30.9[c] | Boutourline-Young and Boutourline-Young (1956) | 26.4 | Milewski and Schumann (1977) |
| | 32.8 | Cohen et al. (1970) | 30.8 | Rooth and Sjöstedt (1962) |
| | 32.0 | Derom (1969) | | |
| | 31.0 | Friedberg (1980) | 33.2 | Rossier and Hotz (1953) |
| | 27.3 | Lim et al. (1976) | | |
| | | | 30.5 | Schlick et al. (1977) |
| | | | 32.1 | Sjöstedt (1962) |
| | | | 33.6 | Stojanov (1972) |
| Bicarbonate | 21.0 | Bartels et al. (1972) | 21.2 | MacRae and Palavradji (1967) |
| (mmol/liter) | 21.0 | Bonica (1974) | | |
| | 22.3 | Derom (1969) | 21.6 | Milewski and Schumann (1977) |
| | 22.0 | Gutsche (1979) | | |
| | 20.0-24.0 | Friedberg (1980) | 20.8 | Prystowsky et al. (1961) |
| | 21.0 | Lucius et al. (1970) | | |
| | | | 21.1 | Schlick et al. (1977) |
| | | | 21.4 | Sjöstedt (1962) |
| | | | 23.0 | Stojanov (1972) |
| Buffer base | 42.0 | Bonica (1974) | | |
| (mmol/liter) | 45.4 | Derom (1969) | | |
| | 43.8 | Sjöstedt (1962) | | |
| | 45.9 | Stojanov (1972) | | |
| Base excess | −3.2 | Andersen and Walker (1970) | −3.5 | Milewski and Schumann (1977) |
| (mmol/liter) | −3.0 | Bartels et al. (1972) | | |
| | −1.2 | Cohen et al. (1970) | +0.2 | Rooth and Sjöstedt (1962) |
| | −2.2 | Derom (1969) | | |
| | −3.5 | MacRae and Palavradji (1967) | −3.3 | Schlick et al. (1977) |
| | | | 0 | Sjöstedt (1962) |
| | | | −1.1 | Stojanov (1972) |
| Lactate | 1.3 | Hendricks (1957) | | |
| (mmol/liter) | 0.8-1.0 | Marx and Greene (1964) | | |
| | 1.7-3.3 | Samueloff et al. (1961) | | |
| | 1.5 | Schmid (1973) | | |

[a]Blood gases, saturation, $O_2$ capacity and pH are from arterial or arterialized blood.
[b]Values rounded off to three significant figures.
[c]$paCO_2$.

Maternal $pO_2$ in pregnancy is sufficiently high to saturate hemoglobin almost completely. Values for the $sO_2$ range between 95 and 97% (Table 2). However, the $O_2$ capacity of maternal blood and consequently its $O_2$ content are reduced compared to nonpregnant women. The relative decrease in $O_2$ capacity caused by a reduction in hemoglobin concentration starts in early pregnancy (Hytten and Leitch, 1964). This is a result of a disproportionate increase in plasma volume with respect to the increase in erythrocytes. Despite physiologically low hemoglobin concentration and hematocrit values in pregnancy, the total amount of hemoglobin in pregnancy is increased (Hytten and Leitch, 1964).

There is no general agreement about changes in oxygen affinity (usually expressed as $p_{50}$, the value of $pO_2$ in millimeters of mercury which is sufficient to saturate 50% of the hemoglobin). This lack of agreement may be due to differences in the conditions under which investigations were carried out. Bauer et al. (1969) and Meier et al. (1978) have reported a significant decrease in oxygen affinity during pregnancy. Our own recent investigations show high $p_{50}$ values (R. Huch and A. Huch, unpublished data) favoring the fetus. However, unchanged affinity during pregnancy or only a slight variation of affinity in comparison to nonpregnant women has also been reported (Prystowsky et al., 1969; Lucius et al., 1970; Wulf et al., 1966b). What is agreed upon is that maternal $p_{50}$ is significantly higher than fetal $p_{50}$ (see Table 4).

Plasma bicarbonate is reduced during pregnancy, probably as a result of the low blood $pCO_2$. There is a reduction in the renal threshold for bicarbonate secondary to diminished reabsorption of filtered bicarbonate (Kellerman, 1976). As a consequence of this, the plasma pH value, mainly dependent on the ratio of $pCO_2$ to plasma bicarbonate, remains relatively stable during pregnancy or shows only a mild alkalotic tendency. Theoretically this can also be calculated by using the known values for nonpregnant and pregnant subjects in the Henderson-Hasselbalch equation:

$$pH = pK + \log\left(\frac{[HCO_3^-]}{0.03 \times pCO_2}\right)$$

$$pH = 6.10 + \log\left(\frac{24}{39}\right) = 7.41 \quad \text{(nonpregnant)}$$

$$pH = 6.10 + \log\left(\frac{21}{32}\right) = 7.44 \quad \text{(pregnant)}$$

As a result of the decrease in plasma bicarbonate and the reduction in hemoglobin concentration, the buffer capacity of maternal blood during pregnancy decreases from a range of 46-47 mmol/liter (Table 1) to 43-43 mmol/liter (Table 2). The base deficit increases from a normal value of about 1 mmol/liter (Table 1) to 4 mmol/liter (Table 2) in uncomplicated pregnancy. There is controversy as to whether it is correct to describe this as an accompanying compensatory acidosis (Crawford, 1975) or to consider this value as the zero point in normal pregnancy (Derom, 1968).

Reduced plasma bicarbonate values and low $pCO_2$ values are a disadvantage when compensation has to be made for any complication of pregnancy causing metabolic acidosis, for example, lactic acidosis with eclampsia. However, in the uncomplicated pregnancy, there is no evidence of a lactic acidosis. The reported values are low and show only a slight tendency, if any, to increase throughout pregnancy (Hendricks, 1957; Marx and Greene, 1964; Schmid, 1973).

## CHANGES IN BLOOD GASES, BLOOD COMPARTMENTS, AND ACID-BASE STATUS IN THE MOTHER DURING LABOR

According to the above-described blood gas changes and alterations in acid-base status, the mother starts labor in a state of almost fully compensated respiratory alkalosis. During labor there are the additional effects upon maternal acid-base balance of pain-induced breathing changes, uterine contractions, physical work (especially in the second stage of labor), and the metabolic and respiratory influences of the fetus, whose homeostasis is significantly altered by the uterine contractions.

With the beginning of labor, the dominant mechanism for maintaining the maternal acid-base balance in pregnancy changes. Ventilation increases markedly, despite the existing hyperventilation of pregnancy (Bonica, 1974, Lehmann et al., 1972). According to the investigations of Lehmann et al. (1972), the mean respiratory minute volume of 11.8 liters at the beginning of labor increases to a mean of 23.4 liters at the end of the second stage. This results in increased hyperventilation with even lower $pCO_2$ values (Table 3). However, to a great extent a compensatory metabolic acidosis which occurs at this time tends to maintain acid-base homeostasis. There is general agreement that the compensated or partly compensated respiratory alkalosis during pregnancy changes to a compensated or partly compensated metabolic acidosis during labor. Base deficit increases during labor, lactic acid increases significantly, and pH becomes more acidotic (Table 3). The extent of metabolic acidosis is positively correlated with the intensity of the contractions (Rooth, 1964, Rooth and Nilsson, 1964).

There is still some doubt as to the extent to which local lactate production from the active myometrium is responsible for the increase of lactic acid, compared to the lactate produced by voluntary muscular activity of the mother. It is reasonable to assume that a significant component of the acidosis stems from the normal increase in lactate that occurs during heavy physical work. A total energy expenditure of about 1200kcal per 8 hr of labor was calculated (Lehmann et al., 1972). This corresponds to the physical work of about 50 W as measured by an ergometer. The results from the investigation of Erkkola and Rauramo (1976) also support this theory. These authors were able to show that the mean pH value in physically fit women was significantly higher than that in women who were physically unfit. Also, the level of lactic acid in the mother after a work test was correlated with the levels of lactic acid in the umbilical vessels.

In the very late phase of the second stage of labor, it is possible that exclusive dead space ventilation with panting or apnea while pushing results in maternal hypoxemia and adds to the lactate production. At the very least, this breathing technique in the very end of labor diminishes the large decrease in $pCO_2$ in some of the investigated populations due to the hyperventilation already described (Table 3).

Despite the consideration of only so-called normal pregnant patient populations, great variations can be seen among the data reported. This is much more notable during labor (Table 3). Explanations for this increased scatter include the different obstetrical situations, the different degrees of pain and fear of the parturient women, varying preparations to labor, different levels of physical fitnesses, and nonidentical times for investigation. Table 3 is an attempt to group the reported data according to uniform criteria, particularly the variables which change acutely during labor. It was especially problematic to group the values within the last column, that corresponding to the late second stage or delivery. Therefore meaningful comparisons of data

Table 3 Values for Blood Gases and Acid-Base Data in Women During Labor[a]

| | Onset of labor or early first stage | | Late first stage | | Late second stage or delivery | |
|---|---|---|---|---|---|---|
| $pO_2$ (mmHg) | 101.9-117.8 | Andersen and Walker (1970) | 104.4-111.8[b] | Andersen and Walker (1970) | 102.5-114.2[b] | Andersen and Walker (1970) |
| | 108.0 | Fisher and Prys-Roberts (1968) | 112.0 | Jouppila and Hollmén (1976) | 118.0 | Jouppila and Hollmén (1976) |
| | 110.0 | Jouppila and Hollmén (1976) | 96.1 | Wulf (1967) | 109.0 | Künzel and Wulf (1970) |
| | | | | | 104.9 | Schachinger (1980) |
| | 90.8 | Künzel and Wulf (1970) | | | 90.7 | Wulf (1962) |
| | 83.0 | Milewski and Schumann (1977) | | | 106.7 | Wulf (1966) |
| | | | | | 96.4 | Wulf et al. (1967) |
| | 90.0 | Newman et al. (1967a) | | | | |
| $SO_2$ (%) | | | | | 95.2 | Bruns (1961) |
| | | | | | 97.9 | Schachinger (1980) |
| Hb (g%) | 12.2 | Jacobson (1971) | | | 14.5 | Jacobson (1971) |
| Hct (vol%) | see Table 2 | (pregnant women) | | | | |
| $O_2$ capacity (vol %) | see Table 2 | (pregnant women) | | | 16.8 | Bruns (1961) |
| $O_2$ content (vol %) | see Table 2 | (pregnant women) | | | 16.1 | Bruns (1961) |
| $O_2$ affinity [$p_{50}$ (mmHg)] | see Table 2 | (pregnant women) | 30.5 | Bauer (1969) | | |
| pH | 7.43-7.46[b] | Andersen and Walker (1970) | 7.42-7.47[b] | Andersen and Walker (1970) | 7.46-7.52[b] | Andersen and Walker (1970) |
| | 7.45 | Cohen (1970) | 7.42 | Cohen (1970) | 7.45 | Bruns (1961) |
| | 7.42 | Fadl (1969a) | 7.43 | Fadl (1969a) | 7.37 | Cohen (1970) |
| | 7.45 | Feige (1977) | 7.42 | Fadl (1969b) | 7.39 | Derom (1968) |

| | | | | | | |
|---|---|---|---|---|---|---|
| pH | 7.48 | Fisher and Prys-Roberts (1968) | 7.50 | Jouppila and Hollmén | 7.45 | Erkkola (1976) |
| | | | 7.46 | Künzel (1976) | 7.41 | Fadl (1969a) |
| | 7.45 | James (1973) | 7.45 | Pearson (1974) | 7.34 | Fischer (1965) |
| | 7.45 | Jouppila and Hollmén (1976) | 7.48 | Wulf et al. (1967) | 7.43 | James (1973) |
| | | | | | 7.39 | Jouppila and Hollmén (1976) |
| | 7.53 | Künzel and Wulf (1970) | | | | |
| | 7.42 | MacRae (1967) | | | 7.45 | Künzel and Wulf (1970) |
| | 7.45 | Milewski and Schumann (1977) | | | 7.46 | Künzel (1976) |
| | | | | | 7.40 | MacRae (1967) |
| | 7.41 | Newman et al. (1967c) | | | 7.43 | Pearson (1974) |
| | 7.43 | Pearson (1974) | | | 7.43 | Rooth (1972) |
| | 7.46 | Rooth (1972) | | | 7.39 | Rooth (1972) |
| | | | | | 7.34 | Schachinger (1980) |
| | | | | | 7.44 | Wulf (1962) |
| | | | | | 7.34 | Wulf (1966) |
| | | | | | 7.45 | Wulf et al. (1967) |
| $pCO_2$ (mmHg) | 30.7-24.6[b] | Andersen and Walker (1970) | 31.2-23.9[b] | Andersen and Walker (1970) | 22.3-16.2[b] | Andersen and Walker (1970) |
| | 30.0 | Cohen (1970) | 31.5 | Cohen (1970) | 26.0 | Bruns (1961) |
| | 31.0 | Fadl (1969a) | 27.5 | Fadl (1969a) | 31.5 | Cohen (1970) |
| | 25.5 | Fisher and Prys-Roberts (1968) | 31.0 | Fadl (1969b) | 27.1 | Derom (1968) |
| | | | 22.0 | Jouppila and Hollmén (1976) | 28.6 | Fadl (1969a) |
| | 28.0 | Jouppila and Hollmén (1976) | | | 27.0 | Fischer (1965) |
| | | | 27.0 | Künzel (1976) | 24.0 | Jouppila and Hollmén (1976) |
| | 31.9 | MacRae (1967) | 28.6 | Pearson (1974) | | |
| | 26.3 | Milewski and Schumann (1977) | 23.7-20.0[b] | Reid (1966)[c] | 27.0 | Künzel (1976) |
| | | | | | 30.3 | MacRae (1967) |
| | 33.8 | Newman et al. (1967a) | 23.7 | Wulf et al. (1967) | 29.9 | Newman et al. (1967a) |
| | 28.3 | Newman et al. (1967c) | | | 27.6[c] | Pearson (1974) |
| | 31.8[c] | Pearson (1974) | | | 25.5 | Reid (1966)[c] |
| | 31.6 | Reid (1966) | | | | |
| | | | | | 25.0 | Rooth (1973) |
| | 26.0 } | Rooth (1973) | | | 27.0 | Rooth (1973) |
| | 25.0 } | | | | | |

Table 3 (continued)

| | Onset of labor or early first stage | | Late first stage | | Late second stage or delivery | |
|---|---|---|---|---|---|---|
| $pCO_2$ (mmHg) | | | | | 27.1 | Schachinger (1980) |
| | | | | | 36.4 | Wulf (1962) |
| | | | | | 20.9 | Wulf et al. (1967) |
| Bicarbonate (mmol/liter) | 21.2 | MacRae (1967) | 25.7 | Wulf (1967a) | 19.8 | Bruns (1961) |
| | 21.6 | Milewski and Schumann (1977) | | | 18.2 | Derom (1968) |
| | | | | | 16.3 | Fischer (1965) |
| | | | | | 20.3 | MacRae (1967) |
| | | | | | 20.9 | Wulf (1962) |
| | | | | | 19.7 | Wulf (1966) |
| | | | | | 22.0 | Wulf et al. (1967) |
| Buffer base (mmol/liter) | | | | | 44.2[d] | Sjöstedt (1962) |
| Base excess (mmol/liter) | −3.1- −5.1[b] | Andersen and Walker (1970) | −3.1- −3.9[b] | Andersen and Walker (1970) | −6.6- −8.5[b] | Andersen and Walker (1970) |
| | −1.8 | Cohen (1970) | −2.5 | Cohen (1970) | −5.2 | Cohen (1970) |
| | −3.6 | Fadl (1969) | −4.7 | Fadl (1969) | −5.1 | Fadl (1969) |
| | −3.5 | Feige (1977) | −3.0 | Fadl (1969a) | −6.6 | James (1973) |
| | −5.5 | James (1973) | −3.1 | Jouppila and Hollmén (1976) | −8.2 | Jouppila and Hollmén (1976) |
| | −2.8 | Jouppila and Hollmén (1976) | −3.1 | Künzel (1976) | −3.2 | Künzel and Wulf (1970) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | −1.3 | Künzel and Wulf (1970) | −2.7 | Pearson (1974) | −3.4 | Künzel (1976) |
| | −3.3 | MacRae (1967) | −1.7 | Wulf et al., (1967) | −4.8 | MacRae (1967) |
| | −3.5 | Milewski and Schumann (1977) | | | −7.4 | Newman et al. (1967b) |
| | | | | | −4.2 | Pearson (1974) |
| | −4.5 | Newman et al. (1967b) | | | −7.0 | Rooth (1972) |
| | −5.1 | Newman et al. (1967c) | | | −7.1 | |
| | −2.1 | Pearson (1974) | | | −2.1 | Wulf et al. (1967) |
| | −4.8 | Rooth (1972) | | | | |
| | −5.4 | | | | | |
| Lactate (mmol/ liter) | 1.4 | Jouppila and Hollmén (1976) | 1.7 | Jouppila and Hollmén (1976) | 3.4 | Derom (1968) |
| | 1.3 | Marx (1964) | 1.9 | Schmid (1973) | 2.1 | Hendricks (1957) |
| | 1.9 | Schmid (1973) | | | 3.1 | Jouppila and Hollmén (1976) |
| | | | | | 2.0 | Marx (1964) |
| | | | | | 3.3 | Rooth (1964) |
| | | | | | 2.8-5.9 | Samueloff (1961) |
| | | | | | 2.8 | Schachinger (1980) |
| | | | | | 3.9 | Schmid (1973) |
| | | | | | 4.3 | Wulf (1966) |

[a]Blood gases, saturation, $O_2$ capacity, and pH are from arterial or arterialized blood.
[b]First value between, second value during contractions.
[c]$paCO_2$.
[d]Without definite labor stage definition.

**Table 4** Fetuses during Labor (Scalp Blood) and Delivery (Cord Blood)

| | Early first stage | Late first stage | Second stage; late second stage delivery | Umbilical vein | Umbilical artery |
|---|---|---|---|---|---|
| $pO_2$ (mmHg) | 22.0 Hobel (1971)[a] | 22.0 Hobel (1971) | 17.5 Low (1979) | 22.4 Beer (1955) | 9.2 Beer (1955) |
| | 24.8 Künzel (1970) | 19.1 Low (1979) | 14.0 Modanlou (1974) | 29.0 Hobel (1971) | 18.0 Hobel (1971) |
| | 24.1 Lumley (1971) | 21.0 Modanlou (1974) | 17.3 Saling (1966) | 31.5 Jouppila (1976) | 22.5 Jouppila (1976) |
| | 17.0 Modanlou (1974) | 24.6 Renou (1968) | 17.2 Wulf (1967a) | 30.6 Künzel (1970) | 17.8 Künzel (1970) |
| | 23.0 Newman (1967b) | 18.4 Saling (1966) | | 31.2 Livnat (1978) | 22.0 Livnat (1978) |
| | 20.4 Saling (1966) | 22.9 Wulf (1967a) | | 26.8 Low (1979) | 15.6 Low (1979) |
| | | | | 28.2 Roemer (1976) | 18.1 Roemer (1976) |
| | | | | 29.5 Rooth (1962) | 18.0 Rooth (1962) |
| | | | | 24.4 Saling (1966) | 14.5 Saling (1966) |
| | | | | 24.2 Schachinger (1980) | 14.8 Schachinger (1980) |
| | | | | 29.3 Sjöstedt (1960) | 18.2 Sjöstedt (1960) |
| | | | | 28.9 Sjöstedt (1960a) | 16.9 Sjöstedt (1960a) |
| | | | | 20.7 Vasicka (1960) | 10.2 Vasicka (1960) |
| | | | | 31.9 Wulf (1962) | 10.6 Wulf (1962) |
| | | | | 30.3 Wulf (1966) | 18.4 Wulf (1966) |
| | | | | 27.3 Wulf (1967a) | 18.9 Wulf (1967a) |
| $SO_2$ (%) | 45.0 Newman (1967b) | 45.0 Fischer (1964) | 25.0 Fischer (1964) | 47.7 Beer (1955) | 13.7 Beer (1955) |
| | 43.3 Saling (1966) | 36.1 Saling (1966) | 36.0 Kastendiek (1979) | 49.0 James (1958) | 22.0 James (1958) |
| | | | 30.4 Saling (1966) | 66.0 Künzel (1970) | 20.0 Kastendiek (1979) |
| | | | 37.0 Towell (1976) | 70.0 Roemer (1976) | 36.0 Künzel (1970) |
| | | | | 61.5 Rooth (1957) | 35.5 Roemer (1976) |
| | | | | 56.0 Rooth (1963) | 34.0 Rooth (1957) |
| | | | | 50.3 Saling (1966) | 18.0 Rooth (1963) |
| | | | | 53.8 Schachinger (1980) | 20.2 Saling (1966) |
| | | | | 60.1 Sjöstedt (1960a) | 23.7 Schachinger (1980) |
| | | | | 61.0 Towell (1976) | 23.7 Sjöstedt (1960a) |
| | | | | | 36.0 Towell (1976) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Hb (g%) | 15.3 | Jacobson (1971) | | | 18.5 | Jacobson (1971) | 15.8 | Jacobson (1971) | 15.1 | Jacobson (1971) |
| | | | | | | | 15.8 | Künzel (1970) | 15.7 | Künzel (1970) |
| | | | | | | | 16.7 | Rooth (1957) | 15.7 | Schachinger (1980) |
| | | | | | | | 16.0 | Schachinger (1980) | | |
| Hct (vol%) | | | | | 54.0 | Towell (1976) | 48.6 | Künzel (1970) | 47.5 | Künzel (1970) |
| | | | | | | | 51.9 | Schachinger (1980) | 51.1 | Schachinger (1980) |
| | | | | | | | 50.9 | Towell (1976) | 50.6 | Towell (1976) |
| $O_2$ capacity (vol%) | | | | | | | 20.7 | Bruns (1961) | 20.7 | Bruns (1961) |
| | | | | | | | 23.0 | Bartels (1966)[b] | | |
| | | | | | | | 22.2 | Beer (1955)[b] | | |
| | | | | | | | 21.0 | Wulf (1967)[b] | | |
| | | | | | | | 22.0 | | | |
| $O_2$ content (vol%) | | | | | | | 10.6 | Beer (1955) | 2.9 | Beer (1955) |
| | | | | | | | 11.4 | Bruns (1961) | 5.6 | Bruns (1961) |
| | | | | | | | 13.8 | Künzel (1970) | 7.6 | Künzel (1970) |
| | | | | | | | 6.5 | Prystowsky (1959) | 3.9 | Prystowsky (1959) |
| | | | | | | | 12.3 | Rooth (1963) | | |
| | | | | | | | 14.3 | Wulf (1967) | | |
| $O_2$ affinity [$p_{50}$ (mmHg)] | | | | | | | 19.7 | Bauer (1969)[b] | | |
| | | | | | | | 22.0 | Beer (1955)[b] | | |
| | | | | | | | 20.0 | Hellegers (1961)[b] | | |
| | | | | | | | 21.0 | | | |
| | | | | | | | 19.0 | Prystowsky (1969) | | |
| | | | | | | | 21.0 | Rooth (1959)[b] | | |
| | | | | | | | 22.1 | Wulf (1966)[b] | | |
| | | | | | | | 22.8 | Wulf (1967)[b] | | |
| pH[c] | 7.29 | Beard (1965) | 7.29 | Beard (1965) | 7.24 | Beard (1965) | 7.29 | Beard (1965) | 7.24 | Beard (1965) |
| | 7.33 | Bretscher (1967) | 7.33 | Bretscher (1967) | 7.30 | Bretscher (1967) | 7.32 | Beer (1955) | 7.24 | Beer (1955) |
| | 7.35 | Caspi (1979) | 7.34 | Caspi (1979) | 7.24 | Caspi (1979) | 7.33 | Bretscher (1967) | 7.27 | Bretscher (1967) |
| | 7.37 | Feige (1977) | 7.30 | Fischer (1965) | 7.18 | Fischer (1965) | 7.41 | Bruns (1961) | 7.39 | Bruns (1961) |
| | 7.36 | Hobel (1971) | 7.36 | Hobel (1971) | 7.32 | Hobel (1971) | 7.31 | Caspi (1979) | 7.25 | Caspi (1979) |

Table 4 (continued)

| | Early first stage | Late first stage | Second stage; late second stage delivery | Umbilical vein | Umbilical artery |
|---|---|---|---|---|---|
| pH[c] | 7.32 James (1973) | 7.36 Jouppila (1976) | 7.25 James (1973) | 7.37 Erkkola (1976) | 7.31 Erkkola (1976) |
| | 7.31 Kubli (1966) | 7.30 Kubli (1966) | 7.37 Kastendiek (1979) | 7.26 Fischer (1965) | 7.20 Fischer (1965) |
| | 7.36 Kubli (1968) | 7.35 Künzel (1976) | 7.27 Kubli (1966) | 7.35 Hobel (1971) | 7.28 Hobel (1971) |
| | 7.40 Künzel (1970) | 7.30 Low (1979) | 7.31 Kubli (1968) | 7.35 Jouppila (1976) | 7.28 Jouppila (1976) |
| | 7.35 Lumley (1971) | 7.31 Modanlou (1974) | 7.37 Künzel (1976) | 7.31 Kubli (1966) | 7.28 Kastendiek (1979) |
| | 7.32 Modanlou (1974) | 7.34 Pearson (1974) | 7.27 Low (1979) | 7.35 Kubli (1968) | 7.24 Kubli (1966) |
| | 7.28 Newman (1967b) | 7.32 Renou (1968) | 7.30 Modanlou (1974) | 7.37 Künzel (1970) | 7.30 Kubli (1968) |
| | 7.32 Pearson (1974) | 7.31 Saling (1966) | 7.31 Pearson (1974) | 7.38 Künzel (1976) | 7.29 Künzel (1970) |
| | 7.37 } Rooth (1972) | 7.37 Wulf (1967a) | 7.30 } Rooth (1972) | 7.37 Livnat (1978) | 7.29 Künzel (1976) |
| | 7.35 } | | 7.28 } | 7.32 Low (1979) | 7.26 Lamberti (1972) |
| | 7.31 Saling (1966) | | 7.28 Saling (1966) | 7.36 Roemer (1976) | 7.27 Livnat (1978) |
| | 7.35 Schmid (1976) | | 7.35 Schmid (1976) | | 7.25 Low (1979) |
| | 7.30 Towell (1976) | | 7.26 Towell (1976) | 7.33 Rooth (1961) | 7.27 Roemer (1976) |
| | | | 7.31 Wulf (1967a) | 7.32 Rooth (1962) | 7.26 Rooth (1961) |
| | | | | 7.30 Saling (1966) | 7.25 Rooth (1962) |
| | | | | 7.30 Schachinger (1980) | 7.25 Saling (1966) |
| | | | | 7.29 Towell (1976) | 7.25 Schachinger (1980) |
| | | | | 7.37 Wulf (1962) | 7.33 Schmid (1976) |
| | | | | 7.35 Wulf (1964) | 7.23 Towell (1976) |
| | | | | 7.27 Wulf (1966) | 7.34 Wulf (1962) |
| | | | | 7.30 Wulf (1967a) | 7.26 Wulf (1964) |
| | | | | | 7.20 Wulf (1966) |
| | | | | | 7.24 Wulf (1967a) |
| $pCO_2$ (mmHg) | 44.0 Beard (1965) | 42.0 Beard (1965) | 44.0 Beard (1965) | 42.0 Beard (1965) | 52.0 Beard (1965) |
| | 40.7 Caspi (1979) | 33.1 Caspi (1979) | 44.2 Caspi (1979) | 44.9 Beer (1955) | 60.4 Beer (1955) |
| | 50.0 Hobel (1971) | 34.1 Fischer (1965) | 40.0 Fischer (1965) | 38.3 Caspi (1979) | 45.2 Caspi (1979) |
| | 38.9 Künzel (1970) | 45.0 Hobel (1971) | 48.0 Hobel (1971) | 42.0 Fischer (1965) | 57.0 Fischer (1965) |
| | 44.3 Lumley (1971) | 39.0 Jouppila (1976) | 49.4 Khazin (1971) | | |
| | 46.0 Modanlou (1974) | 39.0 Künzel (1976) | 39.0 Künzel (1976) | 39.0 Hobel (1971) | 48.0 Hobel (1971) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| $pCO_2$ (mmHg) | 41.9 | Newman (1967) | 47.0 | Low (1979) | 51.0 | Low (1979) | 36.0 | Jouppila (1976) | 55.0 | James (1958) |
| | 46.5 | Newman (1967b) | 44.0 | Modanlou (1974) | 47.0 | Modanlou (1974) | 33.1 | Künzel (1970) | 40.5 | Jouppila (1976) |
| | 52.2 | Pearson (1974) | 49.2 | Pearson (1974) | 45.2 | Newman (1967) | 34.0 | Künzel (1976) | 45.1 | Künzel (1970) |
| | 37.0 } | Rooth (1972) | 44.6 | Renou (1968) | 54.0 | Pearson (1974) | 32.5 | Livnat (1978) | 43.0 | Künzel (1976) |
| | 41.0 } | | 49.1 | Saling (1966) | 46.0 | Rooth (1974) | 39.7 | Low (1979) | 44.2 | Lamberti (1972) |
| | 44.5 | Saling (1966) | 39.7 | Wulf (1967a) | 41.0 | Rooth (1972) | 39.4 | Roemer (1976) | 39.2 | Livnat (1978) |
| | 45.0 | Towell (1976) | | | 51.1 | Saling (1966) | | | 50.8 | Low (1979) |
| | | | | | 50.0 | Towell (1976) | 38.0 | Rooth (1961) | 50.7 | Roemer (1976) |
| | | | | | 44.7 | Wulf (1967a) | 39.6 | Rooth (1962) | 45.0 | Rooth (1961) |
| | | | | | | | 43.4 | Saling (1966) | 48.8 | Rooth (1962) |
| | | | | | | | 37.5 | Schachinger (1980) | 57.0 | Rooth (1963) |
| | | | | | | | 43.0 | Towell (1976) | 56.8 | Saling (1966) |
| | | | | | | | | | 47.7 | Schachinger (1980) |
| | | | | | | | 49.5 | Wulf (1962) | 56.0 | Towell (1976) |
| | | | | | | | 43.4 | Wulf (1964) | 60.5 | Wulf (1962) |
| | | | | | | | 40.8 | Wulf (1967a) | 52.7 | Wulf (1964) |
| | | | | | | | | | 49.6 | Wulf (1967a) |
| Bicarbonate (mmol/liter) | 19.6 | Beard (1965) | 19.3 | Beard (1965) | 17.2 | Beard (1965) | 18.6 | Beard (1965) | 18.1 | Beard (1965) |
| | 21.7 | Caspi (1979) | 17.3 | Caspi (1979) | 18.2 | Caspi (1979) | 19.4 | Caspi (1979) | 19.0 | Caspi (1979) |
| | 18.9 | Saling (1966) | 17.2 | Fischer (1965) | 13.6 | Fischer (1965) | 17.6 | Rooth (1961) | 14.8 | Rooth (1961) |
| | | | 18.9 | Saling (1966) | 16.5 | Saling (1966) | 16.6 | Saling (1966) | 15.6 | Wulf (1964) |
| | | | 23.0 | Wulf (1967a) | 19.7 | Wulf (1967a) | 16.9 | Wulf (1964) | 18.6 | Wulf (1966) |
| | | | | | | | 19.5 | Wulf (1966) | 17.9 | Wulf (1967a) |
| | | | | | | | 19.5 | Wulf (1967a) | | |
| Buffer base (mmol/liter) | 41.2 | Saling (1966) | 43.0 | Low (1979) | 41.1 | Low (1979) | 38.0 | Beer (1955) | 36.9 | Beer (1955) |
| | | | 40.9 | Saling (1966) | 36.9 | Saling (1966) | 41.4 | Low (1979) | 39.5 | Low (1979) |
| | | | | | | | 39.2 | Rooth (1961) | 34.7 | Rooth (1961) |
| | | | | | | | 41.7 | Rooth (1962) | 38.4 | Rooth (1962) |
| | | | | | | | 37.0 | Saling (1966) | 38.0 | Wulf (1964) |
| | | | | | | | 38.5 | Wulf (1964) | | |
| Base excess (mmol/liter) | −5.5 | Beard (1965) | −6.3 | Beard (1965) | −9.1 | Beard (1965) | −6.0 | Beard (1965) | −7.4 | Beard (1965) |
| | −3.8 | Feige (1977) | −9.0 | Fischer (1965) | −14.0 | Fischer (1965) | −4.0 | Hobel (1971) | −5.0 | Hobel (1971) |
| | −0.3 | Hobel (1971) | −1.0 | Hobel (1971) | −1.8 | Hobel (1977) | −6.7 | Jouppila (1976) | −6.8 | Jouppila (1976) |
| | −2.0 | Künzel (1970) | −3.1 | Jouppila (1976) | −6.4 | Kastendiek (1979) | −3.3 | Künzel (1970) | −9.6 | Kastendiek (1979) |

Table 4 (continued)

| | Early first stage | Late first stage | Second stage; late second stage delivery | Umbilical vein | Umbilical artery |
|---|---|---|---|---|---|
| Base excess (mmol/liter) | −3.4 Lumley (1971)<br>−7.0 Modanlou (1974)<br>−7.5 Newman (1967a)<br>−2.5 Newman (1967b)<br>−2.8 Pearson (1974)<br>−4.1 Rooth (1974)<br>−2.7 Rooth (1972)<br>−5.6 Saling (1966)<br>−4.5 Towell (1976) | −3.8 Künzel (1976)<br>−5.0 Modanlou (1974)<br>−2.4 Pearson (1974)<br>−5.7 Saling (1966)<br>−1.2 Wulf (1967a) | −3.3 Künzel (1976)<br>−7.0 Modanlou (1974)<br>−7.4 Newman (1967a)<br>−4.5 Pearson (1974)<br>−3.6, −4.3 Rooth (1972)<br>−10.1 Saling (1966)<br>−5.4 Towell (1976)<br>−5.8 Wulf (1967a) | −5.2 Künzel (1976)<br>−3.4 Roemer (1976)<br>−7.7 Rooth (1961)<br>−6.2 Rooth (1962)<br>−1.0-10.0 Rooth (1963)<br>−4.3 Rooth (1964)<br>−9.5 Saling (1966)<br>−6.4 Towell (1976)<br>−8.1 Wulf (1964)<br>−6.4 Wulf (1967a) | −4.8 Künzel (1970)<br>−6.1 Künzel (1976)<br>−6.8 Lamberti (1972)<br>−8.6 Livnat (1978)<br>−3.7 Roemer (1976)<br>−12.2 Rooth (1961)<br>−9.4 Rooth (1962)<br>−2.0-19.0 Rooth (1963)<br>−5.9 Rooth (1964)<br>−5.7 Towell (1966)<br>−8.8 Wulf (1964)<br>−8.4 Wulf (1967a) |
| Lactate (mmol/liter) | 1.7 Low (1979)<br>2.0 Schmid (1973) | 2.0 Schmid (1973) | 2.9 Schmid (1973) | 2.8 Hendricks (1957)<br>2.8 Jouppila (1976)<br>4.1 Rooth (1962)<br>2.7 Rooth (1964)<br>2.1 Schachinger (1980)<br>3.3 Wulf (1966) | 3.8 Hendricks (1957)<br>2.7 Jouppila (1976)<br>3.7 Low (1979)<br>4.6 Rooth (1962)<br>2.7 Rooth (1964)<br>2.1 Schachinger (1980)<br>3.5 Schmid (1973)<br>3.8 Wulf (1966) |

[a]References are given in the shortest possible form, in this table only, because of space limitations.
[b]No differentiation made between umbilical vein and artery.
[c]pH was rounded off to 3 significant figures, as in previous tables.

obtained in labor, even with only minimal differences with respect to the time of investigation, are impossible due to the varying length of this period. The reported interindividual differences in the various studies, which do not show up when citing only the mean values, are also considerable.

The influence of parity is also well documented. According to Rooth and Nilsson (1964), base deficit tends to be higher in primiparae than in the multiparae with a mean difference in normal cases of 1.1 mmol/liter. Derom (1968) performed detailed studies on the influence of parity and showed that lactate values are highest in primiparae and decrease with each birth. He reported a similar correlation for pH.

The other cause of the great variability in data—already mentioned briefly—is the length of labor, particularly the duration of the second stage of labor. This is clearly one of the factors which influences the extent of metabolic acidosis and contributes to the relationship to parity just described. According to our own studies (Kalinkov et al., 1981), lactate concentration increases in a linear fashion with the duration of the second stage of labor. In uncomplicated cases, we measured a mean of 2.65 mmol/liter after 10 min, 3.45 mmol/liter after 20 min, 4.02 mmol/liter after 30 min, and 4.51 mmol/liter after 40 min of pushing.

An additional very important factor limiting the ability to compare the results from different investigations is the fact that frequently no distinction has been made between blood sampling done between contractions and that done during contractions. Andersen and Walker (1970), who distinguished systematically between blood sampling at the peak of a contraction and that during a period of uterine relaxation, saw great differences (Table 3). Rapid changes in blood gases with consequent changes in acid-base balance are likely to occur in conjunction with the increased maternal ventilation accompanying each contraction. The respiratory minute volume in untrained and unmedicated women increased from a normal of 10 liters between contractions to 20 and even as high as 35 liters or more during peaks of contractions (Bonica, 1974). Andersen and Walker (1970) showed that $paO_2$ values in maternal blood vary by more than 15 mmHg relative to contractions; $paO_2$ values are always higher during a contraction. The latter fact may perhaps be one explanation for the large differences reported in $pO_2$ values in maternal arterial blood.

As previously mentioned, it seems likely that as a consequence of the special lung physiology of pregnant women, rapid blood gas changes happen "physiologically," which implies that a single blood gas analysis does not give reliable information about the normal behavior of blood gas and acid-base variables and that continuous techniques are needed to describe the normal pattern.

## Continuous Blood Gas and Acid-Base Measurements in the Mother During Labor

### Continuous $pO_2$ Measurements

Continuous recordings of $paO_2$ have shown that the $paO_2$ changes with painful contractions are much greater than previously recognized. With a technique described by Fabel (1968), the arterial $pO_2$ in seven women in labor was measured continuously in a brachial artery bypass (Wulf et al., 1972). Figure 1 shows a portion of trace from a continuous recording of $paO_2$. Note that $paO_2$ increases with each contraction, with fluctuations of 20 mmHg or more. In the upper third of Figure 1, $pO_2$ variations are shown in the first stage of labor. The middle tracing shows even greater variations during the second stage of labor without pushing. The lower tracing was recorded just

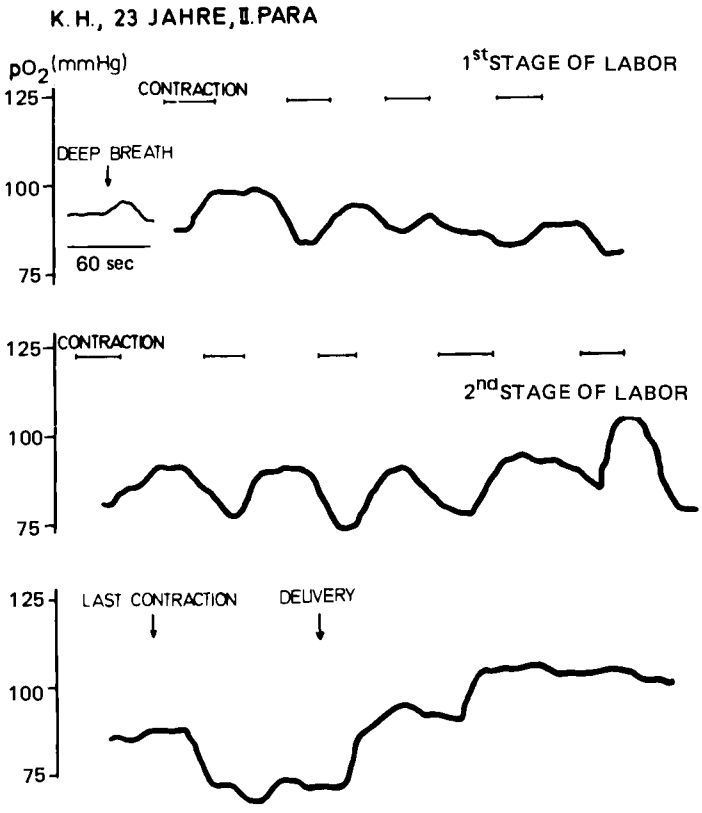


Figure 1 Excerpts of continuous recordings of maternal arterial $pO_2$ during the first and second stage of labor and during delivery. (From Wulf et al., 1972.)

before and after delivery. The $pO_2$ decreases because of the apnea during pushing. After delivery of the infant's head a slight increase in $pO_2$ results, but with the final expulsive effort the $pO_2$ falls again. It then increases steeply after delivery to a level consistent with the postpartum hyperventilation. The fluctuations shown in Figure 1 illustrate how difficult it is to define a "normal" value with a single blood gas value.

Systematic studies of the dynamic behavior of maternal arterial $pO_2$ during labor in a large series of patients (Dudenhausen et al., 1974; Huch et al., 1974, 1977) have only become possible with the development of a noninvasive continuous technique to measure $pO_2$, that is, transcutaneous $pO_2$ ($tcpO_2$). This technique measures the $pO_2$ across the intact skin overlying a hyperperfused capillary bed. Hyperperfusion is obtained by local application of heat through the electrode. Comparative arterial-transcutaneous measurements have previously shown a good correlation in populations of relatively healthy young females (Huch et al., 1974).

Measurements with the transcutaneous $pO_2$ electrode in 120 parturient women have confirmed the above-described patterns in relation to contractions. Typically breathing increases in depth and frequency during a painful contraction. Only when a woman is breathing regularly because of discipline or minimal pain does her $pO_2$ have a stable level. Hyperventilation usually results in a further decrease in $pCO_2$, causing

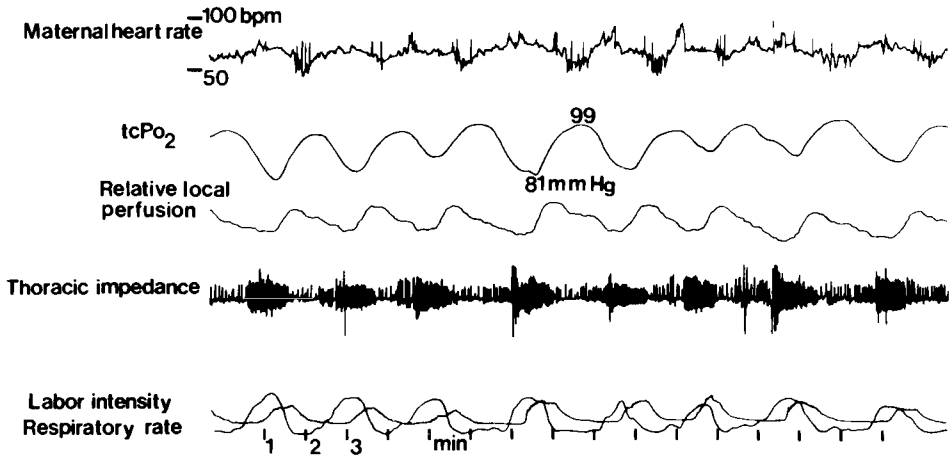


Figure 2 Excerpts of continuous and simultaneous recordings of the maternal heart rate, transcutaneous $pO_2$ ($tcpO_2$), relative local perfusion beneath the $pO_2$ electrode, transthoracic impedance, contractions, and maternal respiratory rate during painful dilatory contractions. Typical $tcpO_2$ fluctuations are due to irregular breathing.

consequent hypoventilation, which is quite normal as the result of chemoreceptorial breathing regulation. Figure 2 shows an excerpt from a $tcpO_2$ recording during painful contractions in the first stage of labor. The fluctuations range from 10 to 20 mmHg. One would not anticipate that these small fluctuations at the part of the hemoglobin dissociation curve where maternal blood is nearly completely saturated would be a significant disadvantage to fetal oxygenation.

According to our investigations, maternal transcutaneous $pO_2$ during labor is high as a result of the hyperventilation of pregnancy and additional hyperventilation and hyperpnea during labor. There is a tendency for maternal $pO_2$ to increase toward the end of labor. In Figure 3, five tracings were synchronized in respect to the time of the delivery of the infants. In all cases fluctuations with the contractions and a tendency for the values to increase are obvious. In addition, there is an impressive stabilization of $pO_2$ with the delivery of the infant. The degree of these fluctuations is considered normal, or physiological, as they were observed in nearly all labors.

When the woman hyperventilates excessively during contractions, there is an unexpectedly large decrease in $pO_2$ between the contractions which would not be apparent from a single blood gas analysis. We have measured in individual single cases arterial $pCO_2$ values down to 13 mmHg at the end of a contraction and have then recorded $pO_2$ patterns like the one shown in Figure 4. The elimination of $CO_2$ during excessive hyperventilation results in a transient apnea which may last throughout the interval between contractions. Hypoventilation or apnea can last until the initial $paCO_2$ level is reestablished. The tendency for a low $paO_2$ to persist during labor is aggravated by the reduced FRC of pregnancy. Pethidine, administered during labor for pain relief, tends to increase the duration of apnea or the tendency to hypoventilate, resulting in a further fall of $paO_2$ decreases (Huch et al., 1974). One must assume that the combination of hyperventilation and central sedation by drugs reduces even more the stimulus to reventilate the lungs between contractions. In

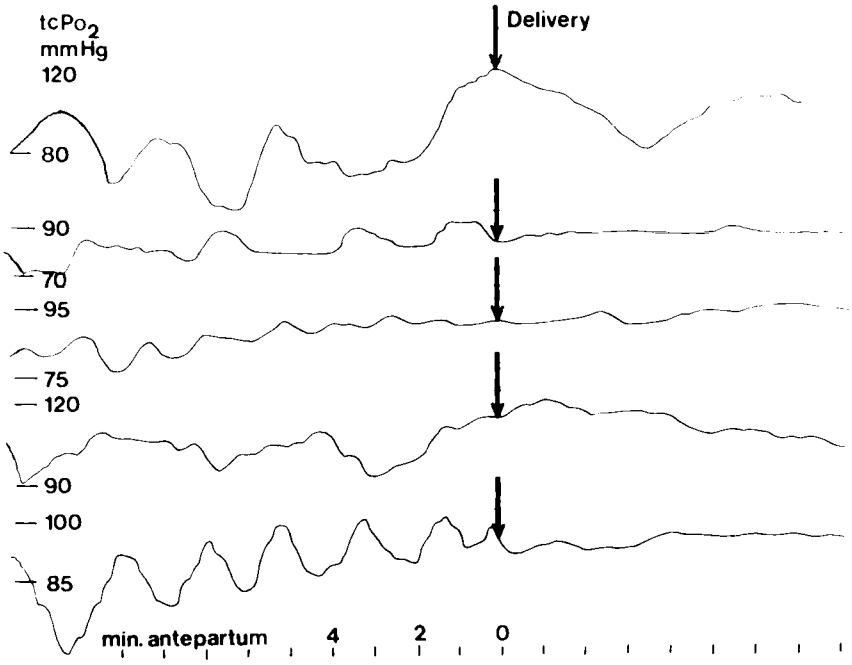


Figure 3 Excerpts of continuous recordings of the maternal transcutaneous $pO_2$ from five women before and immediately after delivery. Note the typical $tcpO_2$ fluctuations before delivery and the high and relatively stable $tcpO_2$ level after delivery.

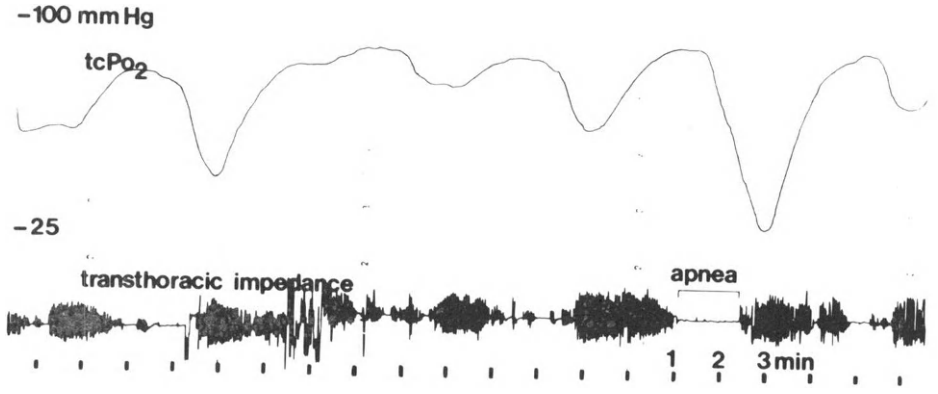


Figure 4 Excerpts of continuous recordings of transcutaneous $pO_2$ and transthoracic impedance from a woman during labor. A long-lasting apnea phase—a result of hyperventilation during the contraction and possibly of the potentiating effect of 100 mg of intramuscularly administered pethidine—leads to a $pO_2$ decrease down to 25 mmHg.

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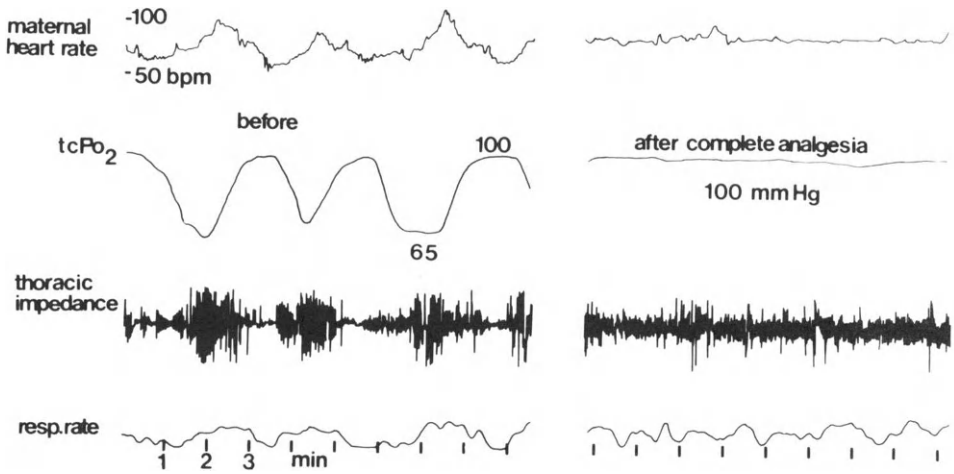


Figure 5 Excerpts of simultaneous continuous recordings of maternal heart rate, tcp$O_2$ transthoracic impedance, and respiratory rate before (left) and after (right) epidural anesthesia.

connection with this, it is worth mentioning that routinely administered doses of pethidine (50-100 mg intramuscularly) relatively seldom relieves pain (Rosen 1975). We will discuss the influence of these large maternal $pO_2$ decreases on fetal oxygenation later.

That pain is the cause of hyperventilation during the contractions and of the subsequent hypoventilation with the resulting $pO_2$ decrease was shown by Strasser et al. (1975). When complete relief from pain was achieved by epidural anesthesia, $pO_2$ was always stable. Figure 5 shows two excerpts from a parturient woman, one before and one after epidural anesthesia. On the left of Figure 5 the previously described $pO_2$ fluctuations are seen together with the varying breathing pattern in relationship to contractions. Constant breathing and $pO_2$ after pain relief are shown on the right of Figure 5. In the above-mentioned study even early fluctuation of $pO_2$ can be taken as a sign of reappearance of pain and insufficient anesthesia. Dudenhausen et al. (1974) confirmed these observations in a similar study.

In addition to complete relief of pain, another possible way to obtain high and stable maternal $pO_2$ values is by breathing instructions given during the interval between contractions. The patient in labor, exhausted from pain and breathing during the contraction and possibly sedated by pethidine or a similar drug, has to be actively encouraged to breathe in the pause between contractions. Even asking her to speak results in a rapid rise in $pO_2$. Figure 6 shows the marked effect of breathing instructions, without which irregular breathing with phases of apnea are seen. A relatively stable $pO_2$ level results when the patient is asked to breathe after each contraction.

### Continuous $pCO_2$ Measurements

Although the initial possibility of transcutaneous continuous $pCO_2$ measurement was reported in 1973 (Huch et al., 1973) only now, after technical problems have been overcome, have the first clinical trials started. Recently, promising results with maternal transcutaneous $pCO_2$ recordings have been reported by Huch's group (unpublished results, 1982). To our

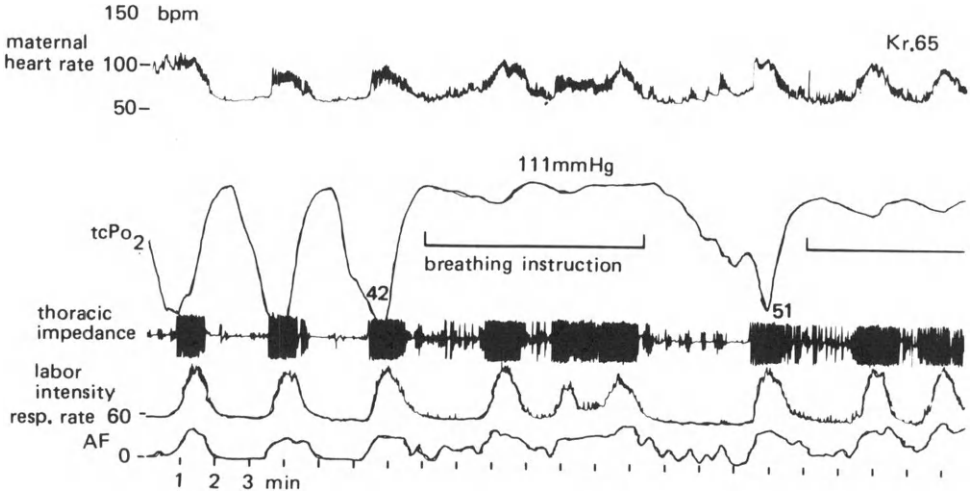


Figure 6 Excerpts of simultaneous continuous recordings of maternal heart rate, $tcpO_2$, transthoracic impedance, contractions, and respiratory rate from a woman during labor. Hypoventilation or apnea in the pause between contractions disappears after breathing instructions to the mother.

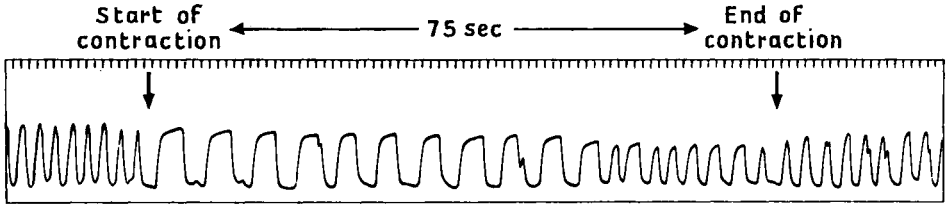


Figure 7 Excerpt of continuous recording of end-expiratory $CO_2$ from a woman during labor. Hyperventilation during the contraction results in a decrease in alveolar $CO_2$. (From Reid, 1966.)

knowledge, no continuous intraarterial $pCO_2$ measurements have yet been made in parturient women.

It is also possible to register semicontinuously end-expiratory alveolar $pCO_2$ with an infrared gas analyzer, with good approximation of arterial values. Reid (1966) published such $paCO_2$ recordings in women during labor. As with the continuous $pO_2$ technique, he saw distinct $pCO_2$ fluctuations related to contractions. At the beginning of labor, with infrequent and irregular contractions, little change in $paCO_2$ was noted. As soon as the contractions became more frequent and painful, there were significant $paCO_2$ decreases with contractions. Figure 7 shows a recording of end-expiratory $CO_2$ changes during a contraction 75 sec long. The plateau of the end tidal breath decreases from 25 to 18 mmHg. With increasing intensity and frequency, greater decreases with the contractions were seen. Reid observed the lowest values at the end of the first stage of labor, when hyperventilation was most pronounced. During the second stage frequent breath holding and less hyperventilation resulted in some return toward normal $paCO_2$ (Reid, 1966).

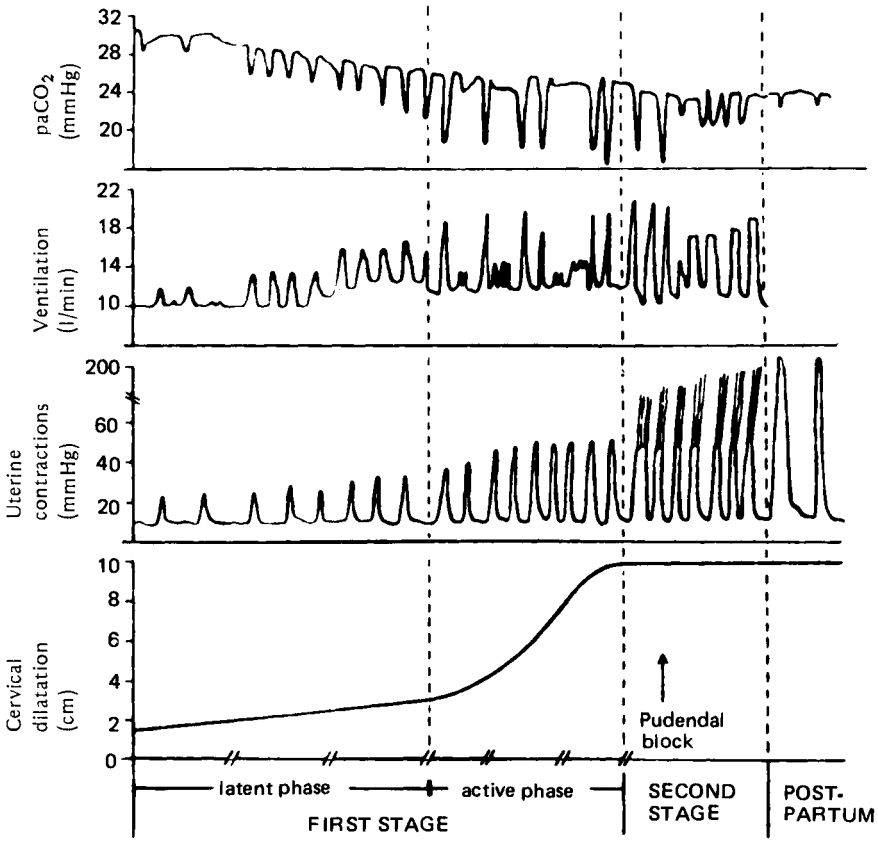


**Figure 8** Schematic presentation of the results from simultaneous recordings of end-expiratory $pCO_2$, minute ventilation, contractions, and cervical dilatation during labor in an unpremedicated primigravida showing the relationship between the frequency or intensity of contractions, changes in ventilation, and alveolar $pCO_2$. (From Bonica, 1973.)

Figure 8 is a schematic presentation of the results obtained from parturient women with the same end-expiratory $CO_2$ technique by Bonica (1973). The data is presented together with the recordings of respiratory minute volume, uterine contractions, and a description of the stage of labor. The longer labor lasted and the more intense the contractions became, the bigger was the parallel increase in ventilation and the resultant decrease in $paCO_2$. The schematic recordings in Figure 9 from Bonica (1973) demonstrate the close relationship between maternal hyperventilation and pain, which is similar to our own findings. The decrease in $paCO_2$ is only slightly reduced by the pain-relieving effect of pethidine. Only total relief from pain obtained by epidural anesthesia leads to a relatively constant respiratory minute volume and a constant $paCO_2$.

"Continuous" Lactic Acid Measurements

Semicontinuous lactate measurements in the parturient woman have been reported by Hendricks (1957). He started from the assumption that a large component of the uterine venous blood is rapidly forced back into the central venous reservoir and later

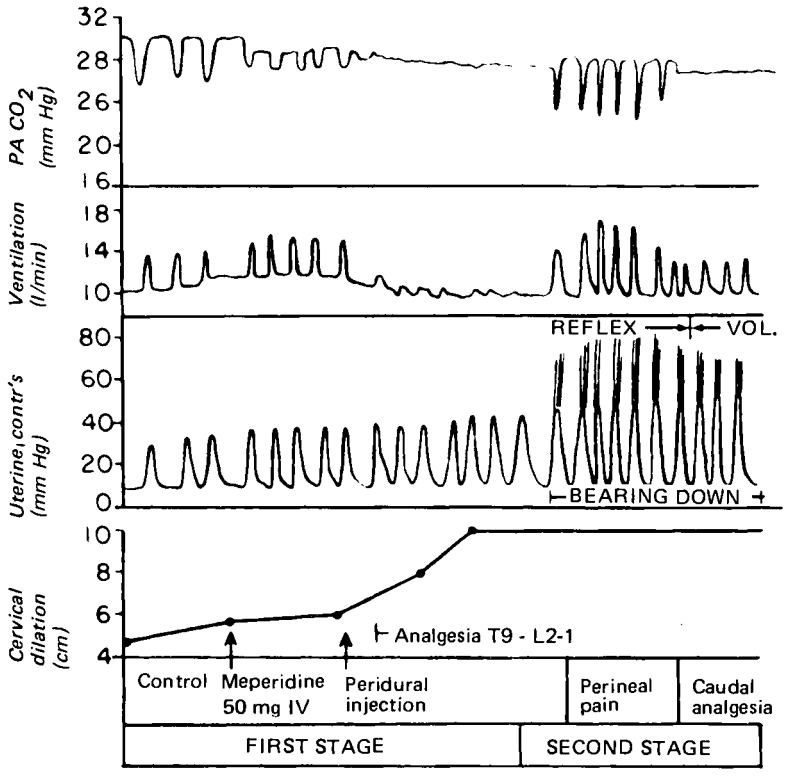


**Figure 9** Same schematic recordings as in Figure 8 showing the influence of pain and measures of pain relief on ventilation and alveolar $pCO_2$. As in Figure 5, complete analgesia leads to constant ventilation. (From Bonica, 1973.)

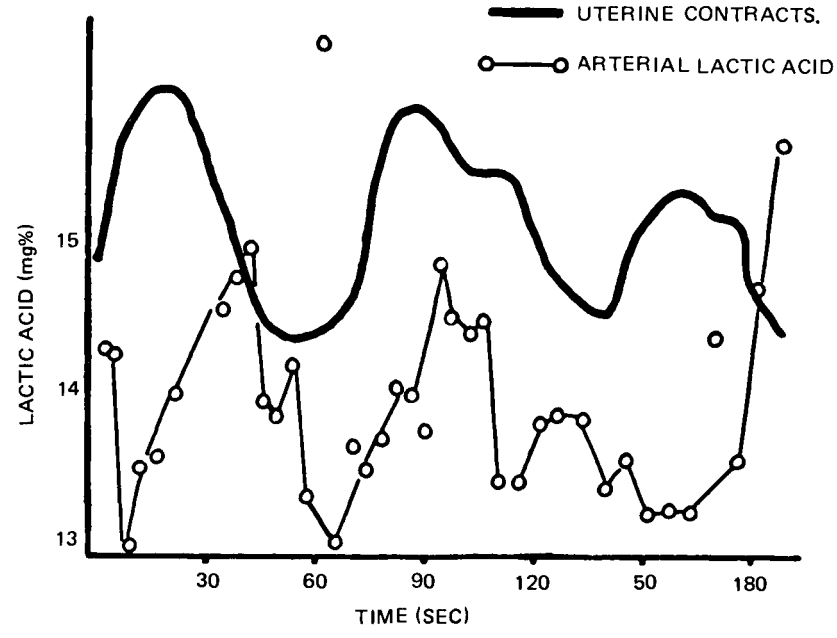


**Figure 10** Excerpt of continuous recording of contractions and semicontinuous registration of maternal arterial lactic acid in the brachial artery from a woman during labor. (From Hendricks, 1957.)

into the maternal systemic circulation with each uterine contraction. He argued that this should result in detectable fluctuations in maternal blood lactic acid. He did recordings in the following way: The brachial artery of women in labor was cannulated and connected to a sample collector. Arterial specimens were taken continuously and analyzed in 4-sec portions. Figure 10 shows a typical result. The curve of arterial lactic acid values mirrors the changes in contractions quite well. Comparable alterations in maternal lactic acid levels were found in each of seven so-called "flush-out" studies. These results are impressive because they show how fast even a metabolic variable can change. They clearly demonstrate that a substantial amount of lactate comes from the uterine circulation, although it is impossible to differentiate how much comes from the fetus, from the placenta, and from the uterus itself. These results are consistent with the results from the other continuous recordings and show quite clearly the impossibility of obtaining consistent values which can be used to define normality.

## CHANGES IN BLOOD GASES, BLOOD COMPARTMENTS, AND ACID-BASE STATUS IN THE FETUS DURING LABOR AND DELIVERY

To write an account of the status and changes which occur in the human fetus during pregnancy and labor is more difficult than for the mother. It is still not possible to study the blood gases and acid-base status of the human fetus in utero before labor under physiological conditions. Blood samples obtained in connection with elective cesarean sections in different gestational age periods may be altered by the manipulation of uterus and cord and by the changes induced by anesthesia or premedication.

Amniotic fluid is the only relatively accessible fetal milieu in pregnancy and analysis of amniotic fluid only gives a limited indication of the situation in utero. Starting with the pioneer work of Sjöstedt et al. (1958), several studies of blood gases and pH have been made with the hypothesis that such values might provide a good reflection of fetal oxygenation (Johnell et al., 1971; Kittrich and Janda, 1967; Schreiner et al., 1961; Schreiner and Bühlmann, 1962; Schreiner and Gubler, 1963; Schreiner, 1964; Sjöstedt et al., 1961; Vasicka, 1966). Although it had been shown that the $pO_2$ measured in the amniotic fluid seems to reflect the $pO_2$ of the subcutaneous tissue of the fetus (Sjöstedt et al., 1958) and that the $pCO_2$ corresponds to the value in the umbilical artery (Sjöstedt et al., 1961; Schreiner and Bühlmann, 1962), there is no agreement as to what extent the amniotic fluid values reflect acute changes in the fetus. Equilibrium between the fetus and the amniotic fluid is slow (Johnell et al., 1971). On the other hand, the $pO_2$ increase in amniotic fluid after maternal supplemental oxygen is no proof of this reflection as long as we cannot exclude that the $pO_2$ increase is not a result of oxygen diffusion from the maternal tissues. The measured pH decrease, the decrease of $pO_2$, and the increase of $pCO_2$ during the course of pregnancy were assumed by the above-mentioned authors to be due to progressive impairment of the supply of oxygen to the fetus toward the end of pregnancy.

We are limited to the results of animal studies, with all their limitations of extrapolations, as the only acceptable data from which the fetal situation can be evaluated. Only the studies in the chronic animal experiment, with indwelling catheters, have allowed sample collection from undisturbed fetuses and reproducible observations in steady-state conditions of the fetus. These results and the data now available from the human fetus at the beginning of labor have led us to question the extrapolation from the data in cord blood at delivery to the intrauterine situation. Such early observations resulted in the

concept of "Mount Everest in utero" (Barcroft, 1946); we now know that the only parallel that can be established is with respect to low oxygen partial pressures. There is no evidence for oxygen deprivation in the normal pregnancy. The fetus has, in comparison to the adult, a higher oxygen-carrying capacity due to a higher hemoglobin concentration (Table 4). Therefore, despite the low $pO_2$ values, the fetus has a relatively high oxygen content. Saturation of fetal blood is facilitated by the higher affinity it has to oxygen (Table 4). However, it should be mentioned that this characteristic is also a disadvantage for $O_2$ delivery in the fetal tissues.

Saling's concept of blood sampling from the presenting part during labor (Saling, 1966) has opened a possibility for new insights. We can now assess the fetal situation with respect to blood gases and acid-base balance before and not only at delivery. There was and still is debate (O'Connor et al., 1979) as to whether capillary blood from the presenting part of the fetus accurately represents the blood of the central fetal circulation. There is the limitation of the source of sampling and there may exist local influences caused by edema, stasis, caput formation, and errors in sampling; however, the majority of both animal and human studies have revealed a good correlation between scalp and central blood samples (Bowe et al., 1970; *Lancet,* 1968; Kubli, 1968; Saling, 1980). Scalp samples have also been correlated with cord blood values and the condition of the infant at birth.

In comparison to maternal blood, the $pCO_2$ is higher, $pO_2$ and $sO_2$ values are strikingly lower, and the pH is about 0.1 pH units more acidotic (Table 4). With a few exceptions, the metabolic parameters base deficit and lactate are similar in the fetus and in the mother. The existing maternofetal pH difference is therefore mainly the result of a higher $pCO_2$ in the fetus. On the basis of this data, the fetal status during labor may be described as a mixed respiratory and metabolic acidosis.

According to the data presented in Table 4, the fetal blood gases and acid-base status remain relatively constant during the entire first stage of labor in normal cases. Significant changes characterized by an increasing metabolic acidosis only become obvious in the second stage of labor, particularly in the late phase. Apart from this constancy in all the reported investigations, however, the great range of values observed in the "normal" fetus (as defined by heart rate and clinical criteria) must be considered. First, the same limitations in the comparability of data, already discussed in the maternal data, are valid, including different lengths of labor, obstetrical situations, and time periods. Also, in the fetus metabolic and respiratory influences change rapidly in the second stage of labor and therefore comparisons are particularly difficult at this time.

Klöck (1974) and Lamberti et al. (1972) reported from fetuses with normal heart rate recordings during the second stage of labor a linear correlation between the number of contractions and the change in base deficit in the umbilical artery. Roemer et al., (1976), and Wood et al., (1973) demonstrated clearly that the fall in actual pH in the second stage of labor is dependent upon time. Great interindividual differences in different studies of different patient populations by the same investigator again show how difficult it is to define a "normal" fetus as far as acid-base and blood gas statuses are concerned. It seems likely that the reduction or interruption of placental gas exchange varies considerably with the length and intensity of the contractions. A wide range of values in physiological situations usually reflect an unsteady state (James, 1973). One must assume that during labor a steady-state condition is only seldom reached, even when blood sampling is done in the interval between contractions. Lumley et al. (1971)

discussed in detail the reasons for the lack of agreement on normal values for fetal scalp blood. In addition to the reasons already discussed, they cited inaccuracies in the measurement and poor arterialization of the fetal scalp blood.

A major source of methodological error occurs in the determination of base deficit and this probably accounts for the wide range in "normal" values for this variable (Table 4). The magnitude of base deficit is overestimated by several millimoles per liter if the dependency on the actual oxygen saturation is not taken into account. This is a frequent source of error in studies where $pCO_2$ is determined indirectly with the Astrup technique and not measured with a glass electrode. In addition, an acute rise in $pCO_2$ can be the cause of a false increase of the base deficit level because of the redistribution of bicarbonate ions between blood and the interstitial fluid compartment (Jacobson and Rooth, 1971).

A base deficit value representative of the entire extracellular fluid is done best if a hemoglobin value of 5% with the Siggard-Andersen nomogram is used (Rooth and Jacobsen, 1971).

This error in methodology has been implicated by Jacobson and Rooth (1971) as the cause of the false impression that both mother and fetus develop metabolic acidosis during labor. They considered that under normal circumstances only the mother develops a true metabolic acidosis. After correction of the fetal blood base deficit values to the ones of the whole extracellular compartment, the authors showed that fetal base deficit only increases slightly.

For the fetus in particular, the normal range of blood gases and acid-base values is not only of academic interest. The definition of what is normal is necessary for the identification of the abnormal state. The obstetrician concerned about adequate fetal oxygenation must know these values. As proposed initially by Saling (1966), there is now general agreement that pH, the variable resulting from both metabolic and respiratory influences, is clinically the most reliable indicator of adequate fetal oxygenation. It is now generally accepted that the critical lower limit of pH lies between 7.25 and 7.20 pH units, tending to be lower in the second stage than in the first stage of labor.

There is ample evidence that in uncomplicated labors, fetal changes reflect those in the mother. Although parallel changes do not necessarily have the same etiology, much of the data supports the view that, with undisturbed placental function, some of the fetal changes are of maternal origin. Many of the cited studies in Table 4 show the obvious dominance of maternal influences in the maternofetal two-compartment system (Hickl, 1966; Künzel, 1974; Rooth et al., 1972; Wulf et al., 1967).

The proof that a close relationship exists between mother and fetus comes also from studies where the mother's blood gases or acid-base variables were changed artificially and these changes resulted in corresponding changes in the fetal blood. Maternal $pO_2$ has been varied by administration of high or low oxygen content gas mixtures (Lumley and Wood, 1973b; Prystowsky, 1959; Newman et al., 1967c; Wood et al., 1971; Wulf et al., 1972). In the fetus higher and lower $pO_2$ values compared to the usual air breathing have been obtained; also, changes in maternal arterial $pCO_2$ are almost immediately reflected in the fetus (Lumley and Wood, 1973b; Newman et al., 1967a; Rooth, 1980) as oxygen and carbon dioxyde exchange through the placenta by passive diffusion. A strong correlation between maternal and fetal values is therefore not surprising, as long as placental gas exchange is not impaired. However, during extreme hypo- and hyperventilation interpretation becomes complicated by simultaneously occurring alterations in placental blood flow resulting from hyper- and hypocarbia (Motoyama et al., 1978).

During attempts to influence fetal acidosis by administration of sodium bicarbonate to the mother (Caspi et al., 1979; Jacobson and Rooth, 1969; Kastendieck and Künzel, 1979; Rooth, 1964) the acid-base relationship between mother and fetus has been studied. Highly significant changes in pH, base deficit, and plasma bicarbonate were observed both in the mother and fetus. However, there was a significant time delay in the fetus (Caspi et al., 1979). The same results were obtained when correcting for maternal alkalosis with $NH_4Cl$ (Newman et al., 1967b). Therefore it has been recommended to measure the maternofetal pH gradient (Jacobson and Rooth, 1971; Rooth, 1964) or the gradient in base deficit (Beard, 1968) to evaluate the significance of a low fetal pH. Monitoring of the maternofetal difference is considered to be superior to evaluation of changes in the fetus alone, because it will discriminate between those changes due to the fetus and those due to the mother (Jacobson and Rooth, 1971). According to Rooth et al. (1973) a difference in maternofetal pH of less than 0.15 pH units is indicative of a well-oxygenated fetus.

Despite the same pH difference, the hydrogen ion concentrations of the mother and fetus may differ considerably, depending on the initial pH value. Furthermore, because fetal pH is influenced by both maternal and fetal $pCO_2$ changes, fetal pH is not exclusively a reflection of metabolic components.

Beard (1968) suggested the measurement of the maternofetal base deficit difference to discriminate between acidosis which is primarily fetal and that which is primarily maternal. He has found that there is normally a base deficit difference between the mother and fetus of about 2.3 mmol/liter, with the fetal value always exceeding the maternal value.

In clinical obstetrics neither of these two suggestions has become a routine, although the arguments are well accepted. One reason might be that even a pure maternal infusion acidosis (Wulf et al., 1967) is not considered to be harmless for the fetus. Rooth (1975) was able to show impressively that parallel to the increase of maternal acidosis, fetal $pCO_2$ increases due to the limited $CO_2$ elimination from fetal to maternal blood. He also showed that a strong correlation between fetal increasing $pCO_2$ values and falling $pO_2$ values exist. When faced with an acute asphyxial episode, the fetus has, without doubt, a smaller margin of reserve.

The fetal situation in labor may be summarized by the statement that in general changes in maternal blood gases and acid-base balance affect the fetal status, but that on certain occasions fetal blood gases and acid-base status may change independently. Situations where the parallel relationship between maternal and fetal status is lost include uterine contractions, which by abnormal intensity, frequency, or length dramatically compromise uterinoplacental blood flow, cord occlusions, or even more pathological situations, for example, abruptio placentae. Furthermore, when considering the relationship between the maternal and fetal state, one should realize that the fetus in utero has some independent abilities to adjust to maternal imbalance for a limited period of time (Dawes 1968) another explanation for the occasionally observed lack of correlation between the maternal and fetal state.

### Continuous Blood Gas and Acid-Base Measurements in the Fetus During Labor

Continuous measurements are particularly difficult to obtain in the human fetus during labor. It is impossible to cannulate a vessel or analyze expired gases. The only access is the presenting part of the fetus, normally the head. Therefore continuous recordings are only possible *in* or *on* fetal skin. It is unlikely that this limitation will ever be

overcome. In addition, application of measuring devices to the human fetus in utero requires more skill than applications to the newborn, and this must be regarded as an unavoidable source of error. Finally, for the reasons already discussed, standard or reference measurements are difficult to obtain accurately. This complicates the interpretation of the validity of a so measured variable as representative of the general condition of the fetus.

### Continuous Subcutaneous pH Measurements

Because fetal scalp blood sampling gives only intermittent information, a method capable of measuring pH continuously would be of great help in the early detection of fetal compromise and in the advancement of our knowledge of fetal acid-base physiology.

In obstetrics, Stamm and his colleagues (Stamm, 1975; Stamm et al., 1974) introduced the concept of continuous registration of a biochemical parameter during labor in the human fetus. The technique described was a miniaturized pH glass electrode placed together with the reference electrode in the subcutis of the fetal scalp. The first reports about excellent correlations between subcutaneous scalp pH and pH values in capillary heel blood in human newborn infants (Stamm, 1975) may have resulted in overexpectations of the technique in utero. Consequently the first results from the human fetus have proved less promising (Kubli et al., 1978). Initial problems were related to sterilization, calibration, fixation, drift, and broken tips which remained in the fetal scalp. As a result of these technical problems the poor correlation between subcutaneous pH and pH from fetal scalp blood was no surprise.

However, with increasing experience (Young et al., 1978; Uzan et al., 1978b) together with the technical improvements by the industry, the rate of good-quality records increased to 60-90% (Weber and Hahn-Pedersen, 1979; Lauersen et al., 1979). It was shown that the more experienced the operator was in the use of the technique, the better the correlation between subcutaneous and scalp pH (Lauersen et al., 1979).

Nowadays the tissue pH electrode is fixed with a double-helix electrocardiograph (ECG) screw electrode. This ECG electrode has a threaded core for the pH electrode. Before the pH electrode is introduced through the already fixed ECG electrode, an incision is made in the center of the ECG electrode with a 2.8-mm blade (Hochberg, 1978). The pH electrode is introduced into this incision in the fetal scalp.

Subcutaneous pH values are lower than scalp blood pH values in most physiological situations (Kubli et al., 1978). In pathological situations this may be reversed according to the first experiences with animal experiments (cited in Saling, 1979). There is evidence that in severe and acute complications the fall in tissue pH lags behind arterial pH (cited in Saling, 1979). In the majority of the studies, the correlation coefficients for subcutaneous and scalp blood pH range between 0.6 and 0.8 when all recordings were taken into account. When the acceptance of a tissue pH recording was based on the initial good correlation to a scalp blood sample done at the beginning of the recording, the correlation coefficient improved (Kubli et al., 1978).

Among the users there is general agreement that the method still needs considerable technical and medical skill, but that with increasing personal experience the effort will decrease and the results improve. Therefore it is too early to speculate about the exact future of the technique. Anecdotally, in some pathological situations during human labor the value of the method in the early detection of early fetal distress has been described (Kubli et al., 1978).

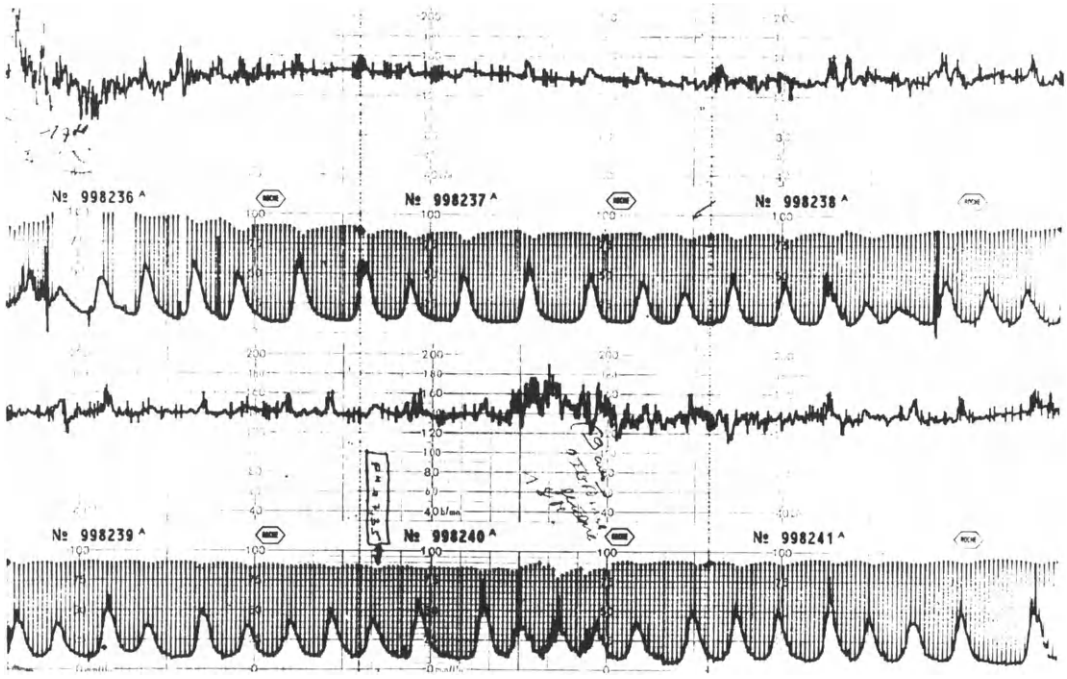


Figure 11 Excerpt of simultaneous continuous recordings of fetal heart rate, tissue pH, and uterine contractions showing frequent pH decreases during and after contractions. (From Uzan et al., 1978a).

Keeping with the main theme of this review, that continuous information might improve our knowledge about the physiological behavior of a variable, Figure 11 is shown to demonstrate the physiological fluctuations of pH in relationship to contractions (Uzan et al., 1978a). Recordings of fetal heart rate and uterine contractions are shown from a fetus during labor. The pH values are indicated by the height of the horizontal lines superimposed on the uterine contraction curve. For pH the contraction channel is calibrated from pH 7.00 to 7.40. Coincidental with the contractions, tissue pH falls by 0.03-0.05 pH units. An occasional rise might reflect maternal hyperventilation during the contraction, with the concomitant change of maternal and fetal pH toward alkalosis (Uzan et al., 1978a).

### Continuous Oxygen Saturation Measurements

For the evaluation of fetal oxygenation oxygen partial pressure, saturation, or content would seem to be the most relevant parameters to measure. However, extensive clinical experience with fetal scalp blood analysis has shown that pH determinations give a much more consistent prediction of the actual condition of the newborn immediately after delivery. It has been pointed out that inadequate scalp perfusion in the presence of a relatively large arteriovenous difference would give a false value and that oxygen partial pressure as well as saturation are much more subject to short-term variations

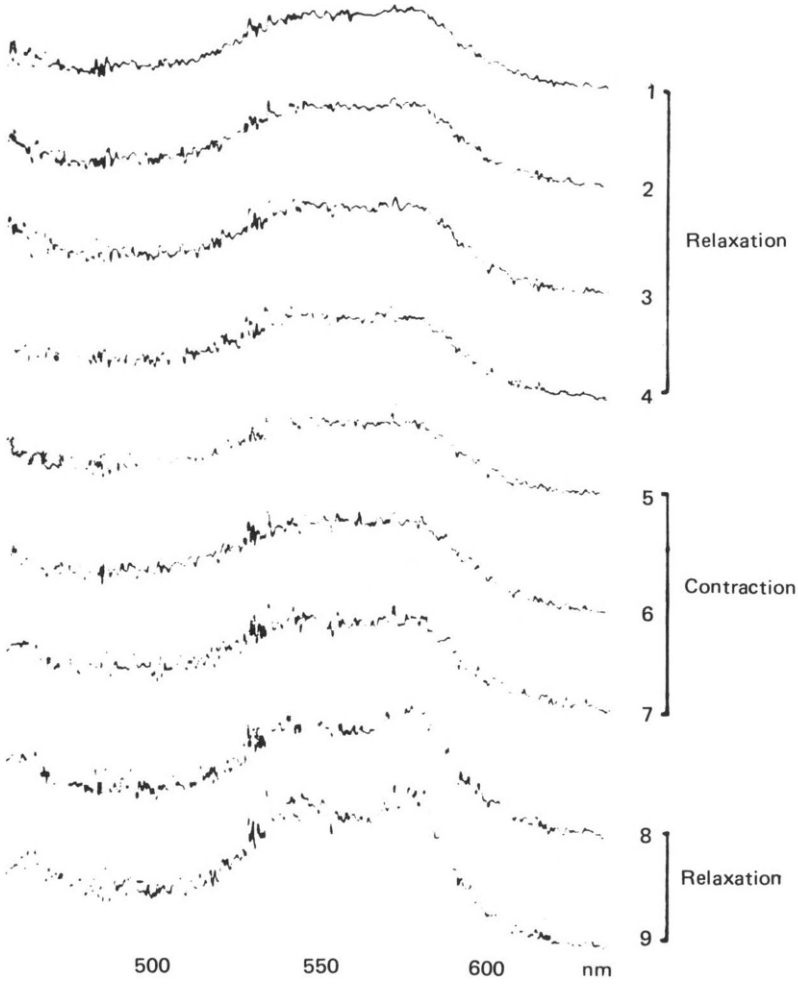


Figure 12 Reflectance spectra from the fetal scalp in between and during uterine contractions. (From Shimuzu et al., 1980.)

than pH. Therefore blood gas analysis is more like a snapshot without great diagnostic or prognostic significance (Lumley et al., 1971). In addition, because of the Bohr effect, changes in fetal pH may further limit the value of oxygen partial pressure measurements either in a single sample or as a continuous recording. In view of the hemoglobin dissociation curve for fetal blood, small variations in pH will lead to significant changes in $pO_2$ and may falsely be read as changes in oxygenation (Lumley and Wood, 1973a). In particular fetal acidosis based on chronic hypoxia may not be fully appreciated because $pO_2$ will be maintained at a relatively high level while saturation is low.

For this reason any technique to continuously measure oxygen saturation deserves special interest. Recently Shimuzu et al. (1980) reported their results with a reflectance spectrophotometer. Fiber bundle light with a wavelength between 450 and 650 nm is

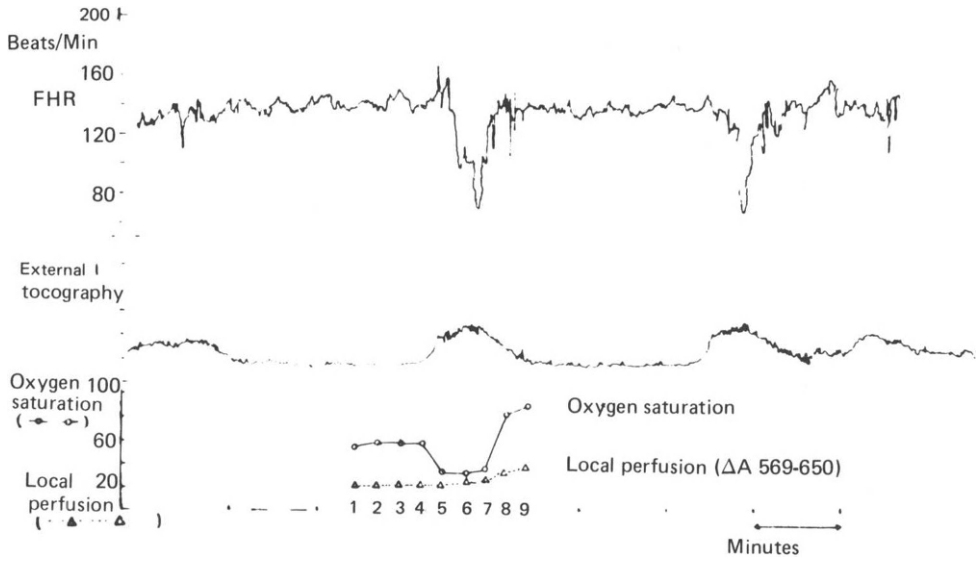


**Figure 13** Excerpt of simultaneous fetal heart rate and contractions together with the oxygen saturation behavior as a result of a contraction calculated from the spectra changes in Figure 12. (From Shimuzu et al., 1980.)

directed on the fetal skin through an amnioscope. Figure 12 shows the change in oxygen saturation in the fetal scalp during a contraction. The spectrum which is analyzed at 15-sec intervals tends to lose its double-peak appearance, which is typical for oxygenated blood. It becomes narrower, indicating a decrease in hemoglobin bound to oxygen. Using an appropriate calibration, the values can be converted into percentage of saturation for each 15-sec interval (Figure 13). In between contractions saturation values vary between 45 and 60% and drop to around 30% during a contraction. Of interest is a considerable overshot after the contraction. Shimuzu et al. (1980) have described this pattern as a characteristic change in the oxygenation of the fetal tissue in relation to uterine contractions when the fetal heart rate is not hypoxic. According to the authors, this technique is not ready for routine clinical application. Apparently the pressure with which the fiber bundle is applied to the tissue is critical for scalp perfusion. The biggest problem, however, with this technique, as with other reflectance measurements on the skin, is seen in the in vivo calibration which becomes necessary in view of a non-homogeneous distribution of hemoglobin in the tissue. Our own attempts with a rapid spectrophotometer (Huch et al., 1970) have been unsuccessful because the results could not be quantified.

### Continuous Oxygen Tension Measurements in and on the Skin

There have been several attempts to continuously record $pO_2$ from the fetal skin using needle electrodes which are all based on the polarographic principle (reviewed in Huch and Huch, 1977). However, these contributions were mostly limited to a description of the electrode and the reproduction of a few recordings. Generally speaking, the problems are related to the drift of these electrodes, their own oxygen consumption (which is comparatively high and related to the caliber necessary to introduce them into the skin),

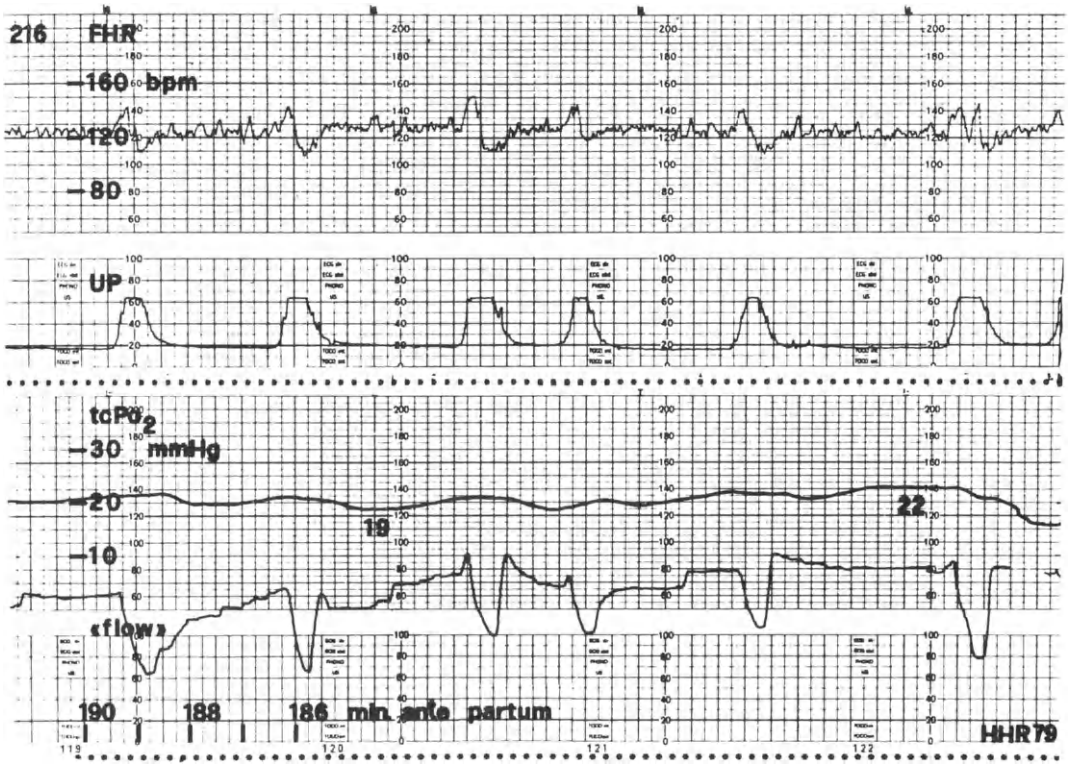


**Figure 14** Excerpts of simultaneous continuous recordings of fetal heart rate, contractions, fetal $tcpO_2$, and relative local perfusion "flow." As a result of contractions, "flow" decreases immediately, whereas $tcpO_2$ shows a slight decrease 40-50 sec after the onset of a contraction. (From Huch et al., 1981.)

unavoidable tissue trauma, and interference with the microcirculation. In addition, there is no way of calibrating the "bare electrode."

The development of the transcutaneous technique, which provides data with a currently widely accepted correlation with arterial $pO_2$ in healthy and sick newborn, for the first time offered the possibility to continuously and noninvasively measure fetal $pO_2$ sub partu, after membranes have been ruptured. The heated Clark electrode is fixed with either tissue glue or by vacuum on a few square millimeters of fetal scalp, which is prepared by removing the hair (Huch et al., 1977). Problems as well as potentials (O'Connor and Hytten, 1979) of the application in the fetus were rapidly recognized by several investigators.

Since transcutaneous $pO_2$ values are not only dependent on the arterial $pO_2$, but also on the need to raise the local perfusion of the skin, there is a tendency to record lower values from the presenting fetal part in labor when compared to central measurements. The occurrence of temporary disturbances of skin perfusion caused by the pressure of the electrodes against pelvic bones or tissues of the pelvic floor, as well as development of edema and stasis in the microcirculation of the presenting part, does present problems of interpretation which are specific for the sub partu situation, especially during the second stage of labor. The simultaneous recording of local skin perfusion is of some

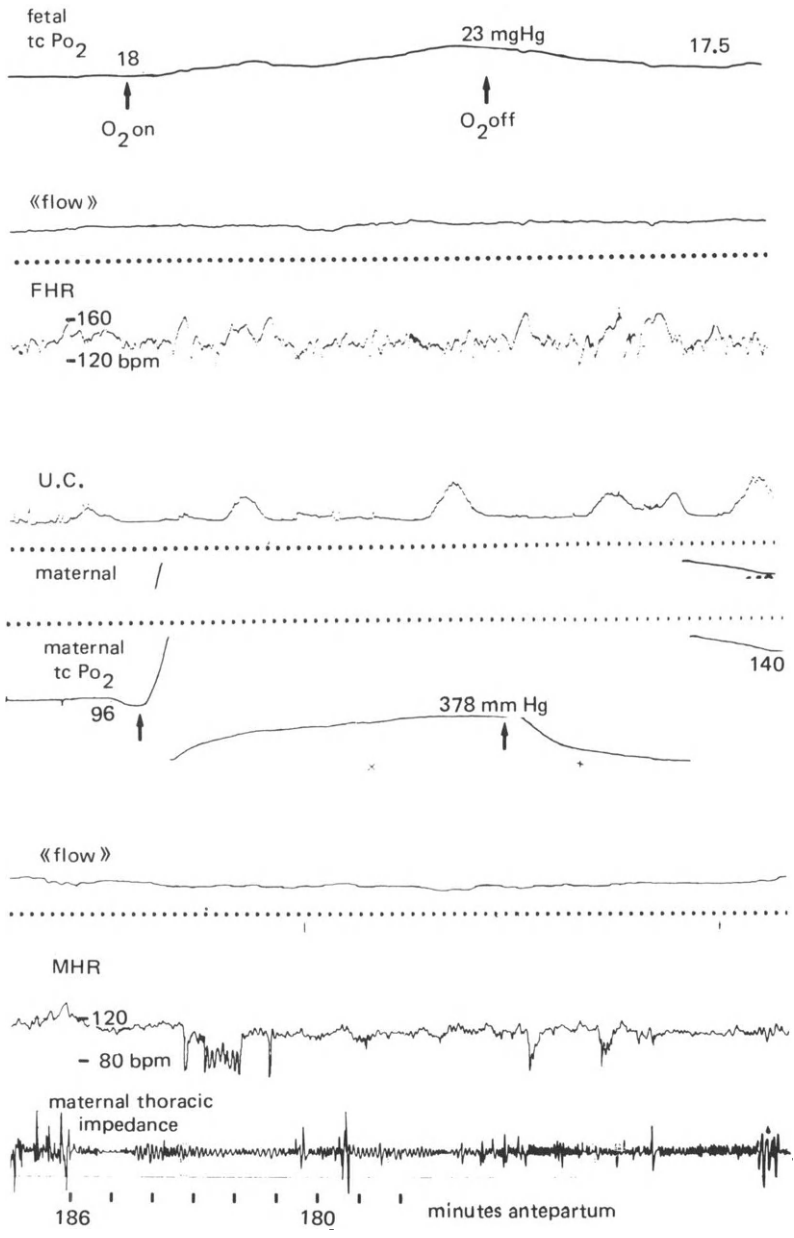


Figure 15 Excerpts of simultaneous continuous recordings of fetal $tcpO_2$ and "flow," fetal heart rate, uterine contractions, maternal $tcpO_2$ and "flow," maternal heart rate, and thoracic impedance 186 min before delivery. During the period between the arrows on the maternal and fetal $tcpO_2$ recordings the mother breathed 100% oxygen. (From Huch et al., 1979.)

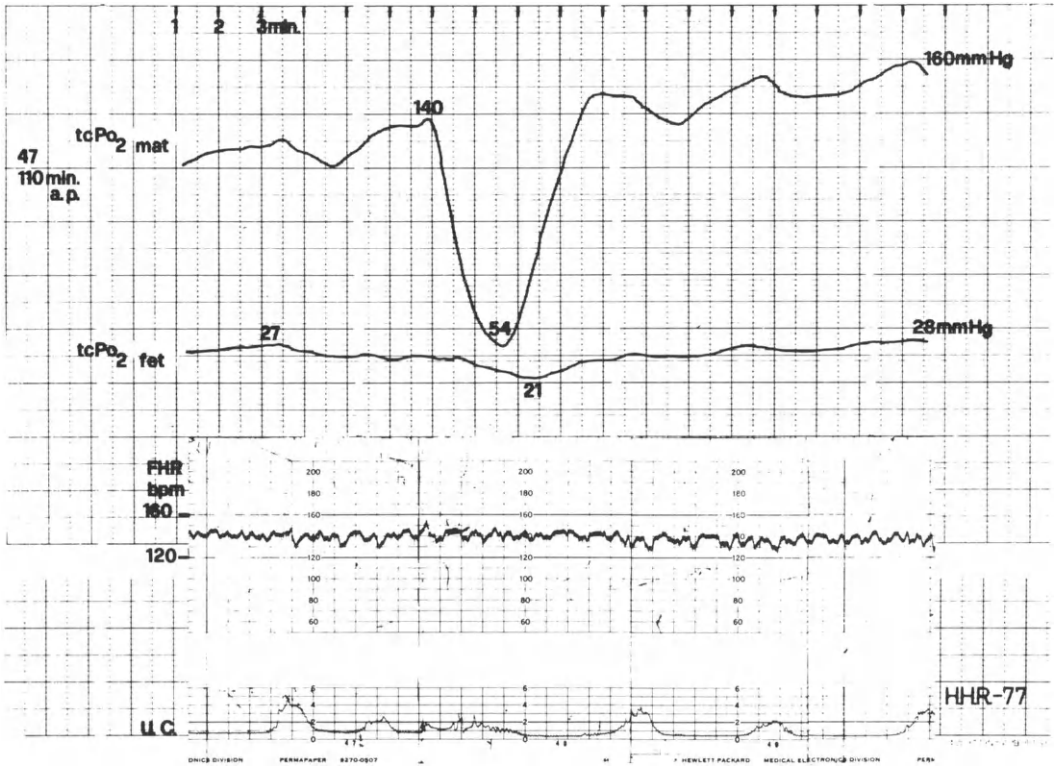


Figure 16 Excerpts of simultaneous continuous recordings of maternal $tcpO_2$, fetal $tcpO_2$, fetal heart rate, and contractions. A maternal $pO_2$ decrease is reflected in the fetal record. The mother was given additional oxygen (air-oxygen mixture) to compensate for frequent hypoventilation. (From Huch et al., 1977.)

help to differentiate between a drop in $pO_2$ during a contraction which is related to increased pressure on the electrode and a real drop in either central or local $pO_2$ (Huch et al., 1977). Current investigation is concentrating on finding ways to simplify handling to make it applicable for routine clinical use.

There is a characteristic pattern of change in transcutaneous $pO_2$ during normal labor, a typical example of which is shown in Figure 14. With a delay of about 40-50 sec following the beginning of each contraction $pO_2$ declines by 1 mmHg to a maximum of 3 mmHg and returns to the original level in between contractions. The highest value, which averages 18-19 mmHg is reached during relaxation of the uterus and during the first half of a contraction. However, there is no increase of $pO_2$ with a contraction, as has been postulated on the basis of microblood measurements (Renou et al., 1968). The delayed drop of 10-15 sec in the fall of transcutaneous scalp $pO_2$ is a reflection of a lag time of transcutaneous values compared to arterial changes. Additionally, it may well reflect the circulatory time for placental blood, which is less oxygenated during a contraction, to reach the scalp. This delay must be taken into consideration for interpretation. It also helps to differentiate between a decrease in $pO_2$ due to a reduced placental oxygen exchange during a contraction from a drop in $pO_2$ caused by pressure on the electrode.

The fall in fetal $tcpO_2$ during and after a contraction has been related to intrauterine pressure by Huch et al. (1981). The lowest pressures which caused a drop in $tcpO_2$ varied between 20 and 45 mmHg. There was a statistically significant correlation between the drop in fetal $pO_2$ and the strength of a uterine contraction. However, with physiological contractions the fall in $pO_2$ did not exceed 1-3 mmHg. Suppression of contractions with intravenous fenoterol for 20 min did not give a significant change in fetal baseline $pO_2$, but the contraction-related fluctuations disappeared (Schneider et al., 1980). In physiological labor the temporary decrease in placental blood flow does not interfere with oxygenation of the healthy fetus.

Simultaneous transcutaneous $pO_2$ measurements in mother and fetus have shown that maternal $pO_2$ changes are immediately reflected in the fetal oxygenation. After supplemental oxygen administration to the mother and increase of maternal $pO_2$, fetal $tcpO_2$ increased independently of the initial $tcpO_2$ level and independently of the duration of oxygen administration to the mother. Figure 15, with simultaneous $tcpO_2$ recordings from mother and fetus during labor, shows the steep increase in maternal $tcpO_2$ during 9 min of supplemental oxygen breathing and the slow but significant parallel rise in the fetus. In 123 periods of oxygen administration to the mother there was a mean rise in fetal $tcpO_2$ of 5 mmHg (Huch et al. 1979). With the mother breathing increased oxygen accompanied by a rise in fetal $pO_2$, there was a drop in the baseline of the fetal heart rate from a mean value of 146 to 141 beats per minute (Huch et al., 1978).

Fetal $pO_2$ will not only reflect a rise in maternal $pO_2$, but will also show a decline when the maternal $pO_2$ drops. Maternal hypoventilation, as described earlier, will affect fetal $pO_2$. Figure 16 shows a significant decline in fetal $pO_2$ beginning 20-30 sec after the maternal $tcpO_2$ started to drop. The overall effect on fetal oxygenation will depend on the extent of maternal $pO_2$ changes and on the fetal $pO_2$ level before the change.

## CONCLUSION

Mother and fetus must be viewed as a unit composed of two compartments which are separated by the placenta. Changes in maternal blood gas values as well as acid-base status will affect the fetus and vice versa. Because of the large difference in size of the two compartments, maternal changes usually will have a more profound impact on fetal values than vice versa. Any evaluation of fetal blood gas as well as acid-base values must take the situation of the mother into consideration. Even today there is no direct way of assessing fetal status antepartum. This can be achieved in various ways intrapartum. Our overall understanding is complicated by the fact that in labor there is no steady-state situation in the mother or the fetus. Under the physiological stress of labor, metabolic as well as blood gas parameters in the mother and fetus are subject to a dynamic change in equilibrium. Today, the major value of a continuous simultaneous recording of several parameters in the mother and fetus should be seen to be a better understanding of these dynamic changes. Further developments in technology as well as improvements of various practical aspects may lead to widespread use of continuous monitoring techniques of blood gas and/or acid-base values in clinical obstetrics in the future.

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# 23

# Prevention of Preterm Delivery

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## INTRODUCTION

Today preterm delivery accounts for the majority of perinatal morbidity and mortality in most countries. With the technological advances of the past 10 years we have observed a significant reduction in the morbidity and mortality associated with this condition. However, preterm delivery remains the leading cause of poor outcome and now accounts for the escalating costs of neonatal intensive care. In the light of recent interest in the prevention of preterm delivery, a chapter directed specifically toward this subject was considered important and timely.

## RECENT ADVANCES

We cannot begin to discuss the prevention of preterm delivery without first reviewing the recent advances which mark our present understanding of this complex problem. Even though our present approach to preterm delivery is crisis oriented, our experiences in dealing with premature labor and the early care of the preterm neonate provide us with a wealth of information about the mother, fetus, and neonate and their complex environment (Hobel, 1978). Advances in pharmacology and biophysics have provided us with new concepts about the manipulation of various perinatal events to improve the outcome of the preterm fetus and neonate.

The steps in the maturation of various fetal and neonatal systems are the primary perinatal adjustments lacking in the high-risk preterm fetus and neonate to which perinatal intensive care in the past 10 years has directed its attention. Recently the special considerations for the management of preterm labor and their effect on outcome were reviewed (Hobel and Oakes, 1980). The primary objective in the treatment of preterm labor with various tocolytic drugs is to prevent delivery until near term in order to achieve a "natural" maturation of the fetus. This approach has not been entirely successful. In a summary of clinical trials Hemminki and Starfield (1978) showed that in only approximately 20% of therapeutic trials using tocolytic drugs was the drug better than a placebo in prolonging the pregnancy. However, short-term tocolysis (24-48 hr) appeared adequate in order to allow time to induce fetal lung maturation "pharmacologically." Utilizing this approach Howie and Liggins (1977) have shown a significant reduction in the incidence of the respiratory distress syndrome (RDS), as well as in morbidity and mortality secondary to RDS.

The greater attention directed toward the management of preterm labor has also provided new information on how to avoid fetal asphyxia, an important component

in the pathogenesis of RDS. It became clear through continuous electronic fetal monitoring and fetal scalp blood sampling that abnormal fetal heart rate patterns and fetal acidosis were associated with a greater risk for development of RDS (Hobel et al., 1972; Martin et al., 1974). Today, safer means of delivering the preterm fetus at risk for asphyxia are a major contribution toward improved outcome (Bowes, 1977).

Even with these significant advances a critical point has been reached when only prevention will allow the next big step toward reducing morbidity and mortality for the preterm fetus to be taken. It is now recognized that the limits of achievable success have been reached in attempts to manage the very early 24- to 30-week pregnancy. Because of extreme immaturity, short-term induction of maturation is less successful. Even with accelerated pulmonary maturation in the very immature fetus, as a neonate it suffers morbidity or death because other systems, for example, the central nervous system or cardiovascular, renal, or gastrointestinal systems, are not capable of supporting life. At these early stages all systems seem to lack anatomical maturity. Neonatal care has become very complex and costly (Pomerance et al., 1977; Stewart, 1977) and a program to prevent the delivery of a majority of these very immature fetuses appears very attractive.

## EPIDEMIOLOGY OF PRETERM LABOR

The study of the patterns of preterm delivery in various populations, especially as to how social and environmental factors affect its occurrence, may provide us with the information required to understand how modern medicine can be used most effectively in preventing this condition.

### Definition

In the past the prematurity rate was defined as the number of infants born weighing less than 2500 g. The reason for this limited definition was the fact that at birth almost all babies are weighed and data on the length of gestation were not always available or accurate. Studies that use this standard definition to define prematurity actually encompass two populations of infants, the truly preterm, those of less than 37 weeks gestational age, and those who are now considered growth retarded at 37 weeks or later but who weigh 2500 g or less at birth. The latter group comprises approximately one-third of all infants less than 2500 g at birth. It is important that this distinction be made as efforts are directed toward predicting the population at risk, because risk factors defining the group at risk for preterm delivery may not apply to the growth-retarded group. In order to establish some uniformity for future studies, the World Health Organization has recommended that the preterm baby be defined as that newborn delivered at a gestational age of less than 37 completed weeks, or 259 days, regardless of weight (World Health Organization, 1969). The upper limit is generally accepted, but the lower limits are still not well established and frequently reported at either 20 weeks (141 days) or 28 weeks (196 days). Since the clinical challenge in caring for newborn delivered at less than 28 weeks gestational age is now accepted, it seems more appropriate that the definition of prematurity (more precisely, immature-premature) extend from 20 to 37 complete weeks (141-259 days) of pregnancy. Hopefully, the expanded use of ultrasound scanning will help in the more accurate estimation of gestational age in pregnancies with "uncertain dates" so that fewer errors will be made.

### Variations in Rates

It is important to look at the international distribution of patterns of preterm delivery, even though it is necessary to use the old definition so that some general indicators of risk or possibly the value of certain methods of health care may surface and provide clues toward understanding the causes of prematurity. Worldwide variation in low birth weight (LBW) was reviewed by Boldman and Reed during the epidemiology of prematurity workshop at the National Institute of Child Health and Human Development in 1976. They showed from available statistics that LBW varies from 4.1 to 45%. Statistical correlations of LBW ratios for 21 countries showed that indicator variables were highly correlated with each other. A stepwise regression analysis identified per capita income as the best indicator. The authors' conclusions were that the causes of LBW are attributable to the environment and that a more clear understanding of these factors and their interactions may provide some new insight into the prevention of prematurity.

In the United States the incidence of LBW infants actually increased from 7.7% to approximately 8.3% during the period 1960-1965. Since then the incidence has been slowly declining and the overall rate as of 1974 was 7.4% (Chase, 1976). Various developments in perinatal care and specific changes in the population are thought to account for these minor changes. I recently reviewed these events which I think account for the general improvement in perinatal care in the United States during the past 20 years (Hobel, 1980).

## ETIOLOGY OF PRETERM LABOR

The mechanism initiating premature labor appears to be multifactoral. Before it is possible to elucidate these mechanisms, a hypothesis must first be set forth. For the first time many of the pieces of this complex puzzle are beginning to fit together. For this reason a hypothesis is proposed which is clinically oriented so that subsequent discussions of prevention will be more relevant. Initially the basic assumption is made that the initiating factors in preterm labor are likely to be different from those of term labor. While the fetus is thought to play a primary role in term labor, it is proposed that other conditions, shown in Table 1, are likely to play a pivotal role in causing preterm delivery; however, this does not exclude the possibility that the fetus may, on occasion, play a primary or secondary role in initiating preterm labor.

Figure 1 outlines the complex interactions of various events which play a role in preterm labor. These events are separated into four stages. As noted, there are points of positive feedback to earlier stages. These positive feedback points play an important role in accelerating the biochemical changes responsible for the cascade of events where clinical intervention is less successful. Thus this hypothesis is clinically relevant.

### Stage I

#### Stress and Low Social Class

Stress* probably plays the most important role in increasing the risk for preterm labor. Numerous studies identify a strong relationship between prematurity and situations

*Stress is a physical or emotional situation that causes bodily or mental tension (low social status, low income, limited education, unmarried status, crowding, strenous work, long commuting time, work outside home, change in residence, low weight, and unfavorable age, i.e., less than 18 or over 40).

Table 1 Risk Factors

| Historical | Developing |
|---|---|
| Stress factors | Assessment by interview |
| Physical | uterine motility |
| age ⩽ 17 | pelvic pressure |
| weight < 45 kg | vaginal discharge |
| height < 160 cm | uterine bleeding |
| poor nutrition | |
| poor hygiene | |
| employed | |
| long commute | Assessment by examination |
| Mental | size-dates discrepancy |
| single | error in date of last menstrual period |
| divorced | multiple pregnancy |
| low social class | hydramnios |
| smoking | thin lower segment |
| Potential | dilated external os |
| travel | dilated internal os |
| relocation | weight loss |
| | poor weight gain |
| Obstetrical-gynecological | |
| previous dilation and curettage | |
| uterine malformation | |
| incompetent cervix | |
| therapeutic abortion | |
| recurrent abortion | |
| short interval | |
| between pregnancies | |
| labor <3 hr | |
| previous preterm | |
| previous stillborn | |

which cause stress (Papiernik and Kaminski, 1974; Wortis and Freedman, 1962; Fedrick and Anderson, 1976; Klein, 1971). Socioeconomic status is the factor which is frequently used to identify the patient at risk for a variety of health problems. The mechanism by which socioeconomic status influences the incidence of prematurity is not well understood, but it is probably related to the interaction of the excessive use of tobacco, suboptimal nutrition, poor personal hygiene, and in some cases certain types of work habits. Inadequate prenatal care, as pointed out by Klein (1971) results in a failure to correct these adverse factors, thus allowing them to have an even greater effect. Recently the work of Timio et al. (1979) has shown that workers on certain types of assembly lines had a significantly increased urinary excretion of adrenaline and noradrenaline, suggesting that the autonomic nervous system can be overstimulated by occupational stress. As a factor in the etiology of preterm labor, the inability of patients to cope with these problems is referred to as psychosocial stress. Recently Newton et al. (1979) found that the levels of psychosocial stress in pregnancy were significantly higher in mothers whose babies were born preterm. Because of these recent

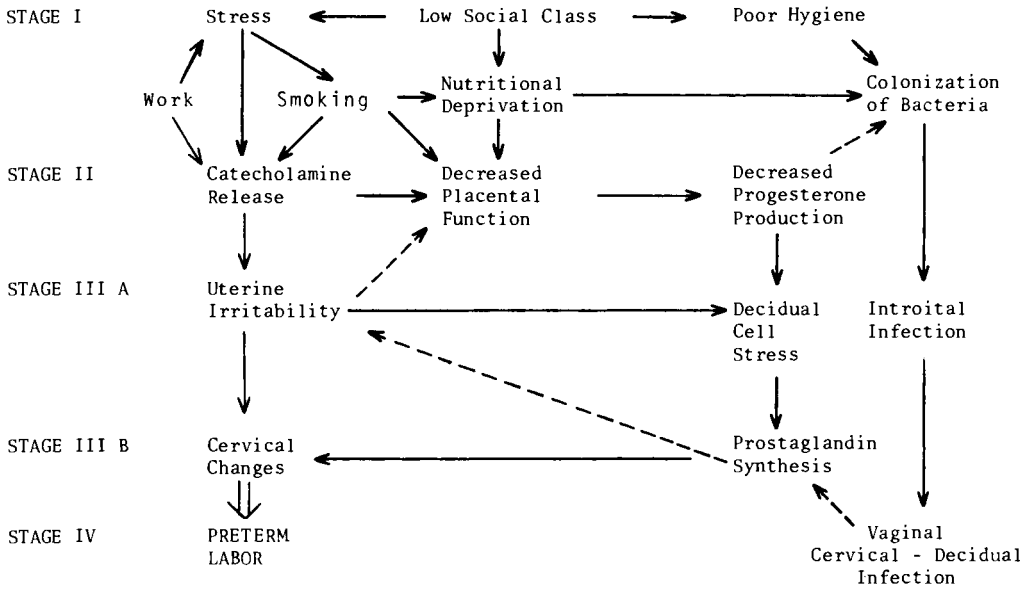



**Figure 1** A hypothesis for the multifactorial etiology of preterm labor. Many of the steps to each stage are supported by various investigations reported in the literature.

findings, stress during pregnancy warrants special consideration in future studies on the prevention of preterm labor.

Numerous reports describe the relationship between smoking and low birth weight infants (Fedrick and Anderson, 1976; Meyer, 1976; Meyer and Tonascia, 1977). However, this relationship exists with both low birth rate and preterm labor, as has been shown by Meyer and Tonascia (1977). The mechanism by which smoking could initiate preterm labor is unknown. Smoking does increase the levels of carboxyhemoglobin in maternal and fetal blood and in its presence in a pregnant animal model studied by Longo showed a reduced oxygen tension in the fetal compartment (Longo, 1976). Reduced oxygen tension could have a direct effect on fetal growth or affect placental production of steroids such as progesterone, which is vital for the maintenance of pregnancy. Quigley et al. (1979) showed that smoking in pregnant women significantly increased the levels of both norepinephrine and epinephrine, causing an acute elevation in maternal heart rate and blood pressure. The fact that both of these amines in low concentrations significantly reduce uterine blood flow in the pregnant ewe, while not affecting heart rate or blood pressure, suggests that the same effect may be produced in women who smoke during pregnancy (Rosenfeld et al., 1976; Rosenfeld and West, 1977).

### Nutritional Deprivation

The causal inference of altered prenatal nutrition on the incidence of low birth weight infants is not well understood. For example, the Dutch famine toward the end of World War II had its greatest effect on the mean birth weight of fetuses and their placentas when they were exposed to the famine during the third trimester, but it did not increase the incidence of preterm labor or intrauterine growth retardation. However, a proportion of those infants exposed to the famine early in gestation were of very low

birth weight, had shortened periods of gestation, and were born prematurely (Stein and Stusser, 1975). The Dutch famine would be an example of the effect of severe malnutrition on pregnant patients who were previously healthy. In a prenatal intervention study in Harlem, New York, nutritional supplementation with a high-protein diet given to women suspected of being at risk for preterm labor was associated with a higher rather than a lower rate of preterm labor. The increase in birth weight (41 g) in the supplemented group in infants delivering at term was not statistically significant (Rush et al., 1974). Data from Guatemala suggest that nutritional supplementation for a population that suffers from chronic malnutrition has favorable effects on preventing LBW infants (Lechtig et al., 1976). In summary, the effects of altered nutrition as a causative factor appears complex. An excellent review of this subject in the human by Susser and Stein (1977) suggests that good nutrition has two major effects: It not only supports fetal growth, but it also affects maternal behavior, which probably has its greatest effect on the pregnancy itself and on the subsequent mental performance of the child.

For a better understanding of the effect of nutrition, it is necessary to learn more about the biochemical alteration secondary to poor nutrition. The effect of chronic malnutrition on placental function is well known (Laga et al., 1972; Winick, 1971). Precisely how these changes occur is not well understood and it is necessary to turn to animal studies for some direction. The recent studies in the rat by Parvez et al. (1980) suggest that starvation is an important stress which activates catecholamine synthesis and also inhibits metabolism. These metabolic changes may well adversely influence the response of the starved animal to stress. It is possible, then, that starvation or inadequate nutrition could be an important component of the stress part of our causal model.

### Poor Hygiene

Currently, it is thought that good personal hygiene is important in the prevention of bacterial infections of the introitus; however, other conditions such as nutrition and its effect on the antibacterial activity of amniotic fluid and host resistance to colonization also play important roles in decreasing the risk of infection. In the past most of the attention regarding infection and the risk of preterm birth was directed toward asymptomatic bacteruria and pyelonephritis (Kass, 1960; Kincaid-Smith, 1968). Recently, Naeye (1977) reported that amniotic fluid infections in the presence of intact fetal membranes is the single most common underlying cause of fetal and neonatal death in the United States. He postulated that shortening of the lower uterine segment, which occurs with early premature labor (stage IIIA and B of our model), increases the possibility of exposure of the fetal membranes to the bacterial flora of the vagina and cervix (Naeye, 1979). Normally amniotic fluid contains an inorganic bacterial growth-inhibitory component, zinc; however, under certain conditions such as poor nutrition, amniotic fluid loses this special activity and the risk of infection may increase (Applebaum, 1979).

## Stage II

### Catecholamine Release

Very little information exists regarding the levels of catecholamines in various pregnancy states, because previous assay systems were not sufficiently sensitive to characterize

baseline levels and large samples of blood were required for the assay. The development of the radioenzymatic assay for the various amines in very small samples of plasma is now providing information which supports our hypothesis that elevated catecholamines could play an important part in the pathogenesis of preterm labor (Johnson and Peuler, 1977). Clinically, activation of the sympathetic nervous system of the pregnant women occurs with the endogenous release of catecholamines secondary to stress and smoking. The precise role that these amines play in preterm labor depends upon a complex interaction between release, receptor type, and number, and their metabolism in peripheral tissues.

The contractile state of the uterus is modified by various physiological and endocrine states. It is well documented that uterine contractions can be initiated by stimulating alpha-adrenergic receptors; however, the response can be modified by the hormonal state of the subject (Pose et al., 1962; Williams and de Schaepdryver, 1966). The early studies of Miller and Marshall in 1965 showed that if rabbits were treated with estrogen, their uteri contracted to both hypogastric nerve stimulation and the infusion of norepinephrine, whereas if the estrogen-primed uteri were exposed to progesterone, the uteri failed to contract in response to either of these stimuli. Recently alpha-receptor studies by Williams and Lefkowitz (1977) have provided conclusive evidence for why different alpha-adrenergic contractile responses were observed under these two different hormonal states in the rabbit. These investigators showed that progesterone significantly reduces the number of alpha-receptor-binding sites in the rabbit uterus. In the rat model, Raz et al. (1971) showed that progesterone facilitated the beta-response (i.e., relaxation) to epinephrine. Thus there does appear to be an intimate relationship between the hormonal environment and the uterine response to adrenergic agents. However, this is only part of the complex interaction between hormones and uterine response. Pregnancy steroids also affect the metabolism of amines. Earlier studies in the rat by Parvez et al. (1975) suggest that exogenous progesterone administration during late pregnancy increases epinephrine stores by reducing monamine metabolism and increases their synthesis by stimulating the production of phenylethanolamine-N-methyltransferase, which is responsible for N-methylation of norepinephrine to epinephrine. These findings are consistent with the observations of Raz et al. (1971) noted above. Thus the end organ response to amines released in the circulation depends on their endocrine environment and metabolic state.

### Decreased Placental Function

Considerable evidence in the animal model exists for the adverse effect of catecholamines on uteroplacental blood flow. The mechanism by which this occurs is by the release of endogenous catecholamines, mainly norepinephrine, and their effect on receptors in the uterine vessels and myometrium. The causal hypothesis (Figure 1) considers alteration in placental function a major component of stage II because the low maternal plasma progesterone and estradiol levels observed in patients at risk for preterm labor could be secondary in part to the effect of endogenous fetal and/or maternal catecholamines. Studies in the nonhuman primate by Adamsons et al. (1971) and Myers (1975) show that either exogenous norepinephrine or maternal psychological stress causes increased uterine motility and fetal asphyxia. Angiographic studies in this same model by Martin et al. (1964) show an impaired filling of placental lobules during uterine contractions. In the human Buster et al. (1978) has shown considerable

short-term variability in maternal levels of hormones produced by the placenta. Could this marked fluctuation be a reflection of the intermittent release of fetal or maternal amines on uteroplacental function? The concept suggests that a primary fetal release of catecholamines and their effect on placental function are yet another mechanism for altering placental production of hormones vital for pregnancy maintenance. The recent studies by Artal et al. (1979) on the increased levels of catecholamine metabolites in amniotic fluid from patients in premature labor would support this hypothesis.

### Decreased Progesterone Production

Progesterone is a key hormone for the maintenance of pregnancy. Plasma levels of progesterone or its metabolites in urine in patients delivering preterm infants have been of interest for several years. The inconsistencies in these various reports were reviewed by Kumar et al. in 1963. These authors reported low placental progesterone concentrations in the majority of premature births when these were compared to normal pregnancies. Most likely the assay and sampling techniques used in these earlier studies prevented investigators from showing a more precise relationship between maternal plasma progesterone concentrations and preterm labor. In more carefully designed studies by Csapo et al. (1974) and those from our own obstetrical service by Cousins et al. (1977) suggest that decreased plasma progesterone levels are indeed associated with preterm labor. A progesterone deficiency is not necessarily a cause of preterm labor but, rather, a significant facilitating factor via the following mechanism. The possibility of how endogenous catecholamines may affect uteroplacental function has already been discussed. Once a progesterone deficiency is established, the deficiency itself increases the sensitivity of the myometrium to various stimuli or renders it more susceptible to spontaneous uterine motility. Secondly, progesterone has a stabilizing effect on decidual lysosomes, as was suggested by Gustavii (1975), which prevents the release of phospholipase $A_2$, which is important for prostaglandin synthesis. Thus a progesterone deficiency could play an important initial step in prostaglandin synthesis by allowing the spontaneous breakup of lysosomes. Finally, the recent studies by Zawaneh et al. (1983) suggest that pregnancy hormone levels affect the adherence of group B streptococci to vaginal epithelial cells. It is thought that while progesterone lowers the adherence of bacteria to the vaginal epithelium, estrogens increase the adherence. Thus when a progesterone deficiency exists, colonization and infection are theoretically more likely. For these reasons decreased progesterone production is a crucial point in the causal hypothesis on the initiating events leading to and complicating preterm labor.

## Stage III

### Uterine Contractions and Cervical Changes

This proposed stage in the evolution of preterm labor is crucial because it is the stage where physical signs and symptoms are of clinical value in recognizing the patient at risk. In concert with these signs and symptoms, significant biochemical changes also take place which are difficult to control during part B of this stage.

### Decidual Cell Stress and Prostaglandin Synthesis

Prostaglandins play a major role in parturition; however, it is unlikely that they play a primary role in the initiation of preterm labor, as we have so far indicated in our causal diagram. In 1972 Gustavii first proposed that the lysosomes of the decidua could play a key role in parturition. He suggested that parturition is initiated by a breakdown of the lysosomes, with a release of phospholipase $A_2$ which then provides arachidonic acid for prostaglandin synthesis (Gustavii, 1975). Previously we mentioned the role of progesterone in stabilizing lysosomes; conversely, estradiol can increase the lability of lysosomal membranes causing a release of enzymes to initiate prostaglandin synthesis. The studies by TambyRaja et al. (1974a) suggest that the onset of preterm labor can also be preceded by a surge in maternal plasma estradiol concentration. In our studies on preterm labor elevated levels of estradiol were not found, although in a few cases of preterm labor not explained by low progesterone levels very high levels of estradiol were observed. TambyRaja speculated that those cases with an estradiol surge may represent cases where the fetus is responsible for initiating preterm labor. Currently it is thought that various conditions such as uterine contractions, cervical-decidual infection, low plasma progesterone or elevated plasma estradiol levels, and uterine distention can cause decidual cell stress and thereby damage lysosomes and initiate prostaglandin synthesis. The synthesis and release of prostaglandins play an important role in the transition from stage IIIA to stage IIIB in preterm labor, which is characterized by cervical ripening and dilatation. Liggins (1978) reviewed the evidence for the role of prostaglandins $F_2$ and $E_2$ by the oral, intracervical, intravaginal, and intravenous routes in cervical ripening. These changes can occur with minimal uterine activity. The precise biochemical changes responsible for cervical ripening (collagen degradation) are unknown at present. It is unlikely that prostaglandins play a primary role in the initiation of preterm labor. TambyRaja et al. (1974b) found elevated levels of prostaglandin F in amniotic fluid only in advanced preterm labor. Likewise, Mitchell et al. (1978) could not find elevated levels of either prostaglandin E or F or 13,14-dihydro-15-keto prostaglandin F in maternal blood in early preterm labor.

### Infection

Once the cervix opens, the intrauterine environment is exposed to pathogenic bacteria and the risk of infection increases. The studies by Naeye (1977, 1979) previously mentioned suggest that an amniotic fluid infection is an important component of preterm labor. It is of special interest that Naeye's most recent publication indicates that the frequency of this type of infection was significantly more common in mothers who reported having coitus one or more times per week during the month before delivery. Coitus itself may be a significant factor via several mechanisms. Not only is there a greater exposure of the membranes to pathogenic bacteria, but seminal fluid contains a high concentration of prostaglandins, as has been suggested by Speroff and Ramwell (1970). It has also been suggested by Mitchell et al. (1977) that stimulation of the cervix causes the release of prostaglandins. Thus these events either singly or in combination could play an important feedback role in perpetuating this proposed stage IIIB part of preterm labor.

### Stage IV

Preterm Labor

We selected a final stage for our causal model and termed it irreversible preterm labor, because once the cervix becomes completely effaced and dilated more than 3 cm, the success of therapy to prevent delivery until the pregnancy reaches term after 37 weeks is less likely. There is ample evidence for us to draw this conclusion from the recent review of the etiology and management of preterm labor by Creasy and Liggins (1979). We have already referred to the review of tocolytic therapy by Hemminki and Starfield (1978), which suggests that most studies do not claim that tocolytic therapy prevents delivery until term. Preliminary data by Whitsett et al. (1980) in the human and in the rabbit by E. S. Diakomanolis (personal communication) suggests that infusions of Vasodilan (isoxsuprine hydrochloride) reduce the number of beta-adrenergic receptors. These data suggest that resistance or tolerance develops, which may account for the fact that beta-adrenergic drugs do not prevent preterm delivery. However, short-term inhibition of labor with tocolytic drugs and the acceleration of fetal lung maturation with glucocorticoids has been a significant step toward reducing morbidity and mortality secondary to RDS in the preterm infant. Future progress in reducing the number of preterm babies will be the elimination of stage IV of preterm labor by first addressing the problems of stage I. Stress and all the factors which produce it probably initiate a series of events that significantly increase the risk of delivering a preterm infant. For those patients at risk during stage II, biochemical tests may be of value in identifying the patient at greater risk for early delivery. Finally, the early symptoms which characterize the patient during stage III can be further identified so that very early intervention can prevent the patient from entering the irreversible stage IV of preterm labor. The concept of identification, intervention, and prevention will be presented in the next two sections.

## IDENTIFYING THE PREGNANT WOMAN AT RISK OF PRETERM LABOR

During the past 12 years there has been a worldwide interest in identifying the pregnancy at risk for morbidity and/or mortality. Only a few investigators directed their attention specifically to the risk of preterm birth (Newcombe et al., 1977). Precise identification systems have not been developed for the following reasons. First, the population data base used in previous studies did not include all the variables which are now thought to be important; second, carefully controlled studies using predictive schemes have not been carried out prospectively. The lack of controlled studies limits the ability to define intervention steps which play a role in prevention. Explained in another way, once intervention occurs, good antenatal predictors lose their power to identify the population at risk. In the light of these limitations various antenatal predictive schemes for identifying the pregnancies at risk for preterm labor will be reviewed in order that a logical scheme can be developed which should be applied in future intervention programs.

Papiernik-Berkhauer (1969) was the first to report a scheme for identifying the pregnancy at risk for preterm labor. His major contribution was the detail in which he outlined and defined his risk factors. From the outset he included the risk factors of fatigue and cervical examination–signs which we believe are very important for making a complete assessment. The fact that his first article was published in French resulted

in limited recognition, but in 1974 he published two articles with Kaminski, a statistician, concerning the frequency of predictive factors and discriminant function analysis of these factors (Papiernik and Kaminski, 1974; Kaminski and Papiernik, 1974). The latter study showed an improvement over Papiernik's original empirical risk coefficient approach. Kaminski, with other French investigators, had previously identified risk factors from a Paris hospital data base, using a similar statistical technique, but the risk factor list was not as complete as that of Papiernik (Kaminski et al., 1973).

In Germany, Saling (1972) proposed a prevention program for both prematurity and dysmaturity called PDP-Program. Preliminary results and experiences using this program were reported to be encouraging Giffei and Saling (1974). Giffei and Saling's predictive list contained 36 risk factors separated into nontreatable and treatable factors. The authors were also careful to differentiate factors related to prematurity from those related to dysmaturity. Other investigators (Weidinger and Wiest, 1974) in Germany have also proposed prematurity prevention lists, but nothing has been published in recent years to indicate their continued use and refinement.

The British Perinatal Mortality Survey of 1958 provided British epidemiologists with an extensive data base from which to identify risk factors for predicting the risk of preterm birth (Butler and Bonham, 1963; Butler and Alberman, 1969). The first article by Fedrick and Anderson (1976) specifically related to the subject of identifying factors associated with spontaneous preterm live births (before 37 weeks, weighing less than 2500 g). Even though this study identified several significant factors, all were based on either past history or information at the start of the pregnancy. Information on weight gain, special examinations, and laboratory tests during pregnancy (except those for anemia) were not available. Fedrick next devised a scoring system to assess the risk of spontaneous preterm birth using the data from which it was derived (Fedrick, 1976). Since so many of the factors studied were based on past pregnancy performance, the score was effective in predicting only a small proportion (29% maximum) of primiparae going into preterm labor, while it was somewhat better for predicting the multiparae at risk (39% maximum). This study was important in that it stimulated others to evaluate this apprach of identifying the patient at risk. Newcombe et al. (1977) tested Fedrick's score by applying it to a different data set, even though it also had several limitations regarding the availability of factors to analyze. These investigators concluded that the application of Fedrick's rule is potentially helpful in multiparae only if it includes information relating to the weights of previous births. These investigators felt that the "shading effect" of other factors is probably relatively unimportant. It is true that some factors are weak predictors, but we feel that it is only through the identification of these minor factors that we can more skillfully apply intervention schemes that will ultimately make the difference.

The father of clinical obstetrics in the United States, Nicholson J. Eastman, was the first to suggest that "only when the factors causing prematurity are clearly understood can any intelligent attempt at prevention be made" (Eastman, 1947). Eastman analyzed the births at Johns Hopkins between 1926 and 1945 and identified many of the same factors that we feel are important today. Based on this study plus his clinical experience, he recommended that practitioners of obstetrics act on his suggestions. However, over the ensuing years only the mortality and morbidity rate secondary to prematurity decreased significantly and the incidence of prematurity has been relatively stable. The problem is that insufficient attention has been directed toward providing obstetricians with techniques for identifying the patient at risk and methods for reducing that risk.

## Equation for Calculating Probability of Preterm Birth

$R$ = ODDS THAT A PATIENT WILL DELIVER PRETERM

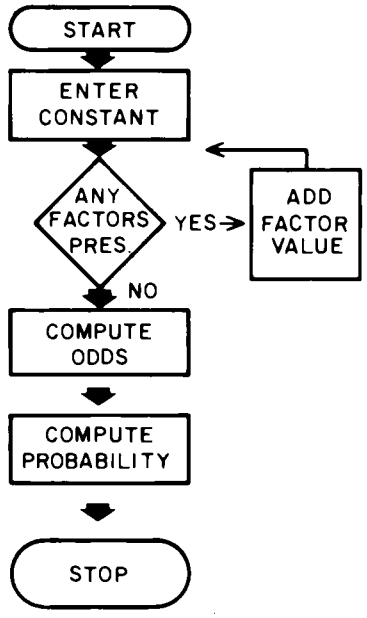
$$R = e^{(B_0 + B_1 + B_2)}$$

WHERE $B_0$ = CONSTANT FACTOR
$B_1$ = FACTOR WEIGHT FOR HISTORICAL CONDITION
$B_2$ = FACTOR WEIGHT FOR HORMONE VALUE

$P$ = PROBABILITY THAT A PATIENT WILL DELIVER PRETERM

$$P = \frac{R}{R+1}$$

## Algorithm for Calculating Probability of Preterm Birth




1. SWITCH UNIT ON*
2. KEY IN VALUE OF THE CONSTANT
3. PRESS [CH S] TO MAKE VALUE NEG.
4. PRESS [ENTER]
5. KEY IN VALUE FOR FACTOR
6. PRESS [+]
7. PRESS [$e^x$]
8. PRESS [ENTER] TWICE
9. KEY IN THE VALUE 1
10. PRESS [+]
11. PRESS [÷]
12. SWITCH UNIT OFF

** Hewlett-Packard – 35 Calculator*

Figure 2 Calculation of the probability (percent) of preterm birth using the factor weight (coefficient) for either the history of the preterm infant and or the progesterone level listed in text. This calculation can be made on a hand-held calculator which has the function $e^x$.

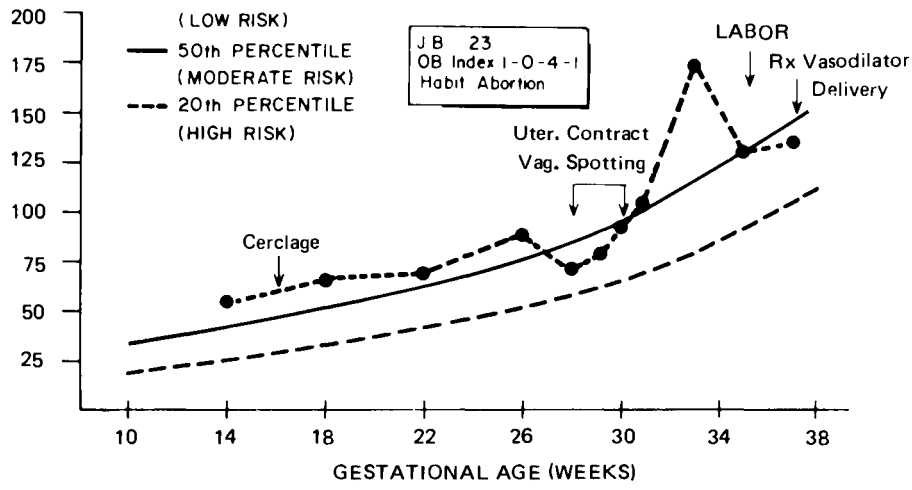



Figure 3 Representation of a high-risk patient (obstetrical index: one term birth, no preterm births, four abortions, one living child) who was monitored throughout her pregnancy with serial plasma progesterone levels. Data points above the fiftieth percentile line (solid line) indicate that the patient is low risk. Points between the solid line and the broken line indicate moderate risk and points below the broken line indicate high risk for preterm delivery. A drop in maternal progesterone levels occurred during two periods when this patient was symptomatic and at greater risk of delivering a premature infant.

In the United States, Donahue and Wan (1973) devised a technique of weighting factors to define the risk of prematurity and neonatal death. Unfortunately, because of limitations within their data sets, their risk score was a better predictor of mortality than of preterm labor; however, their approach was novel. At the same time maternity and infant care projects in the United States were directing considerable effort toward certain high-risk populations (Gold et al., 1969; Sackler et al., 1969). In fact, these programs showed a reduction in prematurity rates. However, because of the lack of precise indicators of risk and intervention schemes for reducing preterm births, the overall incidence of this complication did not significantly change during the 1970s.

Our perinatal group has been interested in risk assessment for several years and, beginning with our endocrine studies of preterm labor in 1967, we initiated a search for the optimal risk factor to predict preterm birth (Hobel et al., 1973; Cousins et al., 1977). A mathematical model for the prediction of preterm birth was published in 1978 (Hobel, 1978). This model was based on a multiple logistic regression analysis of 46 prenatal variables which included an endocrine assessment of the pregnancy. This model and the algorithm used in calculating the probability (percent) of risk of preterm birth with a hand-held calculator are shown in Figure 2. Even though several factors were found to be significantly related to preterm birth, only two factors, previous delivery of preterm infant (coefficient = 1.6506) and low maternal serum progesterone level (coefficient = 1.3899, constant = 2.8652), are necessary to achieve the maximum predictive capability of this model. The uniqueness of this approach to prediction was that it included an endocrine variable which has been shown to be associated with the

risk of preterm birth. Thus we have an example of a predictive model using a historical variable and a laboratory assessment, both of which are powerful predictors, especially when used in combination. Finally, this factor model has value in that it can be used for both multiparae and primigravidae. Currently the model is being expanded to make a series of assessments with serum progesterone measurements at 2-week intervals to characterize the pathway for patients in order to study the effect of intervention on the course of pregnancy, as shown in Figure 3.

## INTERVENTION AND PREVENTION

### Intervention

Heightened interest in preventing preterm labor at an early stage of pregnancy is based on the fact that prevention when labor has started has not been successful and, secondly, the results of a French study by Papiernik at the Antoine Bêclère Maternity Hospital, Clamart, France, near Paris suggest that an intervention program can be successful in reducing the incidence of preterm-labor (Papiernik-Berkhauer, 1979). The purpose of this section is to identify the essential components of a prevention program so that future planning, the design of clinical studies, and implementation of programs can be carried out by others more easily.

The first step in planning a program is the selection of a method for identifying the patient at risk. The method must be complete and should not be limited to historical variables as in Fedrick's model (Fedrick, 1976). A complete assessment-intervention program is shown in Figure 4. It is important that the total obstetrical population within a region be assessed, ideally before a planned pregnancy, and if not, soon after conception. Early assessment is important in order to begin education, counseling, and possibly early therapy as intervention. Table 1 lists the historical and developing factors which should be included in the assessment. Whether or not a scoring system is used is open to choice; Papiernik believed that once the health care team is knowledgeable about risk assessment and scoring, it may no longer be necessary (E. Papiernik-Berkhauer, personal communication). The reader should refer to the papers of Fedrick and Anderson (1976), Papiernik and Kaminski (1974), Kaminski and Papiernik (1974), Saling (1972), Giffei and Saling (1974), Weidinger and Wiest (1974), Fedrick (1976), and Hobel (1978) for further amplification of risk factors and scoring techniques. It is apparent that the first step in assessment and intervention (Figure 4) is directed toward the major factors in stage I of the causal diagram in Figure 1. Intervention is limited to education and counseling. Education of young women is the most important intervention step and a program must direct attention to each risk factor. It is currently thought that education provided during pregnancy may not have its full effect until the next pregnancy or even the next generation. Actually, education to improve the health of the pregnant woman and hence that of her baby must be a continuous process. Therapy when the women is first seen in pregnancy is restricted to psychological support, rest, and nutrition supplementation. Leave of absence from employment for pregnant women as mandated in Sweden and France is important, especially for women who have long distances to commute and who do strenuous work. Work leave establishes pregnancy as a priority. In a similar fashion, the stress of long trips or a change of residence should be avoided during pregnancy. A study of the

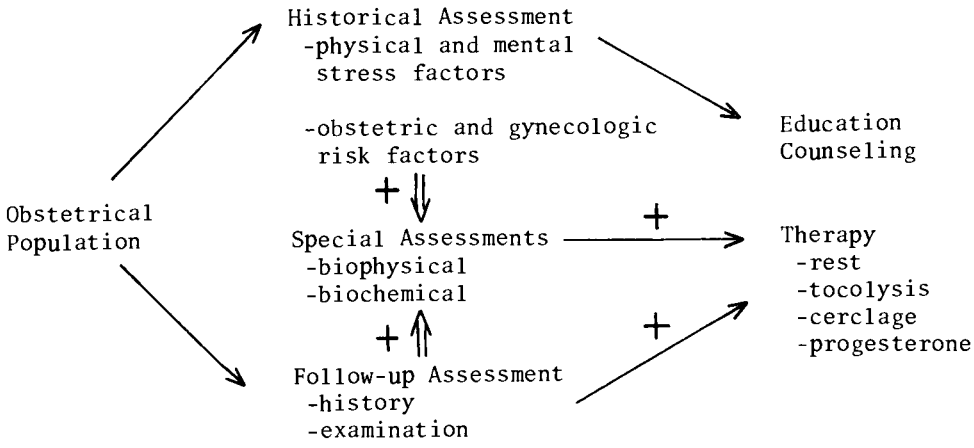



Figure 4 Assessment-intervention scheme proposed for a program to prevent prematurity. Current opinion is that the entire obstetrical population should have a historical assessment and several follow-up assessments. Special assessments are reserved for patients who have historical factors and findings during follow-up assessments. All patients should be provided education and counseling regarding factors which increase the risk of prematurity. Specific therapy is reserved for positive factors discussed in the text.

value of sedatives or tranquilizers to help the pregnant women at risk for preterm labor cope or adjust to stress has not been carried out. Generally speaking, aggressive pharmacological or surgical interventions are reserved for the obstetrical-gynecological historical and developing problems, which are to be discussed next.

A complete obstetrical history also identifies various past obstetrical and gynecological conditions, which frequently require special biophysical or biochemical assessments to further classify patients at risk (Figure 4). Table 1 lists these factors. Once again these special assessments fit in with our causal hypothesis, stages II and IIIA of Figure 1. Types of intervention become more precise with these factors. For example, there is sufficient documentation in the literature to support the role of cervical cerclage in keeping the cervix closed in the patient with a "true incompetent cervix." However, there is a growing trend in the United States and in France to use cerclage in a larger proportion of high-risk patients with questionable incompetence of the cervix. In Papiernik's unit 14% of all patients or 50% of patients at risk for preterm labor had cervical cerclages (E. Papiernik-Berkhauer, personal communication). In the United States attempts are being made to assess the patient "at risk" for incompetent cervix by B-scan ultrasound or, more recently, by real-time sector scanning to identify the early stages of incompetent cervix before placing the cerclage (Sarti and Hobel, 1979). However, this approach may be too conservative.

The next therapeutic measure also being used with greater frequency is oral or intramuscular progesterone. In France 10 mg of chlormadinone acetate (Luteran) administered orally three times daily is commonly used in the patient at risk for preterm labor (Lepage et al., 1970; Breart et al., 1979). In Papiernik's unit approximately

25% of all patients and 100% of at-risk patients received this drug. Cedard et al. (1978) has shown that urinary and plasma estriol (E-3) levels are suppressed by chlormadinone acetate probably via the inhibition of placental sulfatase. It is likely that this drug also suppresses estradiol (E-2), which would alter the progesterone estradiol ratio and thus increase the relative amount of progesterone.

In the United States Johnson et al. (1975) published the results of a double-blind study suggesting that 17α-hydroxyprogesterone caproate (Delalutin), 250 mg/week, is helpful in preventing preterm labor. A second article by this group, published in 1979, again supports the role of progesterone supplementation in high-risk patients for preventing preterm labor (Johnson et al., 1979). The same study also showed that patients who went into preterm labor had lower progesterone and 17α-hydroxyprogesterone levels prior to the onset of labor and treatment was associated with an increase in progesterone levels. These studies on the use of exogeneous progestational agents and the previous studies by Csapo et al. (1974) and Cousins et al. (1977) suggest that it is possible that some patients who go into preterm labor may have either a primary or secondary deficiency of progesterone. Serial progesterone levels in the patient at risk may be an aid to monitoring a patient's course, as in the example shown in Figure 3. As noted, low maternal progesterone levels heralded the onset of symptoms at 28 weeks and admission to the hospital in preterm labor at 35 weeks. Even though this patient was not treated with progesterone, her levels increased with bed rest, but fell again when she was admitted in preterm labor when tocolytic therapy was required to stop preterm labor. To date there is no suggestion that progestational agents have an adverse effect on the developing fetus (Heinonen, 1977). It is generally agreed that treatment with exogenous progestins should be delayed until after 16 weeks gestational age.

Determining the risk for preterm labor is not a one-time assessment based on the history at the first visit. As Papiernik has shown, assessment is dynamic and it must continue at subsequent stages of pregnancy (Papiernik and Kaminski, 1974). Each patient must be assessed for the occurrence of new factors which develop during the course of pregnancy. This is especially true for primigravidae with limited histories. Repeat assessments of all patients may be necessary to identify those patients who progress to stages IIIA and B of the causal hypothesis (Figure 1). A very important part of Papiernik's assessment scheme is the serial examinations to evaluate the cervix, primarily its shortness and state of dilatation. However, station of the presenting part and thinness of the lower uterine segment may also be important. These examinations should be performed at 20, 24, 28, 32, and 34 weeks in all patients, while an additional examination is of value at 16 weeks in the patient at risk for an early loss. Recently E. Papiernik (personal communication) has suggested that some patients may have a congenitally small cervix and uterus which undergoes early effacement and dilation in the primigravida patient. This is probably similar to what Berger and Goldstein (1980) and Cousins et al. (1979) described in women exposed to stilbestrol while in utero.

The final special assessment is external tocography to identify or document the presence of uterine contractions. Women may not perceive uterine contractions and therefore external monitoring may be of value in detecting contractions that may lead to preterm labor. The idea of using a combination of cervical examination and external tocography was first suggested by Wood et al. in 1965. A subsequent article by Anderson and Turnbull in 1969 suggested that cervical changes occurred in most

patients who subsequently delivered at term; therefore these authors were not enthusiastic about examination of the cervix as a means of identifying patients at risk.

It seems likely that examining the cervix and external tocography are two important activities which aid in the ongoing assessment of a patient as she progresses into stage III of the causal diagram in Figure 1. Early cervical changes must be identified and dilation of the cervix prevented if efforts to prevent preterm delivery are to be successful. Currently bed rest and the use of tocolytic drugs and possibly progestational agents are important therapies during stage III. At the present time if contractions are identified, bed rest and oral tocolytic therapy are recommended. Papiernik recommended rectal suppositories (Salbutamol) to be used as necessary (home therapy) in his patients when they recognize uterine contractions which occur less frequently than every 10 min and for more than 1 hr (E. Papiernik-Berkhauer, personal communication).

The importance of recognizing the increased risk of preterm labor in the twin pregnancy has not been mentioned in the foregoing discussion. Follow-up assessments are expecially important to recognize this condition. It is of interest that early studies of Wood et al. (1965) suggested that examination of the cervix and external tocography might be of special value in selecting patients with twins for rest. Recently a matched study by TambyRaja et al. in twin pregnancies suggested that those treated prophylactically with oral Salbutamol delivered larger babies with a reduced mortality rate (TambyRaja et al., 1978). In Papiernik's intervention study special attention was directed toward twin pregnancies in 1977. Prior to this time approximately 40% of twin pregnancies delivered prior to 37 weeks. After implementing the twin intervention program, the prematurity rate for twins dropped to 28% in 1978 and to 19% in 1979 (E. Papiernik, personal communication).

## Prevention

The implementation and success of a comprehensive program of identification and intervention for patients at risk for preterm delivery by Papiernik is sufficient proof for others to implement similar programs. However, at this stage it would be wise for those initiating a program for prevention of preterm labor to ensure that their results can be statistically evaluated against risk factors in both a study and a control population. Papiernik's study at the Antoine Béclère Hospital, Clamart, France, with an annual delivery rate of about 2000 showed a progressive and successive reduction in the preterm rate from 10.1% (1973), 6.2% (1974), 4.9% (1975), and 4.5% (1976) to 3.9% (1977), but the results are difficult to evaluate because of the lack of a control group. In 1978 and 1979 the rates were 3.7 and 3.4%, respectively (E. Papiernik, personal communication). In order to test his prevention program in a different setting, Papiernik in 1975 implemented his risk assessment approach and intervention scheme in the city of Hagenau, approximately 500 k from Paris. In 2 years a fall in the preterm rate from 8.2 to 5.9% was observed (Papiernik, 1977). Initially the study of Papiernik and his colleagues was controlled, with intervention applied to only a portion of the population. Within 2 years there was so much spillover of education and interest in therapy for the control group that the researchers had to abandon the controlled study for ethical reasons (E. Papiernik, personal communication). Some epidemiologists question whether a controlled study can be carried out within the same geographical area.

Information from other parts of the world on changes in preterm rates are limited. In Singapore, where rapid improvements of the socioeconomic status have occurred, TambyRaja and Ratnam have shown a progressive decrease in the prematurity rate

from 5.6% in 1972 to 3.6% in 1978. They considered that these changes were achieved with the improved socioeconomic conditions, coupled with screening and active management of patients at risk for preterm delivery (R. L. TambyRaja and S. S. Ratnam, personal communication). Recently, Herron et al. (1982) published a preliminary report on an identification and intervention program for preventing preterm birth in San Francisco. In a small selected population of patients, during the years 1978-1979 the preterm birth rate incidence decreased from 6.8 to 2.4%. These results are very encouraging. As more attention is directed toward early assessment and intervention, other groups of investigators may also begin to show changes in preterm rates.

## THE FUTURE

Since the etiology of preterm labor appears to be a complex interaction between environmental and social factors, the medical care process must turn to the behavioral and social sciences for direction in dealing with the patient, educators, the employer, and the politician. Countries who strive to reduce significantly the incidence of prematurity must do so through health education, the alteration of lifestyles, and legislation. This in conjunction with medical technology and priority toward prevention will ultimately have its effect on the incidence of this serious complication of reproduction.

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# 24

# Adaptation of the Newborn to Extrauterine Life

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## INTRODUCTION

Healthy survival in the newborn period reflects the adequacy of the maturational changes that have occurred in the fetal tissues before birth and the success of the functional adaptations associated with the beginning of extrauterine life. Survival without damage is, however, also contingent on the awareness of the newborn infant's physiological requirements by those responsible for his care. The immediate establishment of regular breathing, lung aeration, and smooth adaptation of the circulation to extrauterine life are all crucial; in addition, conditions necessary for the maintenance of body temperature, metabolism, "exercise," and growth have to be met by the switch from transplacentally to intestinally derived nutritional sources and by the provision of a favorable environment. Previously nonfunctional or little utilized pathways of detoxification and elimination such as those in the lungs, liver, kidneys, intestine, and macrophage systems suddenly become essential for maintenance of the "milieu intérieur."

Some of the processes alluded to above do not change rapidly as a consequence of birth, but evolve more gradually. It is the intention of this chapter to examine some of those adaptations upon which the immediate survival of the newborn infant depends.

## THE LUNG AND THE ESTABLISHMENT OF EFFECTUAL GAS EXCHANGE

At full-term gestation in the human, the lung and neuronal mechanisms controlling breathing have reached a state of growth and maturity sufficient for successful functional adaptation of the fetus to extrauterine life. Adequacy of function is determined by the anatomical size of the gas exchange area, by the air sac-stabilizing activity of surface-active materials lining these functional units, and by the neurological regularization of breathing mechanisms and controls. The final pulmonary determinant in gas exchange is the matching of pulmonary perfusion with ventilation.

### Lung Growth

Sufficient space, the secretion of lung fluid against a resistance, and diaphragmatic phrenic nerve innervation have been shown to be prerequisites for normal growth and structural development of the fetal lung. Hormonal influences are also of importance. Reduced space, as when developing abdominal organs are present in the chest in association with a diaphragmatic hernia, causes lung hypoplasia (Areechon and Reid,

1963). In utero secretion of lung fluid by the developing fetal lung against a resistance imposed by intermittent glottic closure ensures the maintenance of a positive pressure gradient between the potential air spaces and the amniotic cavity during development, and has been recorded in the fetal lamb (Alcorn et al., 1977). Drainage of lung liquid by tracheal cannulation in the fetal lamb during the last trimester causes inhibition of lung tissue growth and development, the walls of the primitive air spaces remaining thick as in early gestation. Normal diaphragmatic phrenic nerve innervation has also been shown to be essential in the fetal lamb. Thus chronic phrenectomy during the last trimester was associated with lung hypoplasia at term, whereas bilateral vagotomy caused no change in lung size (Alcorn et al., 1980). In man, similar hypoplasia is found in association with the loss of muscle innervation in spinal muscular atrophy (Cunningham and Stocks, 1978).

Postnatally in the rat, proliferation of alveoli can be enhanced by exposure to hypoxia for 3-6 weeks (Burri and Weibel, 1971) and can be influenced by the body's oxygen demands as reflected by the basal metabolic rate. (Bartlett, 1970). Such factors may be involved in the increase in alveolar number in man from an estimated $24 \times 10^6$ at birth to $280 \times 10^6$ at 8 years (Dunnill, 1962).

## The Gas Exchange Unit

From the original observations of Pattle (1955, 1958) on lung-derived surface-active materials and from later studies on their physical properties (Brown et al., 1959; Clements et al., 1961; King and Clements, 1972), it has been generally accepted that during dynamic compression of the surface-active molecules at the gas-liquid interface during lung deflation the increasing pressure within the surface film favors retention of air in the lung. On expansion of the surface-active film, the fall in surface pressure contributes to a rise in intra-air-sac pressure and encourages an even distribution of gas within the lung. In the absence of sufficient surface-active materials, high intra-air-sac pressures arise on deflation which cause uneven ventilation and areas of atelectasis. The above description of surfactant function has, however, been challenged, following more recent studies which have indicated that alveoli unfold on lung inflation; in this situation, the surfactants would be acting as "anti-glues" at low lung volumes and would reduce the work of bringing about deformation on inflation (Sanderson et al., 1976). Whatever the mechanism of surfactant action, the fall in surface pressure at the air-liquid interface in gas exchange units which occurs with the distension of inflation tends to cause gas to be distributed to nonaerated or less well aerated air sacs.

The maturation of the synthetic pathways of surfactants is not the concern of this chapter. However, in the lamb, rising levels of corticosteroids, derived from the fetal adrenal, have been detected in the week prior to the onset of labor and might provide the natural stimulus in that species to surfactant synthesis and chemical remodeling of phospholipids. The surge in the blood levels of catecholamines observed in human cord blood samples at delivery (Sever, personal communication, 1980) may be important in causing the discharge of surfactant from the type II alveolar cells into the lung liquid, as has been shown to occur in the fetal rabbit (Enhorning et al., 1977). Augmentation of pulmonary surfactant secretion may also be brought about by the physical process of lung expansion at birth (Lawson et al., 1979). Structural lung changes induced by steroid administration or by secretion are also considered to be important in allowing lung expansion at birth (Mitzner et al., 1979).

### Breathing Movements and Patterns In Utero. Their Relevance to Neonatal Respiration

Although rapid movements, which were attributed to fetal breathing, were noted by Ahlfeld (1905) on kymographic recordings taken from the abdominal walls of pregnant women, scepticism surrounded the interpretation of such observations until the 1930s, when Barcraft and Barron (1937a,b) made a series of observations on fetal breathing in the lamb. Merlet et al., (1970) later documented episodes of rapid breathing in chronically catheterized fetal lambs and showed that they were associated with negative intrapleural pressures of as low as $-40$ $cmH_2O$. In man, both Doppler ultrasound (Boyce et al., 1976) and the use of the tocodynamometer (Timor-Tritsch et al., 1979) have confirmed the presence of breathing movements in the human fetus. In the lamb fetus Dawes et al., (1972) demonstrated an association between the presence of fetal breathing movements and rapid eye movement (REM) sleep—a link between a behavioral state and breathing before birth. An enigma surrounding fetal breathing patterns is the observation in the human fetus near term that less than 50% of any 24-hr period is occupied by these movements. Extrapolating from the measured fetal blood gas concentrations in other mammalian species and from those recorded in pregnant women, it may be assumed that the gas tensions prevailing in the human fetus would, if responses in utero were similar to those of the neonate, promote an increase in tidal volume and, via consequent activation of stretch receptors (Guz et al., 1966), an increase in respiratory frequency, thereby abolishing the prolonged periods of apnea. Some recent animal data may throw light on this question. Pagtakhan et al. (1971) showed in the fetal lamb that the apparent insensitivity of respiratory drive was not absolute (Table 1). More recently Moss and Scarpelli (1979) demonstrated that nonspecific arousal via somatic stimulation reduced the breathing threshold to $CO_2$ in previously apneic lamb fetuses as did naloxone; naloxone caused initiation of breathing as well as an increased sensitivity to $CO_2$, implying that endogenous opioid peptides might be involved in the physiological suppression of breathing in fetal life. The highest fetal $paCO_2$ recorded by these investigators prior to the onset of breathing movements was about 52 Torr. The same authors have shown that $\beta$-endorphin injected in the cerebrospinal fluid of dogs causes central depression of cardiovascular and respiratory control neurons and facilitation of central vagal projections mediating bradycardia (Moss and Scarpelli, 1981). In newborn rabbits endorphin-induced depression of ventilatory responses has been indirectly shown to diminish with increasing postnatal age (Grunstein et al., 1981).

**Table 1** Arterial Gas Tensions at Which Breathing Was Initiated

| $paO_2$ (mmHg) | $paCO_2$ (mmHg) |
|---|---|
| 5 or less | Less than 40 |
| 6-14 | 40-100 |
| 17-20 | Greater than 100 |

*Source*: From Pagtakhan et al. (1971).

An alternative possibility, that there might be insufficient afferent neuronal traffic to the centers controlling respiration located in the medulla in utero due to relative insensitivity of peripheral chemoreceptors and relative lack of cutaneous or muscle stretch receptor stimulation, was explored in dogs by Sullivan et al. (1978). Sequential removal of afferent respiratory stimuli (wakefulness and vagal, peripheral, and central chemoreceptors) during slow-wave sleep caused progressive depression of respiratory drive until finally an "ideorhythm" of only one breath per minute remained. It is not clear whether this occurred in spite of continued medullary efferent activity which failed to reach threshold stimulatory levels at lower neurons or whether it represented primary medullary suppression. However, in the human fetus, even this basic rhythm is not demonstrable for prolonged periods. The opportunity for fetal chemoreceptor stimulation in normal human pregnancy is diminished by the presence of a fetomaternal gradient for $CO_2$ and by the low maternal $paCO_2$ during pregnancy (31-32 Torr). It would appear that progesterone causes hyperventilation by mechanisms not involving chemoreceptors (Skatrud et al., 1978; Machida, 1981). The lamb fetus is also relatively buffered from changes in maternal $paO_2$ (Matalon et al., 1978). However, because of the position and shape of the fetal hemoglobin oxygen dissociation curve, small changes in fetal $paO_2$ will markedly affect blood oxygen content and, thereby, tissue oxygen delivery.

Further evidence that breathing inactivity in the fetal lamb is not due solely to neural or endorphin depressent effects on the respiratory center has come from the elegant studies of Bystrzycka et al. (1975). These authors have shown continuous medullary efferent activity during apnea, implying that there must be inhibition at a more peripheral site; however, sustained slow, regular respirations of a postnatal type have been induced in lamb fetuses near term by pilocarpine infusion in the presence of intact carotid sinus nerves (Brown et al., 1981). The change in breathing pattern was considered to be due to a central nervous system arousal effect as a consequence of which previously subthreshold afferent stimulation became excitatory. Nonetheless, the importance of afferent neuronal activity is apparent in the changes at birth, although in the lamb intact carotid body chemoreceptor afferents are not essential for the successful establishment of postnatal breathing (Jansen et al., 1981).

### Onset of Breathing

The regularization of breathing movements at birth in the lamb has been shown to be associated with a change in chemoreceptor responsiveness to both $CO_2$ and $O_2$ tensions (Biscoe and Purves, 1965; Purves and Biscoe, 1966). This was ascribed to a documented increase in sympathetic activity; it was speculated that this activity brought about a decrease in blood flow to the chemoreceptors, which would tend to increase the drop in $pO_2$ in blood across the receptor organs and hence, presumably, the chemical stimulus of hypoxia. The gas tension combinations which have been shown to stimulate breathing movements in various mammalian species (Pagtakhan et al., 1971) (Table 1) are rapidly reached in the human at birth. Evidence for a central neural mechanism causing persistence of breathing, beyond that which would be expected following cessation of a stimulus, has been reported in the cat (Millhorn et al., 1980). Changes in the concentration of a long-acting central neurotransmitter could cause an afterdischarge by temporarily changing the excitability of the involved neurons; for example, serotonin depletion in the brains of conscious rats causes hyperventilation (Olson

et al., 1979). However, following administration of selective neurotransmitter antagonists to cats, no effect on respiratory after-discharge patterns was recently demonstrable, thus favoring the suggestion that these after-discharges are due to mutual reexcitement—a reverberation—in the neuronal network involved (Millhorn et al., 1981).

In order to generate the negative intrapleural pressures necessary at birth to surpass the opposing forces deriving from the viscosity of lung fluid and from the low pulmonary compliance, efferent neurological impulses must bring about stabilization of the chest wall; this provides a semirigid frame for effective diaphragmatic action. The synchronous increase in laryngeal cross-sectional area due to the coordinated contraction of cricothyroid and posterior cricoarytenoid muscle groups, as recorded in the rat (Sherrey and Megirian, 1980), ensures anatomical patency of the airway. The low lung compliance probably reflects the initial diminished elasticity of the fluid-filled lung and subsequently the relatively small volume of lung which is aerated in the first days of life.

### Fetal Lung Liquid

Recent measurements in the fetal lamb have shown a decrease in tracheal fluid flow as early as 7 days prior to birth (Kitterman et al., 1979). The rise in plasma cortisol, which in the fetal lamb occurs 48 hr prior to the onset of labor, was associated with a further diminution in production. Inhibition of lung fluid secretion by acinar cells can be caused by exogenous administration of as little as 0.76 $\mu$g/min of epinephrine; the sensitivity of this response increases with advancing gestation and in the fetal lamb at term causes absorption of up to 40 ml/hr of fluid (Walters and Olver, 1978). Very high levels of catecholamines are present in human cord blood following normal vaginal delivery (Sever, personal communication, 1980). Evidence for induced active sodium transport has been obtained and the results are consistent with the hypothesis that net lung liquid absorption occurs as a consequence of the induced efflux of sodium from lung lumen to plasma (Brown et al., 1980; Olver et al., 1981). In theory this mechanism could be activated during labor for removing lung liquid from the newborn.

During vaginal delivery, lung volume diminishes owing to thoracic compression (Karlberg, 1960). In studies on the human neonate, Karlberg recorded positive pressures in the esophagus as high as 95 $cmH_2O$ during delivery which dropped to almost zero immediately after birth. As chest compression ceases on delivery, passive recoil draws air into the nasopharynx and trachea. In three cases studied, volumes of 7, 29, and 42 ml, respectively, were recorded, representing a significant proportion of the expected functional residual capacity of each baby.

With the first breath, which is characteristically short, a negative intrathoracic pressure of up to 70 $cmH_2O$ is generated. This force is dissipated by the inertial and viscosity characteristics of the lung liquid, by the lung tissue itself, and by surface tension forces at the air-fluid interfaces in the airways. Following the first breath, a phase of breath holding is frequently observed in which *positive* intrathoracic pressures of up to 60 $cmH_2O$ have been recorded (Karlberg and Koch, 1962). A second inspiratory effort (Head's paradoxical reflex) may be observed during early breathing, superimposed on individual inspiratory movements, and these surve to further increase lung aeration (Head, 1889). In preventing the generation of large pressure swings by maintaining monotonous low tidal volume ventilation in fetal goats, Egan et al. (1980) found that large volumes of alveolar liquid were retained in the lungs, with only 2% being absorbed per hour.

The composition of lung fluid, as investigated in the lamb (Adamson et al., 1969) derives from the presence of an active transport of chloride ions from plasma which exceed $HCO_3$ movement in the reverse direction; $Na^+$ and accompanying water move down the resulting electrical gradient (Olver and Strang, 1974). Proteins are excluded owing to the small dimensions of the water-filled pores in the acinar walls (Normand et al., 1971). An osmotic gradient of about 25 mmHg from lung liquid to plasma therefore favors movement of fluid from potential air spaces to lung interstitium, lymphatics, and vascular capillaries; this occurs when chloride transport from plasma is inhibited. Some indication of the speed with which lung aeration is achieved was provided by neonatal chest radiographs (Karlberg, 1960). Air becomes progressively trapped in the lung, forming the functional residual capacity (1-10 ml/kg body weight).

When there is delay in lung liquid removal, evidence of worsening respiratory distress is seen. Radiology of the chest may reveal hyperinflation, due to air trapping distal to partial fluid occlusion in the airways, and the presence of pulmonary infiltrates. In a recent study on the pulmonary function in such infants, venous admixture due to the presence of open air spaces with reduced alveolar ventilation to capillary perfusion ratio averaged 8% of the total calculated admixture; an occasional infant was documented as having an extremely poorly ventilated compartment near the end of nitrogen washout (Corbet et al., 1979). It has been suggested that this might represent airway closure with gas trapping in the range of the tidal volume, and might arise from the effects of fetal lung liquid retained in the interstitium or within the lumina of airways (Hanson and Shinozaki, 1970).

The establishment of a newly sited air-liquid interface within 1-10 sec of the first breath due to absorption of lung liquid at the acinar surface probably serves to concentrate the surface-active materials within the fluid at the cell surfaces and thereby helps stabilize the newly inflated lung.

### Rhythmic Control of Breathing

Under steady-state circumstances, involuntary respiration is regulated to balance the uptake of oxygen and the release of carbon dioxide with the rate of metabolism (Olson et al., 1979). Following delivery, the balance between gas exchange and metabolism becomes a function of the baby's respiration. Although the basic mechanism for rhythmic breathing remains unknown, groups of neurons in the brainstem are primarily responsible for the generation of a rhythmic pattern. This activity has been demonstrated in the newborn lamb (Bystrzycka et al., 1975). For a general review of the neural generation of the breathing rhythm, the reader is referred to Wyman (1977). Much of the controversy surrounding the interpretation of investigations into the control of breathing in the newborn arises from the complexity of alterations in brainstem neuronal activity, spinal efferent and afferent inhibition, and paradoxical motion of the upper and lower rib cage that may occur. Certain of these changes are associated with changes in sleep state, that is, from REM or non-REM sleep. For example, as cited by Henderson-Smart and Read (1979), the effects of airway occlusion tests in which air flow is interrupted at the start of an inflation may differ in different sleep states owing to associated alterations in respiratory muscle properties.

In considering the neonate's capability of controlling blood gas tensions, it is necessary to examine the mechanoreflexes involved, the sensitivity of peripheral and central chemoreceptors, the effects of gestation and postnatal age, the functional reserves of the respiratory muscles, and the effects of sleep state on the function under investigation.

There is general agreement that in passing from non-REM to REM sleep, respiratory minute volume rises and $paCO_2$ falls (Hathorn, 1974; Finer et al., 1976). Mean tidal volume is unchanged, but thoracic gas volume falls by about 31% (Henderson-Smart and Read, 1979). The fall in thoracic gas volume has been thought to be secondary to two possible mechanisms: (1) the loss of the neuronal reflex coordination responsible for braking the expiratory air flow which results from the dissociation of laryngeal muscle function from diaphragmatic activity, as has been demonstrated to occur in the rat in passing from non-REM to REM sleep (Sherrey and Megirian, 1980), and (2) to increased prevalence of chest wall distortion in REM sleep (Knill et al., 1976). However, the latter assertion has been disputed, and although distortion is certainly observed more in preterm than in term infants, the frequency of such asynchrony of intercostal muscle and diaphragmatic stimulation may not differ between REM and non-REM sleep (Davi et al., 1979). Davi et al. suggested that their data are more in agreement with the concept that respiration can be either in or out of phase in REM sleep in the newborn.

Chest wall stability and laryngeal occlusion may have important effects on the duration of inspiration ($T_i$) and expiration ($T_e$) and on diaphragmatic fatigue. In normal circumstances, $T_i$ is reflexly interrupted by the phasic increase in lung volume. In the neonate $T_i$ and $T_e$ appear to be independent. By applying a negative pressure to the chest wall to achieve an increase in lung volume, Stark and Frantz (1979) demonstrated an increase in $T_e$ with no change in $T_i$. Only by abolishing phasic lung volume changes by occluding the airway was $T_i$ increased. It had previously been shown that in the preterm infant this maneuver causes shortening of $T_i$ until the tenth postnatal day, after which responses become similar to those found in term infants (Thach et al., 1978). This change in response has been ascribed to the maturation of the Hering-Breuer reflex. Term newborns, breathing within the range of their normal tidal volumes, would appear to be under maximal vagal influence so that any further increase in tidal volume has no effect on $T_i$ via the Hering-Breuer reflex.

The importance of chest wall distortion derives from the demonstration of an inspiratory inhibitory reflex which is activated both by distortion (Knill and Bryan, 1976) and vibration to the chest wall when applied over the expiratory intercostal muscles (Homma, 1980). The recent opportunity to study a quadriplegic neonate (Thach et al., 1980) indicated that intercostal-phrenic reflexes are not essential for REM-sleep-induced changes, and airway occlusion caused $T_i$ shortening in this case. The authors suggested that vagal afferents deriving from receptors in the airways may be mediating the reflex. Activation of reflexes causing $T_i$ shortening on occlusion can be obliterated by administering continuous positive airway pressure, which minimizes any distortion caused by occlusion (Hagan et al., 1977). However, continuous positive airway pressure might equally be preventing activation of airway-related receptors.

Chest wall distortion has marked effects on diaphragmatic fatigue (Muller et al., 1979). Analysis of electromyogram frequency spectra from newborn intercostal and diaphragmatic muscles has revealed a progressive fall in high-frequency power with increasing chest wall distortion. This finding corroborates evidence from histochemical studies (Keens et al., 1978) which have shown the respiratory muscles of newborns to be poorly equipped to sustain high workloads, being relatively deficient in highly oxidative, slow-twitch fibers; preterm gestation newborns are more severely deficient than term infants. Increased intercostal muscle activity is associated with a diminution in fatigue recorded from the diaphragm, while in preterm infants, loss of inspiratory muscle tone causes a fall in functional residual capacity (Lopes et al., 1981). Although

in the earlier study, recovery from fatigue occurred within 5-20 breaths following apnea in normal babies, recovery was prolonged in those with increased respiratory loads. During ribcage distortion, the force generated by the diaphragm is dissipated and fatigue ensues.

The implications of these findings for the neonate are that, although glottic closure might normally cause a reflex increase in $T_i$, if the chest wall is unstable, activation of the inhibitory reflex will shorten $T_i$; if this response occurs at the lower thoracic gas volume associated with REM sleep, the lack of oxygen reserves may place the baby in a critical situation.

### Ventilation and Perfusion Matching in the Lung

From studies on 26 normal newborn under 4 days of age the alveolar-arterial oxygen difference during air breathing was found to average 28 mmHg, approximately three times the adult value (Nelson et al., 1963); however, $N_2$ washout indicated an excellent distribution of ventilation in half of the newborns, implying that other sites of shunting such as the foramen ovale and ductus arteriosus were present.

### Chemoreceptor Responsiveness in the Newborn

In considering the chemoreceptor responsiveness in the newborn, for both term and preterm infants, breath-to-breath respiratory variability is greater in active sleep than in quiet sleep, but the percentage duration of apnea, although greater in active sleep, is significantly less in the term than in the preterm infant (Siassi et al., 1979).

Recent studies in preterm infants have shown that during either REM or non-REM sleep the breathing pattern may change from periodic to regular and that this change can be mimicked by administration of low inspired $CO_2$ concentrations (Rigatto et al., 1980). This change in breathing pattern, which was observed to occur spontaneously or regularly on $CO_2$ administration, was associated with an increase in respiratory minute volume; this was due to a rise in respiratory frequency, there being a small decrease in tidal volume. Such a finding, it is argued by the authors, gives support to the hypothesis that changes in breathing pattern during a given sleep state occur through changes in the chemical control of breathing. It is of interest that the increase in ventilation associated with the change in breathing pattern, whether occurring spontaneously or on administration of $CO_2$, is achieved by an increase in frequency rather than by an increase in tidal volume, as occurs in adults (Davi et al., 1979). However, these latter investigators demonstrated that although the level of $paCO_2$ at which ventilation began to increase was lower in REM than in non-REM sleep, once initiated, there was no difference in $CO_2$ responsiveness between the two sleep states. This is in conflict with earlier studies (Bryan et al., 1976).

Diminished ventilatory responsiveness to chemoreceptor stimulation by reduced oxygen tensions has been demonstrated in the newborn (Cross and Oppé, 1952). An initial increase in ventilation is unsustained. After the first postnatal week, a greater and more sustained response is observed (Brady and Ceruti, 1966). A similar maturation of responses has been demonstrated in newborn monkeys and it is hoped that they may be a useful model for further study of this age-related change (Woodrum et al., 1981). However, the poor response to hypoxia in preterm neonates may indicate central effects of hypoxia and may explain the flatter response to $CO_2$ under hypoxia at early gestations (Sankaran et al., 1979); in preterm infants a sustained increase in ventilation was not

observed until 18 postnatal days (Rigatto et al., 1975). In young beagle puppies the age-related maturation of breathing responses to hypoxia has been found to be slower if the animals were tested in quiet rather than in REM sleep (Haddad et al., 1982), and in the cat, a diminishing threshold for response to $paCO_2$ in the presence of increasing hypoxia has been demonstrated (Lahiri et al., 1978). Hypoxia depressed the central mechanism for resumption of inspiration, inducing more prolonged apnea in the presence of hypocapnia. As cited above, naloxone abolishes hypoxic respiratory depression in the newborn rabbit, provided that apnea is not established (Hazinski et al., 1981).

Although adequate for achieving gas exchange in the normal newborn, the respiratory functional reserves and chemoreceptor responses are clearly in a precarious state should problems arise in neuroventilatory control or function after birth.

## CARDIOVASCULAR ADAPTATIONS

The cardiovascular changes that occur immediately after birth result in an increase in pulmonary blood flow to ventilated acinae. This is achieved by a reduction in pulmonary vascular resistance and functional closure of the foramen ovale. More gradual adaptations include functional followed by permanent closure of the ductus arteriosus, a progressive fall in pulmonary vascular resistance, pulmonary artery and right ventricular pressures, and changes in heart rate and systemic blood pressure.

### Fetal Circulation

Investigation of the fetal circulation has been principally confined to the fetal lamb; the knowledge accumulated from such studies and the possible human neonatal implications have been reviewed by Rudolph (1970), Goodwin (1976), and Strang (1977). Fetal aspects that are of particular interest when considering the adaptations at birth are summarized: Umbilical venous blood from the placenta is relatively well oxygenated ($pO_2 \approx 33$ mmHg) and that proportion entering the heart via the inferior vena cava is diverted by the lower extension of the interatrial septum—the crista dividens—into the left atrium and thence via the left ventricle to the ascending aorta. Blood from the superior vena cava passes into the right ventricle, but only 10-15% of the right ventricular stroke volume reaches the pulmonary circulation, the remainder passing through the large ductus arteriosus to the descending aorta. The coronary and cerebral circulations are therefore assured of a blood supply of higher oxygen content (ascending aorta $pO_2$, 25-28 mmHg) than that to the lower body tissues (descending aorta $pO_2$, 19-22 mmHg). The placenta, with its low vascular resistance, receives 40-50% of the total left and right ventricular output near term in the fetal lamb.

### Fetal Adaptations to Hypoxia

Circulatory adaptations to hypoxia, as might be incurred in labor, have been studied in the fetal lamb (Sheldon et al., 1979). It was found that blood flow to coronary arteries and the brain became a larger fraction of the total cardiac output, thus ensuring a constant tissue oxygen delivery to these sites in the absence of any marked increase in cardiac output. Sheldon et al. pointed out that the weight of the human brain relative to body weight is six to seven times that of the fetal lamb, so that the arterial oxygen

content at which oxygen delivery to the brain becomes inadequate may be considerably higher for the human neonate than for the lamb. In regard to the maintenance of brain oxygen supply, the relative importance in the human under hypoxic stress of blood flow redistribution compared with the overall increase in cardiac output is not known.

Marked elevation of the plasma level of antidiuretic hormone has been found in association with hypoxic stress and, in the human, following head compression (Leung et al., 1980). High levels of catecholamines are also present (Sever, personal communication, 1980). Infusions of vasopressin into fetal sheep (Iwamoto et al., 1979) to achieve values found in stressed newborn lambs cause a rise in mean arterial pressure and a fall in heart rate which is not solely attributable to secondary neuroreflex activity. A fall in circulatory flow to gastrointestinal and peripheral tissues was observed, with an increased percentage of the cardiac output going to the placenta, brain, and myocardium. A potent stimulant of myocardial contractility with marked vasodilator activity on coronary arteries is adenosine. Adenosine markedly increases in hypoxic states following the breakdown of adenosine 5'-triphosphate (Liang and Lowenstein, 1978). Since some degree of increased hypoxia is an almost inevitable consequence of most "normal" deliveries, the distinctness of normality in regard to neurohumoral and cardiovascular findings in the neonate is difficult to define.

The volume of blood passing from placenta to fetus has been shown to be influenced by the time of cord clamping in relation to delivery (Usher et al., 1963) and is increased following intrauterine hypoxia, except where partial occlusion of the umbilical cord results in placental pooling due to umbilical vein obstruction; there is then a reduction in neonatal blood volume (Yao and Lind, 1974; Linderkamp et al., 1978a). The volume transfused can have profound effects on circulatory adaptations, including ventricular systolic pressures and pulmonary vascular resistance.

### Pulmonary Vascular Resistance

The rate of fall in pulmonary vascular resistance on ventilation of the lung is slower in the human neonate (Rowe and James, 1957; Rudolph et al., 1961) than in the lamb, where an immediate marked increases in pulmonary blood flow, fall in pulmonary artery pressure, and fall in calculated vascular resistance have been noted (Dawes et al., 1953). The relative contributions of the rise in oxygen tension, the fall in carbon dioxide tension, and the mechanical effects, which may derive from the changes in surface tension at the newly formed gas-liquid interface in the peripheral lung, to the observed changes in pulmonary circulatory resistance have each been shown to be of about a third of the total (Cassin et al., 1964).

The precise mechanisms by which vasodilation is brought about through changes in $pO_2$ and $pCO_2$ are unclear. It has been suggested (Staub, 1963) that oxygen diffusing from adjacent alveoli would be present in sufficient concentration to cause vasodilation of the pulmonary precapillary resistance vessels. The chemical mediation of this effect in the lamb may in part be via conversion of kininogen to bradykinin (Campbell et al., 1968). A fall in left atrial kininogen level has been shown to accompany ventilation of the fetus with oxygen, as did placing the ewe in a hyperbarric chamber; the latter maneuver gave rise to a mean systemic $paO_2$ of 44 mmHg in the fetus; ventilation of the fetal lung with nitrogen was associated with no change in the kininogen level (Heymann et al., 1969). Neonatal "leukocytes" have been shown to be capable of kininogen activation at oxygen tensions found postnatally, but not at oxygen tensions as found in the fetus (Melmon et al., 1968).

Other substances with demonstrable effects on pulmonary vascular resistance have been reviewed by Strang (1977), but the relative importance in physiological terms of any given compound is unresolved. It does seem probable that the prostaglandins (PG) are of importance in maintaining relaxation of the pulmonary vasculature in utero; inhibition of PG synthetase by indomethacin administration to the ewe, which causes a diminution of fetal PG, is associated with fetal pulmonary vasoconstriction, a rise in pulmonary artery pressure, and increased muscularity of the pulmonary vascular bed (Levin et al., 1979). Fetal infusions of $PGE_1$ temporarily reversed the functional changes. Ventilation of fetal lambs and fetal goats has been shown to cause pulmonary synthesis of $PGI_2$-like material (Leffler et al., 1980) which has local vasodilator activity; in the goat, the slow prolonged decline in pulmonary vascular resistance which follows the onset of ventilation was not seen in animals who had received prior treatment with indomethacin. It is conceivable that the release of bradykinin and the increase in PG synthesis are linked, since such an association has been noted in the adult kidney.

Species differences in the rate of fall in right ventricular systolic pressure postnatally (Rudolph, 1970) (Figure 1) are striking and it is worth emphasizing that extrapolation of findings in one species to another, as in so many other postnatal adaptations, may sometimes be fallacious. The magnitude and rate of fall in pulmonary arterial pressure in the lamb are dependent on the efficacy of lung inflation, the flow to the lungs increasing four- to sixfold after respiration (Assali et al., 1962).

### Closure of the Ductus Arteriosus in Relation to Pulmonary Changes

The removal of the placental circulation results in a marked increase in the overall systemic vascular resistance. This increase, when combined with the fall in pulmonary vascular resistance, produces a reorientation of flow pattern in the circulation. While the ductus remains open, pressures in the pulmonary trunk and systemic circulation remain approximately equal; in the human, pulmonary arterial pressure has been found to be between 20 and 50 mmHg at 6-8 hr age, falling to 20-35 mmHg by 48 hr (Emmanouilides et al., 1964). The rise in pulmonary venous return causes closure of the flap overlying the foramen ovale and an increase in left ventricular stroke volume. If the flap over the foramen ovale is incompetent (Rudolph, 1970), left to right interatrial shunting may continue; the greater systolic emptying of the right ventricle compared with the left once the pulmonary vascular resistance has fallen favors preferential filling from the higher-pressure left atrium. Although in the lamb reversal of ductal flow follows the onset of ventilation and may persist for up to 3 days (Dawes et al., 1955), in the human there is a high incidence of right to left shunting in the first hour after delivery (Moss et al., 1963). Such a shunt was found in 8 of 63 newborn under 6 hr of age. The sensitivity of the ductus to further hypoxic exposure was demonstrated by Eldridge et al. (1955) and Moss et al. (1964). The former demonstrated the development of a right to left shunt in four babies where none had previously been noted, following induction of hypoxia, and the latter authors demonstrated left to right shunts through the ductus in five of six newborns less than 15 hr old in whom administration of 100% oxygen was followed by ductal closure, the ductus tending to reopen on return of the infant to airbreathing. These investigators also demonstrated similar shunting in 1 out of 9 babies studied between 15 and 27 hr of age; 2 of the 8 with no demonstrable shunt developed evidence of a shunt on exposure to a low inspired oxygen tension (13%), and in 12 similarly exposed newborns,

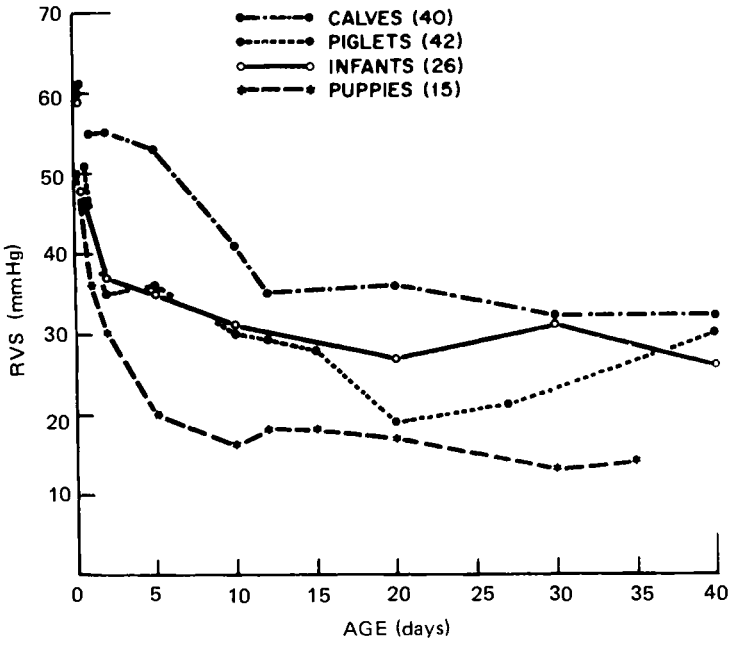


**Figure 1** Decline in right ventricular systolic pressure after birth in the puppy, calf, piglet, and human infant. The difference in the rate of decline of pressure is shown. (From Rudolph, 1970.)

pulmonary artery pressure rose by 15-67%. In contrast to the lamb, the normal human neonate was found, in studies reported in 1963 (Moss et al., 1963), to maintain a high pulmonary arterial pressure which in 24 of 34 babies studied exceeded 50% of the pressure in the aorta up to 15 hr of age. In these investigations, alterations in pressure coincident with changes in the phase of respiration, cardiac cycle, and behavioral state were associated with bidirectional shunting which persisted for as long as 6 hr. The direction eventually became left to right and, after 15 hr, was physiologically insignificant or had disappeared. Adams and Lind (1957) thought the ductus to be functionally patent for as long as 3 days, but the interpretation of their data has been questioned (Moss et al., 1964).

The ductus arteriosus constricts on exposure to a rise in oxygen tension, and relaxes when this falls. It also constricts in response to catecholamines (Born et al., 1956) and to acetylcholine and bradykinin (McMurphy et al., 1972). In vitro studies performed by Kovalcik (1963) on sections of guinea pig and lamb ductal tissue demonstrated that abolition of the vasoconstrictor response to oxygen was produced by prior exposure of the tissue to chlorpromazine. Constrictor responses to noradrenaline, acetylcholine, and bradykinin were all reduced in the absence of oxygen. These observations were taken to imply the involvement of a flavoprotein enzyme in the mediation of the oxygen effect. The evidence for kininogen activation to bradykinin and the documentation of high levels of catecholamines in the lamb and human following normal delivery have been referred to above. Further evidence for linking the ductal response to oxygen with chemical events involving an oxygen-sensing enzyme

has been provided by Fay (1973). Fay showed that in rings of guinea pig ductus, oxidation of cytochrome $a_3$ always preceded contraction; chemical reduction of the cytochrome was followed by relaxation. The very low oxygen tensions (0-2 mmHg) at which oxidation of the cytochrome would be achieved might exist in ductal wall tissues, since no vasa vasorum are present. The final pathway of chemical mediation remains undefined, although Fay speculated that increased synthesis of adenosine 5'-triphosphate might cause a rise in free cytoplasmic calcium, thereby triggering contraction.

Microscopic examination (Gittenberger-De Groot, 1977) of the normally closing ductus has shown intimal cushions protruding into the duct lumen, with fragmentation of the underlying internal elastic lamina and the presence of mucoid material in the media; later cytolytic necrosis is found.

Measurements of mean pulmonary capillary blood flow in 23 newborn humans have shown a progressive increase with time from delivery (Brady and Rigatto, 1971), with mean values of 173 ml/kg min at 24 hr, 182 ml/kg min at 72 hr, and 230 ml/kg min at 14 days. These changes are accompanied by an overall increase in heart rate, from 125 beats/min at 24 hr to 168 beats/min at 14 days, but with no significant change in stroke volume (mean value 1.4 ml/kg). These gradual changes in vascular resistance and blood flow have been indirectly followed using echocardiographic techniques (Riggs et al., 1977a; Halliday et al., 1978). The ratio of the right ventricular preejection period to the right ventricular ejection time decreased with age in term infants at a faster rate than was observed in preterm neonates, in whom the pulmonary vascular resistance is known to be initially lower. The mean right ventricular preejection period was 76.5 msec between 0 and 12 hr, falling to 60 msec at over 48 hr. Correlations between echocardiographic and postmortem measurements of ventricular muscle mass and volume (Warburton et al., 1979) have shown the method to have considerable potential.

### Responses to Hypoxia

Deviation from the normal pattern of ductal closure has been noted in cases of early cord clamping and where hypoxia is present. Hypoxia induces pulmonary vasoconstriction, ductal relaxation, and possibly, if severe, myocardial dysfunction due to subendocardial ischaemia (Rowe and Hoffman, 1972; Rowe, 1977). Evidence that the response of pulmonary precapillary vessels to hypoxia may be mediated by alterations in the redox state of cytochromes which modulate the formation of a chemical mediator has been provided by Miller and Hales (1979). They showed that ventilation of one lung of a dog with 100% nitrogen, with the other lung ventilated with 100% oxygen, caused a 31% drop in perfusion to the hypoxic lung. If hypoxic ventilation was preceded by administration of metyrapone or carbon monoxide, which are inhibitors of cytochrome $P_{450}$, the drop in perfusion in the hypoxic lung was only 10%. In their experiments, the level of oxygen tension in the lung which was associated with vasoconstriction was between the $pO_2$ of blood reaching the lung in the left pulmonary artery (34 mmHg) and that in the nitrogen-ventilated alveoli, where diffusion from blood produced an oxygen tension of 23 mmHg.

Other potential inducers of vasoconstriction of the pulmonary vessels are the precursors of $PGE_2$ and $PGF_2$: $PGG_2$ and $PGH_2$ (Bowers et al., 1979). Both these endoperoxides have been shown in the lamb to be 100 times more potent than their

metabolites in causing vasoconstriction; since $PGE_2$ and $PGF_2$ are released from lungs during hypoxia, their potent precursors must have been formed, but whether they are important in the human is not yet known.

### Postnatal Changes in Cardiac Output

In the fetus the ventricles function in parallel, whereas postnatally they function in series; this change results in an overall increase in the combined ventricular output, which in the lamb increases from 500 to 850 ml/kg $min^{-1}$ at 1 week of age (Klopfenstein and Rudolph, 1978). The increase arises from a marked rise in left ventricular output, which doubles its stroke volume in that time period. The postnatal rise in oxygen requirements may account for the rise in cardiac output. The high resting cardiac output results in a limited capacity for further increase and lambs in the first week of life were restricted in their abilities to tolerate volume loads. Similar considerations may explain the cardiovascular findings in newborns following placental transfusions associated with delayed cord clamping (Yao et al., 1968). Usher et al. (1963) documented that in the first 5 min of life, placental transfusion caused a 61% increase in the neonate's blood volume, two-thirds of which was lost from the circulation in the first 4 hr. Compensating mechanisms in those with delayed cord clamping include a higher urine output and an increased rate of loss of plasma from the vascular compartment (Yao and Lind, 1974).

### Peripheral Circulatory Considerations

In the normal human newborn, the peripheral circulation is characterized by a high blood flow with low perfusion pressure resulting from a markedly diminished vascular resistance as compared with that in adults. Intact vasomotor responses producing an increase in vascular resistance have been demonstrated in association with hypovolemia, hypoxia, and low ambient temperatures and have been discussed by Linderkamp et al. (1978b). These investigators have shown that blood flow, systolic blood pressure, and peripheral resistance are all influenced by blood volume but are also dependent on blood viscosity. Although at a given blood volume, blood flow diminishes with increasing viscosity, this effect is partly compensated at higher blood volumes. At the higher viscosities encountered, peripheral vascular resistance was increased, necessitating more cardiac work.

### Blood Pressure

Apart from a slight fall in systolic blood pressure in term babies during the first 4 hr of life (Kitterman et al., 1969), continuous recording in the newborn has revealed no progressive changes with advancing age or differences between REM and quiet non-REM sleep (Schachter et al., 1976). However, Gupta and Scopes (1965) noted significant falls in blood pressure during deep sleep, with rises occurring in relation to sucking activity. Sleep states were determined by observation.

### Transitional Circulation

The instability of the pulmonary circulation and ductus arteriosus on reexposure to hypoxia after delivery may prejudice survival should a return of right to left shunting and associated hypoxemia cause progressive pulmonary vasoconstriction and systemic

acidemia (James and Rowe, 1957; Roberton et al., 1967; Brown and Pickering, 1974; Riggs et al., 1977b).

Although activation of the sympathetic nervous system has been invoked as contributing to the increasing pulmonary vascular tone (Levitsky et al., 1977), Hales and Westphal (1979) were unable to demonstrate any reduction in hypoxic vasoconstriction in the lung following induction of chemical sympathectomy in dogs. Transient and in some cases persistent pulmonary hypertension is seen in the hypoxic neonate (Levin et al., 1976); in young children living at high altitude, increased muscularity of peripheral pulmonary arteries has been described (Arias-Stella and Saldana, 1962).

The net response to hypoxia depends on the magnitude of local vascular effects, on the responses of peripheral and central chemoreceptors to stimulation, and on the modification of these responses if ischemia becomes profound (Heistad and Abboud, 1980). The relative importance of any individual mechanism in modulating the degree of pulmonary vascular resistance at differing oxygen tensions in relation to postnatal age has yet to be determined.

## EARLY POSTNATAL ENERGY SOURCES

With the cessation in the supply of placentally derived metabolic substrates, the newborn human must mobilize energy sources from stores accumulated in the last trimester of pregnancy (Hull, 1976; Sparks et al., 1980). These stores comprise glycogen and fat and their mobilization enables immediate metabolic requirements to be met. Systemic glucose production in the neonate has been estimated to be between 3.5 and 5.1 mg/kg $min^{-1}$ (Kalhan et al., 1978); this production rate was established by 2 hr of age and would enable glycolysis, which has been shown to be the major pathway for energy production in the infant primate, to proceed (Levitsky et al., 1981).

Initial glucose requirements are met by glycogenolysis; glycogen stores in cardiac and skeletal muscle are 10 and 3-5 times, respectively, those in adults, expressed as grams of glycogen per 100 g of wet tissue (Shelley, 1964). Glycogen stores in liver, muscle, heart, lung, and adipose tissue are rapidly utilized after birth in several mammalian species (Shelley, 1961) and also in man (Shelley and Nelligan, 1966). In man, subcutaneous adipose tissue glycogen levels reach a trough value at 24 hr after birth, total body glycogen at term being about 34 g (Pribylova et al., 1980).

The key hormone in maintaining plasma glucose level at this stage is glucagon. Glucagon, circulating in the presence of low insulin levels, stimulates glucose output from the liver within seconds by the coordinated effects of at least four processes (Park and Exton, 1972): activation of glycogenolysis, inhibition of glycogen synthesis, stimulation of gluconeogenesis, and initiation of adaptive synthesis of some enzymes promoting glucose production.

All the enzymes which are involved in rate-limiting steps in gluconeogenic pathways are present in human liver by 4 months gestation, although phosphopyruvate carboxylase activity is present at only 10% of adult activity in the term neonate, increasing rapidly in the postnatal period (Raïhä and Lindros, 1969). Potential substrates for neonatal gluconeogenesis are lactate, pyruvate, glycerol, and glucogenic amino acids; controlled release of these substrates from peripheral tissue into the blood is achieved by the action of insulin, glucocorticoids, and possibly other hormones. Their release is also determined by the rates of their plasma extraction and conversion to glucose. Glucagon stimulation of gluconeogenesis from lactate and pyruvate is especially enhanced by

catecholamines and glucocorticoids (Park and Exton, 1972). Glucagon does not cross the human placenta (Adam et al., 1972); the plasma level in the newborn increases by 51 ± 8 pg/ml above cord values within 2 hr of birth (Johnston and Bloom, 1973) and the level remains high thereafter (Blázquez et al., 1974). These investigators also recorded a fall in plasma insulin levels in the first 3 hr, the insulin level remaining low thereafter.

An additional important source of glucose production has been demonstrated in the neonatal baboon kidney (Levitsky et al., 1980). Glucose release rates of 0.08, 0.10 and 0.36 mg/min $kg^{-1}$ were found in three newborns studied. The chief substrate for this gluconeogenesis was lactate. If similar renal gluconeogenesis occurs in the human neonate, adequate lactate would probably be available as a product of glycogenolysis in muscle. For further reviews of neonatal glucose homeostasis, the reader is referred to Maniscalco and Warshaw (1978) and Mestyán (1978).

A second important energy source is to be found in fat stores, which amount to about 560 g at term. Interesting interrelations between adipose glycogen stores and lipolysis have been demonstrated in vitro using neonatal adipocytes (Novak et al., 1973). Both glucose and pyruvate promote glycerol release from adipocytes, and the glycogen is rapidly utilized during intracellular reesterification of free fatty acids; adipose tissue glycogen content is higher between 0 and 4 hr than at later ages. Hepatic intramitochondrial oxidation of free fatty acids may indirectly stimulate glucose production from other precursors such as pyruvate by elevating intramitochondrial acetyl coenzyme A and NADH, which activate pyruvate carboxylase. Free fatty acid levels rise postnatally between 0 and 3 hr and remain high (Blázquez et al., 1974); their release is associated with a fall in plasma glycerol (Novak and Monkus, 1972). Following the depletion of glycogen stores, evidence for the switch to utilization of lipid energy stores comes from the documented increases in plasma acetoacetic and β-hydroxybutyric acids and the fall in the respiratory quotient.

Although alanine infusions lead to a rise in plasma glucose in the term newborn, it is not certain whether the rise is secondary to utilization of this gluconeogenic precursor or to its stimulatory effect on pancreatic α-cell glucagon release (Mestyán et al., 1974).

The generation of heat by nonshivering thermogenesis in response to cold stress to which the newborn may be subjected occurs principally in liver, brain, and brown adipose tissue. The oxidation of fatty acids in brown fat can contribute 27 kcal/kg per day; in a 3-kg appropriate-weight-for-gestation newborn, fat stores are probably sufficient for this rate of heat production to continue for 2.8 days. The basal metabolic rate may increase from 35 to 70 kcal/kg per day during cold stress, with brain metabolic activity contributing over half the total heat production (Heim, 1981). The switching on of thermogenic processes is determined by the degree of deviation from that temperature which is regarded as normal for the species. Detailed accounts of thermogenesis and thermoregulation have been recently provided by Hull and Smales (1978) and Bruck (1978).

In summary, to maintain a continuing supply of energy-providing compounds in the postnatal period prior to the acquisition of an adequate nutritional intake, the neonate, having been initially dependent on glucose from glycogenolysis and gluconeogenesis, progresses rapidly to a stage of utilizing products of lipolysis. Although the precise details of these biochemical developments in man are not known, the hormonal environment after birth, comprising high glucagon, low insulin, and high

catecholamine levels, ensures the maintenance of a supply of glucose and energy substrates for cell metabolism.

## NEONATAL SODIUM AND CALCIUM HOMEOSTASIS IN THE HUMAN

Placental transfer of sodium and calcium, with a net positive flux in favor of the fetus, terminates at delivery. The full-term newborn has a marked capacity for renal distal tubular sodium reabsorption (Aperia et al., 1972) and can achieve positive sodium balance soon after birth, even in the presence of a very low sodium intake (Godard et al., 1979). This is in association with a low glomerular filtration rate, as noted by the first group of investigators above, a low creatinine clearance (Weil, 1955), and high plasma levels of renin activity and aldosterone (Dillon et al., 1976). The marked neonatal capacity for distal tubular sodium reabosrption may be due to the high levels of angiotensin II at this age (Pipkin and Smales, 1977), acting either directly on the tubule (Johnson and Malvin, 1977) or mediated via aldosterone secretion. There is a marked rise in aldosterone in the newborn period (Dillon et al., 1976). In spite of the high baseline activity, the renin-angiotensin-aldosterone system is responsive to challenge in the newborn (Sulyok et al., 1980b), as is renal prostaglandin secretion. The latter is normally very low in the neonate (Brouhard et al., 1978); the inability of the neonatal kidney to excrete a sodium load may in part be due to this deficiency, since renal PGA and PGE production are both associated with natriuresis (Lee, 1973). Renal PG secretion does not appear to increase until the third week of life (Sulyok et al., 1980a).

The influence of gestational age at birth on the renin-angiotensin-aldosterone system, sodium conservation, and renal tubular aldosterone responsiveness has been investigated (Sulyok et al., 1979). The increased urinary sodium loss, found particularly prior to 34 weeks gestation, is related to a lack of tubular aldosterone responsiveness (Aperia et al., 1979).

Renal water conservation may also be influenced by the high plasma renin activity. Plasma renin activity has been found to be correlated with urinary osmolality (Godard et al., 1979) and this could be due to enhancement of vasopressin release by angiotensin II (Bonjour and Malvin, 1970). Vasopressin levels, as noted above, are high following birth (Leung et al., 1980).

Factors involved in the early neonatal decrease in plasma total calcium concentration have been reviewed by Tsang et al. (1976). The decrease may be associated with a low level of ionized calcium, the physiologically active fraction (Sorell and Rosen, 1975); it has been ascribed to the relative unresponsiveness of the parathyroid glands to changes in plasma calcium level and to the relative hyperactivity of thyroid C cells, leading to a reduction in calcium resorption from bone as a consequence of the resulting high plasma calcitonin levels (Bergman et al., 1978); however, methodological difficulties have resulted in inconsistencies in the findings in the newborn. These problems have been recently considered (Schedewie et al., 1979), but it would seem that in spite of confirming the findings outlined above in normal term neonates, a definitive description of the interactions of hormonal and nutritional factors in neonatal calcium homeostasis is not currently possible from the available evidence.

The importance of some of the documented acute changes in blood hormone levels during the first days of life in relation to immediate survival has not been clearly defined; sharp increases in plasma pituitary, thyroid, adrenal, and gastrointestinal hormone levels may have survival value.

## CONCLUSION

That the range of variability encountered in the adaptations considered in this chapter is so well tolerated by the newborn is remarkable indeed. However, perinatal practices may have profound effects on normal adaptations; only with increased knowledge can recommendations, based on physiological principles, be soundly constructed so as to achieve a rapid, safe, and optimal perinatal transition. The changes involved in adaptation to extrauterine life are analogous to R. L. Stevenson's assertion that "wherever we are, it is but a stage on the way to somewhere else, and whatever we do, however well we do it, it is only a preparation to do something else that shall be different."

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