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Cartilage

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Preface

This is the third in a series of three volumes devoted to cartilage. Volume 1 dealt with the structure, function, and biochemistry of cartilage; Volume 2 dealt with cartilage's development, differentiation, and growth. This volume covers what I have termed the biomedical aspects of cartilage. Although the topics covered in Volumes 1 and 2 all impinge on the study of cartilage in the human body, the chapters in this volume are more directly related to the inevitable deterioration of cartilage that accompanies aging, disease, or genetic mutations. The introductory chapter discusses the formation of cartilage outside the confines of the skeleton—the so-called ectopic cartilages. It highlights the important but little understood neoplastic and metaplastic processes that produce ectopic cartilage. For a comprehensive overview of mechanisms of cartilage differentiation, this chapter should be read in conjunction with the opening chapter in Volume 2.

Several chapters highlight aspects of age-related changes in cartilage: resorption and remodeling in Chapter 2, lubrication in Chapter 3, and degenerative diseases in Chapter 4. These chapters summarize current theories of how cartilage is maintained under conditions that would be expected to lead to perpetual remodeling, resorption, and functional diminution. Cartilage normally resists invasion by tumors; this is discussed in Volume 1, Chapter 11. Chapter 5 discusses those tumors that do manage to invade cartilage. Two chapters then deal with mutations that affect cartilage. Chapter 6 concentrates on those affecting limb cartilages and highlights the molecular and biochemical bases of some well-studied mutations. Chapter 7 concentrates on mutations that affect craniofacial cartilages and that lead to craniofacial anomalies and growth deficiencies. Here the emphasis is more clinical. Taken together, these two chapters provide a balanced overview of those mutations that affect cartilage and how they might act.

Immunological properties of cartilage, both *in situ* and when used as a graft to stimulate repair, are extensively discussed in Chapter 8, a chapter that emphasizes the need for combined basic and clinical approaches to this important topic. Chapter 9 provides an overview of the role of cartilage in situations involving regeneration and repair of skeletal tissues, and Chapter 10 discusses bioelectrical properties of cartilage and the response of cartilage to bioelectrical stimulation, both *in vivo* and *in vitro*.

Once again it is a pleasure to thank all of the contributors for their willingness to take time from very busy schedules to review their specialized fields within the framework of biomedical aspects of cartilage. It is my hope that this volume, and indeed the three volumes, will serve to emphasize the need for collaborative and coordinated basic and clinical research on cartilage while summarizing current knowledge and pointing toward approaches for the future. Although much has already been done, much remains to be done.

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Volume 1: Structure, Function, and Biochemistry

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1

Ectopic Cartilage, Neoplasia, and Metaplasia

William A. Beresford

I. INTRODUCTION

To pronounce a tissue of an individual as out of its rightful place requires a thorough knowledge of anatomy and its variations. Thus, cartilage is expected in the tongue of a dog, but not in that of a human. Cartilages occurring separately from the principal skeleton, ear, and airway, and as a *normal* part in a particular species, will here be called *extraskeletal,* in distinction to *ectopic* (EC) cartilage, which is not usually found at the site in any member of that species. This convention is necessary here, but contradicts (*1*) the common practice of clinicians to use extraskeletal in the sense of ectopic and (*2*) the skeletal role of the extraskeletal cartilages in the heart, tongue, eye, and elsewhere. Cartilage also arises on or within the skeleton and will be named *osseous* ectopic. For the EC cartilage of soft tissues, *extraosseous* will

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be used. Difficulties and points of interest between the extraskeletal and ectopic categories will be explored.

How easy is it to claim a material in a soft-tissue or bony site as cartilage? Chondroid, pseudocartilage, myxoid, chondroosteoid, and other entities have been hard to distinguish from cartilage, and in some instances the terms may have been misnomers for proper cartilage (see Volume 1, Chapters 1 and 2). True cartilage, of course, has a wide range of forms. How well the ectopic instances meet the morphological and histochemical criteria for cartilage, and the better criteria becoming available will be discussed.

Most EC cartilage falls into Schaffer's (1930) class of *secondary* cartilage, appearing after the establishment of the primary cartilaginous skeleton, and frequently much later in life. The formation of EC cartilage in an already formed and functioning soft connective tissue implies the conversion of one differentiated tissue into another, that is, a *metaplasia* (Virchow, 1884; Willis, 1962). Metaplasia might also happen, were EC cartilage to experience a direct conversion to bone, as is sometimes claimed. In its unexpected late development, the cartilage is a new growth, but when it is normal cartilage, limited in its growth, it is scarcely a neoplasm. However, many cartilage-bearing soft-tissue lesions are without question neoplastic in the modern sense. Consideration will be given to the fit of the concepts of metaplasia and neoplasia to pathological and experimental EC cartilage.

II. EXTRASKELETAL VERSUS ECTOPIC CARTILAGE

Schaffer (1930), Willis (1962), and Beresford (1981) list the many sites of extraskeletal cartilage in various species. Examples are still coming to light, thus the fibrocartilaginous reinforcements in the urethral process of the goat's penis (Ghoshal and Bal, 1976) and the submandibular ventral pouch of rorqual whales (Pivorunas, 1977). The latter cartilage is closely bound to the mylohyoid muscle. A similarly intimate relation to muscle exists for a previously undescribed anterior element of the hyoid skeleton of the finch, *Passer*: the preglossale (Bock and Morony, 1978). This forms late via cartilage, articulates with another bone, and is definitely skeletal. The interest in the preglossale with regard to EC cartilage lies in its absence in all other birds and their possible reptilian ancestors, making it a skeletal neomorph. Bock and Morony suggest that it might have originated phylogenetically as an ectopic splint for its muscle, which developed a joint with the paraglossale and somehow became a constant skeletal element.

This hypothesis of a skeletal element being a genetically incorporated manifestation of an earlier EC cartilage contrasts with the more common, but ill-founded, view of EC mammalian abdominal-wound and scleral car-

tilages as atavistic expressions of crocodilian abdominal ribs and reptilian scleral cartilage. But atavism cannot be cast out altogether. The human os paracuneiforme, accessory to the tarsus, presumably forms in a cartilage, which by its sporadic presence might (but should not) be considered ectopic. Conroy (1978) argues that this element is the vestigial homolog of the primate prehallux.

Returning to the hyoid apparatus, Gentscheff (1934) saw small islands of hyaline cartilage in around 30% of human tongues (including those of newborns) near the tip where the genioglossal muscle inserts into the septum. The position of the cartilage matches that of the carnivores' lyssa, which led Gentscheff to conclude that both instances constitute a relic of the rod in ancestral reptilian tongues. This inconstant extraskeletal cartilage has not confused the categorization of other lingual nodules in man as ectopic (see Section IV,B,1), because its existence is virtually unknown, and the chondromas are off the midline and not so anterior.

Misconceptions have arisen because of ignorance of the partly cartilaginous nature of the cardiac skeleton in many species (Benninghoff, 1930). For dog and human, clinical reports of EC cartilage (James and Drake, 1968; Ferris and Aherne, 1971) ignored fibrocartilage as a normal component of these hearts (Balogh, 1971; Sandusky *et al.*, 1979). The hyaline cartilage in the aortic ring of the rat, although not thought to be ectopic, was construed as an aspect of cardiac aging, until Hollander (1968) showed its presence from the second week of life. On the other hand, truly EC cartilage develops in healing infarcts in the rat's ventricular wall and in the diseased human aortic valve.

The inconstant occurrence of certain cartilage bones might seem to blur the distinction between EC and extraskeletal cartilages, but rarity is not ectopia. The number and form of sesamoids in the hands and feet show how great the anatomical variation can be (Jacobs, 1974; Scranton and Rutkowski, 1980). When the frequency of the accessory bone drops very low, calling it and its cartilaginous precursor extraskeletal recognizes a weak genetic commitment to chondrogenesis at that site that the term ectopic denies.

Constraints are transmitted to an offspring along with the genetic endowment to cells of the ability to respond to proliferative and other stimuli. Ectopic chondrogenesis reflects the persistent responsiveness of fibroblasts and perhaps other cells, and the slackening of controls hitherto keeping them fibroblastic. Extraskeletal cartilages occupy consistent positions because of the constraints. Beyond this simple view is the complex interplay between genome, cells, tissues, and stimuli in the individual and over generations, resulting in the variable presence of some bones and cartilages, the formation of EC cartilage in response to stimuli that are ineffective in other in-

dividuals and species, and the role of mechanical stimuli in the development of extraskeletal cartilages in the heart, tendons, and so on.

III. SPECIOUS ECTOPIC CARTILAGES

Cartilage can grow out of its normal position in such a way that it is obvious why the ectopia exists. In other instances, the explanation is probably one of straightforward displacement. A brief survey of these specious EC cartilages better defines the status of EC cartilage that springs into being by more subtle cellular mechanisms.

Abnormal outgrowth of a permanent cartilage—an *ecchondrosis*—extends it to an unnatural boundary. Bones of the hand may remain fused because cartilage remained where it was due to disappear in the embryonic cavitation for the joints. Cartilage is intentionally transplanted into soft tissues for human plastic surgery and animal experimentation. Local cartilage and cartilaginous emboli result from the injection of fragments of cartilage, limb primordia, chondrocytes, and teratoma cells. Metastases of chondrogenic tumors are obvious natural examples of ectopia. Surgery on cartilaginous bone tumors may seed cells into the overlying tissues, an interpretation made more secure when the tumor is the distinctive chondromyxoid fibroma (Kyriakos, 1979), which seems to have no extraosseous counterpart. Following abdominal surgery, a few of the formations of cartilage (and bone) in the belly wall might start with the accidental transplantation of periosteum, xiphoid perichondrium, or symphyseal fibrocartilage, but Gruber (1921) was already doubtful. An editorial suggested that instrumental abortion may introduce fetal cartilage or primordia into the endometrium (Editorial, 1973). In dislocation or fracture, some paraskeletal EC cartilage might arise from tearing the perichondrium, periosteum, or an insertion structure (Hirsch and Morgan, 1939)—the *Abrisscallus*—although Delorme (1894) and von Dittrich (1926) were unable to cause such a lesion.

Within the skeleton the cartilage is not so well set and can break up in arthritis and avascular necrosis. Aside from this disintegration, loose bodies form in the confines of the joint space. The orthopedic literature on them and their genesis is vast. One of the two major sources is the articular cartilage, where the separation of a fragment in the younger, nonarthritic person constitutes osteochondritis dissecans. Possible causative factors are trauma, normal loading, "anomalous centres of ossification" (Langer and Percy, 1971), genetic endowment, and ischemic necrosis (Green, 1966; Campbell and Ranawat, 1966). Elsewhere, parts of the intervertebral disc can get into vessels and cause disastrous fibrocartilaginous emboli (Khang-Loon Ho *et al.*, 1980).

Taking an elementary view of the "normal," one could reason that ex-

traskeletal cartilages that are present in less than half the population are ectopic. Thus specious EC cartilages come about by outgrowth, failure to disappear, intended and accidental transplantation, injection and metastasis, embolization, traumatic breakup, tearing and displacement, and overregard to simple statistical measures of normality.

IV. TUMORS AND TUMOR-LIKE CONDITIONS WITH ECTOPIC CARTILAGE

Cartilage forming in soft tissues or on bone, unless microscopically small, makes a firm enough lump to invite the names *tumor* or *new growth*. The mature extraosseous chondroma, encapsulated and hardly enlarging, and the ever-growing, invasive, and metastasizing osteosarcoma are examples of neoplasia, but only the latter shows malignant transformation. The clinically critical system of dividing tumors into benign and malignant sorts further notes that histomorphology can mislead on malignancy, especially with cartilage (Ackerman, 1958; Borges *et al.*, 1981). Gradations of clinical behavior may require the additional categories of low-grade malignant and semimalignant (Uehlinger, 1976). At the benign end of the spectrum of new growths are entities that clinicians exclude from the category of tumor (e.g., myositis ossificans). These are included here because the interest is in the untoward differentiation of cartilage common to them, rather than their clinical outcome, and the tumor classification offers a convenient and familiar framework in which to place them.

Table I is adapted from the World Health Organization (WHO) (Schajowicz *et al.*, 1972) classification of skeletal tumors. I include all the major numbered categories, but place in parentheses those unconnected with EC cartilage, omit all specific lesions from which it is absent, and extend the basic table in these ways. In column B I indicate on the same line where a column-A skeletal tumor (e.g., osteosarcoma) has a similarly constituted counterpart in distant soft tissues. Below the matched extraosseous tumors are lists of other benign or malignant tumors of a cartilaginous nature or with some cartilage, found in extraosseous sites. Column C, originally for tumors of bone and its associated soft tissues, is modified to classify tumors of soft tissues in general in which cartilage occasionally takes part. The last category of the column (9. Tumor-like conditions) lists several diverse, but nonneoplastic EC chondrifications.

The plan is to discuss pathological EC cartilage roughly in the order of this table, with several questions in mind. To what extent is there still overlap in the list? How much can the tissues preceding and accompanying the cartilage tell of its origin? What cells contributed the chondroblasts? and What stimuli provoked their differentiation and proliferation? To how few ex-

TABLE I

Human Tumors with Ectopic Cartilage[a]

A. Osseous	B. Extraosseous[b,c]	C. Extraosseous
1. Bone-forming tumors		5. Vascular tumors
Malignant		Intermediate malignancy
Osteosarcoma	Osteosarcoma	Hemangiopericytoma
2. Cartilage-forming tumors		6. Other connective tissue tumors
Benign		Benign
Chondroma	Chondroma	Chondrolipoma[c]
Osteochondroma	Osteochondroma	Fibrogenic chondroma[c]
Chondroblastoma	Chondroblastoma	Juvenile aponeurotic fibroma[c]
Juxtacortical chondroma[c]		Pseudosarcomatous proliferative lesion[c]
		Malignant
	Synovial chondromatosis	Mesenchymoma
	Tracheopathia osteoplastica	7. Other tumors
	Chondromatous hamartoma of lung	Chondroid chordoma[c]
		Neurilemmoma
	Benign mixed tumor	(8. Unclassified tumors)[d]

A	B	C
Malignant		9. Tumor-like conditions
Chondrosarcoma	Chondrosarcoma	Myositis ossificans
Juxtacortical chondrosarcoma		Other posttraumatic chondrifications[c]
Mesenchymal chondrosarcoma	Mesenchymal chondrosarcoma	
	Myxoid chondrosarcoma	Fracture callus[e] of enchondral and membrane bones[c]
	"Chordoid" sarcoma	
	Carcinochondrosarcoma	Nontraumatic chondrifications[c]
	Gliochondrosarcoma	
		Infantile periosteal reaction[c]
	Mixed embryonic tumor	Fibrous dysplasia[c]
	Adenocarcinoma with stromal cartilage	Malformations[c]
	Malignant fibrous histiocytoma with cartilage	
	Teratoma[f]	
(3. Giant-cell tumors)[d]		
(4. Marrow tumors)[d]		

[a] Adapted from World Health Organization (WHO) classification of bone tumors in Schajowicz *et al.* (1972).

[b] Tumors on the same line in columns A and B signify that a skeletal tumor (A) has a similarly constituted tumor in extraosseous soft tissue (B). Items in column C do not match any in columns A or B.

[c] Beresford's additions to WHO list.

[d] Tumors in parentheses are in WHO list but not cartilaginous.

[e] Neither extraosseous nor a tumor.

[f] Cartilage normally benign.

planations can the multiplicity of expressions of ectopic cartilage be reduced?

A. Osseous Tumors

The interest of tumors on or in bone where cartilage is the major component (Class 2) lies in why periosteal and, occasionally, endosteal cells become chondrogenic (or sometimes fibrogenic) instead of making bone. The other aspect of this switching is the production of some cartilage and the intermediate, chondroid bone type I (Beresford, 1981), by the osseous bone-forming tumors (Class 1) in both the juxtacortical/parosteal (Reddick *et al.*, 1980; Banerjee, 1981) and typical osteosarcomas (Dahlin, 1978).

The factors that promote malignant chondrogenesis might emerge from the experimental evocation, by either radioisotopes or viruses, of tumors in sheep and in the facial skeleton of animals. The chondrosarcoma is the common bone tumor in sheep (Nielsen, 1976), and it is osteosarcomas of the jaws that are prone to chondroid differentiation in humans (Dahlin, 1978; Bras *et al.*, 1980). This event, on bones formed mostly in membrane, might surprise those unaware of the widespread ability of periosteal cells to convert to chondrogenesis (see Section V,B). A variant of the chondrosarcoma not listed in the WHO table—the clear-cell chondrosarcoma—also develops on the maxilla (Slootweg, 1980). Among the reasons not to attribute periosteal chondrogenic tumors to embryonic cells held over from any cartilage participating in the development of the bone are the cartilaginous tumors of the cranial vault (see Cianfriglia *et al.*, 1978; Thurner and Lisanti, 1981). Bones here form in membrane with only tiny amounts of secondary cartilage along the sutural margins: an early normal manifestation of the plasticity of periosteal cells.

B. Benign Extraosseous Tumors

1. Chondroma

Chondroma comprises normal cartilage, usually hyaline and sometimes calcified, grading into and enclosed by a perichondrium. It exists as a plaque or nodule, but can be lobulated. The connective tissue in which it lies may be unexpectedly adipose, a fact some observers have merely noted, whereas others have made it the basis for naming the growth a *chondrolipoma* or benign mesenchymoma (see Section IV,E).

2. Osteochondroma

Osteochondroma typically involves a piece of cartilage experiencing calcification and ossification in its interior, with perhaps the addition of some bone to its periphery, making it clear in both places that the cartilage

came first. One may view the osteochondroma as a late, but by no means inevitable, phase in the life of a chondroma, and one that may progress to an ossicle with marrow inside. Thus, the time when the lesion comes to microscopy is paramount for what tissues are seen. However, some *osteomas* never involve cartilage, and chondromas can persist without ossification, in some way resisting the "cascade" of calcification, resorption, etc (Reddi, 1981).

Examples of extraosseous chondroma and osteochondroma are found in the eye (Morrison and Wolter, 1975), orbit (Bowen *et al.*, 1981), brain (Ahyai and Spoerri, 1979), cranial and spinal meninges (Palacios, 1970; Zervas and Berry, 1965), pituitary gland (Shanklin, 1948), skin (Holmes, 1976; Wells *et al.*, 1977), breast (Koischwitz and Helpap, 1976), and subungually (Burgdorf and Nasemann, 1977). More locations are the tongue (Zegarelli, 1977; del Rio, 1978; Wesley and Zielinski, 1978), soft palate (Gardner and Paterson, 1968), tonsil (Weller, 1923), thyroid gland (Weitzner and Appenzeller, 1973), nasal cavity (Yao-Shi Fu and Perzin, 1974), vocal cords (Burtner *et al.*, 1972) and elsewhere in the larynx (Zizmor *et al.*, 1975), lung (Bateson, 1970), synovium (Dahlin and Salvador, 1974; Milgram, 1977), and other nonvisceral soft parts (Chung and Enzinger, 1978).

For the second series of sites rather more than the first, various authors have proposed that the cartilage originates not by metaplasia, but by a displacement of nearby chondrogenic embryonic germs during development. This mechanism may seem to account for certain instances coming to light early in life, but is unsatisfactory where the lesion becomes evident late in accessible organs such as the tongue, or in places like the vocal cords or synovium seen to be cartilage-free during earlier examination or surgery. One may argue that the embryonic rests were microscopic in size, but then why did they long stay dormant? And why have they now grown? In infancy, where there need be no presumption of delayed growth, other abnormalities may aid acceptance of the idea of a disturbed development (see Section IV,D).

Three expressions of benign EC cartilage and bone have clinical identities: tracheopathia chondroosteoplastica, chondromatous hamartoma of the lung and airway, and synovial osteochondromatosis.

3. Tracheopathia Chondroosteoplastica

This state is rare, but more prevalent than the 130 or so reported cases indicate (Lundgren and Stjernberg, 1981). Usually the submucosal connective tissue has more cartilaginous than bony nodules, only a few of which show fusion with the tracheal rings. Dalgaard (1947) summarized the history of ideas on its etiology, including tumor theory, pure infection, infection or mechanical irritation as a trigger, ecchondrosis, perichondrial embryonic

germs, and disturbed development of the connective tissue. Landsberg (1914) favored an indirect metaplasia of cells in the elastic tissue. Although other workers have stained for elastin, only Dalgaard claimed that a significant proportion of the new cartilage was elastic.

Ecchondrotic growth may contribute some of the cartilage, but most cartilage and bone apparently arise fresh from superficial submucosal connective tissue. The metaplastic stimulus remains unknown because chronic inflammation and irritation, and even amyloidosis (Jones and Chatterji, 1977), are far more common than tracheopathia chondroosteoplastic.

4. Chondromatous Hamartoma

Willis (1962) explained that this tumor of the lung is not a hamartoma (i.e., an error of local benign excess in the early development of an organ's tissues) because the condition is acquired in adult life. That the name endures (Hernandez and Reyes, 1980) may be because the correct one is uncertain. Willis thought that proliferating bronchial epithelium in the lamina propria induced cartilage (and other mesenchymal tissues) which joined in a benign mixed tumor. Bateson (1970) disagreed on the initial aberration, proposing that the growth starts as a cartilaginous neoplasm of the bronchial connective tissue in either a large or small bronchus. Then, depending on whether it grows toward or away from the lumen, thereby involving the epithelium to a greater or lesser degree, one of four "cartilage-containing tumours of the lungs and bronchi" develops (endobronchial and intrapulmonary hamartoma and chondroma). From their transmission electron microscopic study, Stone and Churg (1977) concluded that the bronchial epithelium may be more active in the neoplasia. Cartilaginous hamartomas and angiomatous hamartomas with cartilage also occur in the upper airway (Majumder *et al.*, 1977).

5. Synovial Osteochondromatosis

This formation occurs in the synovium as nodules (usually multiple) of cartilage, bone replacing cartilage, and occasionally bone formed de novo (Milgram, 1977). Synovial membranes of joints and tendons are afflicted (Sim *et al.*, 1977; Villacin *et al.*, 1979; McCarthy and Dorfman, 1982). In a few cases the process is *primary* to the synovium, in the remainder it is a secondary reaction to traumatic or degenerative disruption of the articular cartilage, with the production of loose bodies. Since metaplastic synovial cartilage nodules can later work their way free into the joint space, specifying the origin of a loose body requires the microscopy of sections, whereupon a nidus of articular cartilage can often be told from one of new synovial cartilage (Milgram, 1980). The picture is made complex by the reattachment of some loose bodies, and the accretion of new cartilage by the

body while it is free, in which state the loose body is in a kind of organ culture (Barrie, 1978). When seeking to explain the relatively high incidence of synovial chondromatosis by the weak cartilage-inducing properties of gliding-surface cartilage (Urist and Adams, 1968) and the changed mechanical stimulation that free and attached loose bodies could bring to the synovium, one has to say that the primary metaplasia occurs without joint fragments, and synovium in normal use is being worked mechanically.

Chondromas around joints are not always synovial. Some involve paraarticular structures, such as capsule and bursa (Milgram and Dunn, 1980), including bursae newly formed over osteochondromas (Borges *et al.*, 1981). In the hands and feet, the crowding of structures and numerous sheaths make it hard to pinpont origins (Dahlin and Salvador, 1974). Here, Chung and Enzinger (1978) classified some chondromas, admitted to be related to tendon sheaths, merely as *chondromas of soft parts,* because they were not multiple as in the synovium of large joints.

In one case (Heiple and Elmer, 1972), multiple cartilaginous tumors of the hand had not an ectopic soft-tissue beginning, but an extraskeletal cartilaginous one in the volar digital plates. These are normally composed of fibrocartilage of a generally unappreciated thickness. The case was described as a hamartoma for its onset in infancy, but it involved some metaplasia in the partial conversion of the tumorous fibrocartilage to the hyaline kind.

Milgram (1977) attributed the metaplastic cartilage of synovial chondromatosis to "undifferentiated stem cells in the stratum synoviale," but the origin is as questionable as in any connective tissue site. Barrie (1978) proposed another metaplasia in afflicted joints, namely a dedifferentiation or "fibroblastic transformation" of peripheral chondrocytes in articular cartilage-derived loose bodies, followed by their production of fibrocartilage.

6. Chondroblastoma

This is a rare benign chondromatous tumor with chondroblasts predominant, but a scattering of giant cells. Kingsley and Markel (1971) saw an extraosseous example in the ear lobe. However, Chung and Enzinger's (1978) *giant-cell variant of chondroma of soft parts* appears not to be the same entity, for it is less chondroblastic and more lobulated (their Fig. 9).

C. Malignant Extraosseous Tumors

1. Osteosarcoma

Osteosarcoma with areas of cartilaginous differentiation has started as a primary tumor in the pleura (Pearson *et al.*, 1969), great vessels and heart (Yashar *et al.*, 1979), vocal cord (Morley *et al.*, 1973), breast (Schöner and

Gutgesell, 1981), thyroid (see Broders and Pemberton, 1934), liver (Sumiyoshi and Niho, 1971), urinary bladder (Chitiyo, 1973), uterus (Crum *et al.*, 1980), kidney (Salik and Abeshouse, 1962), prostate (Goldfarb and Bertcher, 1960), pelvic (Rachman and Di Massa, 1965) and other somatic soft tissues (Fine and Stout, 1956). Clinical measures that have induced extraosseous osteosarcomas with a component of cartilage are therapeutic X irradiation (Ascenzi *et al.*, 1980) and the injection of thorotrast (Hasson *et al.*, 1975). In extraosseous osteosarcoma, the bone or osteoid and cartilage seem for the most part to arise from the same kind of cell, at much the same time, by a kind of bifurcating metaplasia. Questions are, How many steps are involved? and How do they relate to events in osseous osteosarcoma? Does the malignant change supervene on the metaplastic? In the iatrogenic examples, when and how does the radiation contribute?

2. Chondrosarcoma

These tumors have a wide distribution in the somatic soft tissues (Goldenberg *et al.*, 1967; Wu *et al.*, 1980) but are less common than chondromas in the hands and feet. They arise from bursae, tendon sheaths, and muscle fascia, but are uncommon in joint synovium (Wu and Guise, 1981), and even when seen with longstanding synovial chondromatosis, may not have developed from its cartilage (Dunn *et al.*, 1974). Other places include the pulmonary artery (Hohbach and Mall, 1977), lung (Peter and Maassen, 1980), salivary gland (Gerughty *et al.*, 1969), tongue (Forman, 1967), brain (Alvira and McLaurin, 1978), esophagus (Yaghmai and Ghahremani, 1976), breast (Gisser and Toker, 1975; Beltaos and Banerjee, 1979), kidney (Penschuck, 1979), urinary bladder (Kauffman and Stout, 1963), ovary (Talerman *et al.*, 1981), and scrotum (Angervall *et al.*, 1973). Just as the osteosarcoma has areas of cartilaginous differentiation, the chondrosarcomas infrequently have small foci of osteoid or bone (e.g., Hohbach and Mall, 1977; Gisser and Toker, 1975). The extraosseous chondrosarcoma can also arise where thorotrast has been injected (Schajowicz *et al.*, 1967).

3. Mesenchymal Chondrosarcoma

This consists of a mass of proliferating, small primitive "mesenchymal" cells, in which lie islands of definite hyaline cartilage. The transition between the tissues can be abrupt or, less often, gradual (Guccion *et al.*, 1973). The high vascularity may suggest a hemangiopericytoma, but ultrastructurally basement membrane material and pinocytic vesicles are lacking (Zucker and Horoupian, 1978), and cartilage is very rare in hemangiopericytoma (Rollo *et al.*, 1979). Soft tissues of the limbs and trunk can give rise to mesenchymal chondrosarcoma, but those of the head are far more prone to this (Bloch *et*

al., 1979), with the orbit (Shimo-oku *et al.*, 1980) and the meninges (Kubota *et al.*, 1982) outstanding.

4. Myxoid Chondrosarcoma

This tumor has small cells in an abundant *mucoid* matrix, staining weakly with hematoxylin and eosin (H and E) and trichrome stains. Enzinger and Shiraki's (1972) series of somatic extraosseous examples, mostly in the limbs (Luger *et al.*, 1981), had a matrix giving the hyaluronidase-resistant histochemical staining reactions of sulfated glycosaminoglycans. Only occasionally did the cells lie in lacunae, and there were foci of properly differentiated hyaline cartilage. Mehio and Ferenczy (1978) found similar regions in an intraabdominal myxoid chondrosarcoma to have cells with the ultrastructure of chondrocytes in a matrix with electron-dense granules, but very few crossbanded collagen fibrils. The question is, How cartilaginous is the myxoid tissue in these malignant tumors and the extraosseous myxoid chondroma (Chung and Enzinger, 1978)?

A myxoid appearance can also result from an abundance of epithelial mucin (Greek *myxa*—mucus); a "myxoid degeneration" is one degeneration proposed for cartilage and other differentiated connective tissues, and the brain offers an example of "myxo" misapplied to "loose fibroblastic-type tissue" (Scott *et al.*, 1976). Even as substantial connective tissues formed *de novo*, the "myxoid" ones are made confusing by the uncertain identity (identities) of the formative cell—Is it a fibroblast, an endothelial cell (Morales *et al.*, 1981), a mesenchymal cell (supposing these exist in mature tissues), a stunted chondroblast, or a cell type *sui generis?*

One may speculate that the myxoma reflects fibroblasts producing too much hyaluronate-rich ground substance and too little collagen, whereas the myxoid chondrosarcoma represents chondroblasts making an excess of cartilage-type proteoglycans, but again insufficient collagen. But what then are the natures of (*1*) the myxoid component in the chondromyxosarcoma of the heart (Winer *et al.*, 1977), where the myxoma is the common primary tumor, and (*2*) the chondroid part of the cardiac myxoma that Bell and Greco (1981) describe?

For identifying cartilage, it is only the *combination* of several cytological features not specific in themselves that makes chondrocytes distinctive in electron microscopy (Martínez-Tello and Navas-Palacios, 1982a). Polysaccharide histochemistry, with attention to pH, resistance to enzymes, and so on, reveals only the class of sugar macromolecule present. Better methods to indicate molecular identities, sizes and amounts, along with more ultrastructural detail, will eventually sort out the immature and pathological mucoid connective tissues and the many cartilaginous and quasi-cartilaginous chor-

doid and chondroid tissues. The range of these in nature is extensive, particularly when the lower vertebrates and invertebrates are brought into the picture (Schaffer, 1930; see Volume 1, Chapter 2).

5. Chordoid Sarcoma

Considering ultrastructural and histochemical evidence, chordoid sarcoma is really an inappropriately named extraosseous myxoid chondrosarcoma (Weiss, 1976; Mehio and Ferenczy, 1978; Pardo-Mindan *et al.*, 1981). The chordoid sarcoma is misnamed because it has no vacuolated physaliferous cells typical of chordomas. But to add to the "what is cartilage?" problem, some chordomas, in their usual location at the base of the skull, do have such cells in areas that merge with chondroid regions having cells in lacunae (Heffelfinger *et al.*, 1973; Spoden *et al.*, 1980). These *chondroid chordomas* will be more of a puzzle if their chondroid zones prove cartilaginous. The known power of the embryonic notochord to induce cartilage (Kosher, 1976) would be set against the possibility of notochordal cells themselves becoming chondrocytes, with the related uncertainty surrounding the normal contribution of the notochord to cells of the intervertebral disc (Peacock, 1952). With regard to Cohnheim's ill-regarded hypothesis of embryonic rests as the origin of tumors, the chordoma is exceptional in arising in regions where remnants are common (Ulrich and Mirra, 1982).

D. Composite Extraosseous Tumors

Cartilage forms in neoplasms of nonconnective tissues in four circumstances. It arises in the *stroma* of some carcinomas, adenocarcinomas, and gliomas. Malignant cartilage participates in an intimate mixture with carcinomas and gliomas to form a *carcinochondrosarcoma*. Certain embryonic tumors of liver, kidney, and brain include cartilage—*embryonic mixed tumors*. Finally, cartilage, usually benign, can be one of the multiple tissues of kinds foreign to the site comprising a *teratoma* (Willis, 1962).

1. Stromal Cartilage I

Willis (1962) listed many carcinomas and adenocarcinomas with stromal cartilage or bone, without indicating which tissue took part. More recent cases with cartilage are a squamous carcinoma of the lung (Flanagan *et al.*, 1965) (disproving Willis' contention that such metaplasias do not occur in the stroma of squamous carcinoma), gastric adenocarcinoma (Yasuma *et al.*, 1973), uterine adenocarcinoma (Mauler, 1968), and numerous tumors of salivary and other glands (see Section IV,D,2). Mathews and Moossy (1974) reviewed 17 gliomas containing cartilage and bone, and concluded that in

nearly half the skeletal tissues arose by stromal metaplasia. Cartilage sometimes occurs in the malignant fibrous histiocytoma and the giant cell tumor of soft tissues, but is attributed by Bhagavan and Dorfman (1982) to a metaplastic and benign stromal reaction.

2. *Stromal Cartilage II*

The usually benign mixed tumor, with epithelial and apparently cartilaginous elements, that is common in the parotid is not confined to the major and minor salivary glands. A similar mixed tumor of the salivary type develops in the skin, as the chondroid syringoma (Varela-Duran *et al.*, 1979), in the trachea (Ma *et al.*, 1979), breast (Smith and Taylor, 1969), gallbladder (Higgins and Turner, 1959), Bartholin's gland (Ordóñez *et al.*, 1981), lachrymal gland (Ludwig *et al.*, 1979), and prostate (Manrique *et al.*, 1978). The last two cases were malignant, but this is unusual in the lachrymal gland. These same cases also demonstrate a problem for this whole class of tumors in showing their cartilaginous nature as "myxochondroid," "myxoid," and "chondroid" areas, respectively. Willis (1962, 1967), in particular, doubted the claims for the presence of cartilage in these tumors, although he made an exception for the breast. Tissues less or more like cartilage are not only myxoid (spindle cells) and chondroid (ovoid cells in lacunae) varieties of connective tissue, but loose connective tissues infiltrated with epithelial secretions. Moreover, these "tissues" grade into one another, and their hazy identities are further blurred by the sometimes haphazard application of Marchand's term *pseudocartilage*. Jelso (1974) stood out by distinguishing myxomatous tissue, pseudocartilage, and true cartilage in salivary gland tumors.

That some of the tissue is cartilage is suggested by its endochondral replacement by bone (Yates and Paget, 1952). Although much work is already invested in electron microscopic and histochemical comparisons of the myxoid and chondroid components of tumors with respiratory cartilages (e.g., Hernandez, 1976), the ultrastructures of the chondrocyte and its matrix lack truly unique characteristics, and it is not enough to show that the proteoglycans present belong in the sulfated class. The clear demonstration that cartilage is present awaits immunological and chemical proof of collagen type II, cartilage-specific proteoglycans and link proteins, and appropriate cell-surface glycoproteins, with due regard to their variability in the acknowledged cartilages. For example, human malignant cartilage seems not to make type II collagen (Remberger and Gay, 1977). Methods to show what is unique about the chondrocyte and its products (e.g., using monoclonal antibodies) are now moving into histopathologic use. These

techniques may also help identify the cells making the sulfated proteoglycans and mucins that cause the various confusing tissues to react like cartilage with standard histochemical methods. For example, a polyclonal antiserum to a component of epithelial cells reacted positively with the "chondrocytes" in a chondromatous salivary pleomorphic adenoma (Gusterson *et al.*, 1982).

The neoplastic glandular cells, in whose company the cartilage or a mimic grows, by manifesting one abnormality invite accusation of another. So one has ventured a chondroblastic metaplasia for the epithelial or myoepithelial cells, or the evocation of stromal chondrogenesis by the neoplastic epithelium (Cotchin, 1958). For the cases coming singly to pathology, the last idea has only the support of analogy, whereas the first two routes have the evidence of absent basal laminae, merging tissues, and *transitional cell forms* traced along some of the supposed pathways. The analogy is with the induction of cartilage and bone by transplants of an avian oviduct tumor (Foulds, 1937), and a rat hepatic tumor by Paget *et al.*, (1958), who offered reasons for concluding that the skeletal tissues were of stromal rather than tumor-cell origin. Kawada's (1975) transplants of a murine pulmonary adenocarcinoma may have induced cartilage, but serial transplantations then carried the results beyond interpretation.

While considering the mooted myoepithelial and epithelial metaplasias, the alternative of a stromal-cell origin for any cartilage is to be kept in mind. Bloom *et al.*, (1982), finding two types of chondrocyte, speculate that these originate separately from fibroblasts and myoepithelial cells. Evidence for a myoepithelial source rests first on tracing alkaline phosphatase-positive cells into myxoid areas that merge with cartilage (Pulley, 1973), and second on finding many cytoplasmic filaments in the cells of the apparently transitional myxoid territory (Doyle *et al.*, 1968; Varela-Duran *et al.*, 1979), at the same time as these cells display prominent secretory organelles. Von Bomhard and von Sandersleben (1974) discounted the intracellular fibrils, pinocytotic vesicles, and glycogen as not adequately characterizing cells, but suggested that the production of something like a basal lamina in the lacunae was diagnostic for myoepithelium. General drawbacks to the evidence are first that the variety of cells in the myxoid and chondroid areas is sufficient for several hypothetical sequences, even the dated and improbable notion of a metaplasia of connective to epithelial tissue. Next, the evidence comes from many tumors, each pleomorphic, and various sites, but favoring mixed canine mammary tumors. Finally, measurements of the width of filaments have not always been precise enough to identify actin from intermediate kinds of filaments (Mills and Cooper, 1981). However, the chemical identities of the filaments should be helpful in distinguishing epithelial cells, with cytokeratins (Henderson and Weber, 1981), from connective tissue cells with vimentin, and indicating any conversion between cell types.

3. Carcinosarcoma

As a class these include an intimate mixture of a carcinoma or adenocarcinoma with a cartilage-forming sarcoma. Such malignant mixed tumors arise in the female genital tract as the Müllerian variety, in the uterine tube (Hanjani *et al.*, 1980; Holst and Erichsen, 1981), uterus (Barwick and LiVolsi, 1979; Martinelli *et al.*, 1981), ovary (Hernandez *et al.*, 1977), and also peritoneum (Weisz-Carrington *et al.*, 1977), in the breast (Smith and Taylor, 1969; Kahn *et al.*, 1978), urinary bladder (Delides, 1972; Babaian *et al.*, 1980), gallbladder (Von Kuster and Cohen, 1982), lung (*see* Ludwigsen, 1977), esophagus (Stener *et al.*, 1967), skin (Quay *et al.*, 1981), and perhaps liver (Ludwig *et al.*, 1975). Comparable mixtures of chondrosarcoma and glioblastoma—gliosarcoma—form in the brain (Sarmiento *et al.*, 1979; Richman *et al.*, 1980). The ovarian Sertoli-Leydig cell tumors with heterologous cartilage (Prat *et al.*, 1982) might belong here, although the "mild nuclear atypia" suggests that, as in synovial chondromatosis (DeBenedetti and Schwinn, 1979), the cartilage is not malignant. They might thus go to Section III,D,1, but the variety displayed by Sertoli-Leydig cell tumors makes them difficult to categorize.

The close blending of the epithelial (or glial) and sarcomatous tissues has long bolstered the notion that they share progenitor cells, earlier thought to be from displaced embryonic tissue (Wilms, 1899, 1900, 1902). The explanations now vary with the site, despite the basic similarity of the histopathological pictures. For the female internal genitalia, stromal cells are viewed as pluripotent, a property assigned with more hesitation to the epithelium in most other viscera. In the urinary bladder with a history of bone and cartilage induction by the normal epithelium, the explanation preferred by Delides (1972) for carcinosarcomas is of a dual development from separate cell types (*Kollisionstumor:* Meyer), followed by a metaplasia of the sarcomatous cells to chondrosarcoma. One could extend this argument to the breast and gallbladder, whose epithelia have induced skeletal tissues experimentally in animals (Huggins and Moulder, 1944). Two techniques may separate the contributions of epithelial and connective cells to the mixed tumors. One is the immunostaining of fibronectin, which is present in benign stroma and sarcoma, but absent from carcinoma (Stenman and Vaheri, 1981). The other shows the presence of isoenzymes whereby Matsuda *et al.*, (1980) established that "sarcomatous" regions of a pleomorphic renal cell carcinoma were still epithelial in nature (i.e., the tumor was not a carcinosarcoma).

The other points of caution on these tumors concern once more the certainty of the identification of the tissue as cartilage, and the malignancy of the stroma. Some carcinomas have a richly fibroblastic reactive stroma

which may appear sarcomatous, and have cells with abundant filaments (Schürch *et al.*, 1981). Indeed, these filament-rich cells crop up time and again as participants in the context of ectopic bone and cartilage, for example, in bone-induced osteogenesis (Thorogood and Craig Gray, 1975), myositis ossificans (Povýsil and Matějovsky, 1979), in the chondrogenic parosteal osteosarcoma (Reddick *et al.*, 1980), and in the chondroid regions of benign, mixed parotid tumors (Bloom *et al.*, 1982; Martínez-Tello and Navas-Palacios, 1982b). Whether the filaments are actin, used for mobility and contraction, or have some other nature and significance is unknown.

4. Embryonic Mixed Tumors

Certain mixed tumors of early life represent an aberrant development of an organ's embryonic blastema. Aside from abnormalities in the epithelial components, the mesenchyme in places sometimes forms cartilage (Willis, 1962), as well as other mesenchymal derivatives. To the nephroblastoma (Willis, 1967) and hepatoblastoma (Milman and Grayzel, 1951; Allison and Willis, 1956) behaving in that manner, Mathews and Moossey (1974) would add the embryonal neoplasm of the brain, where, they suspected, "primitive multipotential spongioblastic cells" and "misplaced trapped mesenchymal cells" produced mixed tumors of the fourth ventricle in children.

Willis (1962) separated the embryonic from the other mixed tumors on the grounds that (*1*) the cells had a blastemal multipotentiality and scope for morphogenesis and (*2*) most of the agents thought to promote neoplasms in the adult could not have influenced these cells. He emphasized that a sense of chronology is needed for understanding the potentials of the cells involved, and the mechanisms acting, in tumorigenesis. Thus, he allowed that differentiation to the adult state was long delayed in the skeleton and teeth, and answered affirmatively his rhetorical query: Can embryonic tumors arise postnatally in those structures? On the other hand, there is no doubt about the embryonic nature of the earliest starting tumors with ectopic cartilage—the teratomas.

5. Teratomas

These constitute a multiple ectopia of tissues, which occasionally interact to build structures with a sometimes fair but usually fanciful resemblance to organs. Cartilage is a frequent participant, but in reckoning whether there is a multiplicity of unexpected tissues rather than a mixed tumor (Willis, 1967), all the connective tissues count as one. Other pathologists' requirement that there be, for a teratoma, a tissue representative of each embryonic germ layer overlooks the probable neuroectodermal origin of some cranial bone, cartilage and other "mesodermal" tissues. In general, for a cell in a germ

layer, the limitations that this membership confers on all its eventual progeny have been overstated.

Experiments on strains of mice, using the malignant teratocarcinoma and grafts of genital ridges and embryos (Pierce, 1967; Martin, 1975, 1980), suggest that teratomas owe their many tissues to an origin in pluripotent primordial germ cells or the abnormal development of equally plastic cells in misplaced early embryos. Because of an association between brain and cartilage in some human teratomas, Willis (1962) proposed that early formed neural tissue induces some of the still unspecified cells to chondroblasts: one hypothesis awaiting testing (see discussion in Volume 2, Chapter 5).

E. Miscellaneous Extraosseous Tumors

1. Hemangiopericytomas

These tumors of pericytes have in the orbit (Reeh, 1966) and meninges (Fisher *et al.*, 1958) produced nodules of cartilage-like tissue.

2. Neurilemmoma

This is another rare tumor in which cartilage has been reported a few times (Woodruff *et al.*, 1973; Kasantikul *et al.*, 1982). Willis (1962) noted that Schwann cells, if chondrogenic, would be another instance of skeletogenesis by neuroectodermal derivatives.

3. Mesenchymoma

Mesenchymoma, as a term to identify soft-tissue tumors with a mixture of mesenchymal tissues but no others (Stout, 1948), has had incomplete acceptance, with many pathologists advocating names that specify the components. Benign chondrogenic examples are cartilage in a fibroma (Lawler, 1969) and lipoma of the breast (Dharkar and Kraft, 1981). Chondrolipomas also occur in the lip (Allard *et al.*, 1982) and mediastinum (Lim, 1980), and combined with angiomatous elements in somatic tissues (Dorfman *et al.*, 1980). The fatty association calls to mind Johnson's (1964) proposal that the fat cell is capable of reversible transformations with other skeletal cells, and the mixture of adipose tissue and extraskeletal cartilage in the undertongue of *Galago* (Tokarski, 1904).

It may be disputed whether fibrous tissue counts in naming a tumor mixed for a mesenchymoma, but Dahlin and Salvador (1974) characterized two growths with hyaline cartilage in a fibrogenic stroma as *fibrochondromas.*

4. Cartilaginous (Aponeurotic) Fibromatosis

This occurs in both humans and dogs (Liu and Dorfman, 1974). The fibroblastic lesion expresses itself differently in the two, by a pronounced

tendency to form hyaline cartilage and invade the adjacent calvarium in the dog, whereas in children it affects principally the palms and soles and the fibroblasts make some fibrocartilage, although cranial cases are also known (Corio *et al.*, 1981).

5. Pseudosarcomatous Proliferative Lesion

This lesion of soft tissue (Dahl and Angervall, 1977), affecting subcutaneous and intramuscular (i.m.) connective tissue, has mostly plump fibroblasts in a myxoid matrix, which in one case in five has bone and cartilage lacking the zonal pattern of myositis ossificans (Ackerman, 1958).

6. Malignant Mesenchymomas

These usually have several components, often including skeletal muscle, detectable with antibody to myoglobin. Sites with cartilage-bearing examples are retroperitoneal (Smith and Bennett, 1976), cardiac (McConnell, 1970; Frandsen *et al.*, 1981; Tanaka *et al.*, 1982), mammary (Kay, 1971), cerebellar (Stone *et al.*, 1979), renal (Mead *et al.*, 1982), and somatic (Klima *et al.*, 1975).

F. Tumor-Like Conditions

1. Myositis Ossificans

The formation of bone in some of a muscle's connective tissue characterizes this condition, but cartilage and endochondral ossification sometimes take part in the various forms, including the classic myositis ossificans traumatica, which follows repeated occupational bruising or one severe accidental contusion (Gruber, 1917; Geschickter and Maseritz, 1938). It can be iatrogenic, as ossifying abdominal-wound repair (Mebius, 1924; Gruber, 1926; Orava *et al.*, 1980), and follow tetanus (Ishikawa *et al.*, 1982). Next, the paraarticular connective tissues may ossify after major joint surgery or *immobilization* from burns and a variety of central neural lesions. Cartilage is an occasional sighting in these paraosteoarthropathies (Rossier *et al.*, 1973). Last, neither injury nor disease precedes the few cases of *nontraumatic* myositis ossificans (Gruber, 1917; Caulet *et al.*, 1969; Ogilvie-Harris and Fornasier, 1980).

2. Myositis Ossificans Progressiva

This is special because of its early, nontraumatic onset, hereditary and relentness nature, and associated skeletal malformations in the hand and foot (Connor and Evans, 1982). Again, some cartilage is present (Fairbank, 1950; Bona *et al.*, 1967), but electron microscopy and histochemistry (Hent-

zer *et al.*, 1977; Maxwell *et al.*, 1977) have shown essentially normal skeletogenic processes in abnormal sites. Specific probes for chondrogenesis have yet to be applied to the soft formative nodules, but Maxwell *et al.* did comment, "the abundant mucosubstances... perhaps could indicate a chondrocyte-like identity for these cells." As the disease limits movement, immobilization as a promoter of paraarticular ossification might speed the progression.

The division of the other myositis ossificans into the traumatic and nontraumatic categories (with immobilization as a subcategory) serves for many sites of ectopic cartilage. Inflamed reparative tissue forms cartilage in torn tendons (Ghormley, 1938; Fisher and Woods, 1970), curetted endometrium (Hsu, 1975), injured choroid membrane (Pes, 1903), diseased kidney (Taxy and Filmer, 1975), aortic valve (Seemayer *et al.*, 1973), pericardial sac (Bloor, 1978), fallopian tube (Asami, 1920), and arterial lesions (Marburg, 1902). Synovial chondromatosis is commonly a reaction to implanted or loose fragments. For several of these sites and for myositis ossificans traumatica (Rathcke, 1898; Gruber, 1926), it has been argued that the chondrogenesis is a response of recently proliferated fibroblasts to changing mechanical actions as the organ works. In other sites, such as the tonsil (Weller, 1923), observers of cartilage have not invoked mechanical forces, but have inferred a prior lesion to bring the connective tissue cells to a ready state. Many of the benign chondromas and osteochondromas of Section IV,B were suspected of occurring after damage or infection. Nevertheless, a suspicion is only that, and there are normal fibrous structures which, with functional mechanical stimulation, slowly become cartilaginous without an inflamed proliferative state. Some tendons, ligaments, their insertion sites, and the disc of the temporomandibular joint are known examples of this late, natural, (i.e., extraskeletal) kind of cartilage [to which the vestibular folds of the larynx might be added (Hill *et al.*, 1980; Iyer and Rajago Palan, 1981)]. A direct metaplasia of fibroblasts yields fibrocartilage, which may become more hyaline.

3. Nontraumatic Nonneoplastic Ectopic Chondrogenesis

This broad category is needed:

1. Where there is only a history of *normal use* as in some myositis ossificans and tendon ossifications (Ghormley, 1938), and many chondromas (e.g., of the tongue). If the use is normal, is the latent susceptibility of fibroblasts to chondrogenic stimuli raised in these individuals? If so, is this predisposition ever-present and mirrored in the histocompatibility antigens (Larson *et al.*, 1981)?
2. In immobilization, with drastic change in the pattern of stimulation—

mechanical, neural and chemical—to interact with any individual differences in cellular responsiveness.

3. For chondrogenesis in response to known modestly *increased mechanical stimulation,* for which "trauma" is too strong, for example under loose dentures and in the vocal cords (Cutright, 1972; Burtner *et al.,* 1972), in periosteum hyperstimulated by chafing (Scapinelli and Little, 1970), and maybe in overworked blood vessels.

More cases and information undoubtedly would transfer at least some instances in subgroup 3 into the category of either traumatic EC cartilage or nontraumatic extraskeletal cartilage where, for example, the human nuchal ligament cartilage may belong (Lewinnek and Peterson, 1978).

Thus, whether or not nontumorous cartilage forms depends on more factors than trauma. Although the acute cellular reaction to disruption provides a favorable environment, as in myositis ossificans traumatica, most experimental inductions of cartilage, and perhaps the stromal reaction to some carcinomas, it is not essential to ectopic chondrogenesis.

The firm ectopic nodules are sometimes named chondromas or osteomas, and the speed of their early cellular growth can suggest a sarcoma (Ackerman, 1958), but except for its place their development is too regular and often too much a process of repair for the current conception of neoplasia. Mebius' (1924) term *Granulationsprodukt* describes the traumatic forms and also fits fracture callus.

4. Fracture Callus

On occasion fracture callus has been mistaken for a malignant growth and frequently contains osseous EC cartilage, whether formed by long-bone or membrane-bone periosteum (Hall and Jacobson, 1975). Mobility of the site aids the development of cartilage, but is not a factor in causing the metaplastic cartilage that develops in the nontraumatic infantile cortical periosteal reaction (Caffey's disease; Greer *et al.,* 1981), and the reactive periostitis of somewhat older individuals (Spjut and Dorfman, 1981).

5. Fibrous Dysplasia

Fibrous dysplasia of bone is another affliction of young bones that is not quite tumor or malformation, although it may lead to deformity and occasional osseous EC cartilage. Fibroblasts take the place of bone in the discrete skeletal lesions. In addition to dense collagen, the cells usually form disoriented bony trabeculae, but, in 2–5% of cases, they may make mostly hyaline cartilage, as in Fig. 28-13 of Dahlin (1978) who kindly brought this event and frequency to my attention. The chondroid condition might involve sequential metaplasias—osteoblast or marrow stromal cell to fibro-

blast, fibroblast to chondroblast—or a direct step to chondroblasts. Even without irradiation, the unstable tissue can experience malignant neoplasia, with osteosarcomas outnumbering chondrosarcomas (De Smet *et al.*, 1981; Thivolet *et al.*, 1981).

G. Cartilage in Malformations

Earlier sections dealt with teratomas, embryonic mixed tumors, and myositis ossificans progressiva which, as a hereditary disease, is a *slow malformation*. These leave various other errors of development: some in sites remote from developing cartilage, others where it is less unexpected. Thus, an extra cartilaginous primordium may produce a supernumerary bone, such as a pelvic (Sullivan and Cornwell, 1973) or cervical rib (Connell *et al.*, 1980), or participate in a duplicate limb or digit—all abnormalities more of number than of form. The branchial arches, in contributing structures to the head and neck, may leave EC cartilage in the walls of sinuses, cysts, duplicate auditory canals (Olsen *et al.*, 1980; Belenky and Medina, 1980), the glabella (Sayama and Tagami, 1982), and in ectopic auricles (Bose, 1982). However, the wholesale attribution by Ribbert (1904) and his contemporaries of cartilage in tonsils, salivary glands, and tongue to aberrant fetal primordia was unwarranted.

Cartilage-bearing errors arise as the lower respiratory tract separates from the primitive gut and the lungs develop. The tracheobronchial choristoma of the esophagus (Sneed *et al.*, 1979) sometimes has extensive stenosing cartilage rings and may ossify; occasionally it forms as a polyp (Shah *et al.*, 1975). For the lung, Bateson (1970) listed the true hamartoma (Willis, 1967), congenital cystic disease and adenomatoid formation, and sequestrated pulmonary segments. Bronchiogenic cysts can be widely distributed in the thorax (Agha *et al.*, 1975). Ventricular cartilage occurred in a malformed heart (Zimmermann *et al.*, 1979).

In the eye, cartilage joins in epibulbar teratomas (Wolter *et al.*, 1973) and dermoids, or may be the only constituent of a choristoma on the sclera (Morrison and Wolter, 1975). Cartilage truly set within the sclera or fibrous tissue passing through a coloboma is a characteristic of the malformed microphthalmic eye of 13–15 trisomy, but is an unusual feature of chromosome 18 deletions (Yanoff *et al.*, 1970). Yanoff and Font (1969) also saw cartilage and adipose tissue centrally within a microphthalmic eye of a child with normal chromosomes. Whatever the position in the eye of the cartilage or bone, the temptation to invoke atavism (Mullaney *et al.*, 1970) is to be resisted (Willis, 1962). Finally, cartilage nodules are quite common in congenital polycystic disease and hypoplasia of the kidney (Bigler and Killingsworth, 1949; Salik and Abeshouse, 1962), but lack any particular relation to the pelvic epithelium.

H. Veterinary Examples of Ectopic Cartilage

Although the literature is far smaller, there are enough cases reported to match most of the entities described for humans. For a specious EC cartilage, Pass (1978) found cartilaginous emboli in the pig's spinal cord. Synovial chondrometaplasia is the response to arthritis in some mice (Walton, 1979). Metaplastic cartilage develops in the equine atrial wall (Joest and Schieback, 1924) and ostrich aorta (Bohorquez and Stout, 1972). Ectopic cartilage around cysts in the rat's thymus (Toyofuku, 1910) may be a branchial malformation.

Gleiser *et al.* (1981) report a malignant mesenchymoma in a monkey. The dog experiences extraosseous chondrogenic osteosarcomas (DiBartola *et al.,* 1978), aside from those forming in response to esophageal infection with the nematode *Spirocerca lupi:* a special case, but one leading to the general point that the pattern of neoplasia differs between humans and animals, as seen already with the aponeurotic fibroma. Canine mammary carcinochondrosarcoma (Misdorp *et al.,* 1973) and feline malignant osteogenic mixed tumor of salivary gland (Wells and Robinson, 1975) have no straight human counterparts, and the mammary myoepithelial cell's role may vary (Owen, 1979).

V. EXPERIMENTAL ECTOPIC CARTILAGE

Ectopic cartilage formation needs a loosening of constraints. These accumulate during normal development, so that this review of experimental work will be divided by the age of the responding organism or tissue so as to attempt a measure of the prevailing organization and overall cellular plasticity. Whereas, in an adult tissue, it is hard to specify the least differentiated cell, and this may not be responsible for the cartilage, as in marrow (Ashton *et al.,* 1980), in an embryo one has an idea of the furthest differentiated ones, and hence the kind of metaplasia involved in abnormal chondrogenesis. If differentiated cells dedifferentiate and turn into another specialized kind, this is classic indirect metaplasia, but if stem cells are diverted into an unusual line of differentiation, the tissue as a whole experiences conversion, but the cells only a *deviant differentiation* (Beresford, 1981).

A. Embryonic Cartilage

1. Germ Cells and Very Early Embryonic Cells

The cockerel's testis reacts to injected irritants, such as zinc chlorate solution, by forming teratomas (Maskar, 1972). The mouse teratocarcinoma gives rise to benign EC cartilage upon transplantation and in culture. It provides populations of primitive stem cells, whose commitments to differentia-

tion and malignant growth can be manipulated experimentally with various agents and conditions, for example, 3/A/ID-1 cells from an embryonal carcinoma line, upon subcutaneous injection in syngeneic mice, form cartilage which then undergoes ossification (Nicolas *et al.*, 1980).

2. *Later Embryonic Ectopic Cartilage*

For the construction of the primary, optic, and facial skeletons, cells of mesoderm or ectomesenchyme need inductive stimuli from ectoderm or its derivatives, notochord, spinal cord, retina, mandibular epithelium, etc. in order to chondrify or ossify (see Volume 1, Chapter 5). Experimental confirmations of these interactions *in vitro* or on the chorioallantoic membrane constitute specious ectopia. However, true ectopia arises when the responding tissue is not naturally chondrogenic, for example, lateral somitic mesoderm (Hall, 1977) and mouse tooth germ (Hata and Slavkin, 1978). The difference between these novel inductions and *heterotopic displacement* after normal induction is illustrated by Amprino's (1978) transplants of avian limb bud ectoderm with its deep mesenchyme.

3. *Mesenchymal Switching*

An *in situ* malformation with a deviated mesenchymal differentiation taking place after skeletal induction is seen in the extensive chondrogenesis in the cranial vault, when 11-13 day mouse embryos are heavily X-irradiated (Schmahl *et al.*, 1979).

Another example of a nontargeted agent eliciting a specifically local abnormal cartilaginous response from mesenchyme is that for phallic bone formation in the clitoris of neonatal female rats given excess testosterone (Yoshida and Huggins, 1980). The site-specific, androgen-dependent EC cartilage of antlerogenesis in female deer given testosterone (Wislocki *et al.*, 1947) involves what seems to be the retention of embryonic behaviors by older connective tissue cells, which is related to periosteal switching (see following).

Muscle cells also enter into ectopic chondrogenesis. Urist *et al.*, (1978) attributed the formation of cartilage by cultures of minced late-embryonic rat skeletal muscle, grown on demineralized bone or bone gelatin, to *mesenchymal cells*. However, Nathanson and Hay (1980) find that most of the cells in such cultures are myoblasts that take on the form of fibroblasts before becoming chondrocytes. Satellite cells are not in evidence at this age, and fibroblasts are few.

Embryonic fibroblasts stimulated by bone matrix can form cartilage (Nathanson *et al.*, 1978), although ones from skin are less responsive in changing their synthesis of glycosaminoglycans (Anastassiades *et al.*, 1978), and may not reach cartilage. The involvement of muscle cells in the range of

related expressible phenotypes is further illustrated by mouse embryonic $10T\frac{1}{2}$ and 3T3 cells, usually fibroblastic, which the analog 5-azacytidine inconsistently converts to chondrocytes, more regularly to myoblasts or adipocytes (Taylor and Jones, 1979). [Myoblastic, fibroadipogenic, and fibroblastic clones can be derived from the more primitive T985 mouse teratocarcinoma cells (Darmon *et al.*, 1981).] Lastly, a few myogenic cells become chondrocytes in quail-chick recombinant wing buds (Kieny *et al.*, 1981).

Which cells carry into adult life the phenotypic variability for later ectopic chondrogenesis remains unclear in three situations: (*1*) mature skeletal muscle with its satellite cells, myoblasts of repair, fibroblasts and vascular cells; (*2*) nonmuscular soft connective tissues with their complement of cells, and (*3*) periosteum.

4. Periosteal Chondrogenesis

The cells of the inner periosteum never lose their embryonic capacity for diversion to cartilage formation, exhibited for example when embryonic avian cranial bones are grown in submersion culture (Thorogood, 1979), rat calvarium lies on bone-matrix gelatin (Terashima and Urist, 1975), and endochondral bone periosteum is placed under pressure *in vitro* (Glücksmann, 1939). In maturity, cartilage appears in many osseous tumors and tumor-like conditions, after periosteal scraping in mice (Miller, 1967), and scraping and fracture in the rat's skull vault (Girgis and Pritchard, 1958), in humans and animals when deformity causes rubbing (Storey, 1972), and after giving a molybdenum compound to young rats (Spence *et al.*, 1980). Willis' (1962) idea that bone surfaces stay *embryonic* appears justified, but the term means little without the counterweight of a changed state in maturity. It has meaning for comparison with other tissues, but not for comparing adult and early periostea. Here, the chondrogenic problem remains one of distinguishing a switch by osteoblasts (indirect metaplasia) from the selection and proliferation of either stem cells (deviant differentiation), or any latent chondroblasts (Hall, 1979) (meaning that stem cells are absent, replaced by separate populations of preosteoblasts and prechondroblasts). Once developed past mesenchyme, the cellular composition of dermal, muscular, and other connective tissues becomes equally uncertain, and so makes pointless for now an embryonic-versus-mature distinction.

B. Extraosseous Cartilage in "Maturity"

When judging the contribution to ectopic chondrogenesis in young and adult animals of experimentally introduced biological agents, one may keep in mind two other kinds of study: those where cartilage formed after distur-

bance, with or without the implantation of man-made materials; and others, where, despite disruption and the implantation of materials, neither cartilage nor bone ensued. Examples of the last category are the cutting, squashing, and hammering of muscles in guinea pigs overloaded with dietary calcium (von Dittrich, 1926); injecting alcohol into muscles of rat, guinea pig, mouse, and hen (Bridges and Pritchard, 1958; Bridges, 1959); disturbances to the muscles of mice (Anderson, 1976), and similar procedures used as controls in many studies attempting induction.

Of the exception—the rabbit—Pritchard (1960) wrote:

> This remarkable ability of dead skeletal, cardiac and plain muscle in the rabbit to induce chondrification and subsequent ossification, coupled with the fact that in the vast majority of successful inductions with extracts, trauma, irritants, and alcohol, the tissue involved has been rabbit muscle, leads inevitably to the simple conclusion that damaged muscle is the real inducing agent in all these cases [p. 667].

Bridges and Pritchard did not test vascular smooth muscle, which might contribute both cells and stimulus to aortic chondrifications in rabbits. Minced skeletal and cardiac muscles of mice induce cartilage and bone in the stroma during attempted regeneration (Zacks and Sheff, 1982), but less drastically injured murine muscle is not inductive.

1. Cartilage after Noninductive *Disturbance*

In Table II, listing experiments where cartilage appeared in the absence of an overt inducer, one notes that several instances involve species where crude trauma or the injection of irritants does not evoke i.m. cartilage. None of the sites excludes a contributory mechanical stimulation, and in several—tendon, aorta, heart—such is likely. Whether the cells have a special partiality to chondrogenesis may vary with location, being unlikely subcutaneously and in the ventricle. In the monkey's orbit the authors mentioned cartilage in some control monkeys, but failed to discuss the nictitating membrane. Although tendons in some older rats and rabbits have chondroid regions, Buck (1953) believed paratendinous fibroblasts to have formed the EC cartilage after cutting. However, in the aorta the cartilage appears to result from a direct metaplasia of medial smooth muscle cells, some of which already differ from other smooth muscle in having mainly vimentin filaments, instead of desmin (Schmid *et al.*, 1982). They also react to endothelial injury by synthesizing subintimal collagen (Chidi and DePalma, 1981), indicating a choice of responses. Some inclination to chondrogenesis expresses itself in the occasional aortic cartilage seen in intact older rabbits (Hadjiisky *et al.*, 1979a, b) and rats (see Hodara *et al.*, 1978). (Bone and car-

TABLE II

Extraosseous Cartilage Formed after Experimental Noninductive Disturbance

Species	Procedure	Observation	References
Pig	Implantation subcutaneously (s.c.) of polymethacrylate Hydron sponge	Bone with some cartilage	Šmahel *et al.* (1971)
Monkey	Optic enucleation, with or without implanted prosthesis	Cartilage in repairing connective tissue	Singh *et al.* (1974)
Rat	Implantation s.c. of wide glass tube	Central connective tissue cord had bone and cartilage	Selye *et al.* (1960)
Rat	Cardiac ligature isolating tip of ventricles	Subendocardial cartilage and bone in scar	Somogyi and Kovacs (1969)
Rat	Achilles tendon severance	Cartilage in reparative tissue	Buck (1953); Calhoun *et al.* (1975)
Rabbit	Rerouting of Flexor digit. profund. tendon for rubbing	Cartilage in tendon	Ploetz (1937); Gillard *et al.* (1979)
Rabbit	Injection of formic acid into vitreous body	Cartilage in sclera; bone within the eye	Imai (1930)
Rabbit	Forcible manipulation of immobilized leg	Cartilage and bone in quadriceps muscle	Michelsson *et al.* (1980)
Rat	Systemic excess vitamin D	Aortic medial calcification with cartilage	Bonucci (1975); Hodara *et al.* (1978)
Rabbit	Intravenous papain	Cartilage and bone in aortic media	Tsaltas (1962)
Rabbit	Systemic immunization against heterologous aorta homogenates	Aortic fibrosis, calcinosis, cartilage and bone	Hadjiisky *et al.* (1979a,b)
Cockerel	Carrageenan injected into aortic wall	Aortic medial cartilage	McCandless *et al.* (1963)
Chick	Penetrating steel pin restricting aortic distension	Aortic medial cartilage	Rodbard (1958)

tilage might develop from connective tissue cells arriving with destructive cells eroding old calcified arterial lesions in humans, but calcification precedes chondroid change only in some of the experimental instances of metaplasia of vascular muscle, and by a brief margin.)

2. *Cartilage Induction by Cartilage*

Its inconsistency shows the weakness of this induction. A few nodules appeared late in the fibrous tissue around devitalized cartilaginous callus or growth cartilage implanted into rabbits (Bridges and Pritchard, 1958). Their idea that the cartilage has to be hypertrophic does not hold for the canine auricular cartilage that evoked cartilage in the myocardium (Franco-Browder *et al.*, 1968), and for articular gliding surface, sometimes effective in the anterior eye chamber of rat (Urist and Adams, 1968). Moreover, in the heart, the two controls with Teflon implants were not enough to rule out a mechanical evocation of the new cartilage. Human chondrosarcoma tissue (or cells) more reliably evokes benign host cartilage in nude mice (Hirano and Urist, 1980), raising provocative questions regarding the inductive contributions of matrix materials and cellular identity, malignancy, and debris.

For example, Rous *et al.* (1912) achieved soft-tissue chondrogenic sarcomas in fowl by injecting a filtrate of ground avian cartilaginous tumor, together with granuloma-evoking kieselguhr. They thought that a virus caused the malignant new growth, but its skeletal nature could have been due to the cartilage from which the virus came, constituting separate metaplastic and malignant inductions.

3. *Cartilage Induction by Malignant and Normal Bone and Dentin*

By techniques of decalcification, devitalization, extraction, co-culturing, etc., Urist (1979; and Volume 2, Chapter 1) and his co-workers have shown that bone matrix contains a noncalcific cartilage-inducing factor(s) which is released by cellular erosion. Reddi (1981) and his colleagues are using the reliable response to implanted demineralized bone for detailed biochemical and morphological study of postnatal chondrogenesis, endochondral ossification, and colonization by marrow. Demineralized dentin is chondrogenic in rat, hamster (de Groot and Deshmukh 1975), and guinea pig (Nilsen, 1980). Osteogenesis and sometimes chondrogenesis also occur at sites of erosion in longstanding calcified lesions in man, where what is inductive may be constituents helping the connective or necrotic tissue calcify, not the calcium salts themselves, long thought to be the agent. The relation be-

tween the many pathological soft-tissue calcifications (Dalinka, 1980) and occasional ossification still eludes us.

4. Chondrogenic Induction for Bone Repair

This has been tried, with new bone as the ultimate goal, employing, for instance, fixed skeletal muscle in extracted rabbit dental alveoli (Yamamura *et al.*, 1980), and decalcified bone in rat fibular and parietal defects (Oikarinen and Korhonen, 1979; Mulliken and Glowacki, 1980).

5. Epithelial Inductions of Cartilage

Some of the normal epithelia able to induce bone may also cause a little cartilage to form simultaneously (see also Volume 2, Chapter 5). Canine transitional epithelium does this, when bladder is repaired by fascia (Neuhof, 1917) and cells are injected into cortisone-conditioned mice (Wlodarski *et al.*, 1971a), and autogenous bladder mucosal grafts evoked some cartilage in humans (Welcker, 1950) and guinea pigs (Beresford and Hancox, 1967). Also in the guinea pig, Mestel and Spain (1967) increased the proportion of cartilage by grafting a bladder wall pedicle to the abdominal musculature, thus stimulating it, they suggested, by contractions of the bladder. For the gallbladder of this species, Huggins and Sammet (1933) could not confirm Nakamoto's finding cartilage amid the induced bone; whereas allogeneic i.m. grafts of seminal vesicle and gallbladder mucosae only rarely evoked elastic cartilage (Kobayashi and Kamegaya, 1961).

Various xenogeneic grafts of urinary mucosa into cortisone-treated mice first induce cartilage, which is then replaced by bone (Wlodarski *et al.*, 1971b). This is the usual mode of induction when malignant human cell lines such as WISH and FL (Anderson *et al.*, 1964) are implanted i.m., as discussed by Ostrowski and Wlodarski (1971) and Anderson (1976). The effect is not confined to the mouse, for human K cells are potent in immunosuppressed rats (Hancox and Wlodarski, 1972).

All the epithelial inductions need living, perhaps proliferating, cells, and the distance between the initial cartilage or bone and the epithelial cells is often (but not always) slight. Favoring induction might be the temporary absence of a basal lamina while the epithelial cells proliferate in a granulation tissue, so that the induction is an abnormal display of the power of an epithelium to influence the macromolecular composition of its developing stroma (Merrilees and Scott, 1980). The breakdown of the barrier between epithelium and substrate in invasive carcinomas may assist their occasional stromal metaplasias.

VI. DISCUSSION AND SUMMARY

A. Matrix Synthesis and Variety

The three sides to EC cartilage are its production, responsive precursor cells, and stimuli. Any cartilage is a complex, inhomogeneous and changing mixture of several kinds of macromolecule, requiring fine controls on many cellular activities (Lash and Vasan, 1978). The balance of ingredients known as vertebrate hyaline cartilage is not one tissue, and is not the only kind of EC cartilage. Elastic and fibrocartilage occur, and myxoid, osteochondroid (chondroid bone I) and bone can arise from the same cell population. The chondroid bone spatially linking cartilage and bone seems to signal a direct osseous metaplasia of cartilage, but the evidence indicates that most chondroid bone seen with EC cartilage also arises *de novo,* as yet another product of skeletal cells, and what little is converted cartilage does not progress to osteocytic bone (Beresford, 1981). The sequence of macromolecular production is not fixed, for collagen precedes cartilage proteoglycans in slow nontraumatic chondrifications (e.g., of tendon) whereas in some other sites myxoid tissue seems to come before cartilage.

B. Precursor Cells

All cells are presumed competent by genome for the synthesis and assembly of cartilage-type macromolecules but which cells actually express these abilities in unusual sites? First, there may be nothing queer about the chondrogenic cells except that they are off station, taken by one of the events of specious ectopia (Section III), or by a disturbed migration in some malformations. Next, EC cartilage in embryonic tumors, teratomas, and other malformations involves the turning of primitive embryonic or mesenchymal cells to abnormal chondrogenesis. The cells appear so labile at this time that one tends to attribute the conversion more to a deranged stimulus, than to culpability by the cells (a perhaps misplaced emphasis).

When EC cartilage arises postnatally, it is usual to seize on the least differentiated cell present as its source, because primary orthotopic cartilage forms from multipotent embryonic cells. As pathologists have focused on particular organs, this reasoning has yielded a host of cellular origins, divisible into three groups: (*1*) germ and embryonic cells, for EC cartilage in gonads and on bones; (*2*) in other postnatal contexts, female genital stromal cells, myoepithelial cells, malignant epithelial and glial cells, and a range of ubiquitous cells—pericytes, cells of blood and endothelium, and fibroblasts —of undistinguished morphology and about which little was known to prove that they were fully differentiated; and (*3*) various hypothetical enduring embryonic elements. This last group encompasses Cohnheim's embryonic

rests, Maximow's undifferentiated mesenchymal cell, and, as a current example, persisting mesoderm lining serous cavities (Donna and Betta, 1981): all constructs of the school that resists the idea of a metaplasia by differentiated cells to form ectopic tissues.

The notion that *embryonic behavior* requires *embryonic cells* has hindered study of the cellular side of ectopic chondrogenesis, first, by adding hypothetical entities to the several cell types already present. Second, the view of the event as a "corruption of young innocents" has biased interest into modestly rewarded study of the "corrupting" stimuli and away from the interacting cells. Nevertheless, experimental support now exists for considerable freedom of phenotypic expression among normal skeletal and smooth muscle cells, fibroblasts, osteoblasts, and chondroblasts (see Volume 2, Chapter 1). Cells of the musculoskeletal series constitute a family with an extensive repertoire of fine responses, for which the few tissue categories of histology texts represent little more than stereotypes. What looks like cell instability is the power to respond, usually accurately, to biomechanical, defensive, and other needs in embryogenesis, growth, repair, and regeneration. Even EC cartilage is a successful reconciliation of demands with the cells' own disposition, successful as the elaboration of a complex matrix, and perhaps on occasion truly rewarding. In the phylogenetic perspective, today's EC cartilage may so favorably change the mechanics as to become a future standard skeletal or extraskeletal development. Although of no mechanical benefit, most EC cartilage does not so penalize the bearer that this metaplastic ability of musculoskeletal cells has been selected out. [Around replaced joints radiation can be used to discourage clearly obstructive ectopic bone (Coventry and Scanlon, 1981).] But conspicuously absent from considerations of EC cartilage are its gross form and the biomechanical results, which determine how far it is a hindrance, helpful, or inconsequential.

The many factors the formative cells are taking account of form a screen obscuring the unidentified close-order interactions of cells and their molecular micromilieu that oblige them to make cartilage rather than any other connective tissue. When the participation of even second-order influences is missed, spontaneous chondrogenesis, nontraumatic myositis ossificans and similar terms of etiological vacancy emerge. Experiment has revealed several influences, more secondary than prime, and given some basis for ranking items within each.

Cellular susceptibility to chondrogenic conversion varies with cell type, site, species, the individual, and recent cell experiences. Osteoblasts, meaning inner periosteal cells, transform more readily than fibroblasts. Long-bone osteoblasts produce cartilage more easily than calvarial ones, and fibroblasts from muscle perform better than dermal ones, which surpass

visceral fibroblasts. Rabbit fibroblasts and aortic muscle are prone to make cartilage; fibroblasts from rat and gerbil are reluctant. Individual differences, using for example, fibroblasts from dogs with aponeurotic fibroma or mixed mammary tumors, have yet to be explored. Recent cellular experience involves injury or malignant invasion causing cell proliferation, and the breakdown of basal laminae and phenotype-stabilizing matrix macromolecules. Cell division might facilitate dedifferentiation, and by increasing cell number favors cellular interactions and the chance that variations in cell responsiveness take some cells to threshold. Furthermore, another experience—malignant transformation or a prior dedifferentiation—in epithelial, myoepithelial, chordomatous, and other cells sometimes allows them to produce cartilage, or so histopathology suggests. Some of the programs needed are already in the competence of nonneoplastic epithelial cells, thus notochord makes type II collagen (von der Mark and Conrad, 1979) and hepatocytes at least type I (Karasaki and Raymond, 1981).

C. Stimuli

The biological stimuli are devitalized muscle, demineralized bone and dentin and extracts thereof, malignant and normal living epithelia, and cartilage, (malignant change allowing more epithelia to become inducers). Cartilage follows radiation and other injury, with or without the implantation of man-made sponges, tubes, etc. Increased or normal mechanical loads alone may be chondrogenic, although if the work is normal the cartilage is extraskeletal rather than ectopic. In contrast, immobilization also causes ectopic skeletogenesis. More disconcerting aspects are that the stimuli cannot be followed right up to the responding cell; they are often species specific; and they may interact, since grafting and implants can injure muscle and alter mechanics. In turning to tissue culture to separate cell types and isolate stimuli, one faces the risk that unknown factors *in vitro* could allow an unnatural laxity in phenotypic expression, for example the derivatives of mammary epithelium (Dulbecco *et al.*, 1981), which may occur even if cells are kept *in vivo* in diffusion chambers, where, for instance, chondroid bone is common. Several of the stimuli are, as noted, skeletogenic rather than chondrogenic, in inducing either some or a lot of bone concurrently with the cartilage. Thus, autogenous living bone induces bone with little cartilage (Thorogood and Craig Gray, 1975), decalcified bone the converse (Urist, 1979).

Most EC cartilage apparently forms by metaplasia of specialized cells. Its variety and rarity in pathology and limitations in the scope of experimentation have hampered exact elucidation of its genesis. Remedies will come from (*1*) more chemical and immunologic identification of cells, products, receptors and agents; (*2*) the use of agents to block induction of the cell;

(*3*) attention to the many potentially chondrogenic cells and circumstances pointed to by human medicine and comparative anatomy, and (*4*) guidance from veterinary pathology on suitable animals to exploit better the presently bewildering species differences.

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2

Mechanisms of Resorption and Remodeling of Cartilage

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I. INTRODUCTION

The ability to limit or prevent cartilage breakdown and to stimulate effective remodeling activity continues to be a major unsolved problem in the treatment of patients with musculoskeletal disease. To discuss these questions, this chapter will concentrate on hyaline cartilage, particularly articular cartilage, a tissue long known for its durability, its relative resistance to resorption, and its limited remodeling and regeneration potential.

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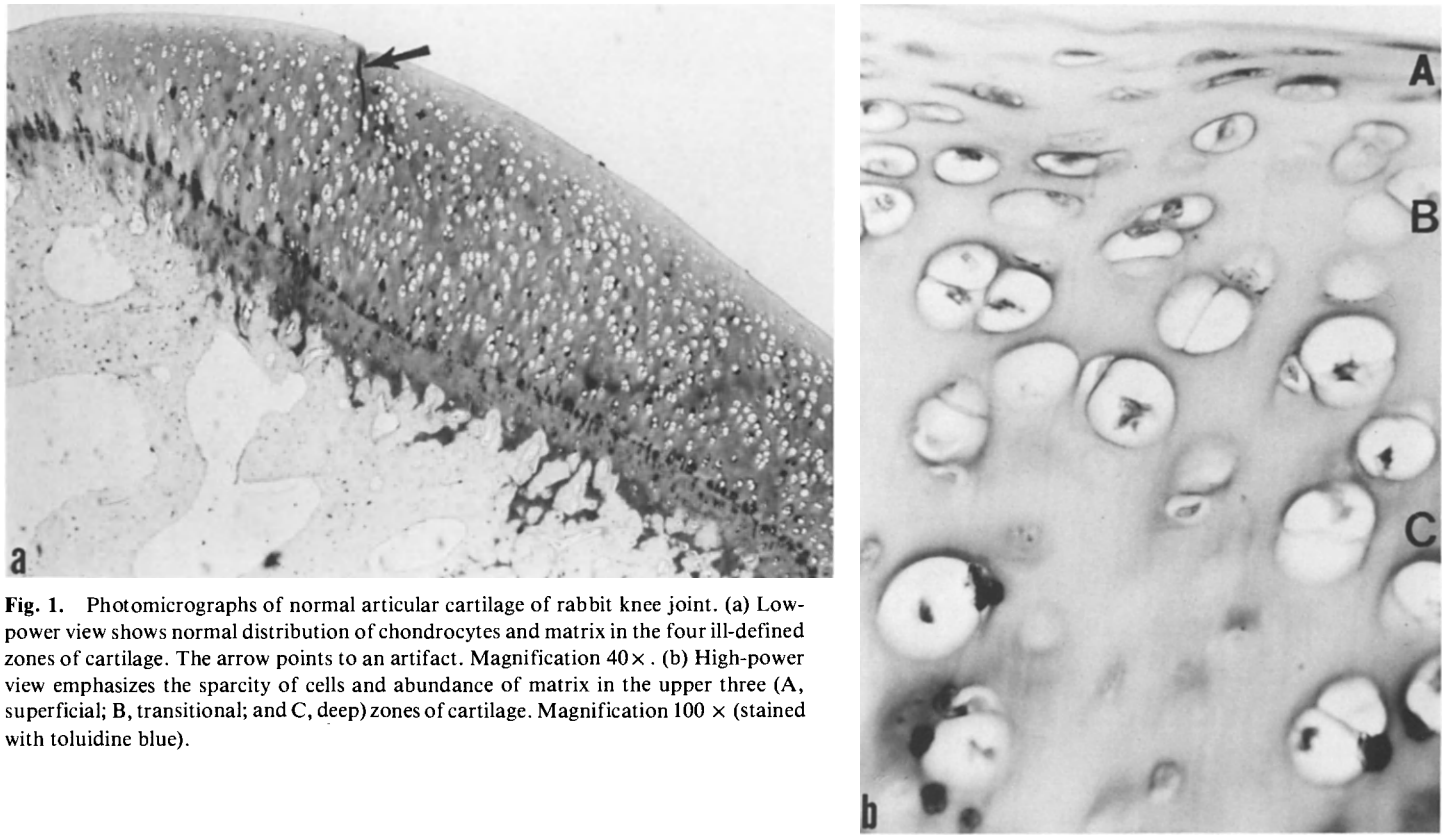


Fig. 1. Photomicrographs of normal articular cartilage of rabbit knee joint. (a) Low-power view shows normal distribution of chondrocytes and matrix in the four ill-defined zones of cartilage. The arrow points to an artifact. Magnification 40×. (b) High-power view emphasizes the sparcity of cells and abundance of matrix in the upper three (A, superficial; B, transitional; and C, deep) zones of cartilage. Magnification 100 × (stained with toluidine blue).

The term *remodeling* will be used to indicate the process whereby cartilage defects are reshaped in the adult animal. The three-dimensional changes that occur during growth are directed by a different set of control mechanisms and will not be included in this discussion (see Volume 2, Chapter 7). These changes are part of a separate subject more properly termed modeling, not remodeling. Since the physical properties of cartilage include only a limited ability to deform elastically and to creep, remodeling must be accomplished primarily through tissue regeneration.

In the healthy adult, cartilage has proven to be a relatively inert tissue containing a sparse number of cells (chondrocytes) that, once formed, are not replaced during the life of the animal (Fig. 1a,b). On a cellular basis chondrocytes themselves are dynamic structures that carry on metabolic activities similar to other types of connective tissue cells. This chondrocyte activity serves a maintenance function with little or no evidence that remodeling of cartilage occurs in the normal state. In contrast, bone remodeling is a continuous process wherein new bone constantly replaces older bone. Therefore, bone remodeling in effect limits the life span of most cellular and structural skeletal elements. Bone, however, not only can renew itself, it demonstrates a remarkable power of recovery from drastic insults, such as comminuted fractures or infections.

In the diseased state, numerous stimuli such as infection, trauma, rheumatoid arthritis, or even prolonged immobilization can lead to the destruction of cartilage. This process probably follows a common pathway at the microscopic and ultrastructural levels, regardless of the precipitating event, the type of cartilage or its location.

The ability of cartilage to regenerate has often been questioned, due in part to a dearth of knowledge on ways to stimulate cartilage formation in diseased joints. It is known that injury to cartilage, whether traumatic or metabolic, often stimulates a limited repair process, but cartilage formation is not directly coupled with resorption as it is in bone. Even so, abundant evidence indicates that cartilage has a definite, if latent, regeneration potential. For instance, a pseudarthrosis may contain hyaline cartilage, growth hormone can reactivate growth in articular cartilage, and exercise can stimulate hyaline cartilage regrowth in surface defects of joints. The overriding problem, therefore, concerns the control of cartilage regrowth in joint surface remodeling, that is, methods of activating and directing the process of articular cartilage replacement.

II. RESPONSE OF CARTILAGE TO INJURY

This section will outline the overall response of cartilage to injury in order to provide background information for more detailed discussions of resorption and remodeling in subsequent sections. The response of osteoarthritic carti-

lage appears to be similar to that described in this section, very likely because trauma is probably the most common cause of osteoarthritis (see Chapter 4 in this volume).

The classical studies utilized linear scalpel cuts into articular cartilage perpendicular to the surface. Subsequently, many investigators have used drill holes through cartilage into subchondral bone. The response of cartilage to such injuries varies dramatically, depending on the depth of the injury and whether or not it violates the deeper layers of cartilage to expose the vasculature in the bone.

A. Superficial Lesions

The response of cartilage to injury can best be seen after a superficial injury that does not reach bone. Such an injury produces no inflammatory response due to the absence of vessels, nor does the cartilage show more than a minimal attempt at repair. Histologically, the first change occurs in the matrix with a loss of proteoglycans along the margins of the injury, as determined with metachromatic stains. This stimulates an attempt at repair with increased glycosaminoglycan synthesis as indicated by enhanced metachromasia adjacent to many superficial chondrocytes and by increased $^{35}SO_4$ uptake (Meachim, 1963). Collagen synthesis has also been found to increase (Repo and Mitchell, 1971). Other than the death of some of the chondrocytes lining the injury, little cellular response is evident histologically, although occasional clusters of cells may be seen in a few weeks. Soon after the injury the cells adjacent to the injured surfaces may show a brief burst of reproductive activity with an increased uptake of tritiated thymidine (Mankin, 1962), or they may fail to respond (DePalma *et al.*, 1966).

The long term response varies depending upon the type of defect, and more important, whether the injury leads to progressive degeneration of the surrounding cartilage. A linear defect often shows little or no long term change, and 6 months to 1 year later may look the same as it did immediately following injury (Fig. 2). Thompson (1975) found that linear defects at the periphery of the patella showed more evidence of healing than those located centrally. In contrast, fibrillation with progressive degenerative changes may develop. When a degenerative process occurs, pieces of cartilage are abraded with motion, and these wear particles produce a synovial reaction with the clinical findings of synovitis. As a result, the cartilage decreases in thickness and in areas it may be lost completely, exposing the underlying bone. Cartilage lost in this manner is often said to have resorbed, however the process is one of erosion and not, as in bone, an active metabolic (cellular) process.

Larger defects show a slightly greater attempt at repair with cell proliferation mainly from the superficial layers of the articular cartilage. A variable amount of new tissue may develop in the defect, at times including hyaline

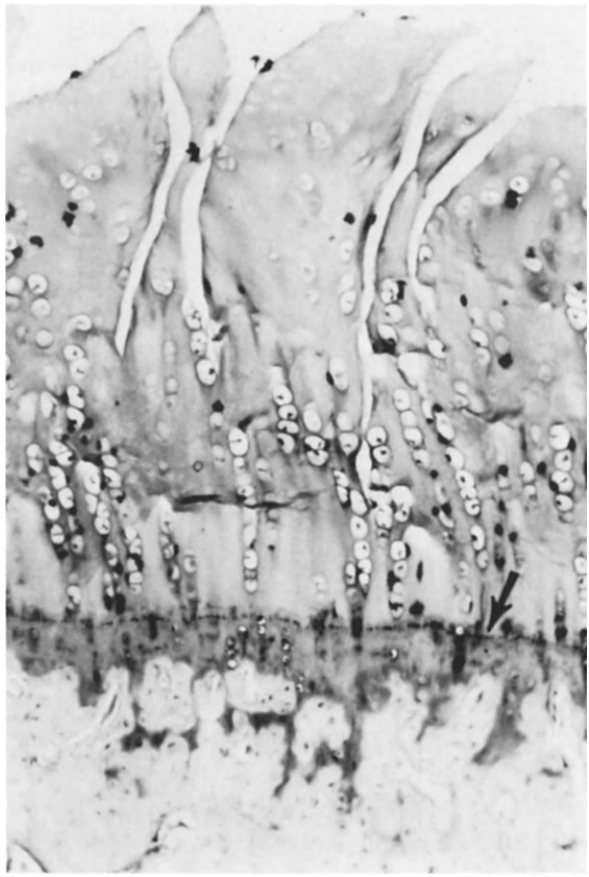
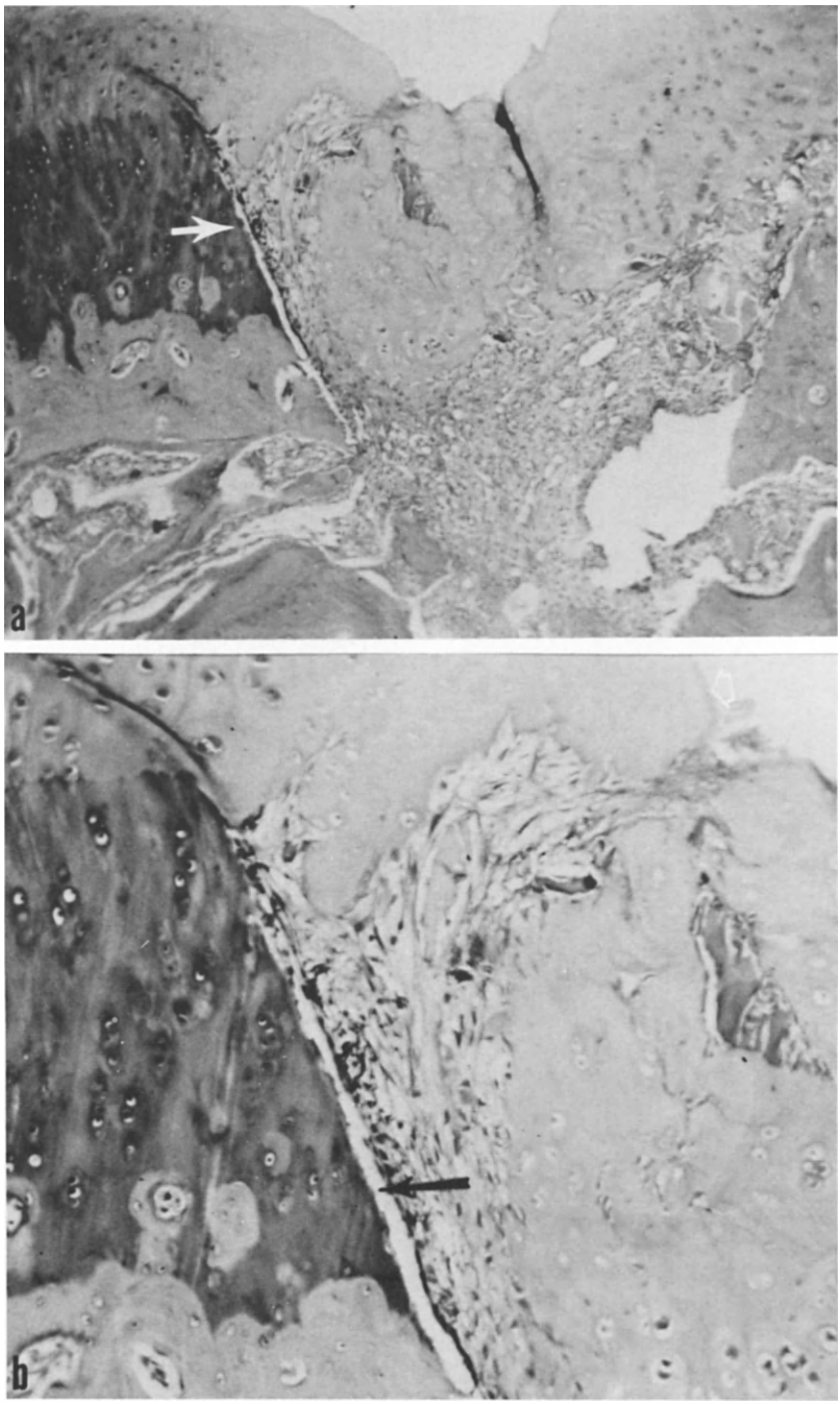


Fig. 2. Photomicrograph of rabbit articular cartilage with superficial scarification. The lacerative defects appear essentially unchanged 6 months after the injury. The tidemark is indicated by an arrow. The superficial zone shows chondrocyte death, leaving behind empty lacunae. Magnification 40× (stained with toluidine blue).

cartilage (Calandruccio and Gilmer, 1962). If the walls of the defect are almost vertical, passive molding of the margins, termed *matrix flow,* may occur to a limited degree.

B. Deep Lesions

The overall response to an injury that extends into subchondral bone differs very strikingly from that seen with a superficial lesion. The reaction of the cartilage itself does not vary much from that following a superficial le-


a
b

sion, except for a greater number of giant lacunae containing multiple cells, but the exposure of vessels permits a much more vigorous response (Mitchell and Shepard, 1976). The defect fills with reparative granulation tissue which bonds to the cartilage surfaces lining the injury. The fibrous tissue undergoes progressive chondrification to produce fibrocartilage which firmly welds the wound edges together and "heals" the lesion (Fig. 3a,b). The sequence of events in the repair process includes: fibrin, granulation tissue, connective tissue, cartilage cells in connective tissue (connective tissue cartilage), fibrocartilage, and hyaline cartilage (Shands, 1931). The development of hyaline cartilage depends on functional stresses from early motion and weight bearing (DePalma *et al.*, 1966). Salter *et al.* (1980) have shown that the majority of full thickness defects in rabbits fill in with hyaline cartilage if continuous passive motion is started immediately, while still under anesthesia. A new layer of subchondral bone develops and separates the cartilage from the marrow spaces. When hyaline cartilage forms, the cartilage layer adjacent to bone calcifies and the tidemark returns (Hjertquist and Lemperg, 1971).

Even though motion, either active or passive, can stimulate the formation of hyaline cartilage in a high percentage of defects, and grossly the articular surface may look normal, the new cartilage may not be durable enough to withstand the stresses of normal activity. In fact, after 6 months to 1 year the newly formed cartilage may show evidence of degeneration (Hjertquist and Lemperg, 1971). Whether the lack of normal durability is due to faulty composition of the cartilage matrix, to incomplete remodeling at the interface between old and new cartilage, to increased stress in the regenerated cartilage because the new subchondral bone is often thicker than normal, or to some other factor, remains speculative.

III. RESORPTION

Cartilage has the reputation of being a durable tissue that is resistant to resorption, as suggested by information from numerous sources. Perhaps the most impressive is the ability of articular cartilage to withstand the stresses of normal joint function, regardless of the level of activity or the weight of the patient, and to maintain its integrity year after year, often throughout the lifetime of the patient. Likewise, the epiphyseal plate acts as a barrier to

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Fig. 3. Photomicrographs of rabbit articular cartilage with *deep* lesion violating the osteochondral junction produced by drilling. A week-old injury shows filling of defect by granulation and fibrocartilaginous tissues arising from marrow at the base of the defect. Note a lack of degenerative changes in the cartilage forming the lateral wall on the left (a—white arrow). A potential space (b—black arrow) still separates the newly formed tissue from the wall. Some *matrix flow* of the surface layers of cartilage (b—white arrow) at the margin is also noted. (a) Magnification 40×, (b) magnification 100× (stained with safranin O and fast green).

infections, as well as to tumors, whether primary or metastatic. Tumors commonly lodge in the metaphysis and may extend into the diaphysis, but rarely violate the epiphyseal plate. Osteomyelitis, which in children typically begins in the metaphysis, does at times break through the plate to erode the bony epiphysis and occasionally produce a purulent arthritis, but such cases generate an undue amount of interest justified only by their scarcity, not their frequency. In addition, the relative resistance of cartilage to certain infections, such as tuberculosis (TB), has become a hallmark of disease.

It is generally believed that all types of cartilage are resistant to resorption, and that normal cartilage is not invaded by capillary vessels (Silvestrini *et al.*, 1979). Apparently, unusual circumstances, such as rigid immobilization, are required for cartilage to become vascularized. This resistance to invasion by vascularized mesenchyme may be due to the presence in cartilage matrix of cationic proteins of low molecular weight which act as proteinase inhibitors (Sorgente *et al.*, 1975; and see Volume 1, Chapter 10).

Other evidence of the durability of cartilage might be its effectiveness in preventing regrowth of a bony bridge across an epiphyseal plate following resection, or possibly its low antigenicity. The absence of blood vessels in articular cartilage and in the epiphyseal plate (other than in the fetus and infant) suggests greater self-sufficiency than afforded other tissues. Not only does the absence of vessels delay the inflammatory response within cartilage and complicate the task of lesions which would enter it from the outside, but in a sense it makes cartilage more independent than tissues that require a ready source of nutrients.

In spite of its apparent durability, cartilage can be destroyed by numerous processes. In fact, the absence of vessels can be a detriment whenever a rapid inflammatory process would be protective, just as the absence of nerve endings serverely limits the response of cartilage to noxious stimuli.

A. Pathophysiology

Resorption of articular cartilage usually occurs as a result of a combined chemical–mechanical process in the absence of inflammatory cells or chondroclasts. Cellular resorption probably occurs at specific sites, most notably in the epiphyseal plate where the transverse and longitudinal septa are handled differently. The transverse cartilaginous plates that separate individual cells do not mineralize and are lysed by cytoplasmic processes from perivascular and endothelial cells. In contrast, the mineralized longitudinal septa are resorbed by chondroclasts that are structurally similar to osteoclasts with a ruffled border as the active surface (Schenk *et al.*, 1967). Active cellular resorption might also occur at other times, possibly during infections or following cartilage transplants, but it does not appear to be an important

method of resorption under normal conditions, especially for articular cartilage.

1. Sequence of Degradation

The process of cartilage resorption seems to follow a specific pattern, regardless of the location or the stimulus, whether pathological or physiological. Silvestrini *et al.* (1979) postulated that the mechanism of resorption of uncalcified cartilage is similar to that which leads to bone erosion. Furthermore, the same sequence occurs in cartilage resorbed during growth as it does in the adult and the process does not require the proximity of vascularized tissue, since similar events occur in articular cartilage when eroded in the absence of pannus.

The resistance of articular cartilage to normal stresses rests on the interaction of two macromolecules, collagen and proteoglycan. The integrity of these molecules must be maintained for normal joint function (Kempson *et al.*, 1976). Although enzymes that can break down either of these molecules have been identified, proteoglycan has been repeatedly shown to be lost first under a variety of pathological and physiological conditions. From a practical standpoint, this permits cartilage to recover from a moderate insult by increasing its proteoglycan synthesis, but once the collagen framework has been destroyed there is little hope of recovering normal cartilage architecture.

Initially, the cartilage matrix loses a portion of its proteoglycan content. This can be detected histochemically with safranin O (Rosenberg, 1971), toluidine blue, alcian blue, and colloidal iron, in addition to a number of other stains. The loss of proteoglycans not only changes the composition of the ground substance, it also exposes collagen fibers which are then more easily attacked by proteolytic enzymes than those surrounded by glycosaminoglycans (Quintarelli *et al.*, 1969). The nature of both proteoglycan and collagen degradation is probably proteolytic. The chondrocytes respond with a compensatory increase in proteoglycan synthesis, but it is often inadequate and the total proteoglycan content decreases. All types of glycosaminoglycans may not be lost in equal amounts. For instance, a relative decrease in keratosulfate and an increase in chondroitin 4-sulfate has been reported in osteoarthritis (Mankin and Lipiello, 1971).

2. Autolytic Degradation

One of the major discoveries in the last 20 years has been the realization that the primary source of catabolic enzymes responsible for degrading cartilage is the chondrocyte. In the recent past, interest was mainly focused on en-

TABLE I

Catabolic Enzymes in Articular Tissue

Tissue	Enzyme	
	Acidic	Neutral
Synovium	Cathepsins B, D, and N	Collagenase[a] Metalloproteases[a] Serine proteases
Articular Cartilage	Cathepsins B, D, and F[b] Metalloproteases[c]	Metalloprotease[c]
Synovial Fluid		Elastase[d] Cathepsin G Plasmin

[a] Werb and Dingle (1976).
[b] Dingle *et al.* (1977).
[c] Sapolsky *et al.* (1976).
[d] Barratt (1973).

zymes produced by the synovial membrane (Table I). These enzymes are synthesized in the synovium and released directly into the extracellular environment, apparently with very little storage in the tissues (Reynolds *et al.*, 1977). Enzymes in the synovial fluid have not received as much attention, possibly due to the presence of enzyme inhibitors (Dingle, 1979).

It is now known that, following injury, the chondrocytes themselves respond by producing enzymes capable of degrading the cartilage matrix (Chrisman and Fessel, 1962). Theoretically, either a hyaluronidase or a protease could act on the proteoglycan to initiate degradation. Although a number of enzymes have been found in cartilage, a cathepsin-like protease, probably a neutral metalloproteinase similar to lysosomal cathepsin but acting at a pH close to neutral, currently appears to be the responsible enzyme (Fessel and Chrisman, 1964; Chrisman, 1969; Dingle and Dingle, 1980). Most of the known tissue proteases, both acidic and neutral, are capable of degrading the polypeptide chain of the proteoglycan molecule (Ziff *et al.*, 1960; Fell and Dingle, 1963; Ali, 1964; Weissman and Spiberg, 1968; Thomas *et al.*, 1960). These enzymes can be found in synovium and in inflammatory cells. Some of them may be responsible for cartilage degradation in specific pathological conditions but none of them are likely to be the initiating agent in osteoarthritis or following trauma. A number of these enzymes, especially the lysosomal enzymes, have an acidic pH optimum and would be unlikely to diffuse into the cartilage matrix and act under environmental conditions *in vivo*. Neutral enzymes, possibly metalloproteinases,

would be more likely to act *in vivo,* but an extrinsically produced enzyme that diffuses into cartilage would be likely to act diffusely throughout the matrix and would not be localized to the matrix immediately surrounding the cells as has been demonstrated by Chrisman (1969; Chrisman *et al.,* 1967). Studies by Dingle with a unique culture technique using cavities in nasal cartilage and proteoglycan aggregate trapped in polyacrylamide beads provide evidence that degradation of the matrix molecule by soluble tissue proteinases is unlikely (Dingle and Dingle, 1980). Latent forms of the metalloproteinases, when activated, show specificity in either gelatin, collagen, or proteoglycan (Sellers *et al.,* 1978). Collagenase attacks the triple helix at neutral pH causing a three-quarter/one-quarter split. At least one other enzyme, neutrophil elastase, is able to attack collagen at neutral pH (Starkey *et al.,* 1977), probably at the cross-link region. Furthermore, elastase readily degrades type II collagen in cartilage, whereas this collagen is relatively resistant to collagenase. The opposite is true for type I cartilage from skin, bone, and tendon.

3. Messengers

Tissues other than cartilage have been found to secrete messengers capable of stimulating chondrocytes to degrade both proteoglycan and collagen (Jubb and Fell, 1980). Dingle and others have identified such a substance that is produced by synovium, and have named it catabolin (Dingle, 1979, 1981, and see Volume 2, Chapter 5). They defined catabolin as a protein, or family of proteins, capable of stimulating intact living cartilage to degrade its extracellular matrix. They also demonstrated that activated monocytes excrete a very active catabolin-like material, and speculate that similar molecules are produced by a wide variety of cells.

Two separate catabolin-like factors produced by macrophages have been found by other investigators (Ridge *et al.,* 1980; Deshmukh-Phadke *et al.,* 1980). These two substances have many similarities, and the fact that each one was described by a different set of investigators raises the question of whether they could be the same substance, yet there appears to be a difference between them (Table II). Production of these factors can be increased substantially by adding lipopolysaccharide to macrophage cultures.

Catabolin-like substances have no direct action on cartilage matrix, as demonstrated by the fact that they affect living cartilage, but not dead cartilage as an enzyme would. The discovery of these messengers may help to explain how cartilage can be resorbed in conditions where no pannus develops. In fact, even in the presence of pannus, catabolin-like materials could be responsible for initiating resorption. Enzymes secreted by pannus have not yet been definitely shown to initiate resorption in either rheumatoid arthritis

TABLE II

Properties of Catabolin-Like Substances

Inducing factor	Chemical composition	Molecular weight	Effect of heat	Effect of trypsin	pH stability	Effect on synthesis						Effect on action (Cartilage breakdown)					
						Cyclohexamide	Indomethacin	Prednisolone	Chloroquine	Gold	Aspirin	Cyclohexamide	Prednisolone	Chloroquine	D-Penicillamine	Gold	Aspirin
Catabolin[a] (synovium)	Protein	20,000	Inactivated at 70°C for 10 min	Stable		↓	None	↓	None	None	None		None	↓	↑	None	None
Macrophage factor[b]	Protein	30,000	Stable at 60°C for 10 min	Stable	Partial inactivation at pH 2	↓	None					↓			↓		
Macrophage factor[c]	Protein	15,000	Stable at 56°C for 30 min	Destroyed													

[a] Dingle, 1981.
[b] Ridge *et al.*, 1980.
[c] Deshmukh-Phadke *et al.*, 1980.

or osteoarthritis, nor have inhibitors of these enzymes been shown to be effective *in vivo*.

4. Lymphokines

Other factors which might be responsible for cartilage degradation form a group of substances called lymphokines derived from lymphocytes, especially T cells. The predominant mononuclear cell infiltrating synovial membranes and present in the synovial fluid in chronic synovitis is a thymic-derived T cell (Van Boxel and Paget, 1975). Lymphokines have been found in pathological synovial fluid, as well as in the culture medium of synovial tissue from antigen-induced joint inflammation of animals (Stastny *et al.*, 1975). Although definitive evidence of the role of lymphokines is not yet available, numerous studies suggest that it may be significant. Repetitive injection of antigen-induced lymphokine causes chronic synovitis in rabbits' knees (Andreis *et al.*, 1974). Human lymphocytes and rabbit splenocytes exposed to T cell mitogens release a factor that stimulates monocytes and/or macrophages to release neutral and acidic proteinase (Herman *et al.*, 1977). Furthermore, the basic matrix components may sensitize lymphocytes (activate T cells), leading to a cycle that perpetuates the process of articular inflammation and cartilage degradation. Lymphokine has been found to decrease glycosaminoglycan, as well as protein, synthesis, although it causes only a slight increase in catabolic activity (Herman *et al.*, 1981). The decrease in synthesis, together with continued cartilage degradation, could have a detrimental effect on cartilage.

5. Retinol

One additional substance capable of stimulating the degradation of cartilage that has been known for many years is vitamin A or retinol (Fell and Mellanby, 1952; Barratt, 1973; Thomas, 1960; Dingle, 1979). Retinol affects living but not dead cartilage by stimulating a depletion of intercellular material. Furthermore, it has little effect on articular explants alone, whereas the presence of adjacent marrow or capsular tissue stimulates extensive degradation. (For a full discussion of vitamins see Volume 2, Chapter 8).

6. Self-Perpetuation

Because of the autolytic response of cartilage to trauma, the process of resorption tends to be self-perpetuating, regardless of the initiating stimulus. This occurs because the loss of proteoglycan changes the physical properties of cartilage and makes it more susceptible to further injury from the stresses

of normal activity. An injury stimulates the release of degradative enzymes from chondrocytes, and these in turn make the cartilage even more susceptible to reinjury.

B. Pressure

In its normal *in vivo* state, articular cartilage is subjected to compression. Physiological compression loads, together with motion, prove beneficial in maintaining cartilage in a state of peak performance. In contrast, excessive compression, as well as immobilization, has a detrimental effect on cartilage. Nevertheless, the relative importance of the two cannot be determined with certainty due to the difficulty of producing compression in a joint without immobilizing it.

Continuous compression leads to localized cartilage breakdown in the area of maximum pressure. These changes can be detected within the first few days. Initially, cartilage necrosis may occur with a loss of normal nuclear staining in the chondrocytes. This is followed by a loss of distinct cell boundaries, decreased cartilage thickness, fibrillation, and ingrowth of vessels from the underlying bone with the replacement of cartilage by fibrous tissue (Trias, 1961; Salter and Field, 1960; Evans *et al.*, 1960; Crelin and Southwick, 1960). Necrosis of the underlying bone may be seen, together with new bone formation. Cysts may develop, similar to those seen in degenerative arthritis. Peripheral to the area of necrosis many large lacunae can be found that contain multiple actively dividing chondrocytes (Crelin and Southwick, 1960), with atrophy of the subchondral bone.

A period of continuous compression followed by removal of the compression for a period of time before sacrifice allows a variable amount of regenerative activity. Little or no attempt at healing may be found in severely damaged cartilage where cell necrosis is complete throughout every level. Where less damage has occurred there may be a significant regenerative response. Cell division of viable cartilage cells, especially of deeper cells, occurs with clustering of chondrocytes. Connective tissue proliferation can be seen in the marrow spaces with metaplasia of the new connective tissue to cartilage. Once the superficial layer of the fibrocartilaginous articular surface has been restored, the vessels disappear.

Intermittent compression for 1-hr periods repeated four times daily produces little or no change in the cartilage. In contrast, 12-hr periods of compression result in severe changes.

C. Immobilization

Immobilization, as might be expected, has detrimental effects on cartilage. Extremely severe changes, including bony ankylosis, have been found in human knee joints immobilized for 1–1½ years or more by pathological processes not involving the joint (Enneking and Horowitz, 1972), and similar

changes have been found in animals (Hall, 1963; Evans *et al.*, 1960; Finsterbush and Friedman, 1975). The basis for these changes is probably the interference with chondrocyte nutrition which results from a lack of motion, whereas destruction results from the nonspecific repair process which occurs.

The more rigid the immobilization, the greater the damage. A cast provides only partial immobilization, thereby partially protecting the joint. Even so, the majority of rabbit knee joints that are immobilized in casts show microscopic evidence of cartilage damage 6–8 months after a 6-week period of immobilization.

The water content of cartilage increases somewhat in the immobilized joint whereas the proteoglycan content decreases. In most cases, clinical function returns to normal once the immobilization is discontinued, but the cartilage is susceptible to injury for several weeks and during this period severe damage can occur if an active exercise program is pursued (Palmoski and Brandt, 1981).

Rigid immobilization requires either internal or external fixation. In rats, the changes that occur are reversible with up to 30 days of immobilization, and after 60 days of immobilization no further changes develop but the severity continues to increase. Fibrofatty connective tissue proliferation can be seen by 2 weeks, and by 4 weeks it completely fills the joint. Adhesions develop between this tissue and unapposed cartilagenous surfaces, as well as with soft tissue surfaces. The cartilage under the adhesions becomes thinner. The superficial cartilage loses its definition and appears confluent with the overlying connective tissue, whereas the deeper cartilage layers show little or no change. The adhesions probably serve as a source of nutrients to the cartilage, in effect substituting for nutrients normally supplied through motion. In areas of cartilage-to-cartilage apposition changes occur which are similar to, but less severe than, those seen with continuous compression. With remobilization, the adhesions persist but adapt by elongating and by developing clefts. Little evidence of healing can be found in the cartilage lesions per se.

D. Relief of Cartilage-to-Cartilage Contact

Not only does a lack of motion cause damage to cartilage, but cartilage that is not apposed by cartilage likewise degenerates. Severe changes have been noted in unapposed cartilaginous surfaces in stiff finger joints (Field and Hueston, 1970). Degenerative changes in the femoral head tend to occur in non-pressure-bearing areas (Harrison *et al.*, 1953), and there is evidence that too little pressure can incite degeneration of the patella (Wiberg, 1941; Wiles *et al.*, 1956).

Relief of contact in animals, either through patellectomy (Garr *et al.*,

1973) or removal of a femoral condyle (Hall, 1969), produces degenerative changes in the remaining cartilaginous surface. Patellectomy led to synovitis with the most severe cartilage changes in areas of pannus. Removal of the lateral femoral condyle caused changes in the lateral tibial condyle with much less severity under the meniscus. The most severe changes show fibrillation with sequestration of small pieces of cartilage, and full thickness loss may be seen with thickening of the underlying bone.

E. Septic Arthritis

The severe damage to a joint that can result from purulent arthritis has been known for years, but the sequence of events leading to the destruction remains in doubt. Hunter (1743) suggested that cartilage could be eroded by "soaking in purulent matter." Phemister (1924) produced gross destruction of cartilage when innoculated with a staphylococcus suspension in normal saline at 55°C. Duplication of these studies by Curtis failed to produce cartilage destruction when incubated at normal body temperature (37°C) (Curtiss, 1969).

Infection initially produces a loss of proteoglycan, but 65% must be lost to produce a visible change, and collagen loss is a prerequisite for visible cartilage destruction. Enzymes capable of destroying cartilage have been found in polymorphonuclear leukocytes (Keiser *et al.*, 1976) and may have an etiological role in adjuvant arthritis (Mohr *et al.*, 1981), as well as in septic arthritis (Janoff *et al.*, 1976; Starkey *et al.*, 1977). Although the damage in sepsis has been attributed to plasminogen or to the direct action of bacterial proteinases, the neutral serine proteinases, elastase and cathepsin G, must also be considered. Elastase and cathepsin G are capable of degrading the proteoglycans and then solubilizing collagen by attacking terminal peptides and/or destroying cross-links. Furthermore, type I collagen from tendon is less susceptible to elastase than cartilage collagen, indicating that different collagenases probably act on these tissues.

The sequence of events in septic arthritis includes the appearance of an inciting agent (bacteria, viruses, antigen–antibody complexes, or microcrystalline deposits, such as monosodium urate) in the synovial space, the presence of chemoattractants in the space with emigration of large numbers of polymorphonuclear leukocytes (polys), the engulfment of the inciting agent by the polys with the release of lysosomal enzymes into the synovial fluid. A possible role for a messenger, such as catabolin described earlier in this chapter, must not be forgotten. One mechanism would be the bacterial stimulation of messenger production by white cells or synovium and the production of cartilage destruction by degradative enzymes released by the chondrocytes. This possibility is more consistent with the fact that the production of inflammation, including polymorphonuclear leukocytes, in a

joint prior to innoculation with bacteria decreases the incidence of infection (Nagel *et al.*, 1965). If enzymes from polys were directly responsible for cartilage destruction, then the presence of polys might make the problem worse, not better. Finally, increased pressure develops within an infected joint (Nagel *et al.*, 1965) and it seems likely that this increase in pressure would aggravate, but not cause, the problem.

F. Antigen-Induced Arthritis

One animal model of rheumatoid arthritis is produced by injecting serum albumin (Sandy *et al.*, 1980) or other substances (Gillard and Lowther, 1976) into an animal's joint. This not only causes accelerated degeneration of cartilage proteoglycans, but it also results in a marked inhibition of synthesis.

G. Chondrocyte Death

Once certain method of producing cartilage "resorption" is to kill the cells. Whatever the cause of necrosis, whether it is excess pressure as described previously, the injection of a toxic agent, or localized freezing *in vivo* (Simon *et al.*, 1976, 1981), the dead cartilage remains in place for a relatively long period of time before it slowly erodes and a meager regenerative attempt begins in the underlying bone. Certainly, the response of cartilage to chondrocyte necrosis emphasizes the crucial role of the cells to the integrity and maintenance of normal cartilage.

IV. REMODELING

Remodeling has been broadly defined to include the structural and biochemical modifications of mature cartilage needed to maintain or restore its normal contour, its dimensions, and its spatial orientation when challenged by noxious biological and mechanical forces. Hunter (1743) and later Paget (1853) noted that, following destruction, the regenerative power of cartilage is usually inadequate.

Cartilage exhibits only a limited, and uniformly ineffective, attempt at cellular and matrix repair of superficial defects. A defect that extends through the calcified zone to communicate with vascularized bone produces a much more vigorous response. In effect, a deep lesion serves as a strong stimulus for cartilage repair, yet in actuality the response of cartilage itself shows little variation between a superficial and a deep lesion. Obviously, the difference between these two lesions represents the contribution of the vascular system, not only that of cartilage per se (Figs. 2 and 3). The morphological changes produced by these lesions were discussed previously and will not be repeated here.

The investigator is faced with two divergent, yet interconnected, avenues

of approach: to stimulate hyaline cartilage from vascularized tissue through metaplasia or to motivate the chondrocyte toward self-renewal. The fact that a deep cartilage lesion generates a strong cellular and vascular response presents a potential opportunity to influence the repair of articular surfaces, once better methods of controlling the regrowth of articular cartilage have been found. Even so, chondrocyte control of articular cartilage repair, whether indirectly through conservation of intact matrix, or directly through enhancement of matrix synthesis, theoretically offers the ideal alternative. Were it possible to adequately stimulate the chondrocyte to repair cartilage defects, then the problem of maintaining the integrity of articular surfaces would be much closer to solution. At the present time little is known about methods of stimulating matrix repair by influencing anabolic activity in chondrocytes, yet successful control of these mechanisms offers the possibility of healing without scar.

The process of formation cannot be easily separated from that of cartilage degradation for a change in one is often accompanied by a change in the other and any combination may occur. Thus an increase in the synthesis of matrix components does not, by itself, indicate that cartilage repair is occurring. Degradation may have increased more than formation, in which case the result will be a net loss of cartilage. Likewise, an attempt to estimate the rates of formation and resorption by examining cartilage morphologically can be equally deceptive. For example, cartilage loss occurs in both rheumatoid and degenerative arthritis and measurements of cartilage degradation show an increase in both conditions. In contrast, the rate of proteoglycan synthesis is usually decreased in rheumatoid arthritis and increased in degenerative arthritis, especially in the earlier stages of the disease.

This section will review many of the known methods of influencing the cartilage repair process.

A. Physical Forces

Pressure and tension, as well as motion, have been found to influence cell migration and alignment, as well as tissue differentiation. For example, uniform tension stimulates the formation of fibrous tissue with its long axis oriented parallel to the lines of tension (Bassett and Herrmann, 1961; Eggers *et al.*, 1949). A similar phenomenon can be observed clinically by placing a fracture, which already displays early callus, in traction to gain length. If successful and bone length increases, mineral accretion will cease, or a portion of the callus may even resorb, leading to a delay in healing.

In contrast, compression stimulates either bone formation or cartilage formation, depending in part on the oxygen concentration (Bassett and Herrmann, 1961). With an adequate oxygen supply compression causes

bone to form from chick embryo explants of the tibia, whereas compression in the presence of a low oxygen concentration (5%) stimulates cartilage formation.

The fact that excercise stimulates the formation of hyaline cartilage in articular cartilage defects of animals has already been noted. A similar response has been found in humans following cup arthroplasty of the hip (Urist, 1958).

B. Oxygen Tension

As noted above, low oxygen tension in the presence of compression stimulates cartilage formation. The production of cartilage in areas of decreased blood supply has been noted *in vivo* during the repair of fibular defects (Lutfi, 1974). Likewise, low oxygen tension in the presence of thyroxin increases the rate of chondroitin sulfate synthesis, whereas low oxygen tension alone has no effect (Pawelek, 1969). These findings are similar to those in the epiphyseal plate where oxygen tension is decreased and compression is present.

C. Tissue and Cellular Factors

Numerous factors produced by cells, or associated with tissues, especially synovium and cartilage, have been found to influence the synthesis of cartilage matrix components and/or chondrocyte growth. Many of them also effect cartilage degradation. Currently, this area is undergoing intensive investigation, but not enough is known to neatly classify the various substances. Some of these factors will be discused briefly.

1. Cartilage

Cartilage and some of its components have been found to stimulate proteoglycan synthesis (Bollett, 1968). Kato *et al.* (1980b) extracted a factor that stimulated proteoglycan synthesis and found that it differed from chondroitin sulfate proteoglycan. They postulated it must be a new peptide. Chondromucoprotein extracted from cartilage has also been found to stimulate chondrogenesis (Kosher *et al.*, 1973). In addition, chondrocytes produce a factor termed *conditioned medium factor* that can increase $^{35}SO_4$ incorporation. This factor is more effective with higher cell density (Solursh and Meier, 1973).

One indication that cartilage itself is capable of stimulating cartilage formation can be found in degenerative arthritis. It is known that pieces of cartilage flake off into the joint in this disease, probably due to the altered mechanical properties associated with the loss of proteoglycans and with fibrillation. It has been found that repeated injections of this cartilage debris in animals produces synovitis with thickening of cartilage at the chondro-

synovial junction. Eventually, bony spurs (exostoses), as well as cysts, develop (Chrisman *et al.,* 1965). Furthermore, the injection of chondroitin 6-sulfate (more commonly found in adult cartilage) produces a greater reaction than does chondroitin 4-sulfate (more commonly found in children). These findings might explain the clinical differences between children with degenerative arthritis and older adults with degenerative arthritis (George and Chrisman, 1968).

Not only does cartilage contain something that facilitates the repair process of cartilage, but cartilage extracts can accelerate healing in soft tissues as well (Prudden, 1964; Herrmann and Woodward, 1972).

2. *Other Factors*

A factor termed *multiplication-stimulating activity,* described as a conglomerate from rat-liver cells, has been found to stimulate proteoglycan synthesis in, and growth of, resting chondrocytes (Kato *et al.,* 1980a). This factor is even more effective when used with certain hormones, such as calcitonin, parathyroid hormone, or insulin. (See Volume 2, Chapters 9 and 10, for a fuller discussion).

Parathyroid hormone and calcitonin stimulate proteoglycan synthesis in growing but not resting chondrocytes (Suzuki *et al.,* 1976). Thyroxin markedly stimulates the synthesis of glycosaminoglycans by chondrocytes (Lash *et al.,* 1973). Growth hormone has been found to facilitate matrix healing in traumatized joints (Chrisman, 1975).

A factor from polymorphonuclear leukocytes stimulates glycosaminoglycan and DNA synthesis in human synovial fibroblasts (Myers and Castor, 1980). Extracts from platelets produce the same result, whereas extracts from other cells, such as human lymphocytes, granulocytes, tumor cells, and spleen cells stimulate glycosaminoglycan production, but not DNA synthesis. Although these changes occur in synovium and not in cartilage, the process might help explain the synovial reaction and hypertrophy that occur with chronic arthritis.

D. Miscellaneous Factors

Potassium ions have been reported to stimulate the synthesis of chondroitin sulfate by chondrocytes (Lash *et al.,* 1973), and uridine diphosphate stimulates proteoglycan production by cartilage both *in vitro* and *in vivo* (Ehrlich *et al.,* 1974). Retinoids have been found to increase heparin sulfate in fibroblast maintenance (Shapiro and Mott, 1981). At least one drug, sulindac sulfide, has been found to increase proteoglycan synthesis (Palmoski and Brandt, 1980). Finally, a deceased calcium ion concentration *in vitro* decreases glycosaminoglycan synthesis of cartilage but has no effect

on [^{3}H]leucine incorporation or on degradation (Palmoski and Brandt, 1979a).

Implants

Artificial materials placed in defects of articular cartilage have been reported to stimulate hyaline cartilage. A high degree of success has been reported with the use of Proplast, a low modulus, 80% porous, stiff sponge composite of polytetrafluoroethylene polymer and graphite fiber (Kessler *et al.*, 1980). Holes filled with Ivalon (polyvinyl alcohol sponge) heal better than unfilled holes, but reconstituted collagen sponge is preferable although it stimulates more fibrocartilage than hyaline cartilage (Speer *et al.*, 1979).

V. CARTILAGE TRANSPLANTS

Cartilage transplants, in contrast to transplants of other tissues, have a high rate of success, at least in certain parts of the body and if performed in accordance with certain principles. This is true for both autogenous and preserved homogenous transplants, probably due to the relative isolation of the chondrocyte in its matrix (McGlynn and Sharpe, 1981). The role of cells in the immune response is critical, but the cartilage matrix acts as a filter to screen out immunological messages, thus in effect limiting the cell's ability to respond. Even so, in most locations allografts have not been as successful as autografts when studied experimentally, an observation that may in part be related to the fact that experimental allografts are usually transferred directly from one animal to another, whereas clinical allografts are usually preserved for a period of time before use, and preservation appears to decrease the immunological response (DePalma *et al.*, 1963). In any case, the fate of cartilage grafts seems to depend in large part on the recipient site.

The transplantation of cartilage has become an accepted method of treating specific clinical problems, such as the restoration of facial contours (McGlynn and Sharpe, 1981), and cleft palates. There is growing interest in cartilage transplants for other purposes, such as restoration of the alveolar ridge (Perri de Carvalho and Okamato, 1979). When transferred to a subcutaneous site, as is done in most clinical applications today, a transplant stimulates only a minimal inflammatory response and it eventually becomes encapsulated.

Regardless of the type of transplant, the cells must remain viable because cartilage that contains necrotic cells gradually disintegrates. It is known that chondrocytes survive for 4–6 weeks when stored at 0–5°C, and in the case of allografts the success rate is higher if the cartilage is stored before implantation. Another critical element determining the success of cartilage grafts in

soft tissue sites is the accuracy of fit. Cartilage grafts cannot survive in a blood-bathed cavity, so if any space remains between the cartilage and the tissue at the host site and if hemostasis is not meticulously obtained, the space will fill with blood making it likely that the chondrocytes will die. It is possible that disregard for this simple surgical principle could be responsible for widely varying success rates of experimental studies that have been reported.

The fate of cartilage transplants into muscle is less certain. They have been found to resorb (Sengupta, 1974), but autografts have been reported to show little change, even at 450 days, and little change is seen in some allografts up to 344 days (Campbell *et al.*, 1963). It may be that when no local hematoma develops, and it the graft becomes encapsulated (Fig. 4), it will survive.

The survival of intraarticular cartilage transplants varies with the site and the method of implantation. Both autografts and allografts of cartilage will survive when used to fill articular cartilage defects, if the surface is level with the adjacent articular cartilage surface or slightly elevated above it. When recessed, the cartilage degenerates, probably because it is unable to receive proper nourishment with joint motion (Pap and Krompecher, 1961). Function appears to be of greater importance in maintaining the survival of transplants to joint surfaces than does tissue compatibility. For a high success rate, it is equally important to transplant a thin (< 5 mm) section of the underlying subchondral bone. The attached bone facilitates handling, permits a better fit, and provides more secure fixation. The bone does not survive and it is gradually replaced. When the transplanted cartilage is thicker than the adjacent articular cartilage at the recipient site, the deeper portion of the transplant ossifies approximately to the level of the adjacent cartilage (Benum, 1974). Eventually, a calcified zone and a tidemark develop, similar to normal cartilage. It is of interest that transplantation of a similar specimen to a nonarticular site within the joint results in ossification of the entire specimen. Large portions of articular surfaces can also be transplanted successfully if a thin rim of subchondral bone is included (Lane *et al.*, 1977; Pap and Krompecher, 1961; DePalma *et al.*, 1963).

A cartilage graft placed loose in a joint becomes a loose body. The synovium is critical to the survival of such a transplant. If the body remains loose, and therefore independent from the synovium, it will survive and may grow or even ossify (Fig. 5). Obviously, a loose body must depend upon synovial fluid to supply its nutrients. The central part of a large loose body may necrose, but cells closer to the surface remain viable. If a loose body becomes attached to synovium it may survive or it may undergo partial, and possibly complete, resorption (Fig. 6). When attached to synovium, homografts are more likely to resorb than autografts (Sengupta, 1974).

The transplantation of isolated chondrocytes has also been attempted

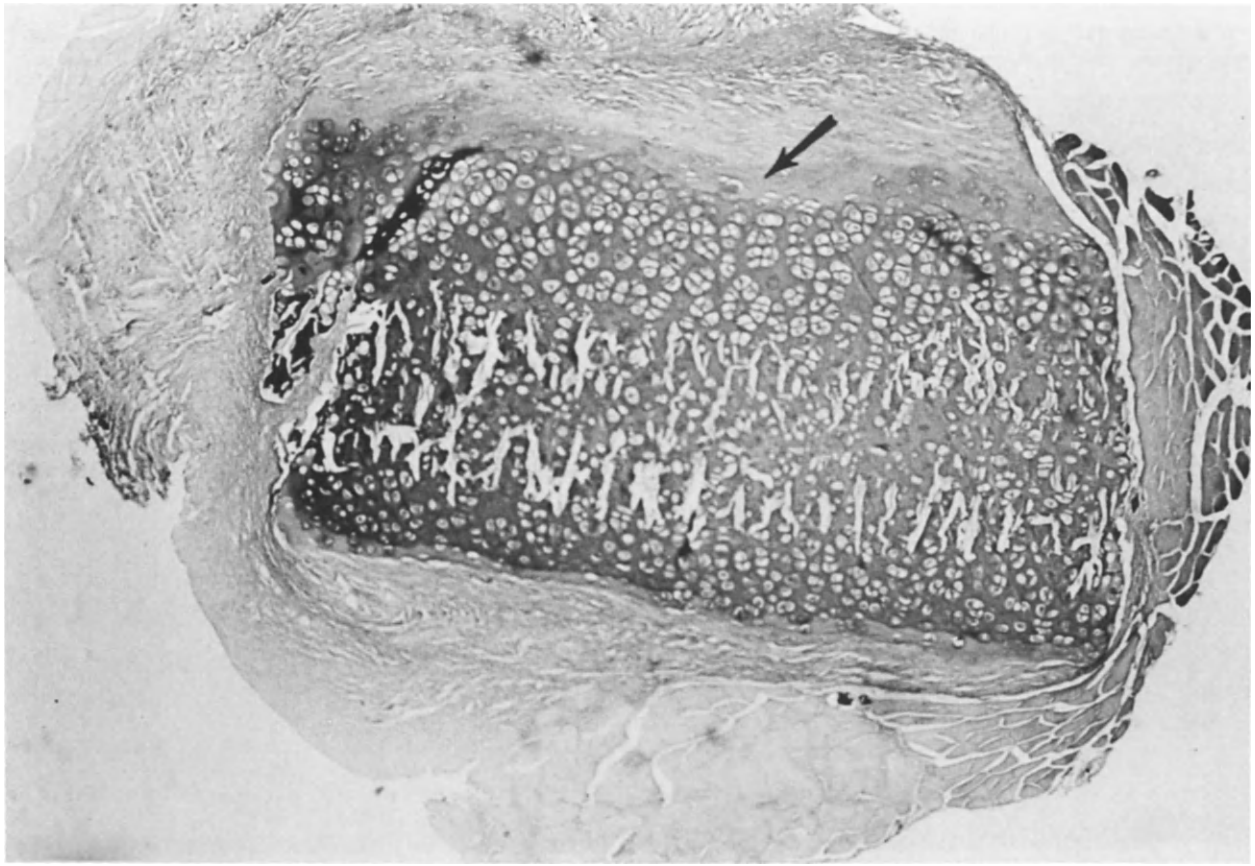

Fig. 4. Photomicrograph of a 1-week-old autografted costal cartilage in quadriceps femoris of a rat. Note the absence of inflammatory response (arrow), except for some infiltration to the left. Magnification 40× (stained with toluidine blue).

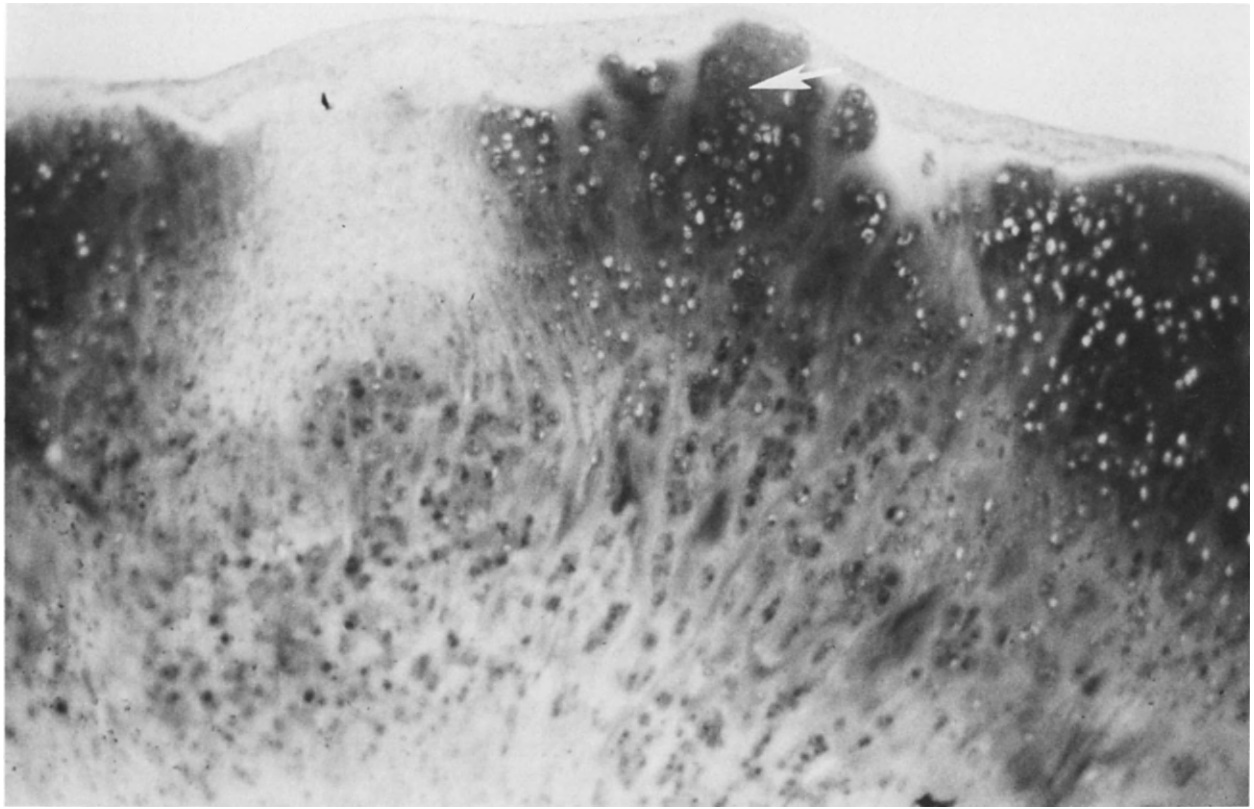

Fig. 5. Photomicrograph of a loose body not attached to the synovium present for several years in a human knee joint. Note the smooth surface layer and growth spurts of chondrocytes (white arrow). Magnification 40× (stained with safranin O and fast green).

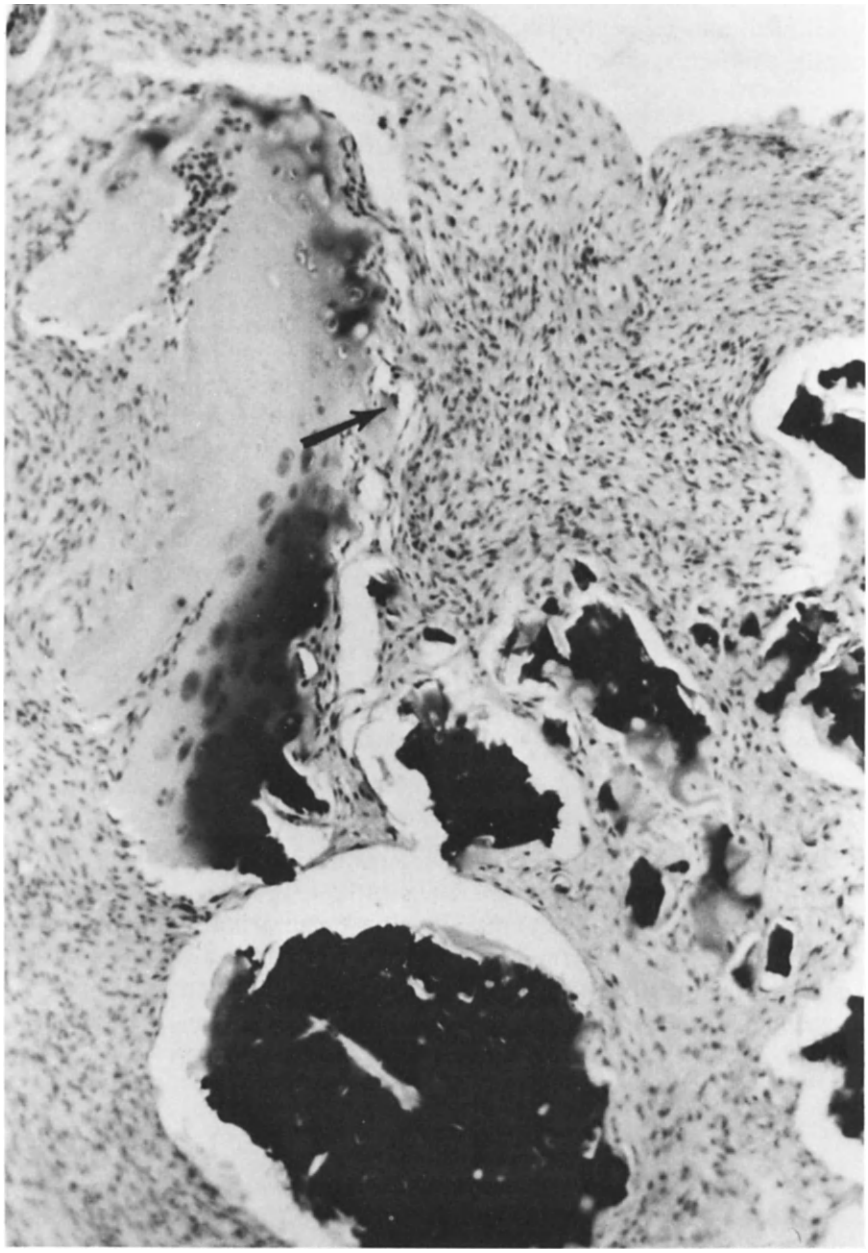

Fig. 6. Photomicrograph of a loose body attached to the synovium in a rat knee joint. Note the exuberant growth of granulation tissue and proliferating synovium eroding the cartilage edges (arrow). Clear cells presumed to be macrophages and other blood cells also participate in this process. Magnification 100× (stained with hematoxylin and eosin).

(Chesterman and Smith, 1968; Green, 1977). Better techniques must be developed, but this method may hold promise for future repair of articular cartilage defects.

VI. HORMONES, VITAMINS, AND DRUGS

In the past, cartilage was thought to be relatively resistant to the influence of systemically administered drugs and biological materials, due principally to a limited transfer of many such agents across diffusion barriers. Within the last decade, clinical and experimental studies have shown that the viability and metabolic activity of the chondrocytes in articular cartilage can be materially influenced by either systemic or topical application of chemical agents, and biological products like drugs, hormones, antimetabolites, etc. Abnormalities of joint physiology have been shown to occur in diabetes (Silberberg and Silberberg, 1964; Sokoloff, 1969), hyperlipidemia (Silberberg and Silberberg, 1950, 1960), acromegaly (Bluestone *et al.*, 1971; Silberberg *et al.*, 1964), gout (Jaffe, 1972; Gardner, 1965), hemochromatosis (Hamilton *et al.*, 1968), and numerous other conditions.

Of more direct concern is the action of such agents when administered by intraarticular injection, as in clinical practice. Knowledge of the effects on cartilage in this situation represents a distinct challenge, since the ability of cartilage to repair itself is limited. In addition, as cartilage is avascular and aneural, it fails to develop the early symptomatology that might draw our attention to it. By the time a symptom is elicited, cartilage may have undergone irreparable damage.

A. Hormones

Hormones cause profound ultrastructural and metabolic changes in chondrocytes and in matrix turnover. These effects, though similar, are more marked in immature cartilage than in adult tissue. All of the hormones investigated so far have shown dose-dependent, ultrastructural signs of cellular regression (see Volume 2, Chapter 9). Based on their effects on cartilage, Silberberg (1968) classified hormones into two broad classes: those causing overdevelopment and those causing underdevelopment of chondrocytes and their cytoplasmic organelles.

1. Hormones Causing Overdevelopment

These include growth hormone (somatotropin), which acts through somatomedin, and hormones that act synergistically with it such as thyroxin, insulin, estrogen, and testosterone.

a. Growth Hormone. This hormone acts through its intermediary product, somatomedin. It increases DNA synthesis and cell division, with up to

tenfold increases in the synthesis of matrix components, proteoglycans and collagen, in normal articular cartilage (Silberberg and Silberberg, 1964; Sledge, 1973; Yablon *et al.*, 1974), costal cartilage (Skottner *et al.*, 1977; van Baul-Offers and Van den Brande, 1979), and in experimental lacerations of articular cartilage (Chrisman, 1975). The administration of human growth hormone and semipurified somatomedin to Snell dwarf mice produces only a transient rise in both sulfate and thymidine uptake in costal cartilage (van Baul-Offers and Van den Brande (1979). After treatment for 4 weeks, no differences in cartilage activity were noted between the treated and nontreated animals. Mammalian growth hormone also stimulates proteoglycan synthesis in avian chondrocyte monolayer cultures (Sokoloff, 1976), possibly due to absence of growth hormone in the birds.

Following growth hormone or somatomedin administration, rough endoplasmic reticulum, Golgi apparatus, mitochondria, and microscopic vesicles become more numerous in the immature chondrocytes (Silberberg *et al.*, 1964). On the other hand, cellular degeneration and death, decreased synthesis of proteoglycans, and fibrosis with microscars of the matrix have also been documented (Silberberg *et al.*, 1964; Silberberg and Hasler, 1971; Smith *et al.*, 1975). This phenomenon was partly attributed to dosage levels. In addition, a strong response to growth hormone initially may be followed by "premature exhaustion." Ashton and Matheson (1979) and Heins *et al.* (1970) noted that chondrocyte response to somatomedin declines with age, possibly due to a change in the number or affinity of somatomedin receptors on chondrocyte membranes. No evidence for an inhibitory substance in serum or in cartilage has been found to explain the end-organ unresponsiveness of aged chondrocytes to somatomedin (Kumar, 1979). Somatomedin reduces chondrocyte adenyl cyclase activity by depressing intracellular cyclic AMP (cAMP) levels (Tell *et al.*, 1973), but the exact role of cAMP in chondrocyte metabolism has not yet been worked out.

b. Somatostatin. Somatostatin, a hypothalmic factor, inhibits the release of growth hormone, and the secretion of insulin, glucagon, and other hormones. In matrix implantation studies under the influence of somatostatin, Weiss *et al.* (1977) observed almost a 50% reduction in cell proliferation, based on [^{3}H]thymidine uptake and ornithine decarboxylase activity. They concluded that somatostatin regulated the proliferation of chondro-osteoprogenitor cells *in vivo*. The exact mechanism is unclear at this time.

c. Thyroxin. The demonstrated effects of thyroxin on cartilage have varied in the past. The general belief at this time is that thyroxin causes cell hypertrophy and primarily affects the maturation process of chondrocytes. Cell death (Silberberg, 1968), depression of sulfate incorporation and synthesis of proteoglycan (Dziewiatkowski, 1964), and neutralization of soma-

tomedin-mediated DNA synthesis (Ash and Francis, 1975) have also been documented after thyroxin administration.

Propylthiouracil causes marked cell degeneration, matrix vesicle formation, and enhanced mineralization (Dearden, 1974).

d. Estrogen. Although estrogen initially stimulates the proliferation of chondrocytes in the growth plate, continued administration leads to increased calcification, thereby accelerating the maturation process with eventual inhibition of linear skeletal growth (Silberberg and Silberberg, 1943; Gardner, 1943). The response varies with the dosage level. With large doses, the epiphyseal zone narrows and the number of cells in the columns decreases, but controlled administration enlarges the epiphyseal zone with swelling and sclerosis of the matrix (Silberberg, 1971; Schiff, 1966). The response also varies between epiphyses, for instance in the radius where a given dose can widen the proximal epiphysis and narrow the distal one (Negulesco and Kossler, 1978). Electron microscopic studies show an initial acceleration of chondrocyte secretory activity as evidenced by the accumulation of stippled material representing protein–polysaccharide complexes in the Golgi cisternae and subsequent secretion into the matrix (Fahmy *et al.*, 1971b). Subsequently, the transport of such molecules is retarded, leading to intracytoplasmic retention and polymerization of precollagenous products. This causes cell hypertrophy and promotes early degeneration of chondrocytes. This sequence of events also explains the reported depression of proteoglycan synthesis by estrogen (Dziewiatkowski, 1964; Minot and Hamilton, 1967).

e. Testosterone. Large doses of testosterone suppress the proliferation of chondrocytes in the growth plate but intensify hypertrophy, hyalinization, and ossification (Silberberg, 1971). Histologically, the cell columns appear to be telescoped with abrupt transition of cell characteristics from one zone to another. This has been attributed to a local change in the ground substance induced by testosterone. In addition, the normal sequences of cellular division, development, and maturation appear to be accelerated.

Electron microscopic studies have shown a zonal increase in dividing cells with large doses of testosterone, and intracellular secretory products accumulate earlier (Fahmy *et al.*, 1971a). The matrix shows premature foci of calcification, as well as thicker and longer collagen fibers. Testicular hormone seems to accelerate the normal sequence of the chondrocytes' life cycle.

Although both estrogen and testosterone accelerate cellular maturation, the mechanism of hormone action differs (Fahmy *et al.*, 1971a,b). Testosterone increases the acquisition of glycogen and lipids by the chondrocytes, as well as their discharge into the matrix through the formation of foot processes, which imparts a "spiny appearance" to the hypertrophic cartilage

cells. Estrogen, on the other hand, retards the transport of protein–polysaccharide complexes out of the cell, resulting in an intracellular accumulation and subsequent crystallization of collagen precursors. The enhanced calcification and sclerosis of cartilage matrix following both of these hormones contributes to the premature degeneration of cartilage.

2. *Hormones Causing Underdevelopment*

These include adrenal corticosteroids and prostaglandins.

a. Adrenal Cortical Steroids. Repeated intraarticular injections of corticosteroids cause dose-related "destructive lesions" of articular cartilage with almost a 50% decrease in the rate of incorporation of $^{35}SO_4$ and of [^{3}H]glycine (Chandler and Wright, 1958; Mankin, 1974; Sweetnam *et al.*, 1960; Zachariae, 1965). A similar change has also been demonstrated with the systemic administration of corticosteroids to rabbits for prolonged (9 week) periods of time (Mankin *et al.*, 1972). Histologically, focal chondromalacia and incipient osteoarthritis occur. Electron microscopy shows a marked decrease in the volume of rough endoplasmic reticulum and Golgi apparatus, a decreased number of chondrocytes microvilli, and decreased thickness of territorial matrix (Higachi *et al.*, 1980; Silberberg and Silberberg, 1971).

Hydrocortisone inhibits the degradation of proteoglycan (Comper *et al.*, 1981), and prednisolone decreases the synthesis of catabolin (Dingle, 1981).

b. Prostaglandins. The actions of prostaglandins are complex and knowledge of these actions as applied to cartilage is far from complete.

Prostaglandins can cause a breakdown of ground substance *in vivo* (Teitz and Chrisman, 1975; Fulkerson *et al.*, 1977). This phenomenon can be prevented by the administration of chloroquine, aspirin, or indomethacin, which suggests that prostaglandin synthetase inhibitors may help control the breakdown of arthritic cartilage by limiting *de novo* prostaglandin synthesis. Recent studies have shown that isolated chondrocytes can synthesize substantial amounts of prostaglandins.

Estradiol stimulates the synthesis of PGE_2 and PGI_2 (Rosner *et al.*, 1982). This results in a marked reduction of [^{35}S]sulfate incorporation with a worsening of clinical pathology. The maximal inhibition of prostaglandin synthetase by indomethacin corresponds to the time at which rate of synthesis is likewise maximal (Kent *et al.*, 1980). In organ cultures of embryonic femoral and tibial rudiments, prostaglandin A inhibits growth and produces chondrocyte degeneration (Kirkpatrick, 1980).

Prostaglandin E_1 (PGE_1) can suppress an induced inflammatory response by inhibiting lysosomal enzyme release (Born *et al.*, 1982).

B. Vitamin A

The action of vitamin A (retinol) has already been discussed. Its effect is probably due to an increased synthesis and extracellular release of lysosomal hydrolases (cathepsin D) (Barratt, 1973).

C. Drugs

1. Nonsteroidal Antiinflammatory Drugs

The action of these agents on cartilage degradation is of particular interest because of the potential for preventing or limiting the breakdown of cartilage in various pathological conditions. Salicylate, indomethacin, phenylbutazone, fenoprofen, and colchicine, but not D-penicillamine, have been found to inhibit proteoglycan degradation to varying degrees (Comper *et al.*, 1981). Although the specific mechanisms of action are not known, salicylate limits the autocatalytic degradation of proteoglycan, but it also decreases its synthesis somewhat. The prostaglandin synthetase inhibitors (aspirin, indomethacin, and chloroquine) do not decrease the synthesis of catabolin (Dingle, 1981). Eventually, other antiinflammatory drugs will probably be found to affect one of the recently discovered messengers, such as catabolin.

The effect of anti-inflammatory drugs on proteoglycan synthesis has also been studied. Fenoprofen and ibuprofen have been found to inhibit proteoglycan synthesis in normal canine articular cartilage *in vitro* (Palmoski and Brandt (1980). The degree of suppression is similar to that produced by salicylate in cartilage culture (Palmoski and Brandt, 1979a,b). The decrease in proteoglycan synthesis appears to be a specific effect, rather than a general inhibition of chondrocyte metabolism. In contrast, indomethacin and sulindac sulfoxide do not affect proteoglycan synthesis, whereas sulindac sulfide stimulates synthesis.

2. Alkylating Agents

Intraarticular injection of alkylating agents such as nitrogen mustard and thiotepa or methotrexate in rabbits leads to definite erosion of the cartilage surfaces (Steinberg *et al.*, 1967) with inhibition of RNA and protein synthesis (Mankin, 1974). Thiotepa produces less damaging effects on articular cartilages than does nitrogen mustard.

3. Amphotericin

Cell toxicity due to a single dose of amphotericin is more marked in the superficial zone. Initially, the injured cells produced considerably less proteoglycan compared to normal, which leads to chondromalacia (Edwards and Michael, 1977). The chondrocytes can recover from a limited dose and the cartilage can regain a normal gross and microscopic appearance.

4. Lathyrogenic Substances

ß-Aminoproprionitrile (BAPN) increases the proportion of soluble collagen without altering total collagen synthesis and decreases proteoglycan. In the embryonic chick, BAPN can prevent the formation of cartilage (Hall, 1972). The effect of BAPN is dose related.

5. Diphosphonates

The diphosphonate EHDP decreases proteoglycan synthesis and increases the amount of aggregated proteoglycans in cartilage. In addition it decreases degradation (Fleisch, 1980).

VII. CONCLUDING REMARKS

Within the last two decades, our understanding of cartilage, particularly the biological behavior of the chondrocyte, and the chemical and physical nature of the matrix, has rapidly broadened. The chondrocyte has assumed a central role in cartilage degradation, as the focus has shifted from extrinsic to intrinsic control of catabolic functions.

The demonstration that degenerative changes in cartilage emanate from the chondrocyte and follow a purposeful, rather than an indiscriminate, pattern has in large part been responsible for this evolution. As a result, increasing attention has been given to the communication systems that control cellular behavior, in particular the signals recognized by the chondrocyte. An increasing number of factors or "messengers," which appear to intercede between the cellular systems external to cartilage and the chondrocytes, have been discovered. The major thrust of current research efforts is now toward unraveling further details of these control systems.

Of perhaps equal importance has been the growing body of information documenting a relationship between mechanical forces and chemical changes in cartilage. This subject has in effect led to a rediscovery of the value (and hazards) of exercise. In the forseeable future, this subject is likely to remain in the forefront as insights gained in the laboratory are given practical application.

In spite of the major advances that have been made, many unknown aspects remain, such as the macromolecular configuration of matrix components and the influence of these molecules on chondrocyte behavior. The specific pathological mechanisms underlying arthritic processes must still be elucidated in the detail needed to control resorption and remodeling in a clinical setting. The challenge for the future will not only be to extract relevant details from the burgeoning information available, but also to mold the fragments received into useful concepts capable of spearheading solid rather than illusory gains.

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3

Lubrication of and by Articular Cartilage

C. W. McCutchen

I. INTRODUCTION

In 1932 M. A. MacConaill said that the observed, if yet to be measured, low friction of joints resulted from hydrodynamic lubrication by the synovial fluid.* He believed that the menisci of the knee worked as Kingsbury–Michell tilting pads. The low friction of joints starting from rest remained unexplained.

Years later, in 1953, Ogston and Stanier noted that the thixotropy of synovial fluid at low shear rates should make it a good hydrodynamic lubricant. It gets more viscous as the shear rate falls, which is when greater

*Modern railroad cars have roller-bearing axles. Older cars, now retired, had plain bearings. In a plain-bearing car the chassis is carried on inverted saddles of bronze, called brasses, that ride on top of the smooth, cylindrical ends of the axles. The axle box around each bearing has an oil reservoir under the axle end. Sitting in the oil is a wick that rubs the underside of the turning axle, leaving a layer of oil on it. Viscosity tries to drag the oil under the brass, and pry the latter away from the axle. The car's weight tries to hold the brass against the axle. The result is a compromise. A film of oil is dragged between the surfaces, but gets thinner in the direction of axle rotation as more and more of the oil is stopped and turned back by the gradient of bearing pressure, or squeezed out the ends of the bearing in directions parallel to the axis of the axle. The taper of the film sets the brass and axle slightly off center with respect to each other (Michell,

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viscosity is needed to hold the rubbing surfaces apart. But the fluid never solidifies, however low the shear rate, so it was still unexplained why a period at rest under load did not squeeze it out, leaving the joint to stick when first moved.

Worse, Tanner (1959) noted that although the *form* of synovial fluid's viscosity versus shear rate curve was favorable, its *magnitude* was far too low. The fluid would not hold the rubbing surfaces far enough apart to keep the shear rate in its thixotropic range. They would come so close together and shear the fluid so fast that it would behave, "as a thin Newtonian liquid," that is, one whose viscosity is independent of shear rate. The calculated film thickness was much less than the likely height of the hills on the cartilage surfaces, which would touch each other and carry most of the bearing load.

Tanner (1966) later decided that even this low viscosity might be sufficient if account were taken of the deformation of the bearing surfaces. Deformation spreads the load and makes the film thicker. It was ignored by most lubrication theoreticians until 1949, when a Russian, A. N. Grubin showed that it was the deformation of the surfaces, combined with the pressure-induced rise in the viscosity of the lubricant that permitted a fluid film to exist between loaded gear teeth. The pressure applied to the synovial fluid by the cartilages is not high enough to cause a noticeable rise in viscosity (Cooke *et al.*, 1978), but deformation alone should greatly increase the film thickness.

Return now to 1934. E. S. Jones measured the friction coefficient of a horse stifle joint (corresponding to the knee in man) with a cheap and subtle friction balance. Friction coefficient is the force that makes the rubbing surfaces slide, divided by the force that pushes them together. It was 0.02, whether the lubricant was synovial fluid or physiological saline. In a different apparatus, Jones exercised a stifle joint under load, and found that if allowed to dry out it got hot and quickly wore out.

When hydrodynamic lubrication is complete (when the railroad car's axle turns fast enough for the brass to be entirely supported by oil) the resistance

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1937, pp. 140–142.). So long as the axles turn, the car is supported on films of oil. Friction is very low. This is *hydrodynamic lubrication.*

Whenever the car stops, the oil films are squeezed from between the axles and brasses, which touch, and very soon carry all the load by metal-to-metal contact. Friction is much higher. It takes a substantial pull to start the car rolling again. This pull, often in the form of a convulsive yank from the locomotive, upset passengers and damaged cargo, and was one reason the railroads adopted roller bearings.

Whereas an axle box supports load primarily at right angles to the shaft axis, a thrust bearing supports load parallel to the axis. A flat collar on the shaft may be run against Kingsbury–Michell tilting pads that automatically incline themselves to produce tapered oil films (Michell, 1937, pp. 151–155). [Oddly, however, thrust collars will run satisfactorily at lower loads against a solid flat that gives no apparent taper at all (Barwell, 1956, pp. 195–198).]

rises with rubbing speed (Barwell, 1956, p. 107ff.). In dry friction—a book sliding on a desk top—or, more generally, when there is zero pressure in any liquid or gas bathing the rubbing surfaces, the resistance usually changes little with speed. This is Amontons' law, which holds in most bearings as long as the speed is too low for hydrodynamic lubrication.

Rubbing surfaces are seldom what they seem unless stringent precautions are taken. Oxygen from the air combines chemically with the surfaces, water gets absorbed on them, and so on. Most contaminants lower friction, but leave it independent of speed. Certain ones are especially good at lowering friction—MoS_2, the soaps found in greases, etc. These are called *boundary lubricants.* Typically the soaps give friction coefficients around 0.1 (Bowden and Tabor, 1950, pp. 176–199).

In 1936, Jones set up a pendulum with a human finger joint as pivot, and observed how its oscillations got smaller with time. With boundary lubrication, the loss in amplitude between one swing of the pendulum and the next should be the same for every pair of swings (Edwards, 1959). With hydrodynamic lubrication the loss between swings falls as the amplitude falls so long as the fluid film is complete. Once solid-solid contact commences, the loss rises and the pendulum soon stops. Because his pendulum's behavior was between the expectations for boundary and for hydrodynamic lubrication, Jones, or rather his engineering consultant, C. Jakeman, of the British National Physical Laboratory, thought that the lubrication was hydrodynamic when the rubbing speed was high, shifting to boundary when the speed was low near the ends of the swings.

In 1959, John Charnley used a friction balance that used human knee joints, and found the friction at very low rubbing speed to be no more than 0.02 with either synovial fluid or saline as lubricant. He also found that a pendulum with a human ankle joint as pivot lost the same amplitude between every pair of swings. Friction neither rose with increasing speed, as happens with complete hydrodynamic lubrication, nor with decreasing speed, as happens at low speed when hydrodynamic lubrication starts to fail. Charnley concluded that joints use *boundary lubrication.*

Boundary lubrication must be remarkable, because man's slipperiest material, Teflon (Du Pont's name for polytetrafluoroethylene) has a friction coefficient of 0.04 when rubbed against itself (Bowden and Tabor, 1950, pp. 165–166).

II. WEEPING LUBRICATION

If necessary, engineers can make bearings with very low friction at all speeds. They use *hydrostatic lubrication.* The bearing carries load on a film of fluid supplied continuously (belying the term *static*) at high pressure by an exter-

nal pump. So long as the pump runs, the film will be present, even at zero rubbing speed.

Joints do not work this way. They have neither external pumps, nor pipes to carry lubricant. But consider a deformable material whose surface consists almost entirely of pockets. If pressed against a smooth, flat surface it ought to carry most of its load frictionlessly, by the hydrostatic pressure of the liquid in its pockets. Friction would not be entirely abolished: The pocket walls would rub against the mating surface. But, since the material is deformable, they should not rub very hard. Because the bearing load itself pressurizes the liquid, the mechanism is self-pressurized hydrostatic lubrication.

The cut surface of "Rubazote" rubber sponge was such a pocketed, deformable material (Fig. 1). Its cells were up to 0.7 mm across. A 100-pound weight sitting on three or four pads of Rubazote 16 cm^2 in aggregate area, lubricated by soapy water, could be towed around a flat glass plate with a letter scale (McCutchen, 1959). The friction coefficient was under 0.003 when the load was first applied, and rose slowly as the lubricant leaked away, leaving more and more of the load to be carried by the pocket walls.

Joints cannot work this way either, at least not exactly this way. Two surfaces with large pockets will lose their liquid very rapidly if run against each other. But self-pressurized hydrostatic lubrication is possible without large, open pockets. A sponge with very fine pores will suffice, so long as it is deformable, and the pores, taken together, contain considerable fluid.

When load is first applied, the surfaces will ordinarily be separated by a film of liquid, called a squeeze film. The load pressurizes the liquid in the cartilages to a value about the same as that in the film. Little liquid flow occurs perpendicular to the rubbing surfaces, but parallel to the surfaces there is rapid flow in the squeeze film and much slower flow within the pores of the cartilages. For a short time, the squeeze film entirely separates the surfaces, but quite soon high spots on opposing surfaces touch each other, bridge the film here and there, and start to carry a little of the load.

With impervious materials, this little would soon become a lot, as the squeeze film liquid continued to escape. Friction would rise rapidly. With porous materials, the reduction in film pressure that occurs when the high spots start to carry load sucks fluid from within the cartilages into the film, largely making up for what continues to be squeezed out of the film by the load. Most of the load is still supported by the now incomplete squeeze film. *Weeping* is a standard term for the exudation of fluid, so I called this mechanism *weeping lubrication*. Lewis and I (1959) showed experimentally that cartilage weeps fluid when compressed.

As the cartilages lose fluid, their solid skeletons get more and more com-

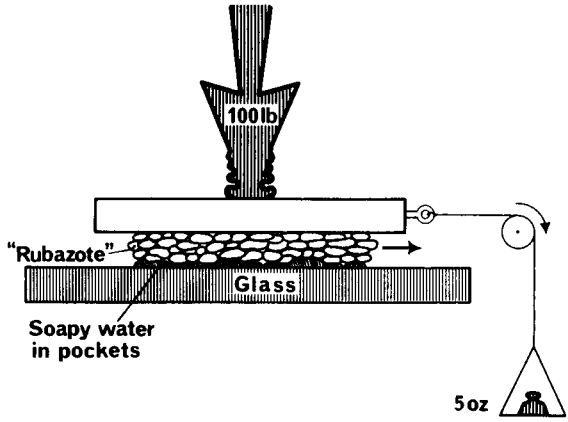

Fig. 1. The cut surface of rubber foam makes a low friction bearing, proving that self-pressurized hydrostatic lubrication is possible. Reproduced from McCutchen (1962b). This first appeared in *New Scientist,* London, the weekly review of science and technology.

pressed, and push harder and harder against each other at the rubbing surfaces, carrying a greater and greater fraction of the load, until eventually they carry it all. The signature of weeping lubrication is thus friction that is very low when load is first applied, and slowly rises to the value for solid rubbing on solid, the same pattern found with the pocketed bearing.

It is inconvenient to measure the friction of cartilage by rubbing it against cartilage. So instead, I rubbed the cartilage-covered ball end of a pig humerus against a glass plate. The load was 5 pounds. It behaved as expected (Fig. 2). The rise in friction was accompanied by a thinning of the cartilage as it lost liquid. The experiment does not prove that cartilage employs weeping lubrication, but provides a strong presumption in its favor.

What other mechanism might be providing the observed lubrication? Not conventional boundary lubrication, which works forever if it works at all. But could it be ordinary squeeze film lubrication? Is it the fluid between the cartilages at the start that causes the lubrication, not that within the cartilages? To settle the question, I waited until friction had risen substantially in the experiment shown in Fig. 2, then lifted the cartilage clear of the glass for a second to form a new film. If a squeeze film caused the low friction at the start of the experiment, a new squeeze film should make the first part of the record repeat itself. The record does not repeat. There is a large drop in friction, but the ensuing rise is much faster than that at the start. To make the initial history repeat, the cartilage must be soaked in liquid for at least 15 min.

Another demonstration that it is liquid from within the cartilage that

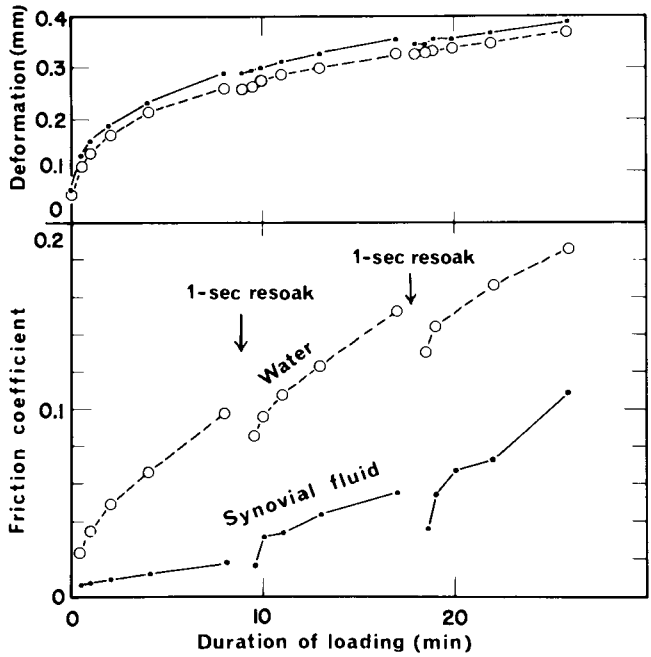

Fig. 2. When the cartilage-covered ball end of a pig humerus is rubbed against smooth glass with a force of 5 pounds, friction is low when the load is first applied and slowly rises. Meanwhile, the cartilage gets thinner, as shown by the deformation record. A short removal of load causes only a short drop in friction. Synovial fluid lubricates better than water. Modified from McCutchen (1962a) with the permission of the publisher.

lubricates, is that lubrication can be sabotaged by stealing the fluid from within the cartilage. Mount a thin slice of cartilage on a porous backstop. The cartilage thins faster, and its friction rises faster because the pore fluid escapes out the back of the sample (McCutchen, 1962a).

One might question the usefulness of any bearing whose friction rises steadily, even if slowly, all the time it is under load. Why do not animals come to a high-friction stop within the first hour of active life? It is because the cartilages in a working joint are able to recharge themselves with fluid. The edges of at least one cartilage cannot help but extend beyond the edges of the opposing cartilage at the extremes of motion. Further, joint surfaces mate with each other so badly that they may not touch everywhere in the region where they oppose each other. Regions not exposed to free fluid at some part of the cycle of motion, if there are any, will be exposed to surfaces that were so exposed, or at least were exposed to those that were exposed, and so on as many times as necessary, to free fluid.

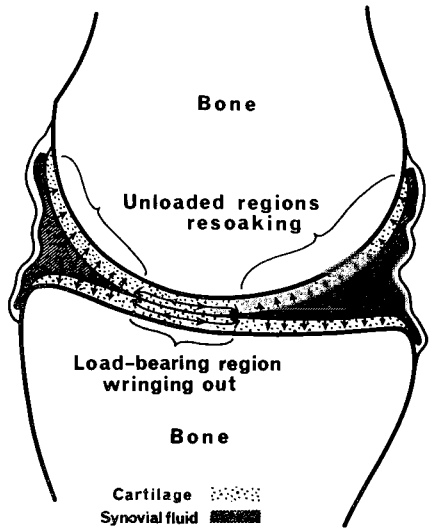

Fig. 3. Fluid has a long and narrow path out of the loaded region of cartilage, and a short, broad return path anywhere there is no load. Modified from McCutchen (1962b). The original version first appeared in *New Scientist,* London, the weekly review of science and technology. In the original the heads of the arrows illustrating the narrow path out of the loaded region did not curl toward the surface, which might suggest that all the escaping fluid stays inside the cartilage. I am indebted to Van Mow for pointing this out to me.

Another apparent objection is that the cartilage is wrung out by the joint load and recharges itself only thanks to its own feeble stiffness that makes it expand. But the fluid path for wringing out, parallel to the joint surfaces, is long and narrow, whereas that for resoaking is short and broad (Fig. 3). This favors resoaking, and allows it to keep up. In an experiment that subjected the pig humerus versus glass bearing to a severe cycle of loading, 1 min of load followed by 1 min with the rubbing surfaces separated, and so on repeatedly, the cartilage got thinner for the first 10 min, but after that the expansion with the load off made up for the thinning while it was on (Fig. 4). The lubricant was synovial fluid.

III. BOUNDARY LUBRICATION BY SYNOVIAL FLUID

As good a bearing as cartilage is when lubricated by distilled water or saline, it is better with synovial fluid—for a while, after which the virtue of synovial fluid progressively vanishes (Fig. 2). Unless the fluid has a clock that tells it how long it has been under load, the loss in lubrication is caused by a change in current conditions. The simplest explanation is that the synovial fluid is a boundary lubricant, but a weak one, and lubricates the solid–solid contact that occurs in a weeping bearing. For some time after load is first applied this contact is gentle, and lubrication successful. Later, when loss of fluid has transferred more of the load to solid–solid contact, lubrication fails.

This interpretation was confirmed by pushing the cartilage against glass with various pressures, and giving it adequate time to wring out completely

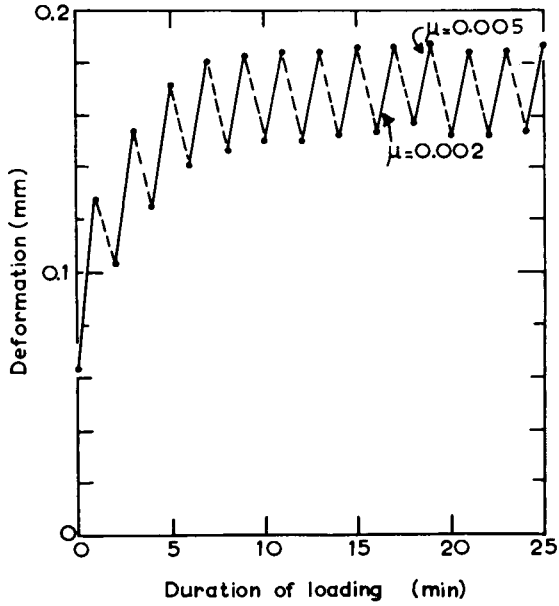

Fig. 4. Equal intervals of loading and unloading leave the cartilage full enough of fluid to be slippery. ——, Loaded intervals; ---, unloaded intervals. Modified from McCutchen (1962a) with the permission of the publisher.

at each pressure (McCutchen, 1967). At 1.5 kg/cm^2 the synovial fluid lubricated indefinitely. At 4.5 kg/cm^2 it hardly lubricated at all (Fig. 5). [The unit kg/cm^2 is more convenient than the pascal, now preferred as the unit of pressure. A practical list of equivalents is, 1 atmosphere $\cong$ 1 kg/cm^2 $\cong$ 10^6 dynes/cm^2 = 10^5 newtons/m^2 = 10^5 pascals.]

In being a weak boundary lubricant, synovial fluid is unlike the boundary lubricants of industry, which remain slippery under enormous loadings.

This weakness is probably why synovial fluid showed little lubricating ability when tested in a plastic bearing by Ropes *et al.* (1947) and in a metal bearing by Tanner and Edwards (1959). At low speeds, such bearings carry load by the contact of high spots that comprise a minute fraction of the apparent bearing area, and squeeze any boundary lubricant very hard. Spreading the load by making one surface of rubber—the other was glass—allows synovial fluid to lubricate (McCutchen, 1966). The bearing pressure was 0.3 kg/cm^2. It lubricates at all speeds down to zero, as expected with a boundary lubricant.

Further evidence that synovial fluid does not lubricate hydrodynamically comes from digesting it with hyaluronidase. It becomes watery, but still lubricates (see Fig. 5; McCutchen, 1967; Linn and Radin, 1968).

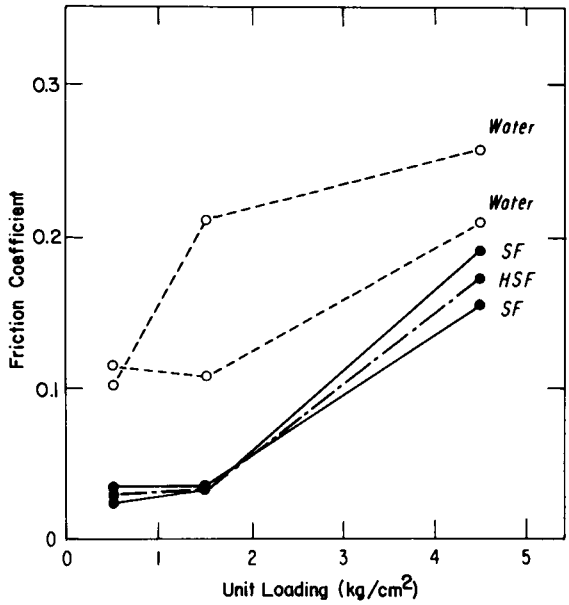

Fig. 5. Synovial fluid lubricates wrung-out cartilage under a pressure of 1.5 kg/cm^2, even after being made watery by hyaluronidase. At 4.5 kg/cm^2, it hardly lubricates at all. SF, Synovial fluid; HSF, synovial fluid digested with hyaluronidase. Modified from McCutchen (1967) with the permission of the Council of the Institution of Mechanical Engineers from *Proc. Inst. Mech. Eng.*

Weeping lubrication and synovial fluid's boundary lubrication complement each other. Weeping lubrication reduces friction tremendously, but permits gentle solid-solid contact. Synovial fluid lubricates this contact, and succeeds only because the contact *is* mild—because weeping lubrication carries most of the load by hydrostatic pressure. Synovial fluid's boundary lubrication could not support the joint loading (10 kg/cm^2 and up) unaided.

IV. HOW DOES SYNOVIAL FLUID LUBRICATE?

No one knows for sure how synovial fluid lubricates, but considerable information has accumulated. When I passed synovial fluid through a 0.22-μm-porosity Millipore filter and tested it for lubricating ability in the rubber versus glass bearing it did not lubricate (McCutchen, 1966). But the residue, redissolved in a volume of saline equal to that of the original charge, lubricated as well as the original fluid. With a 0.45-μm-porosity filter, half the lubricating ability was found in the filtrate, half in the residue, suggesting

that the median molecular diameter was 0.45 μm. With hyaluronidase-treated synovial fluid, a 0.1-μm-porosity filter divided the lubricating ability equally between filtrate and residue. These results suggested that the lubrication was caused by the hyaluronic acid molecules in the synovial fluid. A treatment known to cut them up made the lubricating ability go through smaller holes.

Treating the resuspended residue with proteolytic enzymes destroys its lubricating ability, but leaves it viscous (Wilkins, 1968; Linn and Radin, 1968). Lubricating ability can be destroyed by a treatment that does not chop up the large molecules. (Doing the same experiment on whole synovial fluid has little if any effect on the lubrication. Presumably the enzymes are inhibited by the large amount of protein that is otherwise removed in the filtrate.)

J. F. Wilkins and I (McCutchen and Wilkins, 1969) did a series of experiments in which the resuspended residue was reconstituted in solutions of various salts at various ionic strengths, and tested for lubricating ability in the rubber versus glass machine (Fig. 6). It is odd that KCl, NaBr and $(NH_4)_2SO_4$ all gave lower minimum friction than NaCl, but consoling that with NaCl minimum friction occurs at physiological ionic strength. Friction is high when the residue is reconstituted in distilled water, but low if the rubbing surfaces are washed in distilled water after being soaked in synovial mucin reconstituted in physiological saline. In later experiments (unpublished) we found the friction to be slightly lower after the distilled water wash than before. A saline wash would raise it again, a distilled water wash lower it, and so on through many cycles of alternating washes.

By dissolving the resuspended residue in a strong solution of CsCl and centrifuging it, Radin *et al.* (1970) separated fractions with different densities. Testing the different fractions for lubricating ability in a cow ankle-sized arthrotripsometer (an instrument that measures the friction of an excised joint while putting it through a natural cycle of operation), they found that it was not the hyaluronic acid that lubricated, but instead a protein or *glycoprotein*. Swann and coworkers have since narrowed this down to a glycoprotein of molecular weight 227,500 (Swann, 1978; Swann *et al.*, 1981).

Why, then, did some of the lubricating ability go through a 0.1-μm-porosity filter after, but not before hyaluronidase treatment of the fluid? It might be because the glycoprotein is attached to the hyaluronic acid, and is uncoupled by the high ionic strength of the CsCl solution in the density gradient fractionation (Silpananta *et al.*, 1968). Or perhaps the hyaluronidase used has extraneous activity that attacks the glycoprotein. However, Swann (1978) reports that when small amounts of normal synovial fluid are filtered with a 0.22-μm-porosity filter the lubricating ability goes through the filter; that it is retained only when large amounts are filtered. He suggests that what

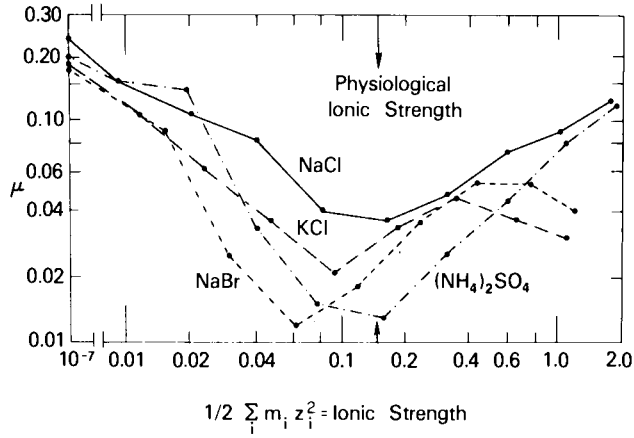

Fig. 6. Synovial mucin lubricates the rubber glass bearing best when reconstituted in dilute $(NH_4)_2SO_3$ at about physiological ionic strength. Reproduced from McCutchen and Wilkins (1969) with the permission of the publisher.

holds back the glycoprotein is not the filter itself, but the mass of hyaluronic acid molecules caught by the filter. When hyaluronidase digestion breaks these into pieces small enough to go through the filter the glycoprotein goes through as well. But Swann (1982) also believes that the glycoprotein molecules are 0.2 μm long. They should not go through the 0.1-μm-porosity filter unless cut up by the hyaluronidase, or unless their protein backbones are more flexible than the picture of the molecule in Swann's paper suggests.

It is not known by what mechanism the glycoprotein lubricates. When it seemed that the hyaluronic acid molecules did the lubricating, I suggested, following Silberberg (1962), that such flexible long-chain polymer molecules could becomes adsorbed to a surface at one or more points on their chains while leaving the rest in solution. This would coat the surface with a fuzz of molecular chains that would resist being squashed down for the same reason a strong solution pushes against the semipermeable membrane that confines it in an osmotic pressure experiment. Because the molecules are in thermal motion there may be no permanent connections between the surfaces. Friction is not inevitable. I called this *osmotic lubrication* (McCutchen, 1966). Unlike the close-packed layers of adsorbed soap molecules of typical industrial boundary lubricants, these openwork layers should get squashed down solid under excessive load, lose their mobility and cease to lubricate.

The glycoprotein molecules now believed to lubricate are chain structures, quite large and with at least some flexibility, spread throughout a large volume of liquid. They might provide osmotic lubrication.

According to the osmotic theory, the adsorbed lubricating molecules hold

the rubbing surfaces apart the same way the stabilizer separates particles of a lyophilic colloid. Roberts (1971) has suggested, instead, that they put an electric charge on the cartilage surfaces which then repel each other like particles of a lyophobic colloid.

Sokoloff (1963) and Maroudas (1967, 1969) have proposed mechanisms that rely on ultrafiltration rather than adsorption. In Sokoloff's mechanism, each time the cartilages expand following a period of load carrying, water and small molecules of synovial fluid enter their pores, leaving the large molecules behind on the surfaces as an ultrafilter residue that provides lubrication when the cartilages are next put under load.

In Maroudas' mechanism the ultrafiltration occurs during the squeeze film period, as the cartilages approach each other and before they touch. As well as flowing in the crack between the surfaces, some of the film fluid will escape by entering the cartilages and flowing through them, after depositing its large molecules on the cartilage surfaces. In McCutchen (1978) I describe possible effects that may make cartilage exude rather than absorb fluid in the squeeze film period. If these turn out to be unimportant, the Maroudas effect ought to occur, though I think Maroudas overestimates the amount of ultrafilter residue. In McCutchen (1978), I, in turn, gave a misleadingly low impression of its magnitude, which has been corrected in McCutchen (1981).

Maroudas proposed her mechanism as the sole explanation of joint lubrication. However, cartilage is very slippery, by ordinary standards, when lubricated by water or physiological saline.

Boosted lubrication (Walker *et al.*, 1968) is like the Maroudas mechanism except that roughness of the cartilage surface is supposed to form trapped pools of synovial fluid, from which water escapes into the cartilages even after they touch. Unless, however, the pockets occupy only a small fraction of the surface area, pockets on opposing surfaces will link together, forming leakage paths rather than traps.

The Sokoloff, Maroudas, and boosted lubrication mechanisms should work with any molecules too large to enter the pores in cartilage, yet of all the materials tested only saliva (McCutchen, 1962a) and polyacrylic acid (J. F. Wilkins, personal communication) seem to lubricate like synovial fluid. All three materials lubricate the rubber versus glass bearing, whose surfaces are impermeable and cannot cause ultrafiltration. Polyacrylic acid lubricates the rubber versus glass system if dissolved in physiological saline, but not in distilled water. But, again like synovial mucin, it still lubricates if the surfaces are washed in distilled water after first having been soaked in polyacrylic acid dissolved in physiological saline.

Adsorption theories of boundary lubrication by synovial fluid, too, suffer from their very plausibility. Adsorption is a common phenomenon. The osmotic mechanism requires only flexible molecules that remain partly in

solution. Roberts' lyophobic mechanism requires only that the adsorbed molecules be highly charged. Each theory predicts that a whole class of molecules should lubricate. What prevents this from happening? What is right about Swann's glycoprotein, polyacrylic acid, and the lubricating constituent in saliva that is wrong about other molecules?

Little *et al.* (1969) note what may or may not be a red herring. Treating cartilage with fat solvents increases its friction, and reduces the amount of lipid revealed in microscopic sections by lipid staining. They believe that the lipid lubricates. In my own experiments (McCutchen, 1962a) I found that the cartilage surface was oily, and that its slipperiness was somewhat reduced by removing the oil. But because joints contain fat pads that are easily damaged on disjointing, I was never sure that the oil was not an accidental contaminant.

V. PATHOLOGICAL SYNOVIAL FLUIDS

Synovial fluids from diseased joints often have subnormal viscosity, suggesting that something thinned the fluids, they ceased to lubricate well, and that was why the joints wore out. Measurements of the lubricating ability of pathological synovial fluids have given contradictory results. Reimann (1976) found that they lubricated poorly, Davis *et al.* (1978) that they lubricated well, Linn and Radin (1968) that they were somewhere between.

VI. LUBRICATION OF SOFT TISSUES

The body is full of lightly loaded bearings. Organs are stuffed in, and allowed to rub against each other. In the joint, the synovial membrane and the fat pads slide over the bone ends and against each other. The thixotropic viscosity of synovial fluid may be sufficient to hold these lightly loaded rubbing surfaces apart. Radin *et al.* (1971) showed that it is the thixotropic part of synovial fluid, the hyaluronic acid, that lubricates the rubbing of synovial membrane against glass. However they believe it serves as a boundary lubricant, because friction did not fall with increasing speed. But friction falls with speed only in the mixed regime, when hydrodynamic lubrication supports only part of the load. Once it supports it all, friction rises with speed. Cooke *et al.* (1976) found it did just that, and it fell with loading, as it should in a hydrodynamic bearing. They concluded that the lubrication of synovial membrane was hydrodynamic.

If the lubrication is solely hydrodynamic, it should fail once the speed is lowered far enough. This experiment does not seem to have been done.

VII. DISPUTES

Weeping lubrication has been disproved again and again. The disproofs are false. Most are based on a misunderstanding of the process of weeping. Typically the disprover explicitly or tacitly defines weeping as an outflow of fluid from a porous, deformable material in response to hydrostatic pressure applied to its surface by a layer of fluid. A correct analysis predicts no outflow, and disproves weeping as thus defined (see Maroudas, 1979). But except for possible effects of the finite extent of the load-carrying region, mentioned above in connection with the Maroudas ultrafiltration mechanism, weeping is not expected to occur until the sponge skeleton of one cartilage touches that of the other.

Remarkably, some analyses have predicted weeping without such contact (Torzilli and Mow, 1976a,b; Higginson and Norman, 1974). These authors did not understand that applying hydrostatic pressure to the surface of a sponge of infinite extent on an impervious backing causes only an equal hydrostatic stress in the sponge pore fluid and in its skeleton. This was pointed out by Harza (1949) in connection with the uplifting effect of pore pressure in dams. In the (good) approximation that the bulk compressibilities of sponge sleleton and pore fluid are small enough to ignore, applying such a hydrostatic stress causes neither strain nor fluid flow in the sponge. To make fluid come out of a sponge, it is necessary to squeeze the sponge, which happens when the cartilages touch each other.

The papers of Torzilli and Mow predicted outflow of fluid from cartilage as the load was in the act of increasing, and a corresponding inflow during reduction of the load. Both flows were expected to be much larger than the inward flow during steady load, which was correctly predicted by Maroudas (1967, 1969). These flows, which they called the mechanical pumping effect, were a consequence of mathematical mistakes, as I have explained in McCutchen (1977). The mistakes were not repeated by Mow *et al.* (1980), in a paper that dealt with experiments in which a porous plate applied load to the surface of a plug of cartilage. The authors found the permeability of cartilage to be several times earlier values (McCutchen, 1962a; Mansour and Mow, 1976; Maroudas, 1979), but this is a consequence of the experiments, not the analysis. The cartilage appears to attain equilibrium faster than expected.

In their closing passage Mow *et al.* (1980) return to the case where load is applied to the surface of cartilage via a full or partial layer of pressurized fluid, and say that "Questions relating to load partitioning and continuity of velocities and displacements at the surface remain unanswered." Harza's paper was 21 pages long, and stimulated 74 pages of mostly unfavorable comment. It was held up 10 years between original submission and publication. Perhaps confusion is to be expected.

Kenyon (1976a,b) gives a theoretical treatment of cartilage and similar

materials that gets this boundary condition right, and has no mistakes that I know of beyond a few sign errors.

Another paper by Kenyon (1980) quotes me as referring to the area over which the rubbing cartilage "skeletons make real contact." The context might suggest that I believed that the size of this area affected the apportioning of stress in cartilage between hydrostatic pressure and skeleton stress. I do not. I routinely define skeleton stress as gross stress minus the pore pressure, which makes it independent of skeleton structure. In the quoted passage I was discussing the relation between friction and the area of real contact, which figured in a dispute that had two positions but one disputant, myself. The contacting high spots on the rubbing surfaces of a weeping bearing are pushed toward each other only by the stiffness of the deformed cartilage skeletons. But they are bathed in liquid at high hydrostatic pressure, which adds an omnidirectional compressive stress to the stress provided by the deformed skeletons. In squeezing the high spot-to-high spot contacts from all directions, does this hydrostatic stress raise their friction? In 1962 I thought it would. The larger was the area of real contact to be clamped together by the hydrostatic pressure, the higher I expected the friction to be. McCutchen (1969) gives the formula.

Later, with the stimulation and technical advice of M. King Hubbert, I applied pressures of up to 100 atmospheres to the water bathing lightly loaded bearings of cartilage and neoprene against glass (McCutchen, 1978). No increase in friction was noted. If this is true, then either the area of real contact in these bearings is far smaller than it ought to be on the basis of their gross deformability, so the local skeleton stress at the high spots is well over 100 atmospheres, or hydrostatic pressure has little effect on their friction.

This barely samples the arguments about joint lubrication. For more complete listings see McCutchen (1978) and Freeman (1979).

VIII. WORK IN PROGRESS

A. Mechanical Properties of Cartilage

Work continues on the mechanical properties of cartilage. Some is less useful than it might be because of the persistent misapprehension that cartilage is viscoelastic. It is primarily poroelastic, which is not the same thing. Parsons and Black (1977, 1979) give *the* retardation spectrum of cartilage, as if the time lag between change in stress and the delayed, creep component of the resulting deformation were a material property, a property of every cubic millimeter of cartilage. In fact, because the lag is primarily the consequence of the viscous flow of fluid through the cartilage, it depends on the material properties, *and on the size of the specimen and on any features of the experiment that determine which way the fluid can flow during the creeping pro-*

cess (McCutchen, 1962a). I have shown (McCutchen, 1982) that the equilibration time is always of the order

$$\frac{(\text{flow-path length})^2}{\text{Young's modulus of cartilage} \times \text{permeability of cartilage}},$$

whatever the relative orientation of the flow and the deformation directions. This relation holds for cartilage or any other poroelastic material. A piece of cartilage that has one or two short dimensions can exhibit a short or long time lag, on compression, depending on whether or not the particular experiment allows fluid to escape by flowing along a short dimension.

In any work where cartilage is compressed or extended this poroelastic time lag should be taken into account. The paper by Woo *et al.* (1979) on the nonlinear behavior of cartilage under tensile straining makes no explicit mention of time dependence, even though the experimenters went to great pains to measure the reduction in sample volume, and thus the fluid loss, that accompanied straining. The strain rate was typically 1% per second and two experiments illustrated took 35 to 50 sec respectively. The cartilage samples were initially 0.025 to 0.0325 cm thick, so the flow path was 0.0125 to 0.01625 cm long. Taking the Young's modulus as 6×10^6 dynes/cm^2 and the permeability as 5×10^{13} cm^4/dyne sec we find that the time lag is between 50 and 84.5 sec. The experiment appears to measure neither the short term properties, when the fluid in the cartilage has had too little time to move significantly, nor the long term properties, when the fluid has flowed until its pressure is negligible everywhere. Instead, it measures properties with the liquid partly equilibrated, properties that would be different if the specimen were thicker or thinner. (The strains in this experiment were so large that neither the Young's modulus nor permeability figure is likely to be accurate, but as the former should go up and the latter down with deformation the formula may still give about the correct equilibration time.)

In a later series of experiments Woo *et al.* (1980) deal specifically with the rate and extent of stress relaxation following very rapid stretching of cartilage. They interpret the time lag as a material property, and nowhere mention the effect of specimen size.

I have stated this objection at length because, not merely is it wrong in itself to confuse poroelasticity with viscoelasticity, it falsifies inferences drawn from creep data. Molecular relaxation is governed by molecular structure, and causes viscoelasticity. From viscoelastic data one might hope to learn about the molecular structure of cartilage. But if creep resulting from liquid flow through the cartilage is lumped with that caused by molecular relaxation, any conclusions supposedly based on the latter alone will be wrong. (To measure viscoelastic lag unadulterated by poroelastic lag one can follow Hayes and Bodine (1978) and subject the cartilage to shear deformation of small amplitude, which should cause negligably small flow of fluid.)

Compressive creep data can be used to predict how fast a joint cartilage will get thinner under load. If the compression tests are done by squeezing small samples of cartilage, the equilibration time will be much smaller than that of a natural joint, because it is proportional to the square of the path length along which the equilibrating liquid flows. By the same rule, the equilibration time for expansion of the cartilage once the load is removed is much shorter than that for thinning under load, because the path length is now only the thickness of the cartilage.

B. Roughness of the Rubbing Surface of Cartilage

Work continues on the roughness of cartilage surface (Sayles *et al.*, 1979; Gardner *et al.*, 1979). The subject exercises a peculiar fascination (see Gardner and McGillivray, 1971, for a historical survey). There is a natural feeling that a bearing that works smoothly ought to be smooth. Smoothness is essential to hydrodynamic lubrication. Cartilage has again and again been declared smooth in spite of conclusive naked-eye evidence that it is not. It has a matte finish. It reflects a point source of light as a splotch, not a point (McCutchen, 1980).

Eventually, when profiles taken of replicas of cartilage showed that its surface was rough, the roughness was enlisted as an essential element of the theory of boosted lubrication (Walker *et al.*, 1968).

Experiments show, however, that even gross changes in the cartilage surface, such as fibrillation caused by arthritis, or removal of the outer layer with a knife leave it quite slippery (Swanson, 1979; McCutchen, 1962a). Presumably the damaged surfaces mold themselves to their mating surfaces under load, and the fissures of fibrillation seal up. Calculations show that the roughnesses of cartilage surface can be many, many times taller than the pore diameter of cartilage skeleton before the resulting leakage along the surfaces has a significant effect on the operation of the bearing (McCutchen, 1975; Kenyon, 1980).

C. Hydrodynamic Lubrication

There continue to be studies on hydrodynamic lubrication (Rybicki *et al.*, 1979; Piotrowski, 1980). The thixotropy of synovial fluid appeals to applied mathematicians and granting agencies, this in spite of many demonstrations that it lubricates about as well after being rendered watery by hyaluronidase as it does in its native gooey state. With or without non-Newtonian synovial fluid, were cartilage smoother than it is, elastohydrodynamic lubrication might suffice during walking or running (McCutchen, 1980). But if joints were slippery only in these conditions, and had high friction every time they stopped, they would be like many other bearings, and attract little interest (except that joint disease would be a major pediatric problem). It is their low friction after long periods of rest under load that makes joints remarkable.

Work continues on the boundary lubricating properties of synovial fluid. Having isolated the lubricating glycoprotein, Swann and his associates are learning more about its chemical structure (Garg *et al.*, 1979). It apparently has side groups identical to those found in one component of saliva, and in the antifreeze glycoprotein that keeps the blood of some species of fish from freezing at a temperature lower than that predicted by a straightforward application of Raoult's law (Feeney, 1974). Other oddities of antifreeze glycoprotein are that it lowers the freezing point of a solution more than it lowers the melting point, and that when the solution freezes the antifreeze gets frozen into the ice rather than being largely excluded, as solutes normally are. However, lubricating glycoprotein has not shown this sort of antifreeze behavior (Swann, 1982). Antifreeze glycoprotein has not been tested for lubricating ability. Perhaps out of chemical exploration of this sort will come the explanation of why lubricating glycoprotein and a few other materials are boundary lubricants, and so many apparently similar materials are not.

IX. SUMMARY

The study of joint lubrication started with procrustean attempts to force joints into one or other category of bearings then familiar to engineers. But their behavior did not match the expectations for either hydrodynamic or boundary lubrication.

It was next proposed that they were members of a new category, weeping bearings, that exploited physical principles not knowingly used by engineers. Weeping lubrication was supplemented by an odd sort of boundary lubrication provided by the synovial fluid.

Since then, other explanations have been proposed, some peculiar to joints, but one, elastohydrodynamic lubrication, reflected advances in the understanding of man-made bearings. In my opinion, weeping plus boundary lubrication remains the correct explanation for the lubrication of joints. A 227,500 molecular weight glycoprotein in synovial fluid is the boundary lubricant.

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4

*Aging and Degenerative Diseases Affecting Cartilage**

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INTRODUCTION

Several semantic matters in this review should be grasped at the outset to avoid the contradictions that plague the literature in this field. *Aging* here is taken to mean the time-dependent deterioration of adult cartilage as distinct from the changes that accompany the development of the skeleton. The boundaries between *aging changes, degeneration,* and *degenerative joint disease* are less well defined. Three types of change are at issue in this regard: *(1)* time-related alterations in cartilaginous tissues that occur independently of structural disintegration; *(2)* mild structural aberrations, such as fibrillation of articular cartilage, that are age-related but not necessarily likely to eventuate in deformation of the joints; and *(3)* overt osteoarthritic deformities associated with clinical disabilities. Byers *et al.* (1976) and others apply the term *aging changes* or *nonprogressive degeneration* to the second category, and *progressive changes* to the third. I prefer to confine the term aging to the first of these categories. An enormous literature deals with the subject. Much of the older information has been reviewed (Sokoloff, 1969) and the interested reader may refer to that for statements not further documented here. The present essay goes on from that point and covers only a small fraction of the available publications. Biochemical aspects of aging and degenerative disease are told in Volume 1, Chapters 7–9, 11, and 12, and in Volume 2, Chapters 8 and 9. The following selections from the burgeoning literature seem particularly useful in the context of what follows: proteoglycans (Bayliss and Ali, 1978; Inerot *et al.*, 1978; Roughley and White, 1980; Vasan, 1980), hyaluronate (Thonar *et al.*, 1978), collagen (Gay *et al.*, 1976; Miller and Lust, 1979; Igarashi and Hayashi, 1980), water content (Amado *et al.*, 1976), metabolic activity (Thompson and Oegema, 1979), and enzymes (Ehrlich *et al.*, 1978; Walton *et al.*, 1981).

II. AGING OF EXTRAARTICULAR CARTILAGES

Age-related degeneration of costal, nasal septum, laryngeal, and tracheobronchial cartilages has been known in many mammalian species for more than a century. The concept of osteoarthritis as degenerative joint disease, formulated originally by Bennett, Bauer, and Waine, was predicated on the view that articular cartilage was subject to senescent changes analogous to those in these extraarticular cartilages. However, there are numerous morphological, chemical, and physical differences between these cartilages (Sokoloff, 1969).

A. Calcification

Focal calcification and endochondral ossification develop in relation to the vascular canals in costal cartilage, and increase with age. Although pres-

ent to a degree in all older people, the extent varies from individual to individual. According to Elkeles (1968), mineralization of costal cartilage is more often seen in individuals who have osteoarthritis than in those with rheumatoid arthritis. Foci of fibrosis and chondromucoid degeneration are also present in costal tissue of aged human subjects. Calcification is found in costal cartilages of laboratory rodents. It occurs in pig laryngeal cartilage by 9 months of age. Ossification is regularly seen in elderly human laryngeal cartilage.

B. Chondrocytes

In an extensive study of human costal cartilage, Dearden *et al.* (1974) found that the earliest age-related changes occurred in the chondrocytes. Accumulation of lipid droplets in the cytoplasm was followed by necrobiosis of the cells. Halos develop in the matrix around the degenerated cells. In these zones, dense bodies of cellular debris persist. The amount of interfibrillar glycosaminoglycan (GAG) that can be extracted with hyaluronidase diminishes. The girth of the collagen fibrils increases. These various changes are more marked in the central than in the subperichondrial portion of the tissue, first appearing during the second decade of life and progressing thereafter.

Histochemical (Sames, 1975) and morphometric (Wobst *et al.*, 1980) studies support the view that alterations of sulfated GAG content in the central portion of costal cartilage are related to deterioration and necrobiosis of chondrocytes, and that the peripheral zone is regenerated from the perichondrium.

According to Moskalewski and co-workers (1980), binucleation is a manifestation of terminal differentiation of rabbit auricular chondrocytes. This process is seen in the first months of life but further progression with age has not been described. I have not found articular chondrocytes of older rabbits to be binucleate. Cox and Peacock (1977) found auricular chondrocytes of rabbits up to 3 years of age to become larger through the accumulation of lipid and microfilaments. The nuclei were obscured by these cytoplasmic components and binucleation was not discerned. As in the case of the costal cartilage, these senescent changes are more marked in the central than in the subperichondrial regions.

C. Amianthoid (Asbestoid) Degeneration

This term refers to a focal conversion of the hyaline matrix of cartilage into a characteristic fibrous structure. Not only are fibrils visible with the light microscope in the matrix, but they are strikingly straight and arranged in parallel sheaves. The fibers are argyrophilic and occur in regions from which the chondrocytes have disappeared. In rodent species, costal car-

tilages undergo extensive fibrous transformation during the first half year of life. The pattern is much more irregular than the amianthoid pattern observed in man. Amianthoid change is seen during the second decade of life in man, but does not increase progressively with age.

Hough *et al.* (1973) demonstrated that amianthoid fibrils are collagenous. Although they have the electronmicroscopic periodicity of collagen, they are coarse, some measuring up to 1 μm in thickness. This thickness is greater than previous estimates of the intermolecular attractive forces of tropocollagen would permit (Bard and Chapman, 1973). The cross bands are in register across the width of the fibrils. The increasing anisotropy of the collagen in amianthoid degeneration has been confirmed by Hukins *et al.* (1976) using high angle X-ray diffraction. These investigators found it remarkable that the aging process is accompanied by an increasing order of the affected molecules. Although "unmasking" of collagen fibrils (i.e., greater visibility of collagenous components) is seen in fibrillated areas of degenerated articular cartilage that have been depleted of stainable ground substance, these areas have an unraveled appearance rather than the asbestos-like configuration of the costal lesion. Ghadially *et al.* (1979) however, observed that individual fibers in microscars of osteoarthritic cartilage have an amianthoid appearance.

The further nature of the asbestoid fibers is not known, neither their molecular species nor their pathogenesis. It is not known whether they are newly synthesized during the aging of the tissue, or whether they represent alteration of previously existing collagen. Against the former possibility is that they are found in acellular areas. Amianthoid fibers are more susceptible to digestion with clostridial collagenase than those of uninvolved cartilage, perhaps because they are not invested by proteoglycan that offers steric hindrance to the enzyme (Hough *et al.*, 1973).

D. Senescent Pigmentation

As human costal cartilages become older, they become increasingly brown. Similar pigmentation is seen in the menisci and *annulus fibrosus.* The color change first becomes distinct during the third decade of life. Pigmentation occurs in tendons but is often not recognized because it is seen only in cross section and not in longitudinal planes. Discoloration of articular cartilage is not as marked and occurs more in the basal than the superficial portions.

Two separate types of pigment are involved. Tsukahara and Nasu (1974) have described a *ceroid* pigment in human tracheobronical cartilages after the fifth decade. According to Thurner and Spinelli (1976) the granular chromolipid is located in matrix as well as perinuclear cytoplasm. The illustration indicates that the extracellular material corresponds to foci of amianthoid

degeneration. This granular pigment should probably be classified as a lipofuscin and is intracellular. Lipofuscin aging pigments occur widely in noncartilaginous tissues as well and are currently believed to represent the peroxidized residues of mitochondrial membranes.

The bulk of the pigment is located in the matrix. This pigment is less well known than the lipofuscins and has only recently become the subject of chemical study. It has no generally accepted name. Van der Korst and coworkers (1977) propose that it be called ABC (aging brown cartilage) pigment. They have released it from the tissue by proteolytic digestion. Unlike lipofuscins, it is water soluble. Chemical analysis indicates that it is a glycopeptide. It is in this context that recent demonstration of nonenzymic condensation of reducing sugars with free amino groups of proteins is relevant (Monnier and Cerami, 1981). This mechanism and certain free radical reactions may lead to cross-linking of long-lived proteins such as the collagen of cartilage. Be that as it may, no correlation has been found between senescent pigmentation of rib cartilages and degenerative changes of knee joint cartilage.

Copius Peereboom (1973) suggested that the pigment of the aging *annulus fibrosus* is a masked lipid located in islands close to giant chondrones. In retrospect, the photomicrographs published suggest that some of these lesions were foci of chondrocalcinosis in which lipids are sometimes components. The histochemical techniques employed in this study required treatment with ethylenediaminetetraacetic acid (EDTA), and so might have obscured their calcific nature.

III. AGING OF JOINT CARTILAGE

A. Remodeling

Remodeling is the alteration of the internal and external architecture of the skeleton dictated by Wolff's law in response to variation in mechanical loading. It involves removal of bony tissue at certain points while it is being laid down elsewhere. The concept has been expanded to changes in shape of joints with age and osteoarthritis by L. C. Johnson and others (Bullough, 1981). Indeed analogous phenomena occur in soft connective tissues although they have barely received consideration. The mechanisms involved have been the subject of much thought in bone. Only recently has the question even been formulated in the case of the remodeling of cartilage (Frost, 1979; see also Chapter 2 in this volume). Changes in the shape of joints according to loading have been shown by Thompson and Bassett (1970). They produced abnormal pressures on the articular surfaces of the knees in rabbits by excising one of the femoral condyles. In the compartment having the

reduced pressure, the calcified layer of the tibial cartilage was resorbed by invading blood vessels. On the side subjected to increased pressure, the articular cartilage proper showed loss of metachromasy and necrobiosis of its chondrocytes.

Much of the older literature on age-related remodeling of joints has been based on radiological appearances or macerated anatomical specimens. This material necessarily can not take into account the cartilaginous component. Riede *et al.* (1973) studied the shape of the human ankle joint as a function of age. Increasing flattening of the frontal profile of the bony talus was observed throughout life. The flattening was not associated with osteoarthritic degeneration. Indeed the degree of flattening was inversely related to the degree of osteoarthritis in these joints.

B. Osteochondral Junction

A sizeable body of information indicates that the interface between cartilage and bone undergoes a turnover throughout adult life, even in the absence of fibrillation or overt osteoarthritis (Green *et al.*, 1970; Bullough, 1981). Numerous small segments of both the calcified layer and the subjacent bony plate are interrupted by osteoclastic fibrovascular tissue and new bone of varying age. The appearance suggests that new bone has formed in response to the resorptive event, and maintains the osteochondral junction in steady state. What initiates the resorption is less clear. Could it be a microfracture of the calcified layer? Minute crevices, running at right angles to the joint surface, are frequently seen in microradiographs of the calcified cartilage, particularly of elderly individuals. One's first intuition is that they are technical artifacts. Why the calcified cartilage should be susceptible to this fairly consistent pattern of artifact—if it is an artifact—is itself an interesting question. Dhem (1971), who raised this question, was not confident that he could persuade anyone that the crevices were real, but he had little doubt about linear streaks of increased radiodensity of the calcified layer of these individuals. Age-related changes in the vascularity of the osteochondral junction have been described in the adult human femoral head (Woods *et al.*, 1970). Comparable vascular gaps are not found in the mature rabbit (Greenwald and Haynes, 1969).

Reduplication of the tidemark is another expression of remodeling of the basal portion of the articular cartilage. The tidemark is the basophilic line seen in histological preparations at the junction of the calcified and noncalcified portions of this tissue. It represents the advancing front of mineralization of the cartilage. Reduplication thus indicates recent encroachment of mineralization on the cartilage and presages endochondral ossification. The process does not lead to appreciable thinning of the cartilage with aging unless osteoarthritic disintegration of the latter supervenes. Thus Meachim

(1971) found that the average thickness of humeral head cartilage was 1.48 mm in subjects 25–53 years old and 1.43 mm between 59 and 75 years. The chondrocytes encased within the calcified matrix undergo necrobiosis. Lane and Bullough (1980) observed a linear decline in the thickness of the calcified layer throughout adult life, whereas the number of tidemarks increased exponentially.

C. Surface Smoothness

The smoothness of the normal articular cartilage must be one factor in the low friction of animal joints. Measurement of the degree of smoothness is fraught with technical difficulties. The literature is replete with artifacts (Ghadially, 1978) and a new method of fixation to minimize these problems in electron microscopy has been proposed (Bloebaum and Wilson, 1980). Longmore and Gardner (1978) have employed reflected light interference microscopy to resolve some of the inconsistencies as they relate to aging. Irregularities attributable to the number and diameter of superficial chondrocyte lacunae change relatively little in the years following completion of skeletal growth. The depth of the lacunar hollows increases. A different set of irregularities apparently arises in the matrix and is the consequence of exposure of superficial fibrillary bundles. These changes progress in adult life and do so in the absence of overt fibrillation. According to Stolpmann and Kraaz (1974) the extent of the cell loss and roughening with age varies in different portions of the femoral head.

D. Chondrocytes

There has been a long-standing interest in "effete" chondrocytes as a manifestation of aging of articular cartilage. The term refers to the empty appearance of lacunae in the tangential layer of this tissue. Some investigators (Vignon *et al.*, 1976) have found a progressive reduction of cellular density of the adult femoral head with increasing age. Stockwell (1979) concluded, however, that although there is no doubt that a massive reduction of cell density occurs during growth and maturation, it is less certain that this trend continues after growth has ceased. In his judgment, the diminished proportion of cells is confined to the superficial portions of articular cartilage in areas subject to fibrillation. Leutert (1980) believes that the number of chondrocytes in the adult does not decline. The cells are larger in the adult than in growing individuals but in later years undergo a degree of atrophy. The situation thus differs from that described above in several extraarticular cartilages. Fine-structure studies (Weiss, 1968) indicate that individual cells undergo degenerative changes and ultimately disappear. Their original position is betrayed by microscars indicated by disruption and ir-

regular arrangement of collagen fibrils as well as deposition of amorphous material and dense bodies.

Less is known about the functional properties of articular chondrocytes in aging. Ashton and Matheson (1979) found that the chondrocytes of aging human articular cartilage are stimulated to a lesser degree to incorporate radiosulfate by a standard somatomedin C-containing plasma than are younger cells. Chondrocytes of three-year-old rabbits and humans over 60 divide actively in monolayer culture (Krystal *et al.*, in press). The older lapine cells, as well as those of three-month-old rabbits, are stimulated by 0.2 m*M* ascorbate to increase DNA synthesis under these conditions. In organ culture, however, [^{3}H]thymidine labeling of chondrocytes *in situ* following preliminary exposure to proteases as well as ascorbate, increases only in the young and not in the old cartilage. The disparity of the data obtained under the two regimens suggests that it is not the ability of the chondrocytes per se that is compromised in the aging but their regulation by changes in the surrounding matrix.

In view of the poverty of cellular turnover in intact articular cartilage, the ability of chondrocytes to repair damage to DNA throughout the course of life is a significant question. Chondrocytes grown in monolayer culture display unscheduled DNA synthesis (UDS) following ultraviolet light irradiation and rapid strand break repair after X irradiation (Krystal *et al.*, in press; Setlow *et al.*, in press). There is no indication of reduction in UDS by articular chondrocytes of older rabbits and humans, or in the repair of X-ray damage.

Several laboratories have observed that antibiotics of oxalinic and pipemidic acid composition result in a curious pattern of cystic lysis of the matrix in the central zone of the articular cartilage of young dogs (Ingham *et al.*, 1977; Gough *et al.*, 1979). The mechanism for this is unknown but the changes do not develop in the joints of adult animals.

E. Amyloidosis

Amyloid-like deposits have been found in sternoclavicular cartilage of aging human subjects with great frequency. They are present in all individuals by the age of 50 and increase in extent thereafter. The material is stained by Congo red but has several atypical histochemical and electronmicroscopic features (Uchino *et al.*, 1980; Gaffin *et al.*, 1981).

F. Crazemarks

Lothe and co-workers (1973) have described a peculiar pattern of matrix streaks in the cartilage of aging human femoral heads. Jagged discontinuities in the anisotropic patterns of the tissue are seen with plane polarized light. They are also visible in paraffin-embedded sections of costal cartilage.

Although they undoubtedly are some sort of mechanical artifact, one must surmise that they reflect a presently unidentified material property of the matrix.

IV. DEGENERATION OF ARTICULAR CARTILAGE

Virtually all accounts of the pathology of degenerative joint disease state that the lesions begin with fibrillation of articular cartilage. Although it is difficult to support this conclusion rigorously, fibrillation is the earliest gross change seen on the surface of the opened joint, either in aging or in osteoarthritis.

A. Fibrillation

This term refers to a focal fine roughening of the surface of articular cartilage. It necessarily involves a local dehiscence of the collagen of the tangential layer. Whether the fibrils are individually ruptured or whether some extrafibrillar cement becomes discontinuous is not known since there is presently no way to determine the length of a collagen fibril. The ground substance in the vicinity of the fibrillation is reduced. The residual collagen in the area becomes visible as a consequence (is "unmasked") and has an unraveled appearance in sections. Many investigators have found the use of india ink to impregnate the crevices in the cartilage surface (Meachim, 1972) helpful in quantitating the extent of fibrillation.

B. Osteophytes

These structures (sometimes also called spurs or lips) are recognized in X-ray films or dry bones as outgrowths of bone from the margins of joints. In reality they are composed of heterogeneous tissues and are always capped by several types of admixed cartilage and fibrous tissue. They arise histologically at the junction of articular cartilage, bone, and capsular tissue. Vascular invasion of the calcified layer of the cartilage from the bone marrow is an early feature. Endochondral ossification of the basal cartilage in the region is often seen without a recognizable counterpart on the surface of the cartilage in that region. This is one of the aspects of remodeling of joints that makes it difficult to document the statement that degeneration of articular cartilage has its inception in fibrillation of the surface.

The heterogeneous histological events are entirely analogous to those seen in callus formation and may be regarded as a nonspecific proliferative remodeling in response to local injury. Comparable changes of mild degree are also seen so commonly at the nonarticular junction of tendons and ligaments with bony structures (e.g., the iliac crest) that they are employed as forensic measures of skeletal age.

Osteophytic cartilage has type II collagen in some areas. Elsewhere the clearly fibrous histological character of the cartilage strongly indicates that it is type I. It seems entirely likely that the finding of type I collagen synthesis in osteoarthritic cartilage by Nimni and Deshmukh (1973) resulted primarily from the sampling of this osteophytic fibrous cartilage rather than from a generalized abnormality of collagen matabolism of the cartilage.

V. DEGENERATIVE JOINT DISEASE

Degenerative joint disease and osteoarthritis (osteoarthrosis, arthrosis) may be regarded as synonymous terms. It is a noninflammatory remodeling of movable joints characterized by two discrete pathological processes: (*1*) deterioration and loss of the bearing surface and (*2*) proliferation of new osteoarticular tissue at the margins and beneath the detached joint surface. These events are similar to those described for degeneration of cartilage but are more severe and complex. Probably the cutoff point between the two processes is the breaching of the osteoarticular junction in osteoarthritis. In these areas, disintegration of the cartilage commonly has progressed to the point that the cartilage has eroded down to bone. In addition, the subchondral bony plate has undergone microfracture. This process is associated with new bone, and often, new cartilage formation in the adjacent bone marrow.

A. Chondrocytes

The cellular changes in the affected cartilage are highly variable and always focal. Some are degenerative and others, proliferative. Analyses based simply on the average cell count or DNA content are likely to miss the point. At one extreme, segmental necrosis of chondrocytes or marked atrophy is conspicuous. This is most clearly seen in juvenile disease (e.g., congenital dysplasia of the hip) in which one properly speculates that high local compressive stress prevents adequate percolation of nutrient fluid to the cells. This situation has been reproduced experimentally by several investigators through prolonged compression of the joint surfaces. The matrix immediately surrounding the chondrocytes appears red in hematoxylin- and eosin-stained sections. The GAGs and perhaps the proteins of the matrix are digested by autolytic enzymes of the chondrocytes. In electronmicrographs, Weiss (1973) found that virtually all the chondrocytes in advanced osteoarthritis ultimately show degenerative changes, although there are evidences of increased antecedent biosynthetic and even replicative activity.

At the other extreme are the so-called brood capsules: clusters of chondrocytes located at the edges of the fibrillated clefts in the matrix. That they represent proliferated clones is shown by their uptake of radioactive

thymidine (Hirotani and Ito, 1975; Havdrup and Telhag, 1980). Autoradiography with radiosulfate demonstrates that these clones also synthesize GAGs. They must therefore be regarded as an attempt at repair by the tissue. Dustmann *et al.* (1974) regard the cell clusters as doomed to fail and die. Little or no collagen is seen within them and it must be presumed that chondrolytic enzymes, including collagenases, have been generated to make room for the new cells. Several types of focal mucoid change are also seen in the matrix. One variety has sharp edges and incorporates stellate cells. Weichselbaum almost a century ago made what now seems a prescient suggestion: the chondrocytes digest the pericellular matrix and themselves become myxoid (See Chapter 1 of this volume for a discussion of neoplasia and metaplasia).

Mitrovic *et al.* (1981) and others have described *activation* of articular cartilage in osteoarthritis, that is a generalized increase of biosynthetic activity in the same joint even at a distance from overt damage to the tissue. These authors recognize that a large part of the cartilage in advanced osteoarthritis, by the time it becomes available at surgery, is of *de novo* immature type. The issue is complicated by the fact that their (and others') normal baseline values are derived from cartilages obtained from surgically resected fractured hips. Although the latter may not show major changes in the first few days after the fracture, they are hardly normal. The joints are filled with blood. Within 2 weeks of fracture overt histological changes are readily seen: necrosis of the superficial chondrocytes and fibrous transformation of the matrix (Hirotani and Ito, 1976). The theme is timely because the idea that a generalized activation of the chondrocytes occurs early in osteoarthritis has gained some currency from experimental studies in dogs (McDevitt and Muir, 1976). It is in this context that a recent contrary report by Santer *et al.* (1981) should be evaluated. They found that in early fibrillation of tibial plateau cartilage, the character of the proteoglycans was not different from the apparently normal areas of the same joint. This controversy is important not only because it leaves unresolved numerous differences in published data on the composition of aging and degenerated cartilage but also because it reflects basic differences in concepts of the pathogenesis of osteoarthritis. In studies designed to clarify the issue, both sorts of control—normal-appearing cartilage from subjects of varying age, as well as normal versus fibrillated cartilage of the same joint—are required. Species differences and topographic variation in the proliferative versus degenerative remodeling should also be entertained as additional sources of the discordant data.

B. Subchondral Bone

The arrangement, thickness, and physical properties of the subchondral bony plate bear an important relationship to the overlying articular cartilage (Pugh *et al.*, 1974; Townsend *et al.*, 1976). This raises a question about the

relationship of osteoarthritis and another extremely prevalent senescent disorder of the skeleton—osteoporosis. The answer is virtually unanimous: osteoporosis does not favor, indeed it militates against, the development of degenerative joint disease (Dequeker *et al.*, 1975; Carlsson *et al.*, 1979).

The bony changes in osteoarthritis vary widely from site to site within the affected joint. Radin and co-workers (1973) have proposed that the initial pathological event in osteoarthritis is microfracture of paraarticular trabeculae.

In advanced osteoarthritis, overt microfractures and osteoclastic resorption of the subchondral plate are seen side-by-side with osteoblastic foci and sclerotic new bone (Lereim and Goldie, 1975; Reimann *et al.*, 1977). Gaps in the subchondral plate are often apertures to pseudocystic structures in the adjacent bone marrow. These abnormalities are more common in the hip than in other osteoarthritic joints and probably arise from transmission of pressure from the joint into the medullary soft tissue. Sclerosis of bone beneath the polished surface gives rise to its eburnated (ivory-like) appearance. Irregularity of the cement lines indicates that the sclerotic process occurs in bursts, some of which, at least, have arisen through repair of microfractures. The lacunae in the superficial layers of the eburnated bone commonly lack nuclei. It seems likely that heating during rubbing of bone against bone generates heat and kills the osteocytes. Larger or smaller islands of cartilaginous proliferation interdigitate with new bone formation, in the subchondral marrow. These changes represent aborted attempts at repair of infractions of the joint surfaces. Osteoarthritis in this sense is not an inherent biological inability of the joint surface to repair itself so much as a failure of the repair to be carried out successfully (Macys *et al.*, 1980).

The osteophytes in osteoarthritis are larger than in nonprogressive degeneration (Hernborg and Nilsson, 1973). It would be an error to conclude that they represent late changes in the evolution of the lesions. In osteoarthritis produced experimentally in dog knee joints by incising the anterior cruciate ligament, Gilbertson (1975) found that remodeling of the bone occurs by the end of the first week—no later than changes in the composition of the cartilage.

C. Osteoarthritic Remodeling

The sequence in the remodeling of osteoarthritis can be reconstructed with only a limited confidence. Three general concepts have their own constituencies but seem to be overly simplistic. They are illustrated in Fig. 1.

1. Osteoarthritis is a degeneration of articular cartilage that progressively leads to denudation of the joint surface. If this were valid, little or no remodeling of bone should occur. Only rarely is extensive eburnation seen in

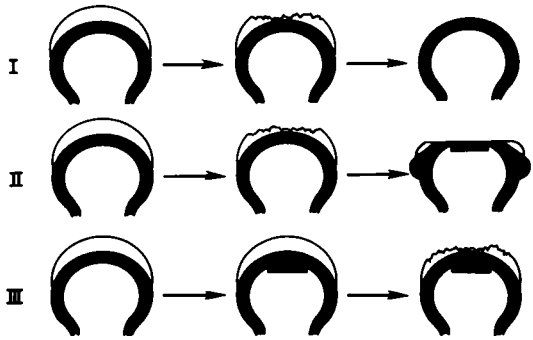

Fig. 1. Divergent concepts of the evolution of osteoarthritis. Bone is represented in black, and articular cartilage as the white cap upon it. Scheme I envisions the process of osteoarthritis simply as a progressive denudation of articular cartilage. In scheme II, fibrillation of the cartilage is viewed as the first step. It leads ultimately to loss of bone at the articular surface, as well as buildup of new bone beneath and at the margins of the joint surface. In scheme III, the increase of subchondral bone precedes fibrillation of the cartilage.

surgically resected femoral heads that retain their sphericity. Solomon (1976) has attributed such instances to antecedent inflammatory lysis as distinct from mechanical overloading of the cartilage. A striking example of this is presented in Fig. 2. By and large the degree of deformity in surgically resected femoral heads is greater in degenerative than in rheumatoid arthritis (Ilardi and Sokoloff, 1981). Lagier (1980) has made analogous observations on the sparing of the contour of the hip joint in ochronotic arthropathy, where the destruction of the cartilage is related to inherent metabolic deterioration of the cartilage rather than mechanical remodeling.

2. *Osteoarthritis begins as fibrillation of articular cartilage that leads to secondary remodeling of the bony components of the joint.* This is a common view. A principal difficulty with the concept is that it is difficult to isolate any individual finding as a unique morphological event that precedes others in the complicated changes seen in the histological section. It does not take into account, for example, the remodeling of the osteochondral junction as an early age-related change in the cartilage. The changes ordinarily coexist.

3. *Osteoarthritis is the consequence of changes in the stiffness of subchondral bone.* The concept proposed by Radin *et al.* (1973) that microfractures precede cartilage damage has already been mentioned. According to this view, it is the bone that normally absorbs most of the energy of impact stress on the extremities. The repair of the fractures leads to a net local increase in stiffness of the bone that in turn causes the overlying cartilage to

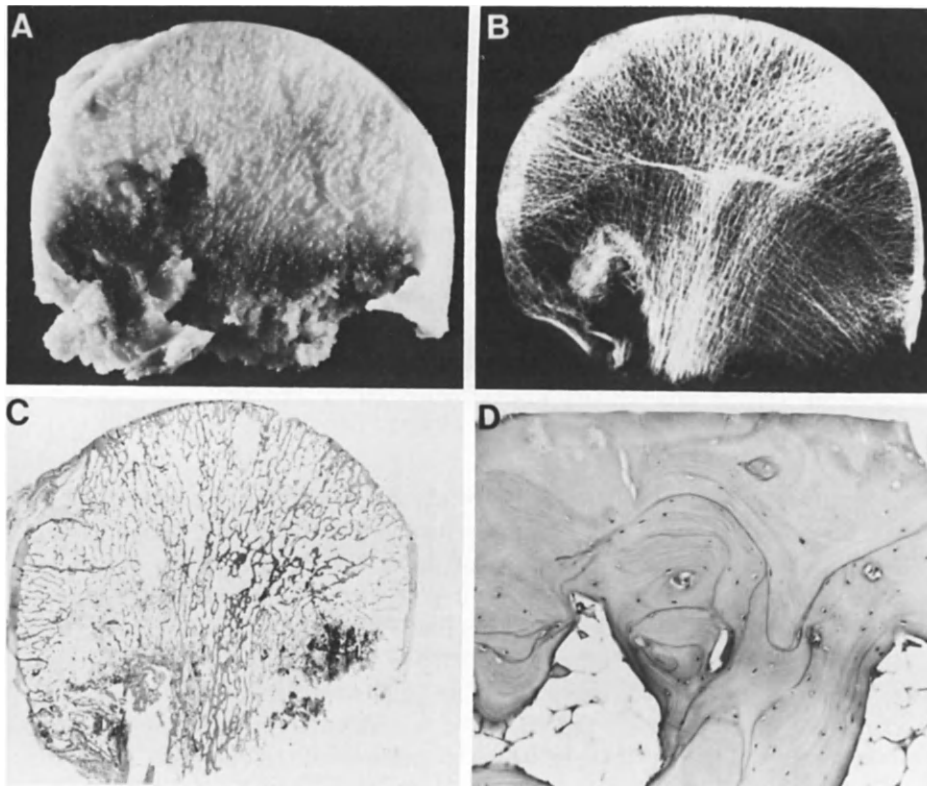

Fig. 2. Nondeforming (concentric) osteoarthritis of the hip. The patient was a 77-year-old man who fell from his bicycle and sustained an intracapsular fracture. There was a poorly documented history of past rheumatoid arthritis. (A) Coronal slab section of femoral head. The articular cartilage is barely visible over the superior surface. The jagged appearance and dark discoloration of the bottom of the specimen reflect the recent fracture. (B) Roentgenogram confirms the paucity of external or internal remodeling of the bone. (C) Photomicrograph discloses the extent of the loss of cartilage. (D) The eburnated surface is devoid of cartilage. Chondrocyte lacunae in the residual calcified layer and osteocyte lacunae are empty in evidence of necrobiosis. Apposition of several seams of new bone is seen beneath the surface. Magnification 180× (stained with hematoxylin and eosin.)

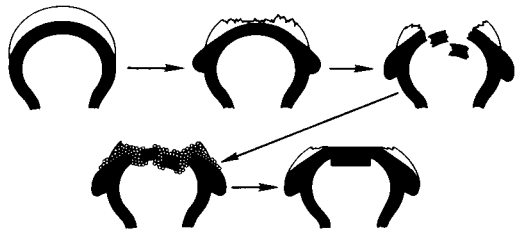

Fig. 3. Preferred view of osteoarthritic remodeling. Here fibrillation cannot be dissociated from remodeling of mineralized tissues even in the earliest stages. The distinctive event is disintegration of the osteoarticular surface, represented as depressed microfractures. It is in the reactive proliferation of new cartilage (the bubbly material) and bone that the productive components of the deformity come about.

absorb excess energy. The latter now, it is argued, leads to the degeneration of the cartilage. This hypothesis has a distinct engineering logic as well as some experimental support but suffers the same limitations as the preceding hypothesis.

The recognition of complexity of the pathological sequences is important because several pharmacologic strategies of treatment of osteoarthritis are based, whether recognized or not, on one or another of these assumptions. The several degenerative and remodeling changes are so intimately associated that it seems unrealistic to attempt to identify a unique initial event in the osteoarthritic process. It is the structural disintegration of the osteoarticular junction and abrasion that lead to the loss of substance of the articular surface. They are also responsible for the proliferative phenomena including the formation of new cartilage at the surface of the osteoarthritic joint (Fig. 3). The generation of new cartilage in defects in the joint surface that penetrate into the subjacent bone marrow has been experimentally documented many times (Cheung *et al.*, 1980).

D. Primary versus Secondary Osteoarthritis

In a certain proportion of cases osteoarthritis clearly develops in the wake of an antecedent structural abnormality or injury of a joint. To these cases the term *secondary* is applied. It is presumed that abnormal mechanical loading is responsible for the deterioration of the joint surface in most of these instances. In other cases, no antecedent abnormality is identified. These are classified as primary or idiopathic types of osteoarthritis. In practice it is often difficult to distinguish between the two. Stulberg *et al.* (1975) are confident that they can identify a structural basis for at least 85% of cases of osteoarthritis of the hip. They further believe that most cases of

osteoarthritis in other joints are also of secondary type. Other investigators place the figure at a much lower level and recognize primary osteoarthritis as a valid and frequent entity (Solomon, 1976; Marks *et al.*, 1979; Cooke, 1980).

E. Time Curve of Degenerative Joint Disease

One way in which it might be possible to assess the relationship between age-related changes and degenerative joint disease would be to plot a time curve that would display their relationships as a function of age. Would there be a linear progression of degenerative changes into osteoarthritis? Would the curve be autocatalytic and follow the Gompertz equation observed in certain other aspects of biological aging? Important methodological difficulties in answering these questions arise both from the diagnostic criteria employed and from statistical sampling. The current success of reconstructive joint surgery introduces its own bias into the population risk.

The scheme for a time curve presented in Fig. 4 is both semiquantitative and fanciful. It confirms older impressions that degenerative changes progress linearly with age in a general autopsy population. The extent of the lesions is greater in the patella than in the hip joint. Disintegration of the cartilage in the patella becomes associated with the erosion down to bone and marginal osteophytosis in the older age group. It is consistent with older views that there occurs a transition from age-related degeneration in the knee into osteoarthritis. Comparable progression into osteoarthritis is not seen in the hip. The lesions found in femoral heads resected for osteoarthritis are far more advanced than can possibly be accounted for simply by the scatter about the mean degeneration score in this structure. One must conclude that local factors other than aging underlie the development of this form of degenerative joint disease. Similar conclusions were drawn from an analysis of the temporomandibular, sternoclavicular, and first metatarsophalangeal joint by Kopp *et al.* (1976).

There is a large statistical scatter in these data. Meachim's values in male subjects examined in Liverpool do not fall so nicely into this pattern. I, too, have been impressed with a wide variation in the severity of patellar degeneration in the New York area. It is thus of interest that Casscells (1978) has concluded that "In patients in the U.S.A., articular cartilage of the knee resists the wear and tear of a normal lifespan remarkably well and infrequently undergoes progressive degradation." Is it possible that joint surfaces of hospitalized patients undergo a degree of repair and so influence the postmortem data?

There is thus a convergence of several sorts of evidence that a mild age-dependent degeneration of joints occurs widespread in the body, and that it progresses to cause complaints in only a relatively small proportion of in-

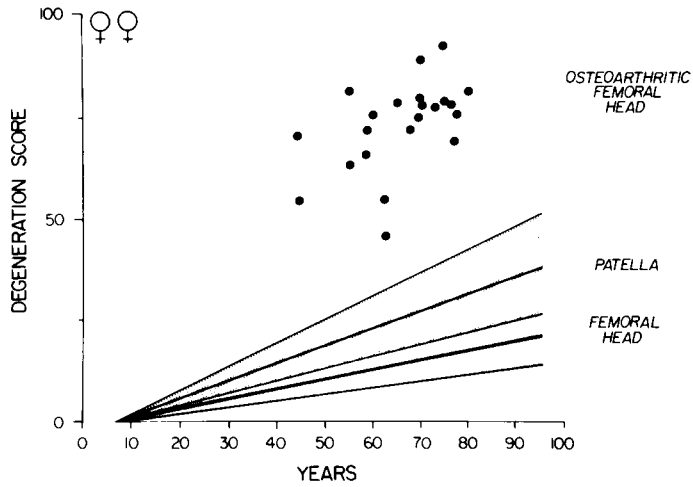

Fig. 4. Time curve of degeneration and osteoarthritis. The values for the patella and femoral head were derived planimetrically from maps of articular surfaces in a necropsy population prepared by Meachim and Emery (1973, 1974). I am indebted to Dr. Meachim for use of the maps. The *degeneration score* in these specimens was computed arbitrarily as follows: one-third of the score was assigned to the percentage of the surface that was abnormal in any way, one-third to the percent that had eroded down to bone, and the remaining third to the (area of osteophyte/area of surface) × 100 × 2. The scores for the surgically resected femoral heads were determined from X-ray films of a central slab section of the specimens. They were based on the percentage of the surface, rather than the area. The values are thus not directly comparable to the degeneration scores and may underestimate the severity.

dividuals (Byers *et al.*, 1976). In instances that go on to crippling deformity a factor other than aging must be inferred. It follows that osteoarthritis should not be regarded so much as a single disease but as a final common pathway of multiple mechanical and biological factors that interact with each other to produce the lesions.

F. Generalized Osteoarthritis

Although minor degenerative anatomical changes occur in many joints at necropsy, controversy continues about the occurrence of severe, symptom-producing polyarticular osteoarthritis.

1. Generalized Primary Osteoarthritis

The concept of generalized primary osteoarthritis was espoused for many years by Kellgren and associates. The association of Heberden nodes (osteoarthritis of the distal interphangeal joints of the fingers) with osteoarthritis

of the hips has been affirmed by some (Dequeker *et al.*, 1975; Marks *et al.*, 1979) but denied by others (Yazici *et al.*, 1975). Discrimination of a subset of osteoarthritis may resolve some of these contradictions. Herberden nodes, particularly those associated with inflammatory manifestations, frequently coexist with the concentric or nondeforming type of osteoarthritis of the hip discussed above. It is this form of hip disease that has been referred to by Solomon (1976) as postinflammatory and as part of primary generalized osteoarthritis by Marks *et al.* (1979) and by Cooke (1980).

2. *Inflammatory Osteoarthritis*

The source of the inflammation in the latter category of generalized osteoarthritis is not known. Cooke (1980) has emphasized the presence of immune complex deposits in the tangential layer of the articular cartilage in them, unlike the secondlary types of osteoarthritis. Doyle (1982) is more interested in calcific crystals as the source of the inflammation. He finds hydroxyapatite and Ca pyrophosphate crystals in the synovium. Calcium pyrophosphate crystals occur more often than hydroxyapatite in the fluid in osteoarthritis (McGuire, 1980). It seems reasonable that the basilar calcification that is an integral part of the remodeling of the cartilage in osteoarthritis should be the source of both types of crystal. The latter may evoke a secondary inflammatory response (Schumacher *et al.*, 1977).

In surgically resected osteoarthritic joints, focal chronic synovitis is the rule rather than the exception. Traditionally this has been considered a foreign body reaction to joint detritus. An autoimmune component may be further generated by inflammatory enzymic modification of the cartilaginous debris. Lymphokines are occasionally found in the synovial fluid and constitute evidence for such a mechanism. Ito and Bullough (1979) find it helpful to distinguish between inflammatory and noninflammatory forms of osteoarthritis on histological grounds. The idea has yet to gain general acceptance.

3. *Progeria*

Several disorders simulate accelerated aging to a varying degree. Progeria of very young children is a caricature of aging but its manifestations commonly include arteriosclerosis, lipofuscin accumulation, osteoporosis, and baldness. The literature contains relatively little systematic information about degenerative joint disease in progeria. Osteoarthritis affecting multiple joints has been reported in a four-year-old-girl, but the changes were poorly documented (Gupta *et al.*, 1976).

4. Endemic Degenerative Joint Disease

a. Kaschin–Beck Disease (Endemic Osteoarthrosis Deformans). This is a common disorder in parts of eastern Siberia and Manchuria. The sparse histological data available (Chu and Tsui, 1978) support extensive clinical impressions that the basic problem resides in the growth plate and epiphyseal cartilage and that articular cartilage is subsequently damaged nonspecifically (Basilevskaja, 1979).

b. Mseleni Disease. This is an endemic arthropathy in northern Zululand that resembles multiple epiphyseal dysplasia (Du Toit, 1979). It, unlike Kaschin–Beck disease, leads to crippling osteoarthritis of the hip. The lesions of the joint surface in resected specimens resemble those of other types of coxarthrosis. Extensive epidemiological studies have failed to identify a genetic or specific basis for Mseleni disease, although Du Toit cites a little-known endemic osteoarthritis in southern India, Handigodu disease, that has some resemblance to it.

VI. MISCELLANEOUS DEGENERATIVE DISORDERS OF ARTICULAR CARTILAGE

Articular cartilage is subject to inflammatory destruction in a wide variety of infectious and rheumatoid arthritides. Chondrolytic enzymes (neutral proteinases and collagenases) are produced by leucocytes in the synovial fluid, synovial lining, granulation tissue in the subchondral bone or pannus that covers the surface of the cartilage, and even by the chondrocytes. The chondrocytes are activated by messenger molecules from mononuclear cells of the inflammatory infiltrate (catabolins). The inflammatory changes in articular cartilage are outside the scope of the present review.

A. Chondrocalcinosis

This is a generic term for several disorders characterized by deposition of calcific crystals in articular cartilage (Fig. 5). By far the most frequent crystal is calcium pyrophosphate dihydrate (CaPP). The deposits are preferentially located in the midzone of the cartilage, and more often in fibro- than hyaline cartilage. The extent and consequences of the crystal deposition vary widely. When CaPP finds its way into the joint space as distinct from the cartilage, it evokes an inflammatory reaction reminiscent of gout. This situation accordingly is called *pseudogout*. There are some who believe that the CaPP crystals in pseudogout crystallize out of the synovial fluid. The more widely accepted view is that they are discharged into the joint space from the cartilage where they formed in the first place.

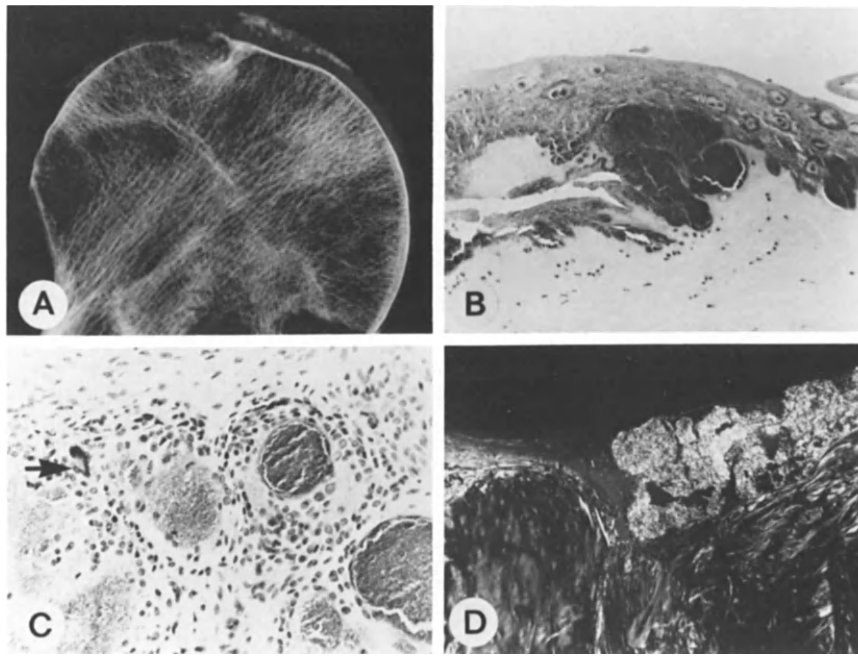

Fig. 5. Chondrocalcinosis. (A) Roentgenogram of femoral head of a 99-year-old man. The chondrocalcinosis was an incidental finding. Punctate radiopaque deposits are located within the articular cartilage rather than on its surface. The contour and thickness of the cartilage are normal. (B) Photomicrograph of intracartilaginous crystal deposits in an 81-year-old woman (case of Dr. Y. C. Lee). (C) The proliferation of cells is a feature of pseudogout as distinct from asymptomatic chondrocalcinosis. Magnification 148× (stained with hematoxylin and eosin). (D) Birefringence of CaPP crystals at the joint surface of part (C) (polarized light).

The frequency of CaPP deposits in peripheral or spinal joints at necropsy in the general elderly population has been variously estimated as 4–25%. In this circumstance, the crystals cause no symtoms or destruction of cartilage beyond their immediate confines. The deposits are punctate and appear as microcystic defects in decalcified sections. The crystals are stubby and have a positive but weak birefringence. These properties and a lesser reactivity with Von Kossa's stain distinguish CaPP from hydroxyapatite crystals histologically.

The pathogenesis of CaPP deposition and pseudogout are uncertain. Inorganic pyrophosphate (PP_i) is a byproduct of many biosynthetic pathways of proteins, nucleotides and nucleic acids, lipids, complex carbohydrates, and glycogen (McGuire *et al.*, 1980). It has been estimated that the amount of PP_i synthesized daily is in the kilogram range. PP_i is short-lived, and hydrolyzed to orthophosphate within the cell of origin by pyrophosphatase.

PP_i is not known to traverse cell membranes and how it gains access to cartilage matrix is thus unknown. One possibility is that PP_i is generated at cell surfaces through the action of adenyl cyclase on ATP in the production of cAMP. A priori one might envision the deposition of CaPP being affected by the levels of Ca^{2+}, PP_i, pyrophosphatases, or by abnormality of a matrix component initiating precipitation of crystals from saturated solution in the interstitial fluid. Several sorts of evidence indicate a close relationship between CaPP and hydroxyapatite deposition. Alkaline phosphatase of cartilaginous fluids possesses pyrophosphatase activity. The remodeling basal potions of articular cartilage and the synovial fluid in osteoarthritis have increased alkaline phosphatase and PP_i levels.

There is a spectrum of metabolic disturbances in which CaPP deposition occurs. Chondrocalcinosis that occurs as a sequela of hyperparathyroidism or hypophosphatasia can be understood in the preceding context. The mechanism is less clear in the arthropathy of hemochromatosis, in which the primary biochemical abnormality involves the storage of hemosiderin Fe^{3+}. Inherited forms of CaPP chondrocalcinosis are often associated with more severe forms of destruction of articular cartilage and osteoarthritis.

The occurrence of a lapine hydroxyapatite chondrocalcinosis is described in Section VIII on comparative pathology. There are rare instances of massive hydroxyapatite calcinosis associated with osteoarthritis of the hip joint in human subjects with or without calcinosis cutis. Gross hydroxyapatite deposition more characteristically occurs in periarticular bursae than in cartilage proper.

B. Hemophilic Arthropathy

Repeated intraarticular hemorrhages lead to crippling joint disease in most persons afflicted by the major bleeding diatheses. The extravasated erythrocytes are phagocytosed by synovial lining cells. In time the capsular tissues become greatly thickened by deposits of hemosiderin and fibrovascular proliferation. In more advanced stages, articular cartilage is also affected. Eburnation and osteophyte formation are conspicuous. The principal difference from ordinary osteoarthritis is that hemosiderin is found in a proportion of residual chondrocytes as well as in the subchondral bone marrow (Hough *et al.*, 1976). In extreme forms, landmarks of the joint are destroyed and replaced by fibrous ankylosis.

Two principal mechanisms for destruction of the cartilage have been entertained: (*1*) release of chondrolytic enzymes by the altered synovial tissue, and (*2*) direct iron toxicity to the cartilage. The latter hypothesis postulates that the Fe^{3+} reacts with the polyanionic matrix to alter its elastic properties and/or to injure the chondrocytes. Ancillary factors include increased intraarticular pressure and loss of motion.

C. Avascular Necrosis of Bone

Bone infarcts account for approximately 15% of reconstructive arthroplasties of the hip carried out at the present time. The lesion evolves in two principal phases: (*1*) infarction of paraarticular bone, and (*2*) at some later time—perhaps months or years—fracture of the dead bone beneath the joint surface. It is at this time that symptoms appear. The articular cartilage, even in the second phase, remains viable for a long time. Its bulk chemical properties are normal (Mankin *et al.*, 1977). The reason for this is that the cartilage, unlike the bone, derives its nourishment from synovial fluid rather than directly from the blood stream. Over the course of months or years, the cartilage becomes avulsed from the collapsed joint surface and disintegrates. The mechanism of the infarction is debated. Vascular occlusions are not ordinarily seen. In a large proportion of cases, treatment of some other disease with corticosteroids is an etiological factor. Many cases, however, remain idiopathic.

D. Ochronosis

This is the pigmentation of cartilaginous tissues that results from alkaptonuria. The latter is a defect in the catabolism of phenylalanine and tyrosine in which the degradative chain is arrested at the level of homogentisic acid (HGA) formation. Deficiency of HGA oxidase results in excretion of large quantities of HGA into the urine. Under alkaline conditions, HGA undergoes oxidation and polymerization in the urine into a melanin-like pigment. Most instances are of genetic origin. Although alkaptonuria is present at birth, ochronotic pigmentation of the cartilage matrix does not develop until several decades have gone by. The pigmented cartilage becomes brittle and the sequences of severe osteoarthritis and spondylosis follow (Schumacher and Holdsworth, 1977). Chondrocytes in the affected cartilage die and also become pigmented. Ochronosis occasionally is seen after excessive loading with phenolic compounds, such as in patients who have been treated for many years for Parkinson's disease with dopamine compounds.

E. Neuropathic Arthropathy (Charcot Joint)

Disintegration of joints follows sensory deprivation. The deficit may be either in proprioception or in perception of pain. Fragmentation of the articular cartilage is a conspicuous feature and shards of this tissue are displaced into the synovial lining. The articulation is highly unstable. Massive destruction of the articular surface and osteophytosis may be considered an extreme form of osteoarthritis.

F. Immobilization

Lack of motion has variable effects on articular cartilage, depending on the conditions of immobilization and the species of animal. The effects attributable to immobilization as distinct from abnormal loading have not al-

ways been clearly delineated. According to Palmoski *et al.* (1979), synthesis of aggregatable proteoglycan in dog knee cartilage diminishes rapidly following external fixation of the joint. The changes are reversible when ambulation is reinstituted. In ten human knees amputated after prolonged loss of motion for a variety of indications, Enneking and Horowitz (1972) found that the principal changes were contracture of the capsular and periarticular tissues. Only later did degenerative changes in the cartilage and ankylosis come about.

VII. DEGENERATIVE SPINAL DISEASE

Degenerative disease analogous to osteoarthritis affects two discrete types of joints in the vertebral column: the intervertebral discs and the diarthroses (apophyseal and neurocentral joints). The term spondylosis applies to involvement of the amphiarthroses and osteoarthritis, to that of the synovial joints.

A. Disc Degeneration

In humans, unlike many quadruped species, notochordal elements disappear from the *nucleus pulposus* during the years of skeletal growth. Physaliferous cells persist to a greater degree in the sacral segments and have been described there in the fifth decade of life. This phenomenon suggests that postural loading may be a factor in this aspect of spinal aging. In the years following maturation, the delineation between *nucleus pulposus* and *annulus fibrosus* become indistinct as the former takes on a fibrocartilaginous character (Buckwalter, 1982). The degenerative changes do not occur uniformly throughout the spines of humans or other vertebrate species. In interpreting data, the segment involved and the pathological character of that particular segment must be identified.

Localized fissures in the *annulus fibrosus,* generally paralleling the direction of the fibers, develop with considerable frequency with aging in lumbar discs. Overt rents in the *annulus fibrosus* make possible abrupt "herniation" of *nucleus pulposus* into the subdural space. The displaced tissue, no longer constrained by taut annular fibers, is able to imbibe water and compress adjacent structures. Most of the rents occur posteriorly and the extruded material impinges on the nerve roots. These episodes do not necessarily occur in older persons and are not associated with multisegmental spondylosis. They may eventuate in spondylotic changes confined to the affected level.

In spondylosis, the development of changes in the disc is more insidious. Fissuring of the tissues is more extensive; disc tissue protrudes anteriorly and laterally. Marginal osteophytes usually appear at the anterolateral margins of affected thoracolumbar vertebrae. In most instances these changes do not

result in discomfort. When osteophytes abut on neural roots, pain and other neurological deficits may arise. The Luschka or neurocentral joints are particularly prone to show this. Luschka joints are not normal structures. They develop as adventitious clefts in the posterolateral aspects of the cervical intervertebral discs during the second and third decades of life.

In severe spondylosis, extensive disintegration and cavitation of the disc ensue. The space between the vertebral end plates is greatly reduced and sclerotic changes analogous to eburnation are seen. Osteoporosis does not increase—indeed it may militate against—the development of spondylosis.

B. Schmorl Nodes

A wholly different pattern of intervertebral disc displacement is seen as Schmorl nodes (Resnick *et al.*, 1978). These structures are protrusions of disc tissue into the vertebral body marrow through gaps in the osteocartilaginous end plates. They are particularly common in the lower thoracic region (Hilton *et al.*, 1976). The gaps are presumed to arise during childhood, perhaps as a result of Scheuermann's disease, but the Schmorl nodes do not appear until adult life. Their frequency does not increase with age. It is not altered by development of osteoporosis (Boukhris and Becker, 1974). The presence of the nodes increases the likelihood of spondylotic changes in the same disc but not elsewhere in the spine, according to Hilton *et al.* (1976).

C. Ankylosing Hyperostotic Spondylosis

The precise nosological status of this entity is undergoing reexamination. It bears the eponym Forestier's disease as well as several other appellations. The hallmark of the lesion is bony bridging of the margins of the vertebral bodies. It resembles severe spondylosis in many ways. The question is often asked whether the lesion doesn't simply represent severe intervertebral disc degeneration with coalescence of massive osteophytoses. The formal features of Forestier's disease, as distinct from spondylosis, are that there is relatively little damage to the intervertebral disc proper, and that the principal location of the lesions is the thoracic rather than the lumbar spine (Vernon-Roberts *et al.*, 1974). In practice considerable overlap occurs. In some lesions, the disc undergoes severe degeneration and bone growth takes place into the intervertebral disc space as well as at the margin. Figure 6 illustrates the appearances of the two forms of ankylosing hyperostotic spondylosis in quadrupeds: one showing preservation, the other, destruction of the discs. The bony bridges are compact and merge imperceptibly with the anterolateral cortex of the vertebral bodies. The appearance suggests that the attachment of the anterior (ventral) longitudinal ligament has become avulsed and then reossified in compact form.

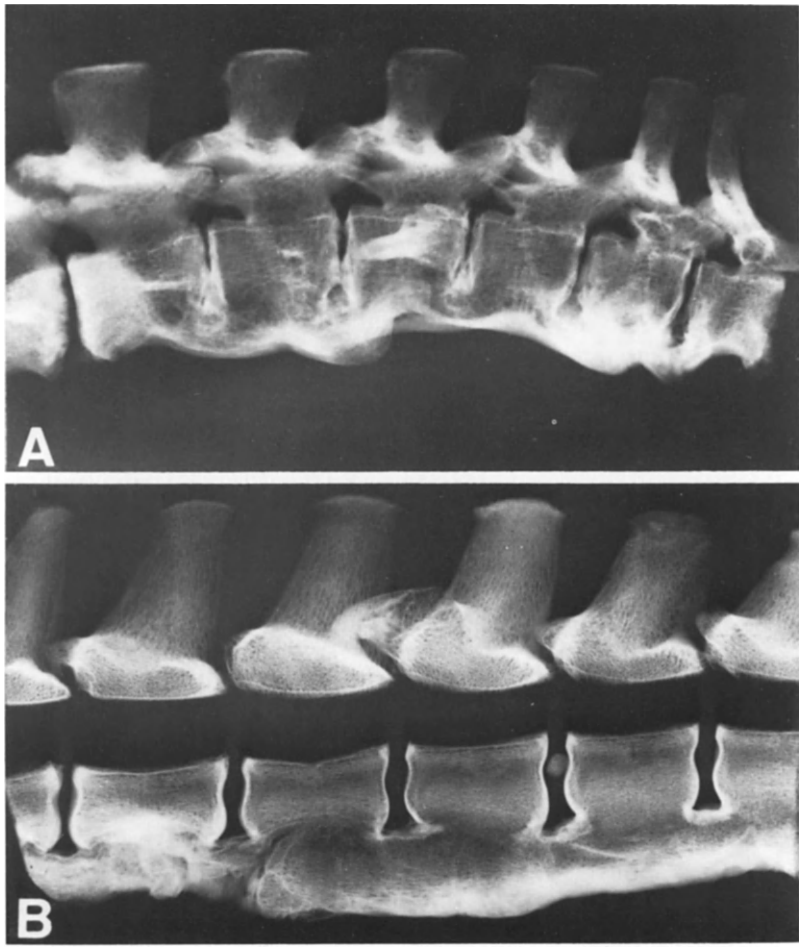

Fig. 6. Different patterns of hyperostotic ankylosing spondylosis. (A) Severe intervertebral disc degeneration with narrowing of the roentgenographic spaces. The ventral landmarks of the vertebral bodies and adjacent discs are lost in osteophytic and ligamentous ossification. (Rhesus monkey.) (B) Intervertebral disc spaces are preserved. (Boxer dog.) Reproduced from Sokoloff (1981) with the permission of the publisher.

Another age-related ankylosing spondylosis occurs in Japan (Ono *et al.*, 1977). The ossific process is confined to the cervical region and affects the posterior longitudinal ligament. It sometimes coexists with ankylosing hyperostosis, but the discs and apophyseal joints are spared.

Unusual patterns of osteophyte formation are sometimes seen in peripheral joints of patients with Forestier's disease. Large ossific tufts appear at

the insertions of the quadriceps tendon into the patella or the triceps tendon into the olecranon process. The joint surfaces proper appear to be preserved. This entity has been recognized only recently and is called the DISH (diffuse idiopathic skeletal hyperostosis) syndrome (Utsinger *et al.*, 1976; Spagnola *et al.*, 1978).

VIII. COMPARATIVE PATHOLOGY

Degenerative joint disease, morphologically comparable to that in man, occurs in many vertebrate species independent of their position in the taxonomic scale. Both appendicular and axial skeletons are involved. Primary and secondary patterns have been described in mammals. The secondary variety is most widely recognized in the hip joint of dogs where it develops as a sequela of acetabular dysplasia. German shepherds are most often affected but other breeds are also prone to it. Lust and Farrell (1977) estimate that the heritability of canine hip disease is about 25% and the remainder of the phenotypic variance is environmental.

Much is known about the occurrence of degenerative joint disease in laboratory rodents (Pataki *et al.*, 1980). A few strains are highly susceptible, the knee and elbow joints being most severely affected, and the hips almost never. Wigley and co-workers (1977) provide further evidence for the contribution of genetic factors in NZB/Bl and NZY/Bl strains. That heritable biochemical defects are major factors to the osteoarthritis as indicated by the frequency of the lesions in blotchy (BLO) mice (Silberberg, 1977). The mutant gene leads to inadequate cross-linking of collagen. In another strain of mouse widely studied for its predisposition to degenerative joint disease, STR/ORT, Walton (1979) attributes osteoarthritis of the knee to spontaneous subluxation of the patella. By containing the subluxation surgically, he prevented development of the osteoarthritis. The implication is that the susceptibility to degenerative joint disease in these animals resides in the mechanical loading rather than in some metabolic peculiarity of the cartilage. The data are impressive but difficult to reconcile with our observations that lesions in STR/1N mice are not confined to the knee but are more generalized. The broader principle is that genetic factors that influence the development of osteoarthritis may operate at many levels—local, generalized, mechanical; and metabolic.

Degenerative spinal disease takes four principal forms in nonhuman species.

1. Degeneration of intervertebral discs often exceeds that found in man. Dorsal displacement of disintegrated tissue into the spinal cord or its roots at times results in overt paraplegia. The propensity to occur in chondrodystro-

phoid breeds of dogs and in the African rodent *Mastomys natalensis* indicate that the phenomenon is genetically influenced.

2. Gross vertebral osteophytes in aged laboratory rats have sometimes been interpreted as evidence of senescent spondylosis (Gloobe and Nathan, 1971). Microscopic analysis demonstrates, however, that this results from idiopathic epiphyseal ischemic necrosis so common in small rodents.

3. Spondylosis does, however, occur sporadically in many quadrupeds (du Boulay *et al.*, 1972). Cats are generally less likely to develop it than are dogs.

4. Ankylosing hyperostotic spondylosis is quite common in marine as well as in terrestrial animals. The condition has often been confused with ankylosing spondylitis in the veterinary literature. Two patterns, as discussed in man, are seen in quadrupeds: one associated with severe degenerative disc disease, and the other not (Fig. 6). There is no involvement of the apophyseal joints or other inflammatory change to suggest that these are spondylitic processes. Of special interest in this regard is the lesion called *osteopetrosis* in bulls (Krook *et al.*, 1971). Morphologically it closely resembles, if it is not identical with, hyperostotic spondylosis. It occurs in bulls fed the high calcium-containing fodder that milch cows receive. Adenomatous hyperplasia of the ultimobranchial glands occurs in these bulls and data have been presented relating the osteopetrosis to nutritional hypercalcitoninemia.

Several metabolic or other noninflammatory lesions affecting articular cartilage are known in nonhuman species. Hemophilic arthropathies in dogs (Swanton and Wysocki, 1975) and other species closely resemble the human counterpart. Chondrocalcinosis of rabbits has some similarities to human chondrocalcinosis but involves hydroxyapatite rather than calcium pyrophosphate deposition (Yosipovitch and Glimcher, 1972). Sporadic instances of ochronosis have been reported. The histochemical features of one case in a bovine (Nilsson and Grabell, 1977) were so different from those occurring in human ochronosis that it is difficult, in the absence of supporting biochemical data, to regard this as a valid model. Keeling *et al.* (1973) have described alkaptonuria but not arthropathy in a laboratory-born orangutan.

IX. CONCLUDING REMARKS

This brief survey amply illustrates the conceptual complexities in the relationship of aging to degenerative diseases of cartilage. Relatively few of the molecular aspects have been considered. The biochemical literature cited in Section I is too massive to be dealt with here. This essay has attempted to construct a framework into which the biological and pathological meaning of the chemical publications must find their place.

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5

Tumors of Cartilage

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I. INTRODUCTION

Tumors of cartilage are broadly interpreted in this chapter to include *(1)* primary benign and malignant neoplasms of cartilage, *(2)* neoplasms in which cartilage is one component, *(3)* cartilage-containing hamartomas, *(4)* tumors that simulate cartilaginous neoplasms, *(5)* conditions with chon-

dromatous metaplasia, *(6)* cartilaginous remnants presenting as tumors, and *(7)* syndromes with cartilage-containing neoplasms. The clinical, radiographic, and histological features of each condition are reviewed in an attempt to increase our knowledge of the behavior of cartilage in general and cartilaginous tumors in particular. No attempt has been made to be encyclopedic or to address the formidable problems of differential diagnosis. For such coverage, the reader is referred to the works of Dahlin (1978), Jaffe (1964), Lichtenstein (1972), Huvos (1979), Spjut *et al.* (1971), and to Chapter 1 in this volume. It should be recognized that the classification of cartilaginous tumors used in this chapter is only one of several possible classifications. It should also be recognized that frequencies given for various lesions refer to those in the literature, which are subject to many ascertainment biases. Such biases are commonly not corrected, frequently not discussed, and sometimes not even recognized.

II. CENTRAL AND PERIPHERAL BENIGN LESIONS

A. Osteochondroma

1. Solitary Osteochondroma

Osteochondromas consist of cartilage-capped bony masses that arise in the metaphyseal area of bones preformed in cartilage. They comprise approximately 50% of all benign bone tumors. Some sources indicate little sex predilection (Spjut *et al.*, 1971) and others show approximately a 65% preponderance in males (Dahlin, 1978; Huvos, 1979). Although the solitary osteochondroma becomes clinically apparent most commonly in the second decade of life, the lesion may be found at any age from the first to the eighth decades (Dahlin, 1978; Huvos, 1979). More than half arise in the distal femur, the proximal tibia, or the humerus. They are exceedingly rare in the facial skeleton. Chondrosarcomas arise in 1–2% of solitary osteochondromas.

The etiology of solitary osteochondroma is unknown. Generally, the condition is regarded as an aberrantly developed epiphyseal cartilage rather than a true neoplasm. D'Ambrosia and Ferguson (1968) found that experimental transplantation of epiphyseal and articular cartilage subperiosteally in rabbit tibiae produced lesions that were similar to human osteochondromas.

Clinically, a palpable, firm mass of relatively long duration is usually present. Pain is not a predominant feature. Radiographically, the tumor appears as a bony excrescence arising from the metaphyseal region (Fig. 1). Microscopically, the mature lesion consists of bony trabeculae surrounding hematopoietic or fatty marrow and covered with an irregular cap of hyaline cartilage. Chondrocytes are frequently arranged in linear fashion mimicking normal epiphyseal plate.

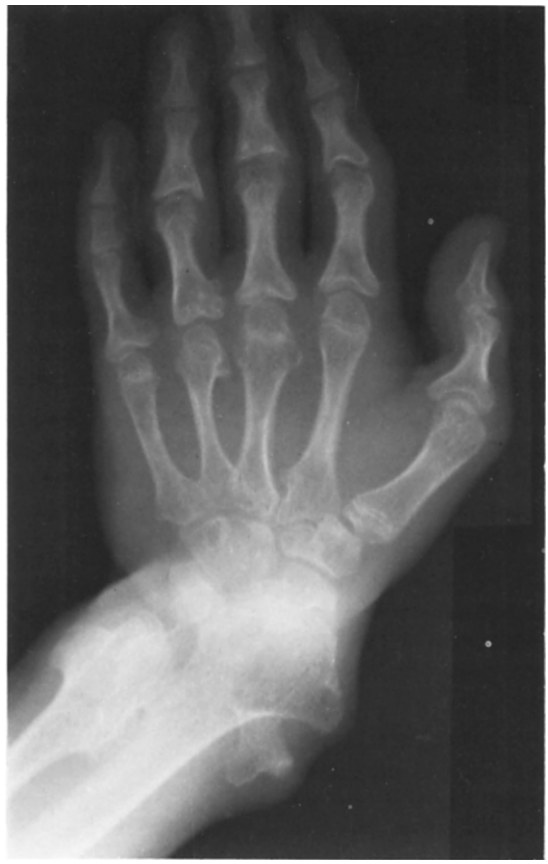

Fig. 1. Multiple exostoses found in hand-wrist roentgenogram. Note cone-shaped epiphyses. Langer-Giedion syndrome.

2. Postirradiation Osteochondroma

Osteochondromas have been known to develop following radiation therapy for a variety of different lesions. The involved bones have been within or adjacent to the field of radiation. Children have been primarily affected from 17 months to 9 years following irradiation (Spjut *et al.*, 1971).

3. Autosomal Dominant Osteochondromatosis

Multiple osteochondromas occur as an autosomal dominant condition with complete penetrance. Although the earlier literature indicated male preponderance, the sex ratio is approximately equal. About 40% of cases arise as fresh mutations. The tubular bones, ribs, pelvis, and scapula are most commonly involved and most patients have recognizable bony defor-

mities—especially bowing of the radius, shortening of the ulna, radiohumeral dislocation, genua valga, clinical deformities, and mild short stature. Most lesions (80%) are recognized during the first year of life. Subcutaneous bursae develop over the exostoses in approximately 10% and chondrosarcomas arise in about 10% of reported patients (Lichtenstein, 1973).

4. Langer–Giedion Syndrome

Multiple exostoses are a constant feature of the Langer–Giedion syndrome. Other features include cone-shaped phalangeal epiphyses, sparse hair, bulbous nose, and prominent ears. Most cases have been sporadic (Hall *et al.*, 1974), although familial instances have been noted (Murachi *et al.*, 1981). High resolution banding of prometaphase chromosomes has revealed interstitial deletion of the long arm of chromosome number 8 in several instances (Zabel and Baumann, 1982). Other cases of the Langer–Giedion syndrome need to be reexamined in this light.

5. Albright Hereditary Osteodystrophy

Albright hereditary osteodystrophy is characterized by short stature, obesity, mental deficiency, rounded facial appearance, short metacarpals and metatarsals, especially the fourth and fifth, and variable hypocalcemia. The condition has been discussed extensively elsewhere (Gorlin *et al.*, 1976). Approximately 10% of the patients reported by Todd *et al,* (1961) had exostoses.

B. Enchondroma

1. Solitary Enchondroma

The solitary enchondroma is a benign cartilaginous growth that develops in the medullary cavity of a bone preformed in cartilage. Enchondromas consist of approximately 10% of the benign tumors of bone in the Mayo Clinic series (Dahlin, 1978). Commonly, the large and short tubular bones of the limbs are affected, especially phalanges and metacarpals, although infrequently enchondromas are encountered in locations outside the limbs. Patients are usually between 10 and 50 years of age at the time the lesion comes to medical attention. Occasionally, a solitary enchondroma may undergo malignant transformation. This is more likely to occur in long bones than in phalanges, metacarpals, or metatarsals (Jaffe, 1964; Lichtenstein, 1972).

Radiographically, an enchondroma appears as an ovoid radiolucency confined to the shaft of a bone but sparing the epiphyseal end. Commonly, the lesion is eccentrically placed and may cause pronounced bulging of the

overlying cortex. It may have a vague trabeculated appearance, and punctate radiopacities representing foci of calcification and ossification may be present.

Microscopically, the cartilage is not highly cellular. Nuclei are small, rounded, dark staining, and retracted in their lacunae. Most cells are uninuclear but an occasional cell may contain two nuclei. Cartilage is often arranged in lobular fashion. Calcification and ossification may also be observed (Jaffe, 1964).

2. *Multiple Enchondromatosis*

Multiple enchondromatosis is characterized by several and sometimes many enchondromas affecting a number of bones. Involvement is often asymmetrical with more severe distortion on one side of the body. The condition is also known as Ollier disease and dyschondroplasia. All cases to date have had sporadic occurrence. The etiology is unknown.

Clinically, swellings are most commonly observed, especially involving the fingers. Occasionally, pathological fracture of an affected bone may be the presenting feature. Bowing and distortion of bones are common. In some cases, skeletal involvement stabilizes after puberty. In others, monstrous deformities develop. Chondrosarcomatous transformation occurs more commonly than with isolated enchondroma (Jaffe, 1964; Lichtenstein, 1972).

3. *Maffucci Syndrome*

Maffucci syndrome is characterized by enchondromatosis, multiple hemangiomas, and phlebolithiasis. The etiology is unknown and all cases reported to date have been sporadic. The cartilaginous tumors tend to have the same distribution as those found in Ollier disease. Unilateral involvement has been observed in 40% of reported cases and chondrosarcomatous changes in 20%. Hemangiomas may be superficial or more deeply situated. Although they generally appear at the same time as the bone changes, they often do not involve the same areas (Gorlin *et al.*, 1976).

Other neoplasms reported in association with Maffucci syndrome include angiosarcoma, cerebral glioma, astrocytoma, and fibrosarcoma. Ovarian teratoma and adenocarcinoma of the pancreas have also been noted (Spjut *et al.*, 1971; Cremer *et al.*, 1981).

4. *Periosteal Chondroma*

Periosteal or juxtacortical chondroma is a benign growth of cartilage that forms subperiosteally but external to the cortex of the affected bone. The lesion is rare, most often being found in the small bones of the hands and feet.

Radiographically, there may be erosion of the affected cortex. Histologically, the chondroma appears as a lobular mass of actively growing cartilage.

C. Benign Chondroblastoma

The benign chondroblastoma or Codman tumor is a rare cartilage-forming neoplasm thought to be derived from immature epiphyseal cartilage cells. The electron microscopic appearance of the tumor supports this origin. The etiology is unknown. Although described in 1931 by Codman, Jaffe and Lichtenstein (1942) first coined the term benign chondroblastoma 11 years later. Although over 500 cases have been reported, the lesion constitutes less than 1% of all primary bone tumors. There is a 2 : 1 male to female ratio (Huvos, 1979; Huvos and Marcove, 1973; Dahlin and Ivins, 1972).

The majority of these neoplasms arise during the second decade of life when immature epiphyseal cartilage cells are active during bone growth. The most common sites, in descending order of frequency, are the femur, humerus, and tibia. Occasionally, cases have been reported from the flat bones of the skull that are presumed to arise from centers of endochondral ossification within these bones.

Radiographically, the tumor appears as a well delineated central destructive lesion. The margin may appear slightly radiopaque. Periosteal reaction and trabeculae within the neoplasm are not usually present. Expansion of the cortex is a feature of larger lesions. Microscopically, the predominant cell type has the ability to form cartilage. Cells have ovoid or round, often indented nuclei and well-defined cytoplasm. Mitotic figures can usually be found but are not numerous. There is little intervening stroma except foci of cartilage which may exhibit calcification. Multinucleated giant cells are also a feature and giant cell nuclei bear resemblance to the nuclei of the chondroblastic cells themselves.

The treatment of choice is curettage since the lesion is usually benign and nonaggressive. Radiation therapy is contraindicated. Occasionally, cases of aggressive chondroblastoma have been reported, such as the case of multiple bone and soft tissue involvement with recurrence noted by Hull *et al.* (1977). Very rarely, cases of histologically proven chondroblastoma have been found to metastasize.

D. Chondromyxoid Fibroma

Chondromyxoid fibroma, a benign neoplasm of chondroid and myxoid elements with a lobular pattern of growth, seems to be derived from cartilage-forming connective tissue. The tumor appears to be less common than the benign chondroblastoma (Huvos, 1979) and accounts for less than 0.5% of all bone tumors in the Mayo Clinic series (Dahlin, 1978). A slight male predilection is usually noted in most reported series of cases.

The lesion appears most commonly during the second decade of life and nearly two-thirds arise within the first three decades. Typically, the chondromyxoid fibroma is located in the metaphyseal region. Rarely, it encroaches on the epiphyseal area. The tubular long bones account for the majority of cases with the tibia being the most common bone involved. The small bones of the feet are also a frequent location for this lesion (Feldman *et al.*, 1970).

The location of the chondromyxoid fibroma in the metaphysis suggests origin from the epiphyseal cartilaginous plate. Ultrastructural study (of one lesion) has shown two populations of cells—one resembling chondrocytes, the other resembling fibroblasts (Tornberg *et al.*, 1973). Fibrous long-spacing collagen has also been reported in this neoplasm. Huvos (1979) discussed the possibility of overlap between chondroblastoma and chondromyxoid fibroma. However, at the present time, definite conclusions are not possible either on the basis of the location of the lesions within bone or on the basis of their light and electron microscopic appearance.

Radiographically, the chondromyxoid fibroma is usually eccentrically located, well-circumscribed, radiolucent, and located in the metaphyseal region. Apparent trabeculations, representing corrugations on the inner surface of the bone, may be seen.

Microscopically, a lobular growth pattern is evident at low power. Higher power may reveal myxomatous areas, fibrous zones, and distinctly chondroid areas. The periphery of these lobules may be more cellular with some cells demonstrating disturbing cytologic characteristics. Osteoclast-like giant cells may be present and delicate argyrophilic fibers may also be observed.

Treatment by block excision seems to reduce the risk of recurrence which may be as high as 25% with curettage. Radiation therapy with its attendant danger of subsequent malignant transformation is contraindicated. To date, spontaneous malignant transformation of a chondromyxoid fibroma has not been convincingly documented.

E. Myxoma of Bone

There is some doubt as to whether the myxoma of bone outside the jaws exists as an entity or whether it represents inadequate sampling of a chondromyxoid fibroma or myxomatous chondrosarcoma. McClure and Dahlin (1977) reported three myxomas of the femur in elderly patients. Another case was noted by Huvos (1979). Myxomas of the jaws are thought to arise from the odontogenic apparatus, an assumption that seems reasonable because of their relatively high frequency of occurrence compared with their rare occurrence in other bones. Myxomas of the jaws do not seem to arise from cartilaginous tissue nor do they show chondroid differentiation.

III. MALIGNANT NEOPLASMS ARISING FROM OR CONTAINING CARTILAGINOUS TISSUES

A. Chondrosarcoma

Chondrosarcoma is a malignant neoplasm of cartilaginous tissue in which osteoid is not produced by the neoplastic cells. When osteoid arises directly from the sarcomatous tumor cells, the lesion should be classified as a chondroblastic osteosarcoma. The differences in behavior and distribution between chondrosarcomas and osteosarcomas make this distinction important.

Chondrosarcomas may be either primary, arising *de novo* from a previously normal bone, or secondary, arising in a preexisting benign cartilaginous neoplasm. Recognized histological variants include clear cell chondrosarcoma and mesenchymal chondrosarcoma (Dahlin, 1978; Huvos, 1979).

The incidence of chondrosarcoma varies with different series. In the Mayo Clinic series (Dahlin, 1978), chondrosarcomas constituted approximately 10% of the malignant tumors. An incidence of 17–22% was reported by Huvos (1979). Approximately 60% of all chondrosarcomas occur in males (Dahlin, 1978; Huvos, 1979; Kreicbergs *et al.*, 1981; Pritchard *et al.*, 1980; Evans *et al.*, 1977). More than half of all lesions arise after 40 years of age. Approximately 30% occur in the pelvis with the femur next most commonly involved. The neoplasm is relatively rare in the hands and feet in contrast to enchondromas which occur most commonly in this location, implying that the vast majority of chondrosarcomas do not arise from solitary preexisting benign cartilaginous neoplasms. In the Mayo Clinic series (Dahlin, 1978), only 12.5% of chondrosarcomas arose in preexisting lesions, most commonly in the pelvis. There was a stronger tendency for chondrosarcomas to arise in patients with multiple exostoses than in patients with single lesions.

Localized pain and swelling are the predominant presenting symptoms. Central lesions produce destruction of the cortex and may expand the surrounding soft tissues. Malignant change in a preexisting benign lesion may be evidenced by an indistinct and fuzzy surface together with loss of clear demarcation from surrounding soft tissues.

The basic histopathologic criteria for the diagnosis of chondrosarcoma (Fig. 2) include *(1)* many cells with plump nuclei, *(2)* more than a occasional binucleate cell, and *(3)* giant cartilage cells with large single or multiple nuclei (Lichtenstein and Jaffe, 1943). Many attempts have been made to grade chondrosarcomas on the basis of mitotic rate, cellularity, and nuclear size (Pritchard *et al.*, 1980; Evans *et al.*, 1977). Grade I chondrosarcoma is characterized by a preponderance of cells with small, densely staining nuclei and a background varying from chondroid to myxoid. These have been

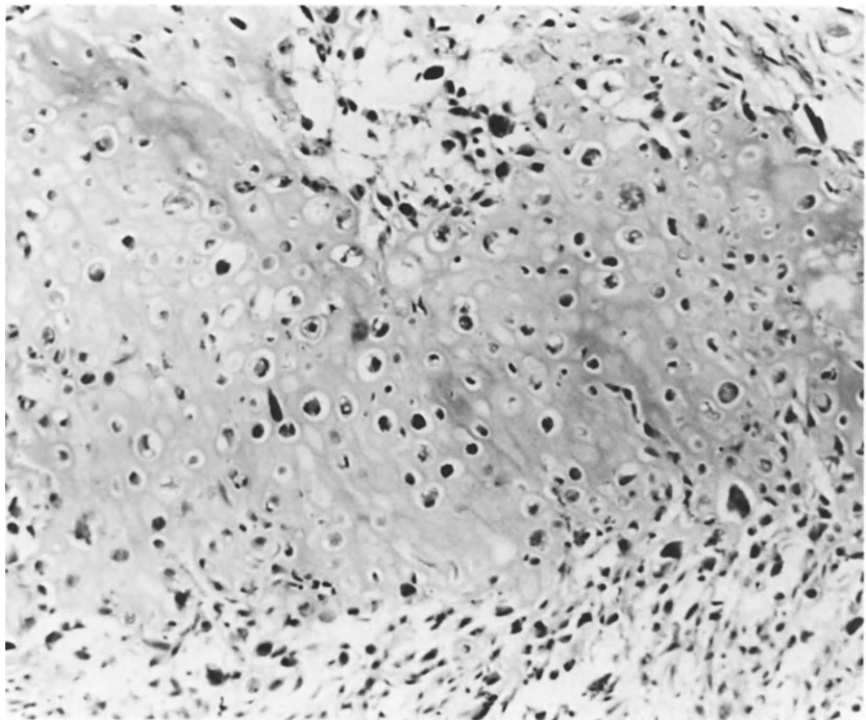

Fig. 2. Chondrosarcoma. Note nuclear pleomorphism (hematoxylin and eosin).

found to be locally aggressive but nonmetastasizing. This group has had a 10-year survival rate of 83%. Grade II chondrosarcoma is characterized by having moderately sized nuclei, but a low mitotic rate. Typically, there is an increased cellularity at the periphery of the tumor lobules. Metastasis occurs in 10% of the cases. The grade III chondrosarcoma is characterized by having two or more mitoses per high-power field in the most active areas and larger nuclei than those found in grades I or II. About 71% of this group shows metastasis and there is a 21% 10-year survival rate. More recently, Kreicbergs and co-workers (1981), using cytophotometrical nuclear measurements, found that nuclear size was the best prognostic indicator whereas cellularity appeared to be the strongest determinant for grading using the conventional grading systems. Dedifferentiation of chondrosarcomas may occur, and in the Mayo Clinic series (Dahlin, 1978), 51 of 470 chondrosarcomas dedifferentiated to a more malignant tumor, usually fibrosarcoma or osteosarcoma. Typically, the cartilaginous component was located at the center with a fairly abrupt transition to fibrosarcoma or osteosarcoma. Only the highly malignant portion was evident in metastases.

1. Clear Cell Chondrosarcoma

On the basis of distinctive microscopic appearance, radiographic appearance identical to chondroblastoma, and better prognosis, Unni *et al.* (1976) separated clear cell chondrosarcoma from other forms of chondrosarcoma. Of the 24 reported cases to date (Unni *et al.*, 1976; Le Charpentier *et al.*, 1979; Faraggiana *et al.*, 1981; Angervall and Kindblom, 1980), 14 have been in males, 9 in females. Mean age was 39 years with a peak incidence in the fifth decade. Most cases have occurred in the femoral head.

Radiographically, clear cell chondrosarcoma is indistinguishable from chondroblastoma with osteolytic expansion at the end of a long bone. Cortices may be expanded but are usually intact unless secondarily involved with a pathological fracture.

Microscopically, the lesion has a lobular appearance at low power. The tumor cells have vacuolated cytoplasm with fine residual cytoplasmic trabeculae. The nuclei may be centrally or peripherally located. Benign-appearing multinucleated giant cells also occur either alone or in clusters and may be located at the periphery of the lobules. Areas of conventional low-grade chondrosarcoma may also be observed.

Electron microscopically the clear cells demonstrate ample irregularly outlined cytoplasm with abundant glycogen (Faraggiana *et al.*, 1981). Histochemical studies demonstrate nonsulfated acid glycosaminoglycans in the cytoplasm of clear cells and sulfated glycosaminoglycans in the intercellular area (Angervall and Kindblom, 1980).

The clear cell chondrosarcoma appears to have a reasonable prognosis, provided that the nature of the neoplasm is recognized and treated reasonably aggressively. Four of the five patients reported by Le Charpentier *et al.* (1979) were alive 13, 9, 13, and 2 years after initial therapy. These patients had experienced recurrence and were ultimately treated by resection.

2. Mesenchymal Chondrosarcoma

Mesenchymal chondrosarcoma is a rare variant of chondrosarcoma that generally manifests different biological behavior and anatomical location from the more common variety of chondrosarcoma (Dahlin, 1978; Huvos, 1979; Lichtenstein and Berstein, 1979; Harwood *et al.*, 1981). Huvos (1979), in reviewing over 80 reported cases, noted that the most common sites, in descending order of frequency, were ribs, mandible, maxilla, skull, pelvis, and vertebral column. There appears to be no distinct sex predilection. Many mesenchymal chondrosarcomas occur in the second and third decades. A review of 17 patients by Harwood *et al.* (1981) showed an average age of 24 years.

Radiographically, the lesion appears as a destructive neoplasm frequently

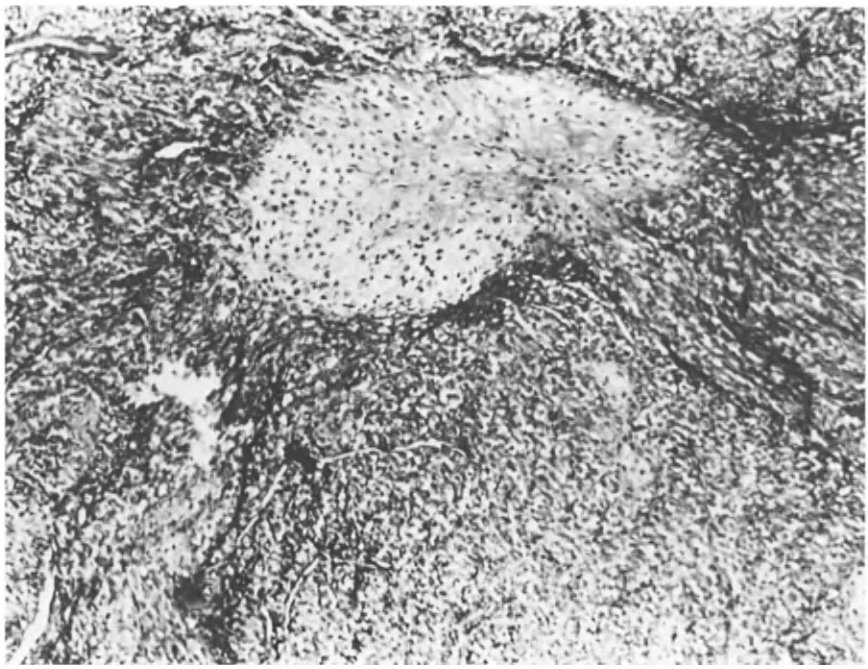

Fig. 3. Mesenchymal chondrosarcoma, showing central area of cartilage surrounded by undifferentiated cells (hematoxylin and eosin).

with extension into the surrounding soft tissues. Some cases have been shown to arise primarily within soft tissues.

Histologically, two patterns are evident. Areas of low-grade chondrosarcoma occur together with sheets of undifferentiated small cells with indistinct cytoplasm that resemble the cells of Ewing's sarcoma (Fig. 3).

Generally, mesenchymal chondrosarcoma has a worse prognosis than routine chondrosarcoma. Of 17 patients reviewed by Harwood *et al.* (1981), 12 were dead. Of these, 8 died within the first year.

Soft tissue chondrosarcoma is considered in the next section on cartilaginous tumors of soft tissue.

B. Chondroblastic Osteosarcoma

By definition, osteosarcoma is a malignant neoplasm that produces tumor osteoid. Among the many recognized histological variants of osteosarcoma is chondroblastic osteosarcoma in which the production of cartilage may dominate the microscopic picture. However, chondroblastic osteosarcoma has the same clinical behavior as fibroblastic or osteoblastic osteosarcomas—that of osteosarcoma and not chondrosarcoma.

C. Malignant Mesenchymoma

Malignant mesenchymoma is a tumor derived from primitive mesoderm and composed of two or more malignant cell types. Fibrosarcomatous, osteosarcomatous, liposarcomatous, and rhabdomyosarcomatous elements may be observed (Lichtenstein, 1972). Cartilaginous elements were observed in the case reported by Blattner *et al.* (1977). Malignant mesenchymoma of bone is aggressive and prone to metastasis. In malignant mesenchymoma of soft tissue, the fatality rate in adults has been estimated to be as high as 60% and in children as high as 40% (Lichtenstein, 1972).

IV. CARTILAGINOUS TUMORS OF SOFT TISSUE

A. Soft Tissue Chondroma

Benign cartilaginous tumors of soft tissue are uncommon. They are sometimes difficult to distinguish from extraskeletal chondrosarcoma, especially if they are highly cellular in microscopic appearance. They occur most commonly during the third and fourth decades, most frequently in the extremities (85%), especially the fingers (Chung and Enzinger, 1978).

Microscopically, soft tissue chondroma is usually well circumscribed and lobulated. It is primarily composed of adult hyaline cartilage. Calcification is frequently observed and on occasion may dominate the picture. Cellular and myxoid areas may also be observed. Although mitotic figures may occur, they are not atypical.

Treatment consists of surgical excision. A single recurrence was recorded in 10 of the 104 cases reported by Chung and Enzinger (1978).

B. Osteocartilaginous Tumor of the Tongue

This is usually known in the literature as osteocartilaginous choristoma of the tongue. The average age of reported cases is approximately 30 years with half of all cases arising in the third decade. Clinical signs and symptoms include a slowly enlarging mass in the tongue and sometimes dysphagia or gagging and nausea. Microscopically, bone as well as cartilage may be observed (Wesley and Zielinski, 1978).

C. Chondromatous Hamartoma of the Lung

The chondromatous hamartoma is thought to arise from a bronchial bud. It occurs within lung tissue near the pleural surface and is found more commonly in males than in females. Grossly, the lesion has a lobulated smooth surface. Microscopically, islands of cartilage are observed together with fatty tissue and cleftlike spaces lined by ciliated epithelium, lymphoid tissue, and muscle (Spencer, 1977).

D. Carney Syndrome

The Carney syndrome consists of chondromatous hamartoma of the lung, gastric leiomyosarcoma, and functioning extraadrenal paraganglioma. To date, all cases have been sporadic and a predilection for females has been observed (Carney *et al.*, 1977).

E. Bronchial Chondroma

Bronchial chondroma is a rare tumor that is thought to arise from fully formed bronchial cartilage. The lesion is primarily composed of cartilage which may show myxoid areas (Spencer, 1977).

F. Soft Tissue Chondrosarcoma

There are many well documented cases of chondrosarcoma arising primarily in soft tissue with no evidence of bony involvement (Goldberg *et al.*, 1967; Korns, 1967; Enzinger and Shiraki, 1972; Wu *et al.*, 1980). Many of these lesions have been found to involve muscle, tendons, or ligaments. The presumptive cartilaginous origin is based on the close resemblance between the tumor cells and developing chondroblasts. Electron microscopic findings together with the presence of large amounts of sulfated mucopolysaccharides are additional evidence of cartilaginous derivation.

Soft tissue chondrosarcomas usually arise in the extremities, the thigh and knee accounting for 50% of the cases (Enzinger and Shiraki, 1972). The neoplasm may occur at any age and show no evidence of calcification radiographically. Grossly, the tumor is a lobulated or nodular mass that is distinctly gelatinous. Microscopically, the multinodular tumor is composed of uniform, rounded, or elongated cells with a narrow margin of eosinophilic cytoplasm. The cells are usually arranged in cords, strands, or small nests in a myxoid, weakly basophilic ground substance. Although some soft tissue chondrosarcomas have a myxoid appearance, others more closely resemble typical chondrosarcomas. The overall behavior of extraskeletal myxoid chondrosarcoma tends to be less aggressive than primary chondrosarcoma of bone (Enzinger and Shiraki, 1972).

V. TUMORS OF SYNOVIAL ORIGIN SHOWING CARTILAGINOUS DIFFERENTIATION

A. Synovial Osteochondromatosis

Synovial osteochondromatosis is an uncommon benign lesion consisting of cartilaginous and osteocartilaginous nodules of the synovium. Although cartilaginous rests, joint trauma, and inflammation have been suggested as the basis for the lesion, metaplasia and neoplasia are more likely explana-

tions. More than 50% of all cases involve the knee, although synovial osteochondromatosis also affects the elbow, temporomandibular, metacarpophalangeal, wrist, shoulder, hip, and ankle joints. Men are affected twice as commonly as women and the mean age for involvement is in the fifth decade (Lichtenstein, 1972; Spjut *et al.*, 1971).

Clinical signs and symptoms include joint pain, swelling, stiffness, and limitation of motion. Radiographically, numerous radiopaque bodies may be found in the affected joint and bursa when calcification and ossification have taken place.

Grossly, the synovium may be thickened with polypoid projections that appear as glistening white nodules when calcified. In many cases, the nodules are so abundant that they form a solid mass. Histologically, a range of osteocartilaginous features may be observed, including masses of cartilage or bodies in which cartilage and bone intermix, in some instances with a fatty marrow being present. Calcification is common. The synovium may reveal metaplasia of the lining and minute cartilaginous bodies. The cytological features of the lesion often cause concern since atypical nuclei may be observed in cartilage cells. However, the degree of atypia is compatible with a benign lesion (Lichtenstein, 1972; Spjut *et al.*, 1971).

B. Synovial Chondrosarcoma

Synovial chondrosarcoma is a rare tumor arising from synovial membranes rather than bone. Grossly, the tumor is a lobular mass involving the synovium, soft tissues, and at times bone. The cut surface is grey and shiny and frequently has areas of focal calcification. Microscopically, the lesion is similar to chondrosarcomas of bone that invade surrounding soft tissue as lobular masses. Cytologic features are those of malignant cartilage with plump nuclei, bizarre nuclei, and numerous multinucleated cells (Spjut *et al.*, 1971).

VI. NOTOCHORDAL AND NOTOCHORD-LIKE TUMORS

A. Chordoma

Chordomas are rare malignant tumors that arise from primitive notochordal tissue. Of 31,099 neoplasms of all kinds, chordoma accounted for only nine instances and represented only 5% of 1,639 bone tumors. Chordomas may occur in the posterior cranial fossa and at various locations along the axial skeleton down to the coccyx. The most common locations are the sacrococcygeal region (45%) and the cranial base (39%) (Steegmann, 1971). On occasion, chordomas can be found in locations such as the orbit, frontal sinus, maxilla, mandible, rectum, sigmoid, prevertebral region, intrameningeal membranes, and extrachordal cervical regions. In many instances,

they appear to represent secondary extensions, although direct connections are not always observed at autopsy. Benign ecchordoses, representing heterotopic notochordal tissue, are found only in approximately 2% of all autopsies, most commonly in the region of the sphenooccipital synchondrosis, but occasionally elsewhere along the path of the original notochord. Chordomas may occur at any age, most commonly during the third, fourth, and fifth decades. Males are affected more commonly than females (3 : 2) (Jaffe, 1964; Lichtenstein, 1972; Steegmann, 1971).

Clinical signs and symptoms are commonly detectable earlier in chordomas at the intracranial level because of the location near the cranial nerves and brainstem tracts. In sacrococcygeal chordomas, the tumor may grow to a considerable size before being detected. The incidence of trauma preceding chordomas of the sacrococcygeal region seems to be more frequent than can be attributed to chance.

Radiographically, chordomas in the region of the clivus are usually found limited to the area of the midline initially but with further growth may be found to extend into the sphenoidal sinuses or beyond. Erosive destruction of the sphenoid may be accompanied by scattered radiopacities representing calcification within the lesion. Chordomas appear as expanded, rarified, destructive lesions which may have some evidence of calcification (Jaffe, 1964; Lichtenstein, 1972).

Grossly, chordomas vary widely in size, extent, and consistency. Usually they are much softer than cartilage. They may present a lobular pattern and may be solid or gelatinous with large cystic cavities filled with a mucoid-like material (Jaffe, 1964; Lichtenstein, 1972; Steegmann, 1971).

Microscopically (Fig. 4), masses or chains of epithelial-appearing cells with indistinct boundaries are observed. Some of these may have densely granular cytoplasm. Cells may also be trabeculated or appear as cavities lined by cuboidal cells. Vacuolated cells filled with mucinous material within the cell cytoplasm and in the intercellular spaces produce the characteristic "soap-bubbly" or physaliferous appearance of chordoma. Some islands of cells may be surrounded by myxoid-appearing tissue resembling chondroma or chondrosarcoma. Other areas of dense cellularity may suggest carcinoma. Mitotic figures tend to be observed only in instances that are very malignant (Steegmann, 1971).

Chordomas tend to be slow growing, being locally invasive. They do not metastasize except for an occasional aggressive malignancy that produces widespread metastasis. The only palliative therapy for chordoma is wide surgical excision. Although roentgen therapy is not very effective, it is frequently utilized when the tumor cannot be removed surgically, or sometimes in conjunction with surgical removal. New surgical techniques and treatment with radioactive materials have been used more recently (Steegmann, 1971).

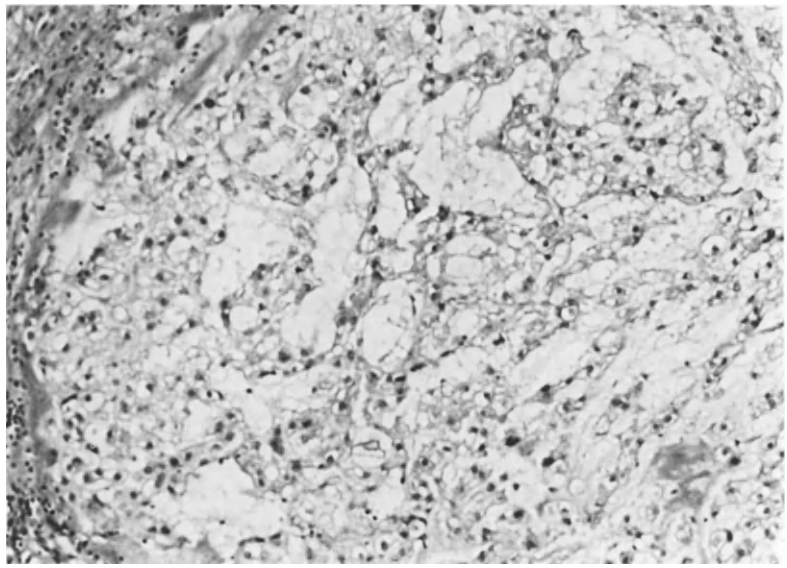

Fig. 4. Chordoma. Note chords of tumor cells and physaliferous appearance (hematoxylin and eosin).

Unusual Aspects of Chordoma

Several reports have noted unusual aspects of chordoma or chordoma-like lesions. Although the tumor almost always appears sporadically as an isolated lesion, it has been reported in a brother and sister, both having the same age of onset (Steegmann, 1971). In two other instances, chordoma has been present at birth in association with neurocutaneous syndromes—neurofibromatosis in one case, tuberous sclerosis in the other (Nix *et al.*, 1978). Ackerman and co-workers (1974) reported a spectacular case in an infant with 18 subcutaneous nodules; the histological, histochemical, and electron microscopic features were those of chordoma, but many of the lesions regressed and disappeared spontaneously, and there was no evidence of bony or systemic involvement.

B. Chordoid Sarcoma

Chordoid sarcoma is a rare tumor that arises from soft tissue or bone and exhibits less aggressive behavior than classic chondrosarcoma of bone. The tumor has been reported under a variety of different names including chordoma peripheric um, giant chordoma-like tumor, chondroid chordoma, and parachordoma. At the light microscopic level, chordoid sarcoma tends to resemble chordoma, although it may sometimes bear similarities to clear cell

chondrosarcoma or extraskeletal myxoid chondrosarcoma. The histochemical and ultrastructural findings reported by Pardo-Mindan *et al.* (1981), although in need of confirmation, suggest that chordoid sarcoma represents a variety of chondrosarcoma.

VII. OTHER CONDITIONS WITH CARTILAGINOUS FOCI, CHONDROMATOUS METAPLASIA, AND CARTILAGINOUS REMNANTS

Cartilage may arise by heteroplasia in which the tissue arises developmentally in an unusual location by differentiation *in situ,* not by dislocation of the tissue. Heteroplastic cartilage has been observed in the tonsils and peritonsillar tissues, uterus, and especially in hypoplastic and cystic kidneys (Willis, 1962, and see Chapter 1 in this volume).

Ear tags sometimes containing heterotopic cartilage together with fibrous and adipose tissue may be observed anywhere from the ear to the corner of the mouth. They may also be observed below the angle of the mandible or on the anterior neck just medial to the sternocleidomastoid muscle. They represent persistent, or in some instances, supernumerary branchial arch remnants. They may occur as isolated anomalies, singly, multiply, or bilaterally, or with a variety of other abnormalities making up various branchial arch syndromes. Solitary ear tags and branchial arch syndromes are etiologically heterogeneous and sometimes hereditary (Gorlin *et al.,* 1976).

Cartilaginous foci may appear in various tumors, either as primary tumor components or as stromal elements. Cartilage is observed in some teratomas, most frequently in association with respiratory-like cavities. In mixed hepatoblastomas, immature hepatic cells are found in association with fibrous tissue, cartilage, osteoid tissue, or bone as primary components, not as stromal elements (Willis, 1962). Congenital midline dorsal hamartomas are composed of mesodermal elements including cartilage, bone, fat, fibrous tissue, and muscle. The well-differentiated cellular components are mature, lacking the primitive organoid structures and neoplastic characteristics of teratomas. The lesion is a distinct clinicopathologic entity that is sometimes confused with meningomyelocele or teratomas in the newborn infant (Tibbs *et al.,* 1976).

Cartilaginous foci may be present in a variety of other tumors. Pleomorphic adenomas of salivary gland origin have foci of mature cartilage, the origin of which has been the subject of prolonged debate (Fig. 5). At present, it is unresolved whether the cartilage arises as part of a genuine mixed tumor or whether the myoepithelial cells have the capacity to produce cartilage

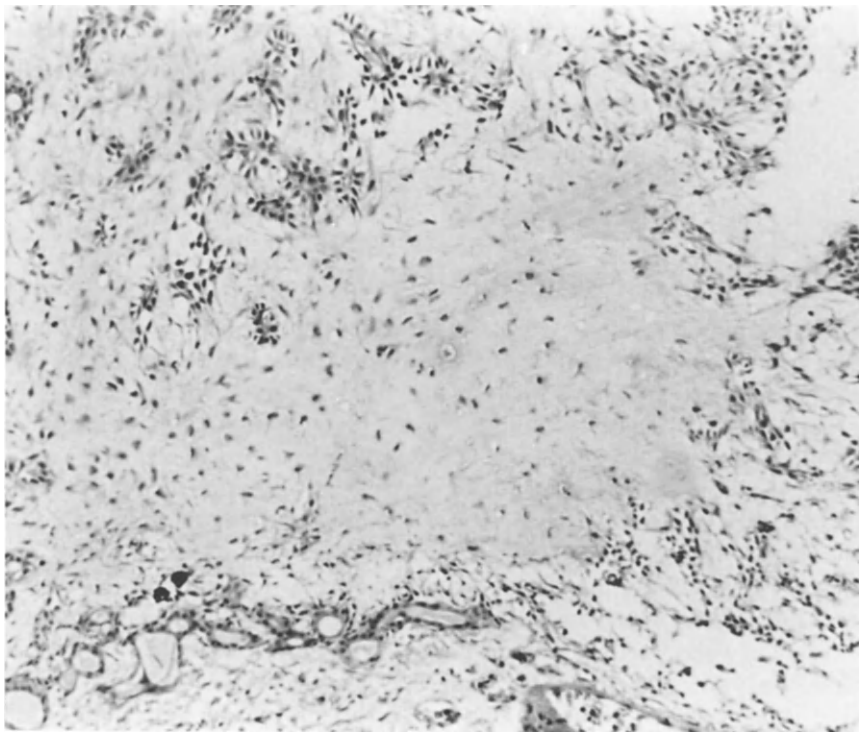

Fig. 5. Pleomorphic adenoma from parotid gland, showing area of chondroid differentiation (hematoxylin and eosin).

(Evans and Cruickshank, 1970). Cartilaginous foci are frequently observed in juvenile aponeurotic fibroma. The presence of cartilage can be explained by the well known capacity of periosteum, tendons, and aponeuroses to demonstrate focal cartilaginous differentiation (Keasbey, 1953; Corio *et al.*, 1981). Cartilaginous metaplasia has been observed in the stroma of many tumors including sweat gland neoplasms and carcinomas of the stomach, intestine, gallbladder, pancreas, endometrium, and prostate. It occurs rarely in carcinoma of the breast, carcinoma of the kidney, and cystadenocarcinoma of the ovary (Willis, 1962).

Chondromatous metaplasia may arise on the basis of irritation or inflammation. It may be observed in the soft tissue or periosteal areas of the oral cavity in association with prolonged irritation, such as that found in some instances of ill-fitting dentures (Cutright, 1972). Focal metaplastic cartilage may also be observed with myositis ossificans and with chronic inflammation of the kidney (Taxy and Filmer, 1975).

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6

Mutations Affecting Limb Cartilage *

Paul F. Goetinck

I. INTRODUCTION

A large number of hereditary abnormalities affecting the limbs have been described in humans and animals. The conditions in humans have been analyzed from a medical point of view and have been described using clinical and radiological criteria (Rimoin, 1975; Sillence *et al.*, 1979). The conditions in the animals have been viewed mostly as developmental anomalies that can be used to study the role of specific genes in developmental processes. In the animal models the analyses of the mutants have been instrumental in establishing or confirming developmental events of the limbs and have led to the elucidation of the molecular basis of certain hereditary developmental diseases (Zwilling, 1956; Goetinck, 1966; Goetinck *et al.* 1981).

When analyzing the underlying causes of skeletal abnormalities, it is important to take into consideration the sequence of developmental events preceding the formation of the skeleton as well as those taking place during its formation. In the limb the early developmental events are characterized by a number of reciprocal interactions between the mesoderm and the ectoderm, as well as among mesodermal cells (Ede *et al.*, 1977; Kelley and Fallon, 1981; Fallon *et al.*, 1982). These interactions lead to the outgrowth of the limb and

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dictate many of the final morphogenetic properties of the limb. This phase in limb development was designated by Zwilling (1968) as the morphogenetic phase. During this phase the mesoderm is histologically homogeneous, although differences in the mesodermal cells can be demonstrated by other means. There are a number of examples in which the cartilaginous skeleton of the limb is abnormal as a result of mutant genes during the morphogenetic phase. Included in this group are the mutant genes that alter the normal interactions between the mesoderm and the ectoderm and thereby lead to limbs that have either excessive or deficient limb parts (Zwilling, 1956; Goetinck, 1966; MacCabe *et al.*, 1975).

The first microscopically visible event in the mesoderm is the condensation of the cells in its central core (Fell and Canti, 1934). This event precedes the phase of cytodifferentiation designated by Zwilling (1968) as the second phase in the development of the limb. The cells that are present in the central core of the limb will undergo chondrogenesis during this phase of cytodifferentiation. The formation of cellular condensation in the central mesoderm has also been shown to be affected by certain mutations. The work from Elmer's laboratory (Elmer and Selleck, 1975; Hewitt and Elmer, 1978; Duke and Elmer, 1977, 1979) has clearly shown that the altered gene activity in brachypodia leads to abnormal mesodermal condensations which in turn lead to the abnormal development of the cartilaginous skeleton. It is evident, therefore, that abnormal morphogenesis of the cartilaginous limb skeleton can result from mutant gene action expressed in the morphogenetic phase. The chondrocytes that do differentiate in these mutants may be completely normal in their ultrastructure and in the biosynthesis of their tissue-specific macromolecules. These cartilaginous skeletal mutants are being distinguished from another class in which the cartilaginous skeleton is defective because the mutant genes are expressed in the differentiated chondrocytes. The present discussion will be restricted to this second class of inherited abnormalities of cartilage.

II. CARTILAGE

A. Chondrocytes

Most chondrocytes differentiate from cells that are of mesodermal origin. The most studied of these chondrocytes are those of the sternum, vertebrae, and limb. In each of these organs the phase of cytodifferentiation is preceded by condensation of the mesodermal cells.

In contrast to the chondrocytes that are of mesodermal origin, the chondrocytes of Meckel's cartilage and the hypobranchial skeleton are derived from the neural crest that originates in the ectoderm (Goetinck *et al.*, 1981; and see Volume 2, Chapter 5).

B. Extracellular Matrix Macromolecules of Cartilage

The changes in the biosynthetic patterns of macromolecules associated with chondrogenesis involve both repression and activation of genetic material. The differentiated chondrocytes are characterized by the synthesis of a set of unique extracellular matrix macromolecules. These are cartilage-specific proteoglycan, proteoglycan link protein, type II collagen, and chondronectin.

1. Proteoglycans

a. Structure. The current model for the structure of sulfated proteoglycans of cartilage has been derived mainly from studies on proteoglycan from bovine nasal septum, pig laryngeal cartilage, Swarm rat chondrosarcoma, and chick limb-bud chondrocytes (Hascall, 1977, and see Volume 1, Chapter 8). The proteoglycan monomer consists of a core protein of about 2.0×10^5 daltons to which are attached two types of sulfated side chains and two types of oligosaccharides. A diagrammatic representation of a cartilage proteoglycan molecule is given in Fig. 1.

The sulfated carbohydrate chains in cartilage proteoglycan are chondroitin sulfate and keratan sulfate (Rodén and Horowitz, 1978). Chondroitin sulfate, a repeating unit of glucuronic acid and sulfated *N*-acetylgalactosamine, is linked to core protein via a linkage region consisting of glucuronic acid–galactose–galactose–xylose. The linkage to the core protein is through an O-glycosidic bond between xylose and the hydroxyl group of serine. Sulfation can be either at the 4- or the 6-position of the *N*-acetylgalactosamine. Keratan sulfate, a repeating disaccharide of *N*-acetylglucosamine and galactose, is linked, in cartilage, to core protein by an O-glycosidic bond between *N*-acetylgalactosamine and hydroxyl groups of serine or threonine. Keratan sulfate occupies the 6-position of the linkage *N*-acetylgalactosamine and position 3 of this linkage amino sugar is occupied by a disaccharide, galactose-*N*-acetylneuraminic acid. The *N*-acetylglucosamine and variably the galactose residues are sulfated.

A third type of sulfated glycosaminoglycan, dermatan sulfate, has been reported in some cartilage proteoglycans. This glycosaminoglycan is a chondroitin sulfate in which the principal uronic acid is L-iduronic acid. Dermatan sulfate has been reported in minor cartilage-proteoglycan fractions (Kimata *et al.*, 1978), in proteoglycans synthesized by dedifferentiated human-chondrocyte cultures (Oegema and Thompson, 1981), and in a proteoglycan found in bovine fetal epiphyseal cartilage (Pal *et al.*, 1981).

In addition to the glycosaminoglycans, two classes of oligosaccharides have been described in proteoglycans from chick limb-bud chondrocytes and Swarm rat chondrosarcomata (DeLuca *et al.*, 1980; Lohmander *et al.*, 1980). One class, made up of three structurally related oligosaccharides of different

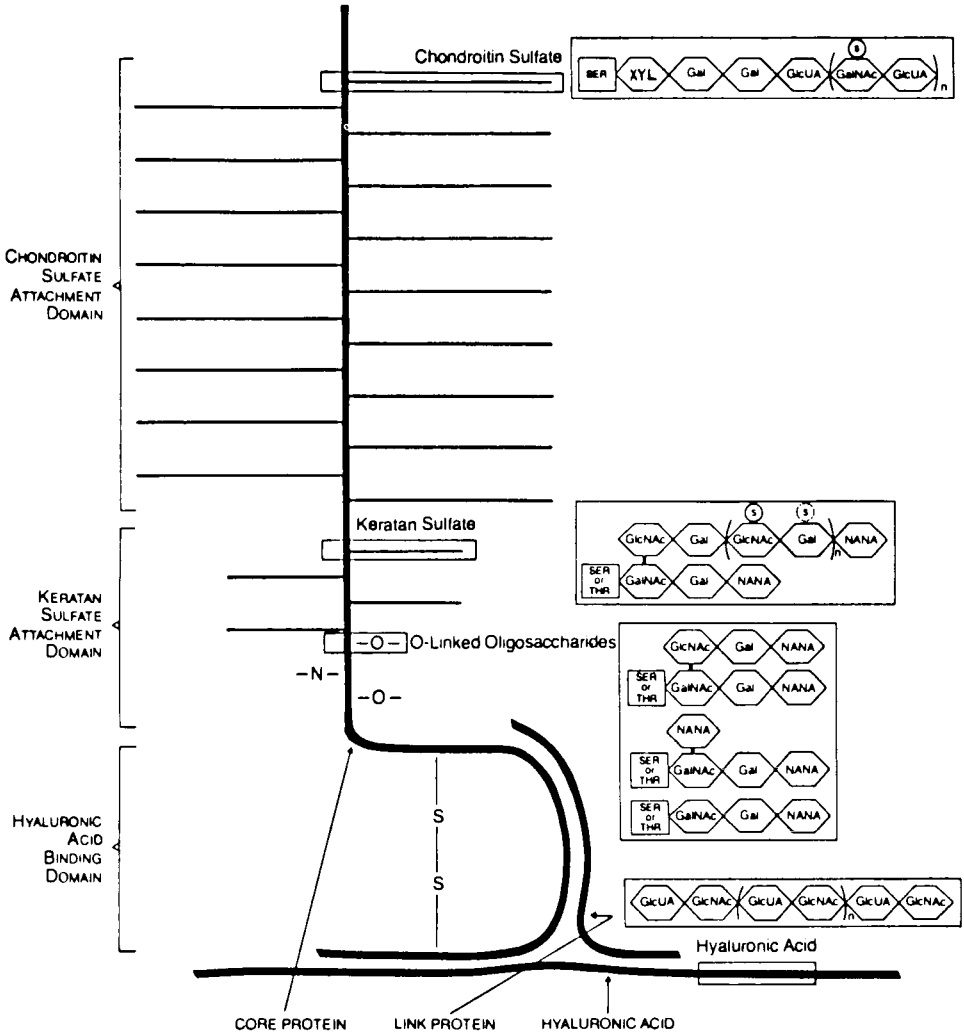

Fig. 1. Schematic representation of a proteoglycan monomer interacting with hyaluronic acid and link protein. A compositional diagrammatic representation of chondroitin sulfate, keratan sulfate, the O-linked oligosaccharides, and hyaluronic acid is presented in the insets. Gal, galactose; GalNac, *N*-acetylgalactosamine; GlcNac, *N*-acetylglycosamine; GlcUA, glururonic acid; NANA, *N*-acetylneuraminic acid; S, sulfate; SER, serine; THR, threonine; XYL, xylose. Reproduced from Goetinck (1982) with the permission of the publisher.

sizes, is a neuraminic acid-rich oligosaccharide which is linked to core protein via the same linkage as keratan sulfate. The second class, a neuraminic acid containing mannose-rich oligosaccharide appears to be linked to asparagine residues through *N*-glycosylamine bonds to *N*-acetylglucosamine.

The proposed generalized model of proteoglycan separates the molecule into three domains: a hyaluronic acid-binding domain which is relatively free of carbohydrate represents one end of the core protein through which the proteoglycan interacts with hyaluronic acid to form aggregates; adjacent to the hyaluronic acid-binding domain is the keratan sulfate-attachment domain of the core protein; and finally, the other end of the core protein represents the chondroitin sulfate-attachment domain. The hyaluronic acid-binding domain can also interact with link protein. The interaction of link protein with the proteoglycan and the hyaluronic acid serves to stabilize the aggregate structure.

b. Synthesis. The synthesis of proteoglycans involves a large number of biosynthetic steps. The earliest events studied so far are those associated with the translation of the core protein mRNA. No information is presently available about either the structure of the genetic material that codes for core protein or any of the possible modifications of the primary product of transcription.

The cell-free translation product of chick embryonic chondrocyte core protein is estimated to be about 340,000 daltons (Upholt *et al.*, 1979). A considerable amount of protein processing must take place between the initial translation product and the final proteoglycan core protein which is estimated to be about 200,000 daltons. One of these steps is likely to be the removal of a signal peptide, but this modification may not be enough to account for the entire difference between the molecular weight of the cell-free translation product and the final core protein as determined by biophysical methods.

The synthesis of the core protein is followed by a series of posttranslational modifications involving the synthesis of the glycosaminoglycans and oligosaccharides. Chondroitin sulfate synthesis begins with the transfer of xylose to serine residues of the core protein followed by the sequential addition of galactose, galactose, and glucuronic acid residues and the synthesis of the repeating disaccharide. The addition of these monosaccharides occurs from the appropriate nucleotide diphosphate-donor form through the action of specific glycosyltransferases. Keratan sulfate and the O-linked oligosaccharides that possess structural similarities to keratan sulfate are also the result of the activity of glycosyltransferases resulting in the transfer of *N*-acetylgalactosamine, galactose, and neuraminic acid. The N-linked oligosaccharides that are rich in mannose presumably require the enzymatic machinery for the synthesis of dolichol intermediates and the synthesis of the mannose complex generally found in the synthesis of glycoproteins.

Sulfation of the glycosaminoglycans involves the synthesis of 3′-phosphoadenosine 5′-phosphosulfate (PAPS) and the transfer of the sulfate from this donor form to the appropriate acceptor.

A general sequence of the steps involved in the biosynthesis of cartilage proteoglycans consists of

1. Transcription of the gene for core protein
2. Posttranscriptional processing and modification of primary product of transcription.
3. Synthesis of core protein
   A. Initial translation of core protein mRNA
   B. Posttranslational processing of core protein
4. Posttranslational modification of core protein
   A. Glycosylation, involving synthesis of nucleotide diphosphate sugars, synthesis of the glycosaminoglycans chondroitin sulfate (synthesis of carbohydrate–protein linkage region and repeating disaccharides; sulfation, see Section IV,B) and keratan sulfate (synthesis of carbohydrate–protein linkage and repeating disaccharides; sulfation, see Section IV,B), and synthesis of O-linked and N-linked oligosaccharides
   B. Sulfation, involving synthesis of PAPS and transfer of sulfate to acceptor

This sequence of the steps involved in the biosynthesis of a proteoglycan is not intended to be complete. Rather, it is designed to indicate the progression of and the complexities in the steps involved in the synthesis of this macromolecule. Clearly the posttranslational modification of core protein requires the activity of a large number of enzymes. These include the enzymes involved in the synthesis of the various nucleotide diphosphate sugars, the glycosyltransferases, and those involved in the activation and transfer of sulfate.

The synthesis of a complex macromolecule such as a proteoglycan, therefore, requires the coordinated activity of a large number of genes. Any one of these genes is a potential site for developmental regulation whereby the structure and, as a consequence, the function of the proteoglycans may be altered. Furthermore, mutations affecting either the structure or the regulation of any of the many genes involved in the synthesis of proteoglycans may lead to the absence of the macromolecule or to a proteoglycan with altered properties. Of the several cartilage-specific macromolecules known, proteoglycans are the only ones to have been shown to be affected by specific mutations. These will be described in more detail later.

2. Link Protein

Proteoglycans from all cartilaginous sources have been shown to interact with hyaluronic acid to form aggregates. The binding of proteoglycan monomer to hyaluronic acid is stabilized by the addition of one or more link

proteins (Hardingham, 1979; Tang *et al.*, 1979). Link proteins have been shown to bind to both proteoglycan monomer and hyaluronic acid (Caterson and Baker, 1978). The number and relative amounts of link proteins in a cartilage extract varies depending on the source. As many as three link proteins have been described in bovine nasal-septum cartilage (Caterson and Baker, 1979) and juvenile xyphoid process (McKeown-Longo *et al.*, 1982). The major band in avian embryonic cartilage has a molecular weight of approximately 48,000. No mutations affecting link protein have been described.

3. *Collagen*

The predominant type of collagen synthesized by chondrocytes is genetically distinct from that synthesized by other tissues (Miller, 1977; and see Volume 1, Chapter 7). Cartilage collagen (type II) contains three identical polypeptide chains [α1(II)] in contrast to the collagen of skin and bone (type I) which consists of two [α1(I)] and one α2 chain. Other major types of tissue-specific collagen are made up of yet other genetically distinct collagen molecules. In the limb, precartilaginous mesenchyme synthesizes type I collagen. The synthesis of this type ceases in chondrocytes and type II collagen synthesis is initiated. When cartilage is replaced by bone, type II collagen synthesis ceases and type I collagen synthesis is resumed (von der Mark, 1980). A number of minor collagen types have been reported in cartilage in addition to the major type II component (Butler *et al.*, 1977; Rhodes and Miller, 1978; Bergeson and Hollister, 1979; Shimokomaki *et al.*, 1980; Ayad *et al.*, 1981; Reese and Mayne, 1981).

The biosynthesis of collagen beginning with the primary transcriptional product of the collagen gene involves a large number of posttranscriptional and posttranslational modifications. This aspect of collagen metabolism has been thoroughly reviewed by Olson (1981; and see Volume 1, Chapter 7). The reader is also referred to a review on collagen metabolism by Minor (1980) which contains a description of a number of heritable diseases of collagen. None of these diseases, however, seem to be specifically related to cartilage.

4. *Chondronectin*

Chondronectin (Hewitt *et al.*, 1980) is a cartilage-specific glycoprotein (MW = 180,000) that upon reduction yields subunits with a molecular weight of approximately 80,000 (Kleinman *et al.*, 1981). Chondronectin plays a role in the attachment of chondrocytes to collagen. Cartilage proteoglycans are directly involved in this attachment process (Hewitt *et al.*, 1981). The nature of the molecular interactions in the attachment process are not

known. No mutations affecting this component of the extracellular matrix have been described.

III. MUTATIONS AFFECTING LIMB CARTILAGE

Several micromelic conditions have been described in animals. In the chicken there is chondrodystrophy (Lamoreux, 1942), Creeper (Landauer and Dunn, 1930), micromelia-Hays (Hays, 1944), micromelia VII (Bernier, 1951), micromelia-Kawahara (Kawahara, 1956), micromelia-Abbott (Landauer, 1965a), and nanomelia (Landauer, 1965b). In the Japanese quail there is chondrodystrophy (Collins *et al.*, 1968) and micromelia (Hill *et al.*, 1963). A chondrodystrophy has also been described in the turkey (Gaffney, 1975). In mice there is chondrodysplasia (Seegmiller *et al.*, 1971), cartilage anomaly (Johnson and Wise, 1971), cartilage matrix deficiency (Rittenhouse *et al.*, 1978), brachymorphism (Lane and Dickie, 1968), and disproportionate micromelia (Brown *et al.*, 1981). The effect on cartilage-specific macromolecules has been established in only a few of these mutations.

Quantitative effects have been described in disproportionate micromelia where the content of collagen is reduced and where the distribution of the collagen in the extracellular space is also abnormal. Proteoglycan content is also reduced in this mutant as it is in the chicken mutant micromelia-Abbott (Quintner and Goetinck, 1981). Although the levels of these macromolecules are reduced and their conditions may vary with developmental age, as in the case with proteoglycans in micromelia-Abbott, the portion of the extracellular matrix macromolecules that is present seems to be normal. Cartilage anomaly and mouse chondrodystrophy also have reduced levels of proteoglycans. In the mouse chondrodystrophy this reduced level may be the result of increased solubility of the proteoglycans. The ultrastructural appearance of the collagen fibrils in the cartilage of this mutant is also abnormal.

Qualitative changes in matrix macromolecules as a result of mutations have only been reported with respect to proteoglycans. Such mutations are brachymorphism in the mouse (Schwartz *et al.*, 1978; Sugahara and Schwartz, 1979), chondrodystrophy in the turkey (Leach and Buss, 1977), nanomelia in the chicken (Palmoski adn Goetinck, 1972; Goetinck and Pennypacker, 1977; Goetinck *et al.*, 1981), and cartilage matrix deficiency in the mouse (Kimata *et al.*, 1981). The information of these mutations will be reviewed in the following pages. They represent examples of mutations that affect this tissue-specific macromolecule at each of the three levels of the synthesis of proteoglycans, that is, sulfation (brachymorphism), glycosylation (turkey chondrodystrophy), and core protein (nanomelia and cartilage matrix deficiency).

IV. ANALYSIS OF MUTATIONS AFFECTING PROTEOGLYCAN STRUCTURE

Although all the cartilage hereditary conditions that have been analyzed in detail at the molecular level are in animals, it is reasonable to assume that some of the human hereditary cartilage diseases have a similar biochemical basis. Since, in most instances, one is dealing with mutations that are expressed during embryonic development and are often lethal, one is limited by the amount of material available for analysis. It is, however, possible to obtain information on the content and structure of proteoglycans from relatively small samples of cartilage.

In order to carry out these tests one does not have to depend on the presence of live chondrocytes in the cartilage. However, the measurements made on such tissues are representative of the total accumulated material, which reflects both the synthetic and degradative processes of the tissue.

If, on the other hand, the cartilage contains live chondrocytes the synthetic capacity of the cells can be screened with relative ease. By exposing the tissue to radiolabeled precursors of proteoglycans, information can be obtained on the synthetic ability of the chondrocytes and on the structural characteristics of the proteoglycans that are being synthesized. A general strategy that has evolved in the analysis of a number of animal embryonic mutants permits one to narrow down the hereditary defect to one of the many steps involved in the synthesis of proteoglycans. (References to the methods used in this strategy will be given when specific examples are discussed.)

This approach is particularly suitable for the analysis of chondroitin sulfate and core-protein synthesis. In this approach the tissues are exposed to radiolabeled inorganic sulfate and incorporation into proteoglycans is quantified. A reduction in sulfate incorporation would be indicative either of a reduced synthesis of normally structured proteoglycans or of the synthesis of a defective proteoglycan.

Information on the quality of the intact proteoglycan can be obtained from its sedimentation on sucrose or glycerol density gradients under dissociative conditions or by molecular-sieve chromatography. The structural integrity of the proteoglycans can also be measured by testing their ability to interact with hyaluronic acid to form aggregates or by challenging them with antibodies directed against normal proteoglycans.

Reduced sulfate incorporation associated with the synthesis of a defective proteoglycan could be the result of either a defect in the sulfation pathway or an alteration in the number or size of the glycosaminoglycans that act as acceptor molecules for the sulfate. A number of assays can be used to decide between these two possibilities.

Information on the structure of the glycosaminoglycan side chains can be obtained by analyzing the isolated chains by either ion-exchange or molecular-sieve chromatography. Undersulfation would be reflected in an altered behavior of the glycosaminoglycans on ion-exchange chromatography. If such a change were indicated, the enzymes in the sulfation pathway could then be analyzed directly. A reduction in either the size or the number of carbohydrate side chains that act as sulfate acceptors could be the result of a defect in a number of the steps in the enzymatic machinery that synthesizes the glycosaminoglycans. This could be at the level of the synthesis of the nucleotide diphosphate sugars, or in the activity of the xylosyltransferase or the glycosyltransferases that function distally to the xylose residues. The use of xylosides as exogenous acceptors for the synthesis of chondroitin sulfate can distinguish among some of these possibilities. Finally reduced sulfation can result from a complete absence of glycosaminoglycans as a result of an absence of core protein.

In summary, defects in proteoglycan structure can be narrowed down, with relative ease, to one of three general compartments in the biosynthesis of proteoglycans (i.e., sulfation, glycosylation, or core-protein synthesis). Examples of mutations acting at each of these levels follow.

A. Aberrant Sulfation

Brachymorphism in mice results from homozygosity for an autosomal recessive gene (Lane and Dickie, 1968). These mice are viable and are characterized by having severely shortened limbs. Histological studies have indicated that the extracellular matrix of the cartilage of these mutants reacts poorly with stains specific for sulfated glycosaminoglycans (Orkin *et al.*, 1977). Ultrastructurally, the proteoglycan matrix granules of the mutant are smaller than normal and are present in reduced number. A network of thin filaments associated with collagen can be seen in the extracellular matrix of the mutant cartilage. This filamentous network can be extracted with 4 *M* guanidine hydrochloride and is thought to represent the defective proteoglycans.

The first biochemical evidence that the proteoglycans of brachymorphic cartilage are defective came from studies indicating that the cartilage contained normal levels of undersulfated glycosaminoglycans. This reduction in sulfation was reflected in the behavior of the glycosaminoglycans on ion-exchange chromatography and electrophoresis which indicated that they were less negatively charged than those of normal. Incorporation of radiolabeled sulfate in chondroitin sulfate is also reduced in the mutant chondrocytes (Orkin *et al.*, 1976).

These observations suggested a defect in one of the enzymatic steps in the sulfation pathway of glycosaminoglycans (Fig. 2). When sulfation of desul-

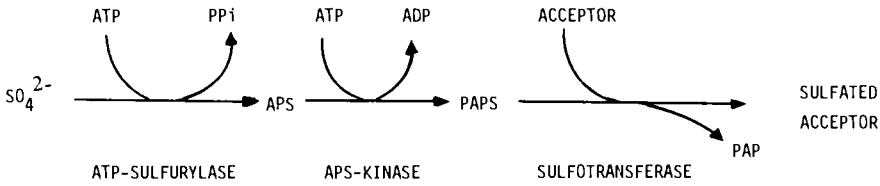

Fig. 2. Sulfate activation pathway. APS, adenosine 5′-phosphosulfate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; ATP-sulfurylase, ATP: sulfate adenylyltransferase; ATP-kinase, ATP: adenylylsulfate phosphotransferase.

fated chondroitin sulfate is initiated with [^{35}S]sulfate and ATP, brachymorphic cartilage extracts catalyze the transfer of the radioactivity onto the acceptor at levels that are 30% of normal. This difference between normal and mutants is not observed when sulfation is initiated from [^{35}S]PAPS (Schwartz *et al.*, 1978). These results indicate that the sulfotransferase activity is normal in the mutant and that the defect lies in the synthesis of PAPS. Initial results suggested that the mutation affected the APS-kinase (Sugahara and Schwartz, 1979) but more recently it has been reported that both ATP-sulfurylase and ATP-kinase are affected (Schwartz *et al.*, 1982). In order to explain the effect of one mutation on the two enzymes, Schwartz and associates (1982) have suggested that the mutation may affect one subunit shared by both enzymes.

The mutation affects only proteoglycans in the cartilage. Normal quantities of type II collagen with normal ultrastructural morphology are present in the mutant (Orkin *et al.*, 1976, 1977). Although it is a cartilage-specific macromolecule that is affected by the mutation, the sulfate-activation pathway is not thought to be specific for this tissue. Indeed, the altered enzymatic activities are also observed in mutant liver extracts (Sugahara and Schwartz, 1979). The phenotypic expression of the gene in cartilage may result from the fact that the small amount of PAPS synthesized may not be sufficient for the complete sulfation of large quantities of proteoglycans. In other tissues, the low levels of PAPS may be sufficient for the complete sulfation of the relatively lower levels of acceptor molecules synthesized. Supporting this view are the recent findings of Pennypacker *et al.* (1981) who reported that sulfation of glycosaminoglycans of brachymorphic skin fibroblast and prechondrogenic limb-mesenchymal cells was normal, although the PAPS-synthesizing activity was reduced in these tissues. In addition, the synthesis of glycosaminoglycans under the influence of ß-D-xyloside could not be stimulated to the same extent in the mutant cultures as in controls.

An alternative explanation to the generalized biochemical defect in brachymorphic mice has been suggested by Sugahara and Schwartz (1979). These authors have proposed that there may be genetically distinct and tissue-specific sulfation pathways because they could not detect a decrease in

TABLE I

The Effect of the Recessive Lethal Gene Chondrodystrophy (*ch/ch*) on Turkey Cartilage Proteoglycan

		[^{35}S]Sulfate incorporation	
	Galactosamine[a]	DPM/sternum	DPM/10^5 cells
Normal	1.80	27,139	5994
ch/ch	0.58	7,788	1570
$\frac{\text{Mutant}}{\text{Normal}} \times 100$	32.2	28.7	26.2

[a] Mg/gm of dry cartilage (Leach and Buss, 1977).

PAPS synthesis in skin extracts from brachymorphic mice. The glycosaminoglycans from skins of brachymorphic mice have also been shown to have electrophoretic properties similar to those from normal skins (Orkin *et al.*, 1976). More detailed studies of the enzymes involved in the sulfation pathway will be required before a definitive conclusion can be reached.

B. Aberrant Glycosylation

In collaboration with E. G. Buss from Pennsylvania State University we have begun an examination of the action of the recessive lethal chondrodystrophy (*ch/ch*) mutation in the turkey (Gaffney, 1975). The results obtained so far suggest that this mutation may affect the glycosylation of cartilage proteoglycans. The cartilage of this mutant contains 32% of the galactosamine found in normal cartilage (Leach and Buss, 1977; Table I). [^{35}S] sulfate incorporation by the mutant chondrocytes is reduced to the same extent (Table I), indicating that the reduced levels of galactosamine are not the result of excessive degradation but rather of a reduction in synthesis. The incorporation of [^{35}S]sulfate into sulfated glycosaminoglycans can be increased in the mutant by the addition of *para*-nitrophenyl-ß-D-xyloside to the medium in which the chondrocytes are cultured (Fig. 3). Xylosides can stimulate chondroitin sulfate synthesis in the absence of core protein (Brett and Robinson, 1971; Levitt and Dorfman, 1973; Schwartz *et al.*, 1974; Galligani *et al.*, 1975; Robinson *et al.*, 1975) and therefore can be used as an indirect measure of the activity of the glycosyltransferases distal to xylosyltransferase. If [^{35}S]sulfate is used as a measure of synthesis the test also measures indirectly the enzymes in the sulfation pathway. Since [^{35}S]sulfate incorporation in this mutant can be increased by a xyloside to the same levels as in similarly treated normals we conclude that the mutation does not affect the synthesis of chondroitin sulfate.

Analysis of the sulfated glycosaminoglycans by molecular-sieve chromatography indicates that the size of the mutant glycosaminoglycans is similar

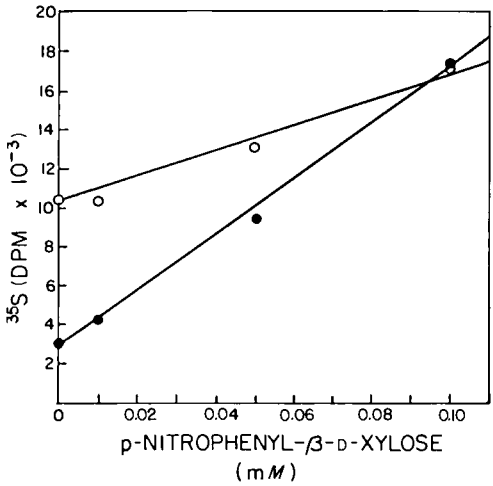

Fig. 3. Stimulation of chondroitin sulfate synthesis in normal and chondrodystrophic turkey chondrocytes by *para*-nitrophenyl-ß-D-xylose. ○, Normal; ●, mutant.

to those of normal proteoglycans (Fig. 4). However, the structure of the proteoglycan in the mutant is clearly not normal as indicated by its sedimentation rate in dissociative 5–20% sucrose density gradients. The mutant proteoglycan sediments more slowly on such gradients than do normal proteoglycans (Fig. 5). As approximately 90% of the molecular weight of proteoglycans is contributed by the carbohydrate components of the molecule these results are interpreted to mean that there are fewer sulfated glycosaminoglycan chains per proteoglycan molecule. Such a situation could exist either on a core protein of normal length or as a result of a shortened core protein.

In order to test these two alternatives we labeled chondrocytes with [^{35}S]cysteine and electrophoresed the intracellular proteins on polyacrylamide gels. The results from this test indicate that there is an intracellular protein in the mutant chondrocytes that is recognized by antibodies to juvenile chicken proteoglycan monomers (Sparks *et al.*, 1980). The molecular weight of this protein in this mutant is identical to that found in the chondrocytes from normal turkeys and normal chickens, approximately 246,000 (Argraves *et al.*, 1981). Taken together these results suggest that the turkey mutant proteoglycan has fewer carbohydrate side chains per core protein of normal length. The present working hypothesis is that the mutation may affect the synthesis of UDP-xylose or the xylosyltransferase reaction. Experiments are being planned to test this hypothesis. Additional tests on the proteoglycans from the chondrodystrophic turkey indicate that they can

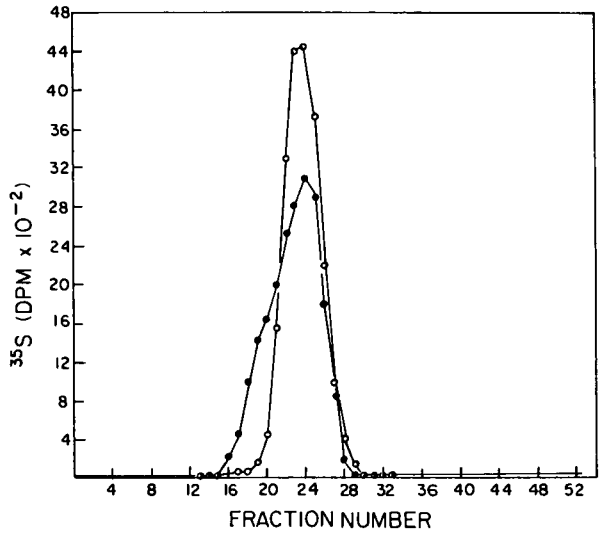

Fig. 4. Molecular-sieve chromatography on controlled-pore glass (CPG 500) of [^{35}S]sulfate glycosaminoglycans isolated by alkaline hydrolysis from a cartilage extract that chromatographed in the void volume of a CPG 1400 column. ○, Normal; ●, mutant.

exist as an aggregate and that they are immunologically indistinguishable from normal turkey proteogylcans in their interaction with antibodies elicited against juvenile-chicken proteoglycan monomer.

C. Core-Protein Mutations

1. Nanomelia in Chickens

Nanomelia is inherited as a single recessive lethal autosomal gene (Landauer, 1965b). The nanomelic embryos are characterized by having shortened limbs and a parrot-like beak. Initial studies on this mutant indicated that its cartilage contains about 10% of normal levels of chondroitin sulfate (Mathews, 1967). These reduced levels of chondroitin sulfate were shown to result from decreased synthesis and not from increased degradation (Fraser and Goetinck, 1971). A comparison of the proteoglycans of normal and nanomelic chondrocytes by molecular-sieve chromatography revealed that there exist two populations of proteoglycans in normal cartilage and that the major population, which makes up 90% of the total, is lacking in the mutant. The restriction of the defect in this mutant to cartilaginous structures led to the suggestion that the major proteoglycan was a cartilage-specific macromolecule (Palmoski and Goetinck, 1972). This suggestion has been

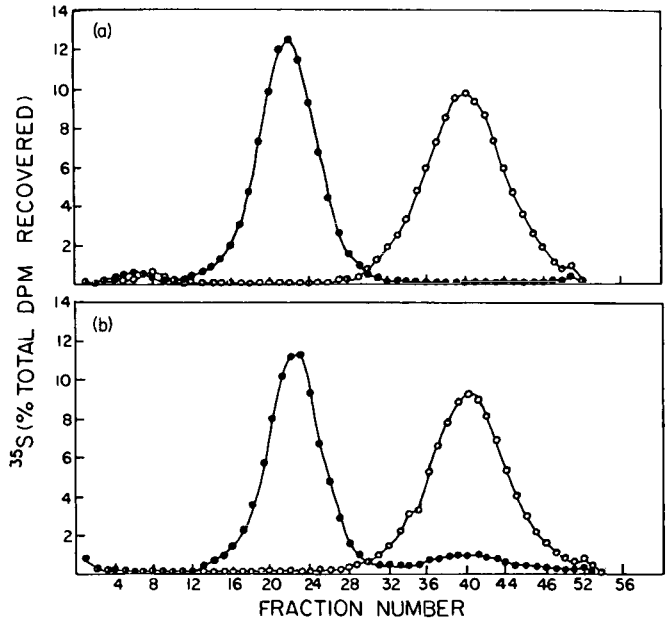

Fig. 5. Dissociative sucrose density-gradient profiles of ^{35}S-labeled proteoglycans from normal (○) and chondrodystrophic (●) turkey cartilage. (a) The extract was sedimented directly. (b) The extract was first chromatographed on CPG 1400 and the void volume material was sedimented. Direction of sedimentation is from left to right.

substantiated by the finding that proteoglycan synthesis in skin fibroblasts and in limb mesenchymal cells is not affected in the mutant (Goetinck and Pennypacker, 1977; Sawyer and Goetinck, 1981; Goetinck, 1982).

Specific action of the mutation on cartilage proteoglycans is also evident from studies indicating that the mutant gene does not affect other cartilage-specific macromolecules. Type II collagen is synthesized by the mutant chondrocytes in normal quantity and with a normal ultrastructural morphology (Pennypacker and Goetinck, 1976). The mutant chondrocytes also synthesize link protein which is present in normal quantities and is functionally and immunologically indistinguishable from link protein of normal chondrocytes (McKeown-Longo, 1981). The nanomelic mutation is expressed in Meckel's cartilage as well as in sternal or limb cartilage (Fig. 6). This indicates that the different developmental histories of the cartilage precursor cells have no effect on the expression of the mutant gene in the differentiated tissue (McKeown and Goetinck, 1979).

An analysis of the capacity of nanomelic chondrocytes to synthesize chondroitin sulfate by exposing them to *para*-nitrophenyl-ß-D-xyloside indicates that the mutant cells are fully capable of such synthesis if an appropriate ac-

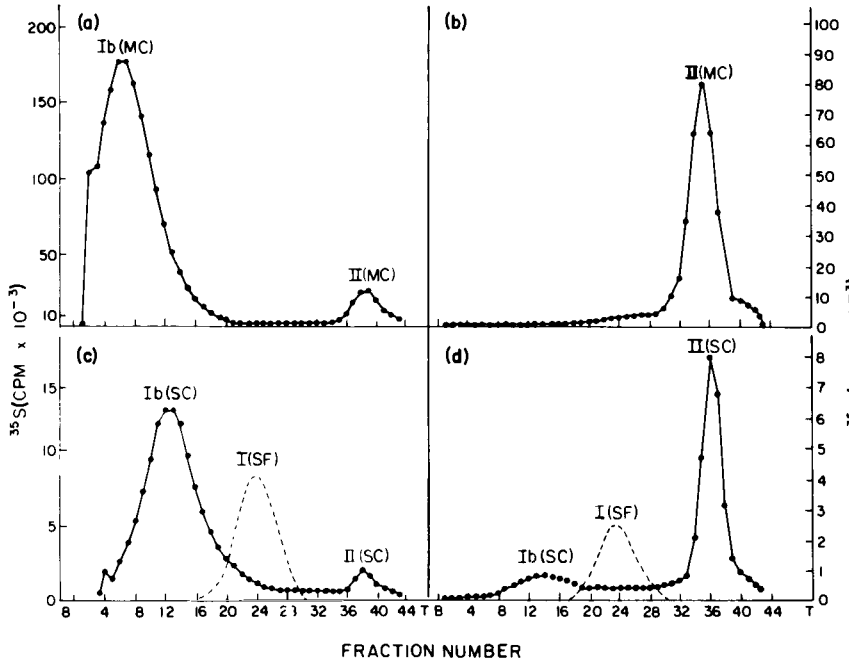

Fig. 6. Dissociative 5–20% sucrose density-gradient profiles of [^{35}S]sulfate-labeled proteoglycans from extracts of (a) normal Meckel's cartilage, (b) nanomelic Meckel's cartilage, (c) normal sternal cartilage, and (d) nanomelic sternal cartilage. For comparison, the sedimentation profile of the main components of skin fibroblasts (SF) is indicated. B, bottom; T, top. Reproduced from McKeown and Goetinck (1979) with the permission of the publisher.

ceptor is provided. The chondroitin sulfate synthesized by the mutant chondrocytes in the presence of the xyloside is of the same size and composition as that synthesized by normal cells under similar conditions (Stearns and Goetinck, 1979). All these tests, combined with the finding that xylosyltransferase in the mutant is also normal, suggested that the mutation acted at the level of the synthesis of the core protein. Direct evidence supporting this suggestion was obtained from the analysis of the radioactively labeled intracellular proteins of cultured normal and mutant chondrocytes by polyacrylamide-gel electrophoresis (Argraves *et al.*, 1981). Normal extracts contained a radiolabeled band which was absent in the mutant (Fig. 7). This protein band had a molecular weight of 246,000 and could be specifically precipitated with an antiserum directed against normal cartilage proteoglycan monomer (Fig. 8).

Figure 6d indicates that the mutant sternum synthesizes extremely small quantities of proteoglycan (about 1% of normal) which sediments to the

same position as normal cartilage-specific proteoglycans. This material also chromatographs in the void volume of a controlled-pore glass 1400 (CPG 1400) column (Fig. 9a). Antibody-binding experiments on the CPG 1400 V_0 material reveal that the serum binds more than 90% of the normal proteoglycan but only 60% of the comparable mutant fraction (Fig. 9b). In order to make a distinction between the monomeric and the aggregated form of the cartilage-specific proteoglycans the CPG 1400 V_0 material was chromatographed on CPG 2500 where the aggregate chromatographs in the void volume and the monomer is included. Most of the normal material is aggregated whereas only a small fraction of the mutant exists in the aggregated state (Fig. 9c). Antibody-binding measurements on the void volume fraction again indicate that only 60% of the mutant material can be bound compared to more than 90% for the normal. The antibody binding for the included material (monomer) is, again, more than 90% for the normal but less than 20% for the mutant (Fig. 9d; McKeown-Longo and Goetinck, 1982).

Taken together the data on nanomelia indicate that the mutation acts at the level of the core protein of cartilage-specific proteoglycan. No detectable intracellular levels of core protein are evident in the mutant. The mutant does synthesize small quantities of a proteoglycan that shares some properties with cartilage-specific proteoglycan of normal. Whether this is an abnormal form of cartilage-specific proteoglycan or whether this represents a minor population of proteoglycans present in both normal and mutant, which has only become detectable in the mutant because of the absence of the large quantities of cartilage-specific proteoglycans found in normal, remains to be determined.

2. Cartilage Matrix Deficiency in Mice

Mice homozygous for the recessive lethal mutation cartilage-matrix deficiency (Rittenhouse *et al.*, 1978) are very similar in their defect to nanomelic mutation of chickens. Only the cartilage-specific proteoglycan is deficient in the cartilage of this mutant. Normal quantities of type II collagen are present. The addition of ß-D-xyloside and a direct assay for the xylosyltransferase indicate that these chondrocytes have the potential for the synthesis of chondroitin sulfate. Antibodies against rat chondrosarcoma proteoglycan monomer that stained normal extracellular matrix failed to stain that of the mutant. Antibodies to type II collagen stained the mutant cartilage without prior hyaluronidase treatment whereas in normal this treatment is necessary to remove the proteoglycans in order to stain for collagen. Although no direct measurement for the absence of core protein has been performed, the data strongly suggest that the cartilage matrix deficiency is defective in the

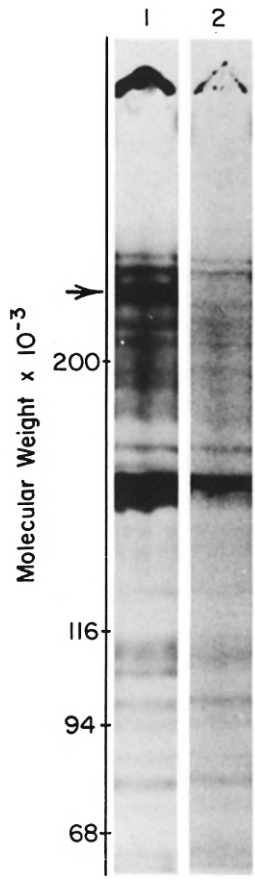

Fig. 7. Fluorograph of [^{35}S]cysteine-labeled chondrocyte extracts electrophoresed on a 4–15% gradient acrylamide SDS slab gel. The normal cell extract (lane 1) contains a radioactive band (arrow) which is missing in the nanomelic chondrocyte extract (lane 2). Reproduced from Argraves *et al.* (1981) with the permission of the publisher.

synthesis of core protein of cartilage-specific proteoglycan (Kimata *et al.*, 1981).

V. CONCLUSIONS

The structure of the skeleton is the result of a series of developmentally regulated events. In the limb, the developmental history includes a temporal appearance of mesenchyme, cartilage, and bone. Each of these tissues has its own tissue-specific properties which reflect the genetic activity at specific times of development.

The analysis of mutations that affect the structure of proteoglycans of cartilage has contributed to the understanding of the role of these macro-

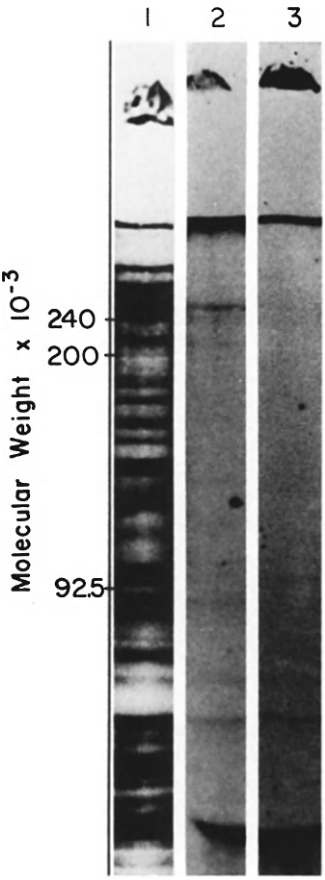

Fig. 8. Fluorograph of [^{35}S]cysteine-labeled normal chondrocyte extracts electrophoresed on a 4–15% gradient acrylamide SDS slab gel. The total extract is shown in lane 1. The material precipitated from the extract with antiserum to cartilage proteoglycan monomer and sheep antirabbit IgG is shown in lane 2. The normal rabbit serum, sheep antirabbit IgG control is shown in lane 3. Reproduced from Argraves *et al.* (1981) with the permission of the publisher.

molecules during cartilage development and morphogenesis. Examples were examined of mutations that alter the biosynthesis at the level of sulfation, glycosylation, and the core protein of proteoglycans. In spite of their diversity of action at the molecular level, the different mutations lead in each case to a cartilage with reduced extracellular space. This, in turn, results in the development of a tissue in which the cells are packed much more closely than in normal and the cartilaginous rudiments are reduced in size. An extreme example of such increased chondrocyte density is shown in the low-power electron micrograph of cartilage from the nanomelic mutant (Fig. 10, upper right). The ultrastructure of the mutant chondrocytes is normal but the extracellular matrix lacks the proteoglycan granules.

In order to investigate further the developmental regulation in the synthesis of tissue-specific macromolecules such as proteoglycans, specific probes

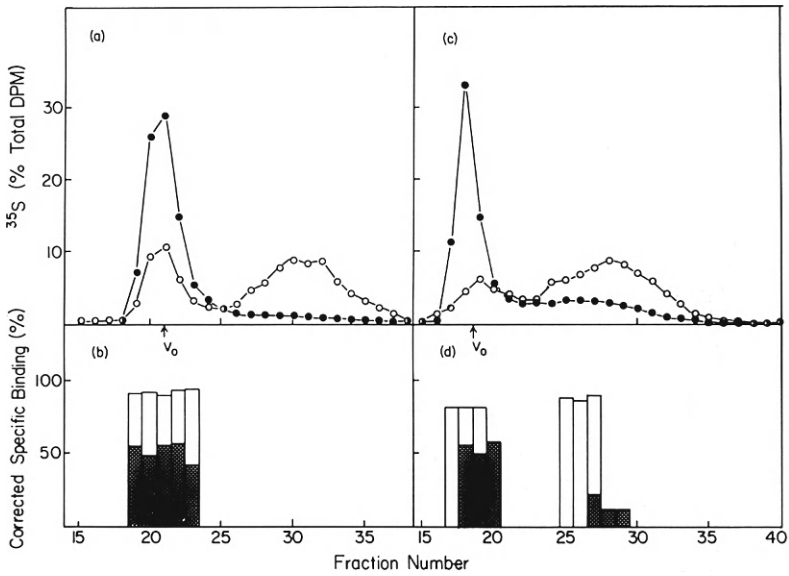

Fig. 9. Chromatographic and immunochemical analyses of proteoglycans synthesized by normal and nanomelic sternal cartilage. (a) Molecular-sieve chromatography of sternal extracts on CPG 1400. (b) Antiproteoglycan monomer serum binding of cartilage proteoglycan. (c) Molecular-sieve chromatography of proteoglycans that chromatograph in the CPG 1400 V_0 on CPG 2500. (d) Antiproteoglycan monomer serum binding to the various fractions that chromatograph as aggregates and monomers. ●, Unshaded areas, normal; ○, shaded areas, nanomelic mutants. From McKeown-Longo and Goetinck (1982).

will have to be developed using recombinant DNA technology to study the structure and the regulation of the genetic material involved.

Molecular defects similar to the animal models analyzed here may also exist in some of the human chondrodystrophies. The strategy for the investigation of embryonic cartilage proteoglycans reviewed in this chapter is suitable for the analyses of these diseases. Such analyses have to be carried out on cartilage because the proteoglycan is a tissue-specific macromolecule. Although the enzymes involved in the sulfation and glycosylation of the proteoglycan may also function in noncartilaginous tissue, the tissue specificity of the proteoglycan is dictated by its core protein. From a point of view of prenatal diagnosis, therefore, such diseases cannot be screened by analyzing skin fibroblasts on cells obtained from the amniotic fluid until the molecular basis of each disease is thoroughly understood or until specific recombinant DNA probes can be developed which could detect the hereditary condition in any cell type of an organism.

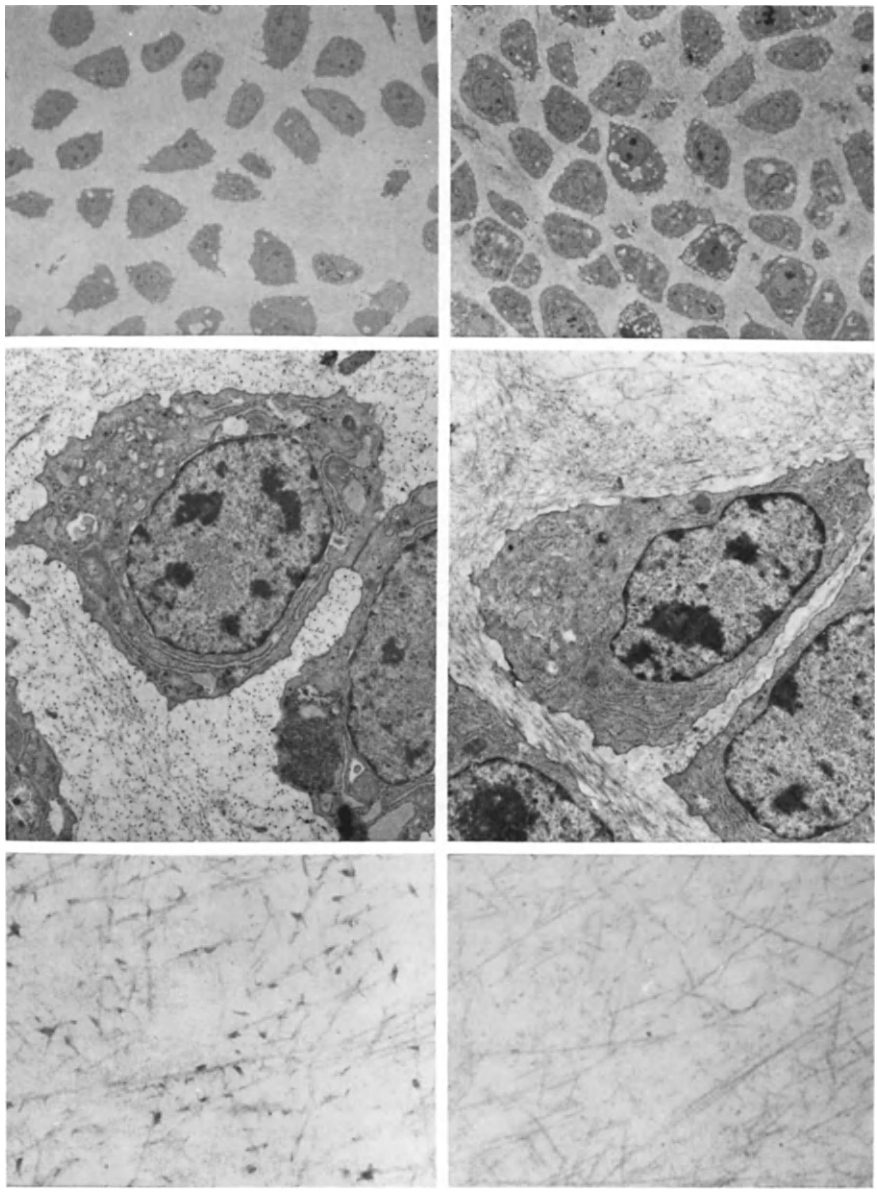

Fig. 10. Ultrastructural features of normal (left) and nanomelic (right) sternal cartilage. The upper, low-power electron micrographs reveal the higher density of the mutant chondrocytes compared to normal. No differences are observed between normal and mutant chondrocytes (middle), whereas the extracellular matrix (lower) of normal cartilage possesses electron-dense matrix granules, which are absent from the mutant matrix.

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7

Mutations Affecting Craniofacial Cartilage

M. Michael Cohen, Jr.

I. INTRODUCTION

The number of genetic mutations, malformations, and pathological conditions that affect craniofacial cartilage are legion. In this chapter, conditions to be discussed are grouped under four headings: (*1*) primary central nervous system malformations, (*2*) chondrodysplasias, (*3*) craniosynostoses, and (*4*) miscellaneous disorders. Although craniofacial cartilage is affected by a variety of mutations in different animal species, only human pathological conditions are considered here, with the exception of achondroplasia. The general features of each condition are presented together with what is known about etiology; description of the altered craniofacial cartilage follows. Although many of the important craniofacial anomalies are discussed in this chapter, no attempt has been made to be all inclusive. For more complete access to the literature on craniofacial anomalies, the reader

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is referred to the works of Bosma (1976), Cohen (1981b), and Gorlin *et al.* (1976).

II. CENTRAL NERVOUS SYSTEM MALFORMATIONS

A. Anencephaly

Anencephaly is characterized by an open neural tube in the cephalic region with an exposed mass of degenerate neural tissue at birth. The cranial vault is absent, producing characteristic bulging of the eyes and absence of the neck. Both the membranous neurocranium and the chondrocranium are grossly malformed. Anencephaly is classified anatomically as meroacrania if the defect does not involve the foramen magnum, holoacrania if the defect extends through the foramen magnum, and holoacrania with rachischisis if spina bifida accompanies anencephaly (Lemire *et al.*, 1978).

The etiology of anencephaly is not known. Most cases occur sporadically, but a tendency toward familial aggregation of anencephaly and spina bifida has been observed in some pedigrees, sometimes with instances of hydrocephaly and encephalocele as well. Although genetic factors are important, Mendelian ratios are seldom observed. Various geographic aggregations of neural tube defects may be explained by common ancestry as well as by unknown environmental factors. Several hypotheses have been proposed to explain the pathogenesis of neural tube dysraphism. Most evidence seems to implicate failure of fusion of the neural tube, the primary defect being either in the neuroepithelium itself or in the surrounding mesoderm. Some lesions may be caused by reopening of a closed neural tube, the primary defect being either an increase in the intraluminal pressure or in the neuroepithelium itself (Lemire *et al.*, 1978).

The cranial floor in anencephaly is different from normal, and such changes are probably caused by alterations in size, form, or duration of the neural functional matrix (Fields *et al.*, 1978). Changes within the body of the sphenoid result in a reduced cranial floor angle and a more vertically oriented clivus. Schematic drawings of the cranial fossae in meroacrania and holoacrania are compared with normal in Fig. 1. In both types of anencephaly, the anterior and middle cranial fossae are constricted laterally. The posterior cranial fossa is increased in lateral extension and anteroposterior constriction in both types of anencephaly. Schematic drawings of individual bones that make up the cranial base in anencephaly are compared with normal in Fig. 2. In anencephaly, the lesser wings of the sphenoid have an anteroposterior orientation, the greater wings of the sphenoid have a reduced transverse dimension, and the lateral end of the petrous portion of the temporal bone is positioned more anteriorly (Fields *et al.*, 1978).

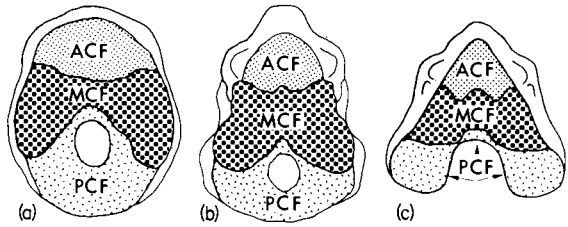

Fig. 1. Schematic drawing of the cranial floor and configuration of the individual fossae in (a) a normal fetus, (b) a fetus with meroacrania, and (c) a fetus with holoacrania—superior view. Note the lateral constriction of the anterior cranial fossa (ACF) and middle cranial fossa (MCF) in the anencephalics. Note increased lateral extension and anteroposterior constriction of the posterior cranial fossa (PCF). In holoacrania, the posterior cranial fossa exhibits schisis (arrows). From Fields *et al.* (1978).

B. Holoprosencephaly

Holoprosencephaly is a malformation complex in which impaired cleavage of the embryonic forebrain is the basic feature. The prosencephalon fails to cleave sagittally into cerebral hemispheres, transversely into telencephalon and diencephalon, and horizontally into olfactory and optic bulbs. The condition can be graded according to the degree of severity as alobar, semilobar, or lobar holoprosencephaly (DeMyer *et al.*, 1964; DeMyer and Zeman, 1963; Cohen, 1982a; Cohen *et al.*, 1971a).

In alobar holoprosencephaly, a monoventricular forebrain lacking interhemispheric division is present. In semilobar holoprosencephaly, rudimentary cerebral lobes and, in some cases, a posterior interhemispheric fissure are present. In lobar holoprosencephaly, the brain has well-formed lobes and a distinct interhemispheric fissure which may be interrupted anteriorly if the frontal neocortex is in continuity across the midline. At the mild end of the holoprosencephalic spectrum are malformations such as absence of the corpus callosum and absence of the olfactory tracts and bulbs (DeMyer and Zeman, 1963).

In a classic article in 1964, DeMyer *et al.* discussed a graded series of facial anomalies found with holoprosencephaly (Fig. 3). The faces that comprise this spectrum include cyclopia, ethmocephaly, cebocephaly, premaxillary agenesis, and less severe facial dysmorphism (Cohen *et al.*, 1971a; Cohen, 1981b). Although the face-brain correlation within this spectrum is strongly positive, DeMyer *et al.* (1964) noted that the correlation weakens at the less severe end of the spectrum.

The most severe facial dysmorphism is cyclopia wherein a single median eye occurs with varying degrees of doubling of the intrinsic ocular structures.

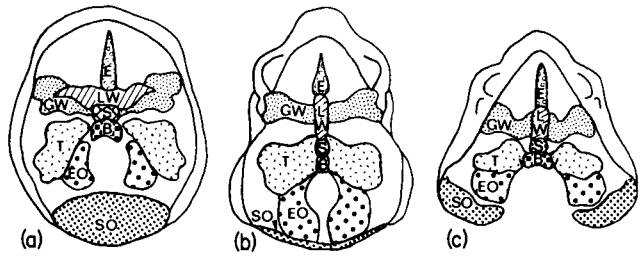

Fig. 2. Schematic drawing of the bones of the cranial floor in (a) a normal fetus, (b) a fetus with meroacrania, and (c) a fetus with holoacrania. Note the anteroposterior position of the lesser wings of the sphenoid and the reduced transverse dimension of the greater wings of the sphenoid (b and c) relative to the normal (a). The more anterior position of the lateral end of the petrous portion of the temporal bone is also evident in anencephaly. In holoacrania, the supraoccipital components are nonunited and widely divergent (c). From Fields *et al.* (1978).

Usually a blind-ended proboscis occurs above the eye. In some instances, the proboscis may be absent. In hypognathic cyclopia, the mandible is completely absent. In these cases, the proboscis is usually missing (Sedano and Gorlin, 1963). In ethmocephaly, severe ocular hypotelorism occurs together with a blind-ended proboscis located between the eyes. In cebocephaly, ocular hypotelorism is found in association with a blind-ended single nostril nose. In premaxillary agenesis, ocular hypotelorism is associated with a flat nose and a median cleft of the upper lip.

Holoprosencephaly is known to be etiologically heterogeneous, having a variety of chromosomal and monogenic causes (Table I). Most cases of holoprosencephaly, however, are sporadic and in the overwhelming majority of instances, the causes remain unknown. Although environmentally induced holoprosencephaly has been observed in a variety of experimental animals, further epidemiologic studies are necessary to prove or disprove various environmentally induced causes of holoprosencephaly at the human level. A number of holoprosencephalic infants have been born to diabetic mothers. Several instances are known of fetal hydantoin syndrome in association with holoprosencephaly. Viral infection, toxoplasmosis, and various drugs, including salicylates, have been suggested as having possible teratogenic effects in man (DeMyer, 1977; Cohen, 1982b).

The severity of holoprosencephaly and its attendant facial dysmorphism are correlated with the degree of hypoplasia of the craniofacial cartilages. In cyclopia, the anterior cranial base is dramatically shortened and roofs a medially placed orbital cavity (Fig. 4). The ethmoidal cartilage and all its skeletal derivatives—the cribriform plate, perpendicular plate, superior and

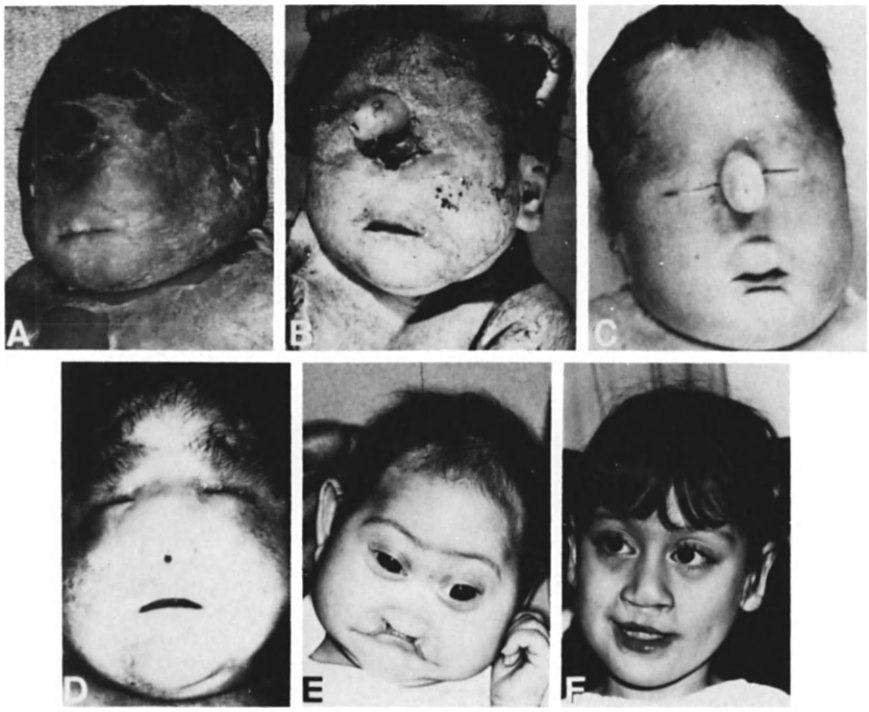

Fig. 3. Spectrum of dysmorphic faces associated with variable degrees of holoprosencephaly. (A) Cyclopia without proboscis formation. Note single central eye. (B) Cyclopia with proboscis. (C) Ethmocephaly. Ocular hypotelorism with proboscis formation. (D) Cebocephaly. Ocular hypotelorism with single-nostril nose. (E) Premaxillary agenesis, flat nose, and ocular hypotelorism. (F) Ocular hypotelorism and surgically repaired cleft lip. (a) From Cohen (1976a); (b), (c), and (d) from Cohen *et al.* (1971a) and Cohen and Hohl (1976); (e) from DeMyer and Zeman (1963); (f) from Cohen (1976b). Montage from Cohen (1982a).

middle nasal conchae, medial orbital walls, and nasal septal cartilage—fail to develop. The nasal cavity is absent and the two posterior halves of the maxillae are directed superiorly, medially, and with the vomer merged in the midline as a thick mass of bone. The right and left alveolar parts of the maxillae are joined superiorly in the midline and the right and left medial pterygoid plates merge medially with the pyramidal processes of the palatine bone, obstructing the junction between the oropharynx and nasopharynx (Kokich *et al.*, 1982a).

C. Encephalocele

The terms cranium bifidum and cephalocele properly describe the spectrum of malformations that includes encephalocele and cranial meningocele. However, the term encephalocele has persisted most commonly for all such

TABLE I

Conditions with Holoprosencephaly[a]

Condition	Comments
Chromosomal	
Trisomy 13	Holoprosencephaly common, full spectrum of facial dysmorphism; most common of chromosomal syndromes with holoprosencephaly
13q-	Holoprosencephaly common, semilobar or lobar holoprosencephaly without extremely severe facial dysmorphism
18p-	Holoprosencephaly uncommon, full spectrum of facial dysmorphism
Trisomy 18	Holoprosencephaly uncommon
Trisomy 21	Holoprosencephaly rare
Triploidy	Holoprosencephaly uncommon
47,XX, + C	—
46,XY/47,XY, + frag	—
47,XY, + B	—
46,XX,t(3p;?)	—
Partial monosomy 22pter → q11	—
Partial trisomy 1q	Holoprosencephaly uncommon
46,XY/45,XY,-G	—
Monogenic	
Meckel syndrome	May have holoprosencephaly with median or lateral cleft lip; autosomal recessive inheritance
Autosomal recessive holoprosencephaly	Full spectrum of facial dysmorphism; may vary within families
Autosomal dominant holoprosencephaly	Incomplete penetrance with remarkably variable expressivity; widely affected individuals may have only slight ocular hypotelorism, anosmia, hyposmia, or single maxillary central incisor
Agnathia-holoprosencephaly	Variable expression of both holoprosencephaly and agnathia; autosomal recessive inheritance likely
Holoprosencephaly-endocrine dysgenesis	Holoprosencephaly, facial clefts, genital hypoplasia, other abnormalities; autosomal recessive inheritance likely

[a] Modified from Cohen (1982b).

lesions. Intracranial structures herniate through a defect in the cranium to form a skin-covered sac which contains cerebral tissue, cerebellar tissue, or only meninges (Warkany *et al.*, 1981). Depending on location, encephaloceles may be classified as occipital, parietal, or anterior. Anterior encephaloceles are further divided into visible or sincipital lesions and basal or clinically invisible lesions (Table II). Encephaloceles may occur alone or as part of a broader pattern of anomalies making up various syndromes and associations (Cohen and Lemire, 1982). The striking features of these condi-

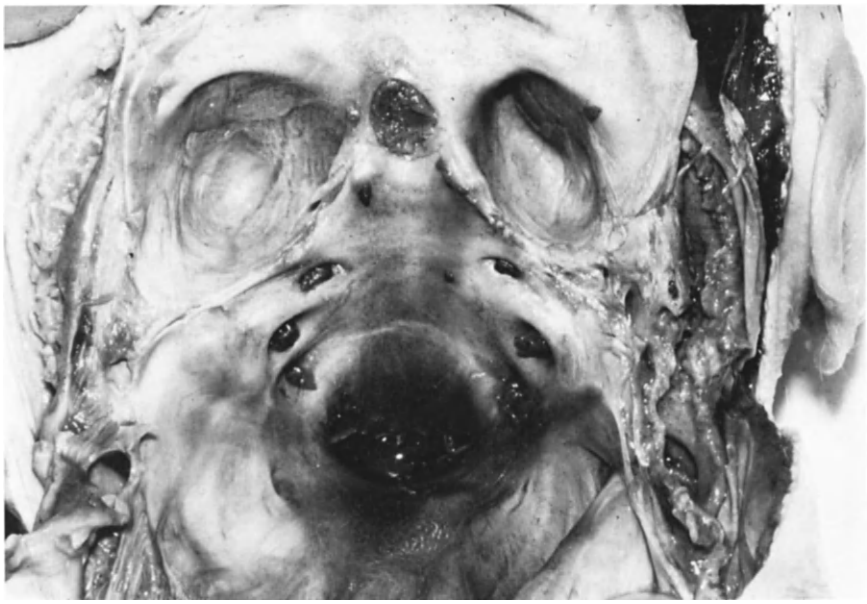

Fig. 4. Cranial base in cyclopia with holoprosencephaly. See text.

tions together with the sites of encephalocele formation and what is known about etiology are summarized in Table III.

Frontonasal dysplasia is characterized by a flattened nasofrontal encephalocele, ocular hypertelorism, widow's peak, and wide-set nostrils with lack of elevation of the nasal tip (Fig. 5). Occasionally encountered are notching of the nostrils or an unusual form of median cleft lip. The etiology is unknown and most instances occur sporadically, although familial instances have been noted. Cohen *et al.* (1971b) postulated that in frontonasal dysplasia, the nasal capsule failed to develop properly allowing the primitive brain vesicle (encephalocele) to protrude into the space normally occupied by the capsule (Fig. 6). The result is a morphokinetic arrest in the placement of the eyes and nostrils which tend to maintain their embryonic positions. Rarely, the same pathogenetic sequence may be caused by frontonasal lipoma or frontonasal teratoma.

III. CHONDRODYSPLASIAS

A. Achondroplasia

Achondroplasia is a rhizomelic form of short-limbed dwarfism in which the head is enlarged with frontal bossing and depression of the nasal bridge (Fig. 7); the hands are short, stubby, and usually trident; extension at the

TABLE II

Classification of Sincipital and Basal Encephaloceles[a]

Type	Boundaries (bones)	Presentation
Sincipital		
Frontoethmoidal		
Nasofrontal	Cribriform plate of ethmoid Nasal process of frontal Frontal process of maxillary	Midline at root of nose
Nasoethmoidal	Ethmoid Frontal Nasal	One one or both sides of nose
Nasoorbital	Lacrimal Frontal	Superior medial angle of orbital cavity; displaces eye laterally; may be bilateral
Interfrontal		
Craniofacial cleft		
Basal		
Sphenoorbital	Superior orbital fissure	Enters orbit, causing exophthalmos
Sphenomaxillary	Superior orbital then inferior orbital fissure	Pterygopalatine fossa
Transethmoidal	Defect in cribriform plate	Anterior nasal fossae
Sphenoethmoidal	Sphenoethmoidal suture	Posterior nasal fossae
Sphenopharyngeal	Defect in sphenoid	Rhinopharynx; sphenoid sinus

[a] From Warkany *et al.* (1981).

elbows is incomplete; the legs are frequently bowed because of lax knee ligaments; the lumbar spine is lordotic, the buttocks prominent, and the abdomen protuberant. These features may not be present at birth, in some cases, but disproportionate growth of the head occurs during the first two or three years of life and then parallels the normal curve. More than 80% of the recorded cases of achondroplasia are sporadic, representing point mutations. Less than 20% of recorded cases are familial, showing an autosomal dominant mode of transmission. Increased paternal age at the time of conception is associated with sporadic cases as in other autosomal dominant mutations (Cohen, 1979a; Rimoin, 1975).

TABLE III

Conditions with Encephaloceles[a]

Condition	Site(s) of Encephalocele	Reported frequency of encephalocele in condition	Striking features	Etiology
Syndromes				
Aberrant tissue band syndrome	Multiple, predominantly anterior	Uncommon	Ring constrictions and amputations of digits or limbs, distal syndactyly, irregular or asymmetric encephaloceles, microcephaly, microphthalmia, bizarre orofacial clefts, other facial disruptions, tissue bands, various other anomalies	Aberrant tissue bands
Chemke syndrome	Occipital	3/6	Hydrocephaly, agyria, absent cortical laminar structure, cerebellar dysgenesis, retinal dysplasia, corneal opacities, cataracts	Autosomal recessive
Cryptophthalmos syndrome	Occipital	10%	Extension of forehead skin to cover one or both eyes, unusual hairline, ear anomalies, notching of the nasal wings, soft tissue syndactyly of hands and/or feet, genital anomalies	Autosomal recessive
Dyssegmental dwarfism	Occipital	2/10	Lethal dwarfism, short broad tubular bones with metaphyseal widening, accelerated carpal bone maturation, bowing of legs as well as thighs and forearms, short broad pelvis with widely flared iliac wings, vertebral anomalies, small thorax, cleft palate, micrognathia	Autosomal recessive
Frontonasal dysplasia	Frontal	Constant	Ocular hypertelorism, widow's peak, anterior cranium bifidum occultum, widely set nostrils with lack of elevation of the nasal tip, notching of nostrils, other abnormalities	Most cases sporadic; some familial; probably etiologically heterogeneous
Knobloch syndrome	Occipital	4/5	High myopia, vitreoretinal degeneration, retinal detachment, meningocele, normal intelligence	Autosomal recessive (presumed)

(*continued*)

TABLE III (*continued*)

Condition	Site(s) of Encephalocele	Reported frequency of encephalocele in condition	Striking features	Etiology
Meckel syndrome	Occipital	80%	Polydactyly, polycystic kidneys, holoprosencephaly, microphthalmia, retinal dysplasia, cardiac anomalies, orofacial clefting, ambiguous external genitalia, other abnormalities	Autosomal recessive
Pseudo-Meckel syndrome	Occipital	?	Arhinencephaly, absent corpus callosum, Arnold-Chiari defect, no evidence of retinal dysplasia, cleft palate, congenital heart defects, accessory spleen, clubfoot, hallucal hammertoes	t(3p+;?)
von Voss syndrome	Occipital	2/2	Aplasia of corpus callosum, hypoplastic olives and pyramids of the medulla oblongata, phocomelia, urogenital anomalies, thrombocytopenia	?
Warfarin syndrome	Occipital	Uncommon	Nasal hypoplasia, bone stippling, limb shortening, low birth weight, optic atrophy, mental retardation, seizures, hydrocephaly	Warfarin during pregnancy
Associations				
Encephalocele/ absent corpus callosum	Parietal Transsphenoidal Other	4/13 3/10 —	Absent corpus callosum; optic nerve abnormalities may occur with transsphenoidal encephalocele; encephalocele/absent corpus callosum association can occur with various syndromes including holoprosencephaly	—

Encephalocele/ clefting	Transsphenoidal Other	4/8 —	Cleft lip, cleft palate, both cleft lip and cleft palate; less commonly oblique facial cleft; common and bizarre orofacial clefts can occur with various syndromes	—
Encephalocele/ craniostenosis	Occipital Frontal	2/20 Rare	Craniostenosis; association can occur alone or with other anomalies and syndromes	—
Encephalocele/ Dandy–Walker; Arnold–Chiari	Occipital Parietal	Uncommon Uncommon	Dandy–Walker defect or Arnold–Chiari defect or both	—
Encephalocele/ ectrodactyly	Occipital	2/2	Ectrodactyly	?
Encephalocele/ hemifacial microsomia	Occipital	Uncommon	Unilateral dysplastic ear, ear tags and/or pits, unilateral hypoplasia of mandibular ramus, various other anomalies especially ocular, skeletal, and cardiac defects	Most cases sporadic; some familial; probably etiologically heterogeneous
Encephalocele/ hypothalamic-pituitary dysfunction	Transsphenoidal	?	Different patterns of hypothalamic/pituitary dysfunction; sometimes optic nerve abnormalities, ocular hypertelorism, cleft lip/palate	Presumed embryonic secondary
Encephalocele/ iniencephaly; Klippel–Feil	Occipital	Constant with iniencephaly apertus; rare with Klippel–Feil anomaly	Iniencephaly apertus, Klippel–Feil anomaly, various other anomalies	?
Encephalocele/ meningomyelocele	Occipital Frontal	Rare	Meningomyelocele	?

[a] From Cohen and Lemire (1982).

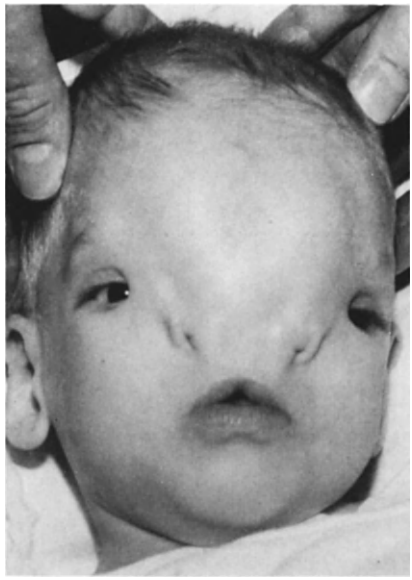

Fig. 5. Frontonasal dysplasia with cranium bifidum, flattened frontonasal encephalocele, wide-set nostrils, and failure of elevation of the nasal tip.

1. The Basic Defect

Although endochondral bone is affected in achondroplasia, the basic defect is unknown (see Chapter 6, this volume). Early histological studies, which suggested gross disorganization of endochondral ossification, were misleading because they described patients with thanatophoric dysplasia, metatropic dysplasia, and achondrogenesis (Parenti-Fraccaro type) rather than true achondroplasia.

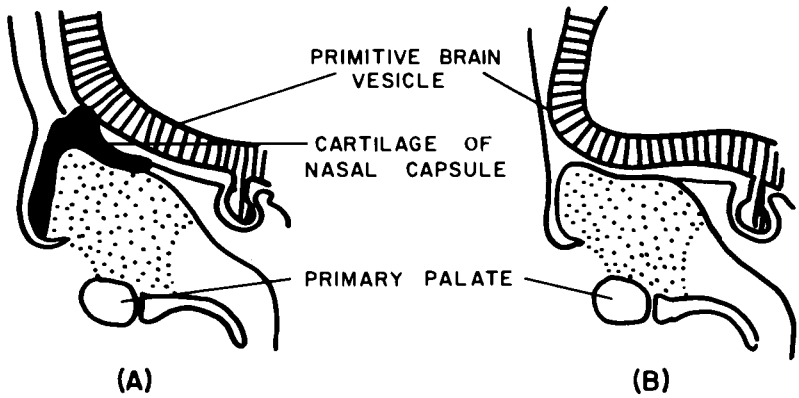

Fig. 6. (A) Normal development of the nasal capsule. (B) Faulty development of the nasal capsule allowing the primitive brain vesicle to fill the space normally occupied by the capsule. From Cohen *et al.* (1971b).

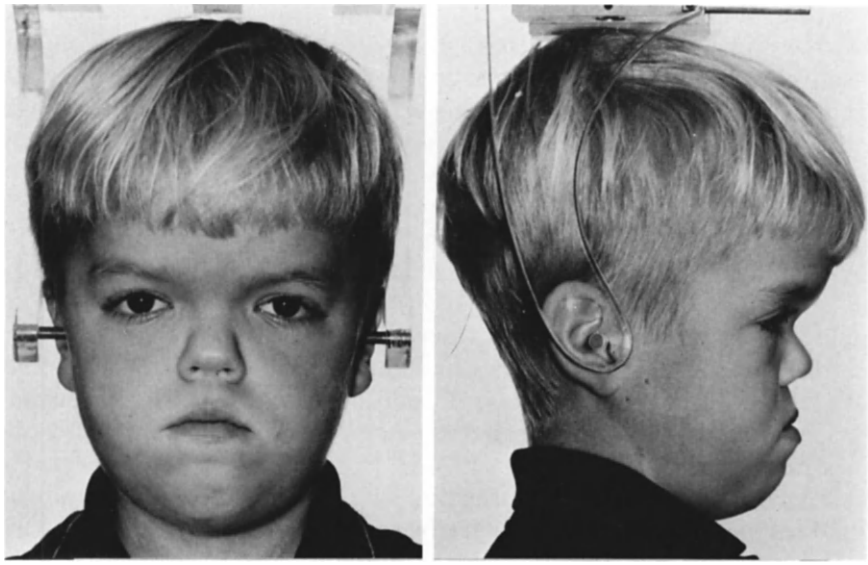

Fig. 7. Achondroplasia. Large head, frontal bossing, and depression of the nasal bridge and midface.

Recent histological studies of endochondral bone formation are somewhat at variance, although the differences are more semantic than real. Stanescu (1971) found that cartilage cells of the tibial growth plate were arranged in clusters, separated by wide septa of fibrous matrix. Primary trabeculae were either absent or very short. Histochemical alterations included weak RNA activity, no alkaline phosphatase or adenosine triphosphatase activity, and no glycogen in proliferative and hypertrophic chondrocytes. Mucopolysaccharides were confined to the perichondrial rim and increased collagen was found in the matrix.

Ponseti (1970) described similar histological findings in the fibular growth plate, but found histologically normal iliac-crest cartilage with increased amounts of galactosamine and glucosamine. Since protein polysaccharides were difficult to extract from achondroplastic iliac-crest cartilage, Ponseti suggested that protein polysaccharides might form large aggregates which could interfere with formation of the growth plate. Since collagen organization, cartilage matrix, and manner of ossification are known to differ in tubular bones and iliac crest, protein polysaccharide aggregate interference might have a greater effect on tubular bones, producing a short, squat shape (see Chapter 6, this volume).

Rimoin and his associates (1970) found well-organized endochondral ossification with longitudinal columns of cartilage cells in chondroosseous rib junctions. Iliac-crest cartilage also appeared normal. The number of cells

per column did not differ significantly from that of age-matched controls, but the primary trabeculae were slightly wider. Periosteal ossification was relatively increased, with periosteal bone extending beyond the growth plate into the perichondrium of the resting cartilage. The periosteal overgrowth produces the cupping appearance observed radiographically at the rib ends and presumably at other growth plates.

Ultrastructural analysis of achondroplastic cartilage reveals normal-appearing chondrocytes and normal-appearing matrix. The only abnormality observed is a relative increase in the number of dead cells surrounded by microscars containing focal aggregations of collagen fibrils. These observations suggest that achondroplasia may be associated with a quantitative decrease in endochondral ossification and a normal rate of membranous ossification. The decreased rate of endochondral ossification in achondroplasia may be due to defective somatomedin-binding to specific chondrocyte receptors (Rimoin *et al.,* 1976).

Defective oxidative energy formation with decreased phosphorylation at site I (the NADH dehydrogenase region of the terminal respiratory system) was demonstrated in mitochondrial preparations isolated from muscle biopsy specimens of subjects with achondroplasia. Since achondroplasia is dominantly inherited, defective oxidative phosphorylation was expected to be present in the mitochondria of all tissues and not just limited to muscle mitochondria. The clinical manifestations might conceivably result from disturbances in the growth of tissues such as cartilage in which oxygenation is usually low. Thus, oxidative energy formation might become rate limiting for growth and development when the capacity for oxidative phosphorylation is reduced (Mackler *et al.,* 1973).

Histological, histochemical, ultrastructural, and biochemical studies of growth plates from different anatomic locations (including both weight-bearing and non-weight-bearing areas) in different age groups would further elucidate the pathogenesis of achondroplasia.

2. *Animal Chondrodysplasias*

Achondroplasia has been observed in dogs, cattle, mice, rabbits, and goats. However, the mere existence of congenitally short limbs has been sufficient to evoke this diagnosis, and it is not certain whether any of these conditions are analogous to human achondroplasia. The *achondroplastic* rabbit has been particularly well studied as a model of human achondroplasia, but this may be questioned for three reasons (Cohen, 1979a). First, the condition is clinically severe and death ensues shortly after birth, suggesting that the disorder is more analogous to thanatophoric dysplasia than to achondroplasia. Second, achondroplasia in the rabbit follows an autosomal recessive mode of transmission and heterozygotes are not affected, unlike

heterozygotes in human achondroplasia. Since it appears probable that recessive traits involve enzymatic defects and dominant traits involve defects in structural or specialized protein, it is doubtful that the basic defect in the achondroplastic rabbit and the human achondroplast are identical. Bargman *et al.* (1972) demonstrated defective energy formation with the absence of phosphorylation at the cytochrome oxidase region (site III) of the terminal respiratory system in mitochondrial preparations from achondroplastic rabbit livers. However, preliminary evidence indicates that Site I may be involved in human achondroplasia. Third, because reported differences in the histological findings in human achondroplasia have not yet been completely resolved, there is no assurance that endochondral ossification is the same in the achondroplastic rabbit and human achondroplasia.

Because Rimoin and his associates (1970) found well-organized endochondral ossification in human achondroplasia, they suggested that dwarfed animals with similar endochondral bone formation such as the brachymorphic mutant in the mouse and the African dwarfed goat might prove to be better models of human achondroplasia. Because the *cn/cn* mouse is known to have disproportionate dwarfism and well-organized endochondral ossification, it too should be studied as a possible model of human achondroplasia.

3. Craniofacial Complex

In achondroplasia, the abnormal craniofacial complex can be viewed as the effect of abnormal endochondral bone formation on the development of the skull as a whole. Significant findings in the achondroplastic skull include enlargement of the calvaria, frontal bossing, large frontal sinuses, occipital prominence, normal anterior cranial-base length, strikingly shortened posterior cranial base length, an acute cranial-base angle, a short nasal bone that is deformed and depressed, short upper facial height, recessed maxilla, posterior tilt of the nasal floor, and a prognathic mandible that is anteriorly displaced but of normal size with a normal gonial angle and a high coronoid process (Fig. 8); (Cohen, 1979a).

The finding of normal anterior cranial-base length in achondroplasia is surprising since the cranial base is preformed in cartilage and hypoplasia and shortening would be expected. Because the brain is enlarged in achondroplasia, the expanding frontal lobes may influence the growth of the anterior cranial base, as it is known to follow a neural pattern of growth (Cohen, 1979a; and see Volume 2, Chapter 7).

It has been observed that trunk length in achondroplasia is nearly normal. Although the vertebrae are preformed in cartilage, the essentially normal trunk length has been attributed to numerous growth centers that are available in the vertebral column and allow more adequate growth to take

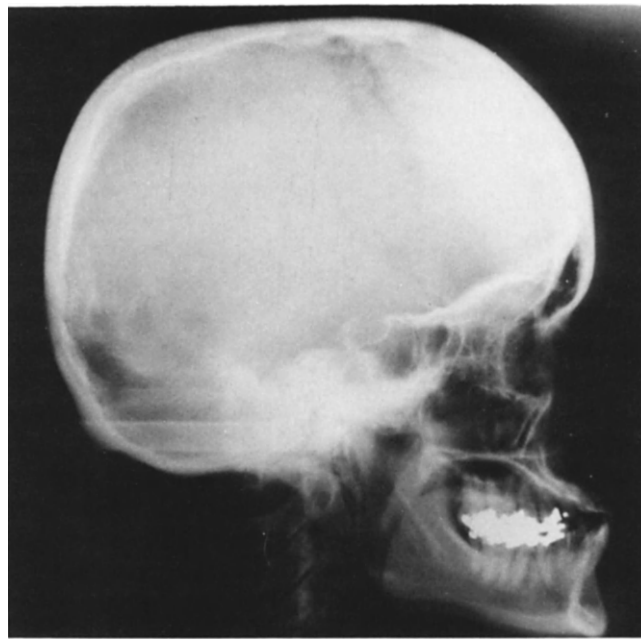

Fig. 8. Lateral cephalogram of an achondroplastic dwarf. See text.

place (Sicher, 1957). It has also been suggested that the number of growth sites along the anterior cranial base compared with the small number available along the posterior cranial base might play a role similar to that of the vertebral column by allowing more adequate bone growth response to the growing brain in the anterior cranial fossa (Pedersen, 1970). Cohen (1979a) found that cribriform-plate length was strikingly reduced, but anterior sphenoidal length was strikingly increased, compensating for the shortened cribriform-plate length and suggesting that growth in the length of the anterior cranial base takes place primarily by adaptation at one site—namely the sphenoethmoidal synchondrosis.

Strikingly short posterior cranial base length results from hypoplasia of bone that is preformed in cartilage with possible early closure of the spheno-occipital synchondrosis. The exaggerated closure of the cranial base angle in achondroplasia may be related to an increased brain size and possibly earlier than normal closure of the intersphenoidal synchondrosis. The acute cranial base angle and the increase in the foramen magnum angle strongly suggest that the natural balance of the achondroplastic head on the spinal column tilts the face downward. The finding of an exaggerated external bony protuberance on the occipital bone may also be related to head balance. Radiographically, the protuberance corresponds to the areas of muscle at-

tachment of the rectus capitis posterior minor and rectus capitis posterior major muscles. These muscles aid in holding the head upright and in extension against resistance or gravity. With the heavy head load in achondroplasia and the tendency of the face to tilt downward, increased neck muscle use may be responsible for the exaggerated bony occipital convexity (Cohen, 1979a).

Shortened maxillary length and upper facial height are probably related to hypoplastic growth of the nasal capsule which is preformed in cartilage and would be expected to be affected in achondroplasia. The shortened, deformed, and depressed nasal bones are membranous in origin and would not be expected to be affected. The configuration of the nasal bone in achondroplasia probably results secondarily from two opposing tendencies—to dip in superiorly because of the hypoplastic nasal septum and to pull out inferiorly to uplift the nose. The posterior tilting of the nasal floor suggests an adaptation to a narrow nasopharyngeal airway. Because the acute cranial-base angle constricts the nasopharynx, the posterior tilt allows a wider opening of the nasopharynx (Cohen, 1979a).

The normal mandibular length in achondroplasia is probably related to the condylar cartilage which grows appositionally instead of interstitially as the chondrocranium does. If the achondroplasia gene only produces an effect on interstitially growing cartilage, then it does not affect the mandible (see Volume 2, Chapter 7). The mandibular prognathism found in achondroplasia, in spite of the finding of normal mandibular size, can be related to the more acute cranial-base flexure. Thus, the mandible, of normal dimensions, is more anteriorly positioned than usual. The large coronoid process may be related to increased muscle stretch of the temporalis from an anteriorly placed mandible. It is also possible that the hyperextension of the achondroplastic head, necessary for interaction with other adults, results in a natural opening of the jaws which is counteracted by increased use of the temporalis muscles among others (Cohen, 1979a).

B. Thanatophoric Dysplasia

Thanatophoric dysplasia is characterized by marked shortness and bowing of the extremities and a narrow trunk of normal length (Fig. 9). The condition is incompatible with life. What has been called thanatophoric dysplasia in the literature represents a heterogeneous group of disorders. Most cases of classic thanatophoric dysplasia occur sporadically. Thanatophoric dysplasia with cloverleaf skull malformation, however, has been observed in affected sibs and autosomal recessive inheritance has been suggested (Gorlin *et al.*, 1976).

Cultured fibroblasts in thanatophoric dysplasia can be distinguished from achondroplastic and normal fibroblasts on the basis of total intracellular

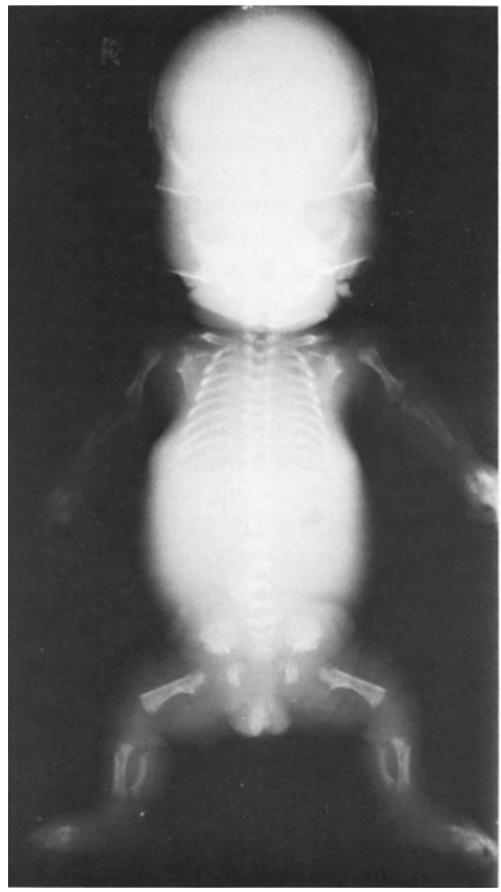

Fig. 9. Thanatophoric dysplasia. Note narrow thorax, short ribs (flared at the costochondral junctions), platyspondyly, narrow sacrosciatic notches, horizontal acetabular roof with spurring, shortening of the long bones, and bent femurs. Cloverleaf skull malformation is also evident.

mucopolysaccharide content and the relative proportion of dermatan sulfate (Danes, 1974). In thanatophoric dysplasia, small vacuoles are observed within individual resting chondrocytes, but these cannot be differentiated from the vacuoles of normal chondrocytes. Within the growth plate, some large areas lack cell proliferation, maturation, and column formation; resting cartilage is in direct contact with metaphyseal bone. Transformation of cartilage into bone is very irregular. In some areas, chondrocyte proliferation occurs, and on occasion, normal appearing cartilage columns may be

observed. In still other areas, poorly organized fibrovascular material is scattered along the growth plate; such material tends to occur more extensively in thanatophoric individuals with cloverleaf skull malformation than in those without, suggesting a similar or identical pathogenetic process. The difference may represent either variability within a single disorder or two distinct disorders with similar pathogenesis (Horton *et al.*, 1979).

The cranial base is completely united into one bone with absence of articulations normally present between the presphenoid, basisphenoid, basioccipital, exoccipital, and supraoccipital components of the cranial base. Thus, the cranial fossae are grossly abnormal in thanatophoric dysplasia with cloverleaf skull malformation. The anterior cranial base is smaller and shallower than normal. The cribriform plate is triangular in shape with prominence of the crista galli. The middle cranial fossa is extremely enlarged, extending laterally and posteriorly to completely surround the posterior cranial fossa which is greatly reduced in size. The petrous crests of the temporal bones are distorted into arcs which enclose the underdeveloped occipital bone. The clivus is reduced in length and the foramen magnum is smaller than normal (Kokich *et al.*, 1982b). The cloverleaf skull malformation is discussed further in Section IV.

C. Chondrodysplasia Punctata

Chondrodysplasia punctata is an etiologically heterogeneous group of disorders with punctate calcifications of bone, usually most marked in the epiphyses of the long bones. The autosomal recessive rhizomelic form is characterized by severe shortening with metaphyseal cupping and splaying of the humerus and femur, small head circumference, depressed midface, cataracts, and death usually within the first year of life. Endochondral bone formation is severely disturbed with focal disruption of the growth plate by fibrous tissue (Gorlin *et al.*, 1976).

The fetal warfarin syndrome is characterized by stippled epiphyses, hypoplasia of the terminal phalanges, and midface hypoplasia. The condition is teratogenically induced by maternal ingestion of sodium warfarin. It has been suggested that pathogenesis may be based on focal bleeding in fetal or embryonic cartilage, leading to abnormal growth of cartilage and scarring which presumably calcifies, resulting in stippling. However, Barr and Burdi (1976) have shown foci of disordered chondrogenesis in the facial cartilages. The lack of hemosiderin deposition in or around the abnormal areas indicated that focal hemorrhage was not responsible for disordered chondrogenesis. They suggested that the stippled appearance observed radiographically might be caused by advanced calcification of the disordered cartilaginous foci.

IV. CRANIOSYNOSTOSES

Craniosynostosis is the premature fusion of cranial sutures. Deformity of the head depends upon which suture or sutures are synostosed and the time during development that fusion occurs. When the coronal suture is involved, a brachycephalic skull shape results. When the sagittal suture is prematurely closed, a dolichocephalic skull shape results. Unilateral involvement of the coronal or lambdoidal suture produces plagiocephaly. Multiple sutural synostosis is also known to occur in some patients. Isolated craniosynostosis occurs more commonly that syndromic synostosis. Some instances of craniosynostosis are associated with abnormalities of the craniofacial cartilages.

A. Etiology and Pathogenesis

Three classic theories have been advanced to explain craniosynostosis. Virchow (1851) believed that craniosynostosis was a primary malformation and that the associated cranial base deformity was secondary to craniosynostosis. The converse was postulated by Moss (1959); the cranial base malformation was the primary anomaly, resulting in secondary premature fusion of the cranial sutures. In speculating on the pathogenesis of the Apert syndrome, Park and Powers (1920) postulated a primary defect in the mesenchymal blastema that led to both craniosynostosis and an abnormal cranial base (see Volume 2, Chapter 4).

Currently, Moss's theory is the most popular of the three. Unfortunately, most discussions of craniosynostosis assume that there is a single pathogenetic mechanism that remains to be elucidated, and that once this is done, alternative hypotheses will be shown to be incorrect. Syndrome delineation and clinical evidence to date strongly suggest that craniosynostosis is pathogenetically heterogeneous. Some 57 different syndromes have been recognized in which craniosynostosis is a feature (Table IV). Some are known to be and others are presumed to be etiologically heterogeneous. Such etiologic heterogeneity, of course, suggests the possibility of pathogenetic heterogeneity. Thus, all three theories are probably correct; each may be implicated in some, but not all, cases of craniosynostosis.

According to Moss's theory, spatially malformed lesser sphenoidal wings in coronal synostosis, and spatially malformed cribriform plate and crista galli in sagittal synostosis are viewed as primary abnormalities which, at the points of dural attachment, transmit aberrant tensile forces upward through the dura, leading to premature fusion of the overlying sutural tissues.

Many familial instances of isolated (nonsyndromic) craniosynostosis have been observed (Cohen, 1979b). Most instances are compatible with autosomal dominant transmission. In some families, involvement is variable —some family members having fusion of the sagittal suture, some having fu-

TABLE IV

Summary of Syndromes with Craniosynostosis[a]

Type of syndrome	Number of syndromes
Chromosomal syndromes	11
Monogenic syndromes	26
Autosomal dominant	12
Autosomal recessive	12
X-linked	2
Teratogenic syndromes	2
Total syndromes with known genesis (all categories above)	39
Unknown genesis syndromes	18
	57

[a] From Cohen (1979b).

sion of the coronal suture and still others having synostosis of both coronal and sagittal sutures. It is difficult to conceive of dramatically different primary abnormalities of the cranial base occurring in the same family as a dominant trait. Thus, a different pathogenetic mechanism than the one proposed by Moss may be operative in such families.

A primary abnormality of the cranial base as the cause of craniosynostosis in the Apert syndrome has been proposed by Moss (1959) and supported by Stewart *et al.* (1977). However, clinical evidence to date indicates that the pathogenetic mechanism for craniosynostosis in this syndrome may be unique. Features of the Apert syndrome include progressive calcification and fusion, with time, of the bones of the hands, feet, and cervical spine (Schauerte and St-Aubin, 1966). Progressive generalized bony dysplasia with ankylosis of joints as well as progressive limitation of motion at these joints have been documented (Harris *et al.*, 1977). Finally, progressive calcification of the cartilaginous nasal septum and stylohyoid ligament has also been observed (Harris *et al.*, 1977). The most frugal hypothesis would be that whatever mechanism is responsible for progressive calcification throughout the body is also responsible for premature craniosynostosis in the Apert syndrome. Because neither the cranial base not the points of dural attachment can be invoked to explain progressive calcification elsewhere, it is probable that they have nothing to do with craniosynostosis in the Apert syndrome either.

Persson and co-workers (1979) showed that craniosynostosis occurred

after the experimental application of methyl cyanoacrylate adhesive to the coronal suture in 9-day-old rabbits. The resultant immobilization produced constraint of growth stretch across the sutural area which, presumably, caused craniosynostosis to occur. Graham and colleagues (1979) hypothesized that, in similar fashion, human prenatal head constraint may be responsible for some cases of craniosynostosis. They noted that some mothers of infants with isolated sagittal synostosis gave a history of early descent of their abdominal silhouette and severe pelvic pressure during the last one to three months of gestation. These symptoms were interpreted as a sign of early descent of the fetal head ino the lower pelvis.

Lack of growth stretch at the sutures may also be implicated in three malformations in which premature craniosynostosis may occur as a complicating feature. First, sutural fusion may accompany some cases of microcephaly (Duggan *et al.*, 1970). Lack of central nervous system growth may result in lack of growth stretch across the sutural areas, producing secondary craniosynostosis. Second, several reports have linked shunted hydrocephaly to craniosynostosis (Kloss, 1968). Low pressure systems may be implicated, in which growth stretch at the sutural areas suddenly becomes totally deficient. Finally, some cases of encephalocele have been associated with craniosynostosis (Lorber, 1967). Such "blow-out" lesions may sometimes result in lack of growth stretch across sutures.

Premature craniosynostosis may result from several dysmetabolic states. It has been observed to accompany hyperthyroidism during childhood (Johnsonbaugh *et al.*, 1978). Sutural fusion may be caused by primary thyroid hyperplasia, but more commonly results from excessive thyroxine treatment for congenital hypothyroidism.

Premature sutural fusion may also occur in the Hurler syndrome, which is characterized by α-L-induronidase deficiency. Craniosynostosis involves the sagittal and lambdoidal sutures (Gorlin *et al.*, 1976).

Craniosynostosis has been observed in various etiologically distinct forms of rickets, including vitamin D-deficiency rickets, simple hypophosphatemic rickets, hypophosphatemic-hypocalcemic-aminoaciduric rickets, hypophosphatemic-aminoaciduric-cirrhotic rickets, azotemic osteodystrophy, and hypophosphatasia (see Volume 2, Chapter 8). The extent of synostosis is related to the severity of the rachitic process. Premature sutural fusion was observed in a third of 59 rachitic children under 9 years of age in the study of Reilly and associates (1964).

Craniosynostosis may occur in various hematologic disorders. Hyperplasia of the marrow with compensatory bony overgrowth of the calvaria can "lock" the sutures. Conditions known to result in premature fusion of sutures include the thalassemias, sickle cell anemia, congenital hemolytic icterus, and polycythemia vera (Duggan *et al.*, 1970).

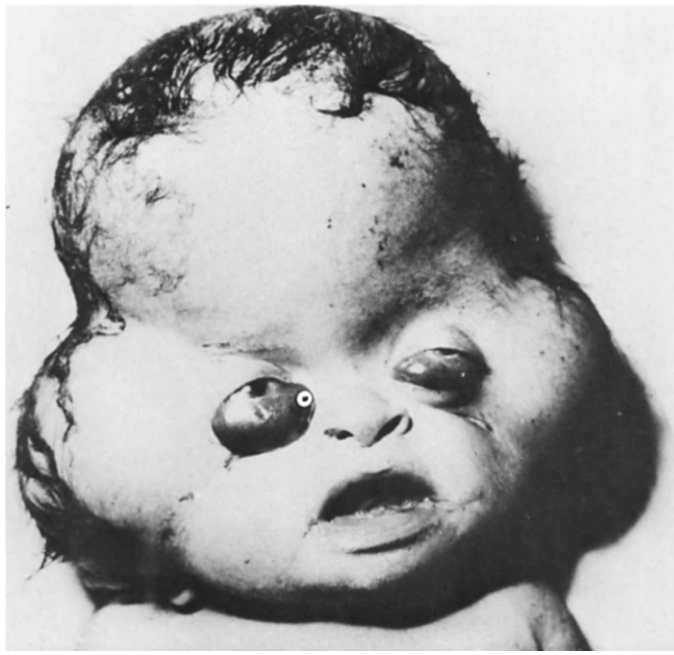

Fig. 10. Cloverleaf skull malformation. From Cohen (1975).

Premature fusion of sutures may occasionally accompany a variety of miscellaneous disorders as an abnormality. These conditions include among others, ataxia-telangiectasia, the epidermal nevus syndrome, and Job syndrome (Cohen, 1980). The pathogenesis in such instances is completely obscure.

B. Syndromes

Of the 57 known syndromes with craniosynostosis (Table IV), the most common are the Crouzon syndrome, the Apert syndrome, and the Saethre–Chotzen syndrome. All 57 syndromes have been reviewed elsewhere (Cohen, 1979b). Only a few can be discussed here.

1. Cloverleaf Skull Anomaly

In the cloverleaf skull malformation (Figs. 10 and 11) the skull is trilobular in shape with premature fusion of cranial sutures. Variability in the degree of severity occurs, and different sutures may be involved in different patients. Synostosis may involve the coronal, lambdoidal, and metopic sutures with bulging of the cerebrum through an open sagittal suture or, in some cases, through open squamosal sutures. Synostosis of the sagittal and squamosal

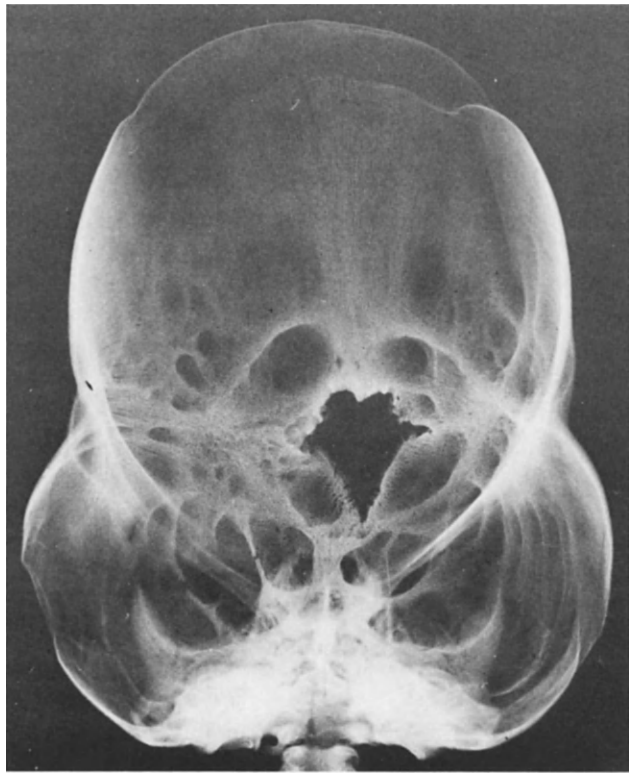

Fig. 11. Radiograph of a cloverleaf skull. From Partington *et al.* (1971).

sutures with cerebral eventration through a widely patent anterior fontanel may also be observed. A trilobular skull may occur with complete synostosis of all the cranial sutures. Finally, some instances are known in which the sutures are widely patent with no evidence of craniosynostosis at birth. The cloverleaf skull malformation is both etiologically and pathogenetically heterogeneous, occurring most commonly as an isolated malformation, with one form of thanatophoric dysplasia, or with Pfeiffer syndrome (Cohen, 1979b). Conditions with the cloverleaf skull malformation are summarized in Table V.

2. Crouzon Syndrome

The Crouzon syndrome is characterized by craniosynostosis, maxillary hypoplasia, and shallow orbits with ocular proptosis (Fig. 12). The condition follows an autosomal dominant mode of transmission, 67% of the cases be-

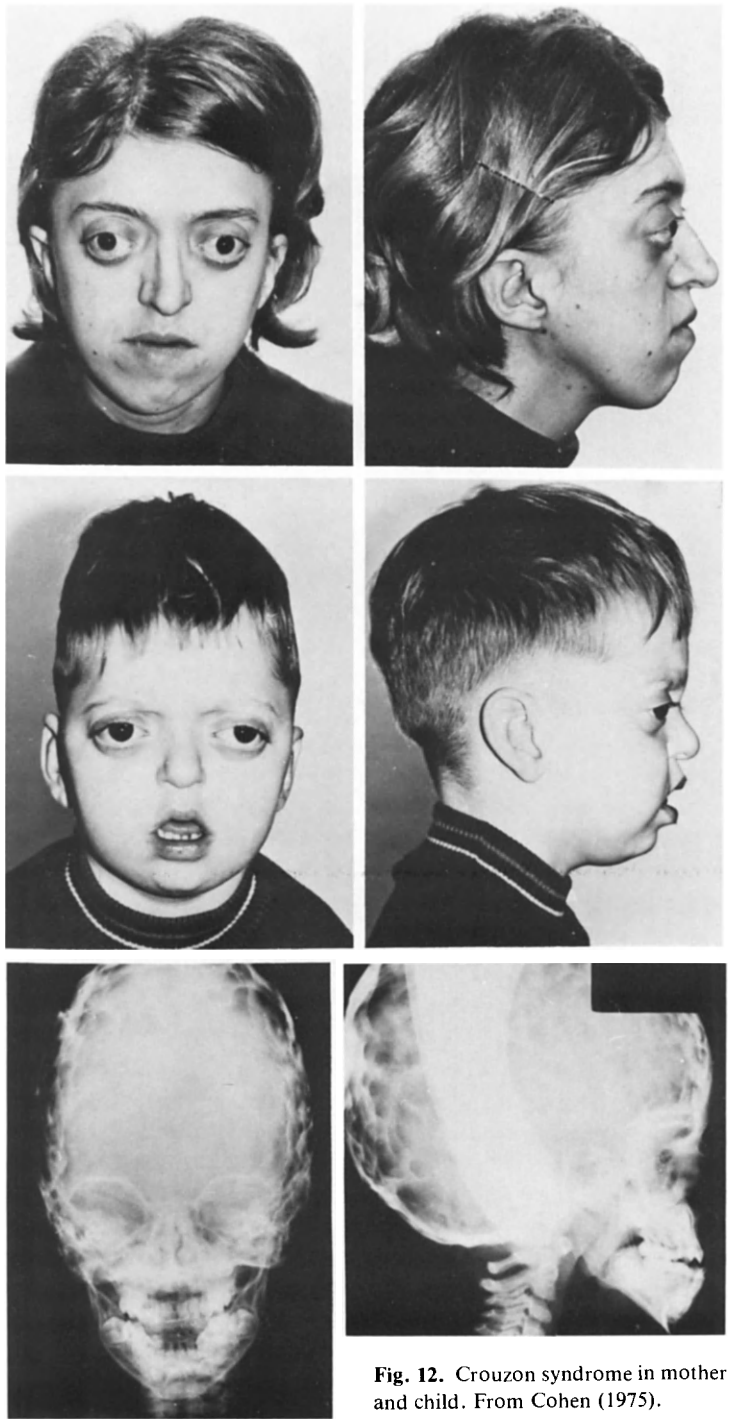

Fig. 12. Crouzon syndrome in mother and child. From Cohen (1975).

TABLE V

Conditions with the Cloverleaf Skull Anomaly[a]

Condition	Striking features	Frequency of cloverleaf skull anomaly with condition	Etiology
Aberrant tissue band syndrome	Ring constrictions and amputations of digits or limbs, variable distal syndactyly, facial clefts, encephalocele	Rare	Amniotic bands presumed
Antley–Bixler syndrome	Craniosynostosis, midface hypoplasia, low-set, rolled-down ears, tower-shaped rib cage, radiohumeral synostosis, ligamentous contractures, hypoplastic distal phalanges, tapering fingers, vertically slanted iliac bones, femoral bowing, femoral fractures, clubfoot	Common	Autosomal recessive
Apert syndrome	Craniosynostosis, ocular proptosis, downslanting palpebral fissures, midface deficiency, symmetrical syndactyly of all four limbs minimally involving digits 2–4	Rare	Autosomal dominant; most instances sporadic
Carpenter syndrome	Craniosynostosis, mental deficiency, short stature, preaxial polysyndactyly of feet, brachydactyly and clinodactyly with variable syndactyly of hands, congenital heart defects	Rare	Autosomal recessive

Crouzon syndrome	Craniosynostosis, ocular proptosis, midface deficiency	Uncommon	Autosomal dominant
Iatrogenic anomaly	Cloverleaf-shaped skull	Rare	Bilateral subtemporal decompression procedures for hydrocephaly
Isolated anomaly	Cloverleaf-shaped skull	All cases	?
Partial trisomy 13q syndrome	Craniosynostosis, meningomyelocele, mental deficiency, short neck, asymmetric chest, micropenis, cryptorchidism, other anomalies	Uncommon	Partial trisomy 13q
Pfeiffer syndrome	Craniosynostosis ocular proptosis, midface deficiency, broad thumbs and great toes, variable soft tissue syndactyly of other digits	Common	All cases with cloverleaf skull are sporadic; Pfeiffer syndrome without cloverleaf skull has autosomal dominant inheritance
Thanatophoric dysplasia	Large skull, short thick limb bones, curved radii and fibulae, narrow thoracic cage	Common	Affected sibs have been reported with thanatophoric dysplasia and cloverleaf skull anomaly; all instances of thanatophoric dysplasia without cloverleaf skull anomaly have been sporadic
Various syndromes of unknown genesis	Cloverleaf-shaped skull with various anomalies making up unrecognized patterns	?	Causes unknown but probably heterogeneous

[a] From Cohen (1982a).

ing familial and 33% being sporadic, representing fresh mutations. Penetrance is either complete or extremely high (Cohen, 1979b).

Cranial malformation in the Crouzon syndrome depends on the order and rate of progression of sutural synostosis. Brachycephaly is most commonly observed, but scaphocephaly, trigonocephaly and, as already indicated, the cloverleaf skull malformation may be observed. A characteristic feature of the Crouzon syndrome is pronounced ocular proptosis secondary to extremely shallow orbits. The combination of basilar kyphosis and arrested maxillary growth in the Crouzon syndrome may lead to occlusion of the epipharynx and respiratory distress (Cohen, 1979b).

3. Apert Syndrome

The Apert syndrome (Figs. 13–15) is characterized by craniosynostosis, midfacial malformations, and symmetric syndactyly of the hands and feet. Although most cases of the Apert syndrome are sporadic, dominant transmission with complete penetrance has been reported. The four familial cases, the equal number of affected males and females, and the increased paternal age effect in sporadic instances strongly suggest autosomal dominant transmission. The rarity of familial cases is explained by the reduced fitness of affected individuals (Cohen, 1975).

A middigital hand mass minimally involving the second, third, and fourth fingers is always observed. Associated synonychia is variable in degree. The first and fifth fingers may be joined to the middigital hand mass or may be separate. When the thumb is free, it is broad and deviates radially. In the feet, syndactyly involves the second, third, and fourth toes. The first and fifth toes are sometimes free and sometimes joined by soft tissue union to the second and fourth toes, respectively. Toenails may be separate or partially continuous. The great toes are broad and hallux varus is commonly observed (Cohen, 1975).

In the Apert syndrome, there is irregular early obliteration of cranial sutures, especially the coronal suture. The anterior fontanel may remain open longer than normal. Accentuation of digital markings may be observed with age. Because sutural involvement is variable, craniofacial appearance and degree of asymmetry is also variable. Turribrachycephaly is commonly observed and the occiput is flattened. The forehead is steep and during infancy a horizontal groove which disappears with age may be present above the supraorbital ridges. Bulging at the bregma or slightly anterior to the bregma may be noted in some cases. The middle third of the face is hypoplastic, resulting in relative mandibular prognathism. The nose has a parrot-beaked appearance. In the relaxed state, the lips frequently assume a trapezoidal configuration. The palate is highly arched, constricted, and may have a median furrow. Cleft soft palate or bifid uvula may be observed in

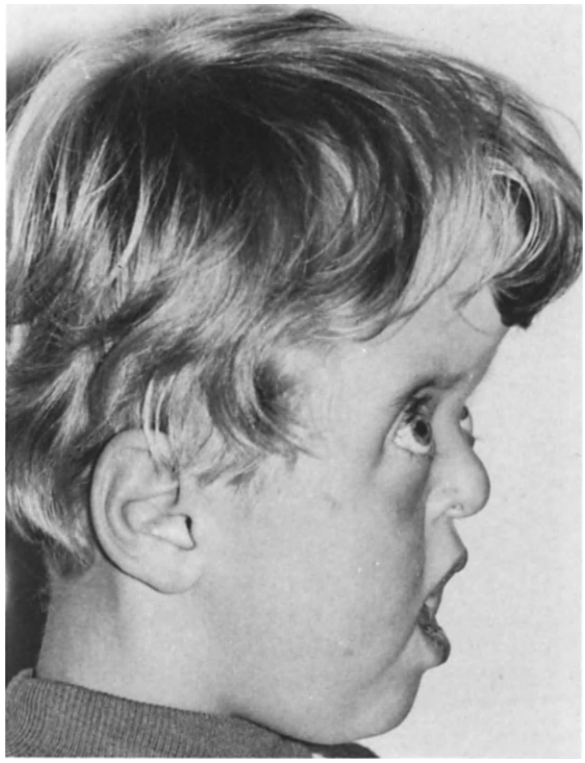

Fig. 13. Craniofacial appearance in Apert syndrome.

over 30% of the cases. The maxillary dental arch may be V-shaped with severely crowded teeth and bulging alveolar ridges. Class III malocclusion is usually present, with anterior openbite or crossbite and unilateral or bilateral posterior crossbite. Retarded dental eruption is a common finding. Hypertelorism, proptosis, downslanting palpebral fissures, and frequently, strabismus may be observed (Cohen, 1975).

4. Pfeiffer Syndrome

The Pfeiffer syndrome consists of craniosynostosis, broad thumbs and great toes, and partial soft tissue syndactyly of the hands and feet, which is a variable feature. The condition has autosomal dominant inheritance with a high degree of penetrance and variable expressivity (Cohen, 1975).

5. Saethre-Chotzen Syndrome

The Saethre-Chotzen syndrome is characterized by craniosynostosis, low-set frontal hairline, facial asymmetry, ptosis of the eyelids, deviated nasal

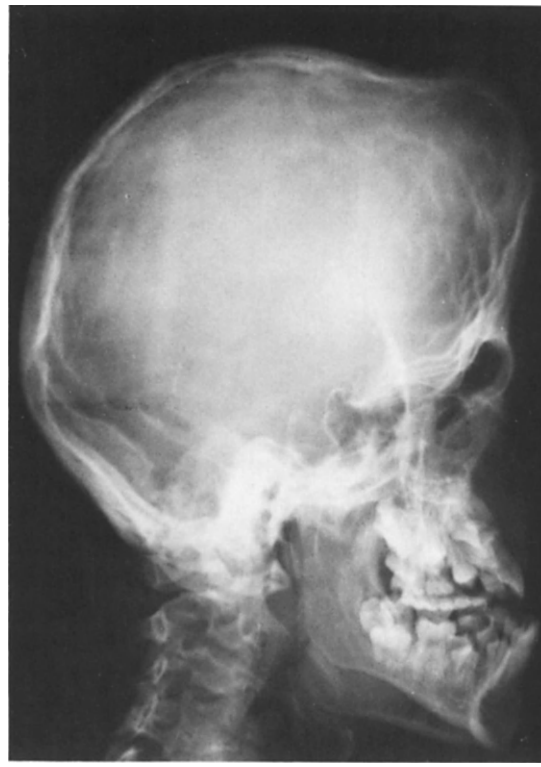

Fig. 14. Roentgenogram of the skull in Apert syndrome.

septum, and, variably, some degree of brachydactyly and partial cutaneous syndactyly, especially of the second and third fingers. Autosomal dominant inheritance is evident with a high degree of penetrance and variable expressivity (Cohen, 1975).

6. Carpenter Syndrome

Striking features of the Carpenter syndrome include craniosynostosis, preaxial polysyndactyly of the feet, brachydactylous hands with clinodactyly and variable soft tissue syndactyly, short stature, obesity, and various other abnormalities. The occurrence of mental deficiency and congenital heart defects is of special concern. The condition follows an autosomal recessive mode of transmission (Cohen, 1975, 1979b).

C. Craniofacial Cartilages

In isolated sagittal synostosis, with growth and development the lesser sphenoidal wings and the cribriform plate of the ethmoid become elevated relative to the planum sphenoidale, and basilar kyphosis occurs with down-

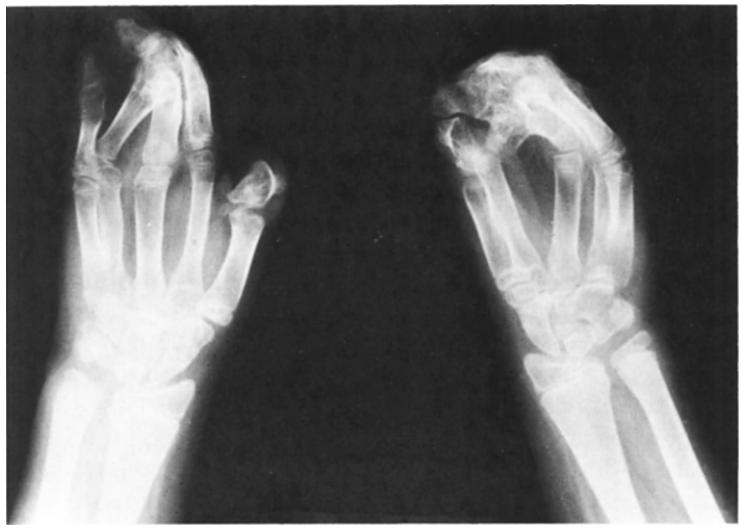

Fig. 15. Syndactyly in Apert syndrome.

ward rotation of the posterior cranial fossa. In isolated coronal synostosis, both the anterior and posterior portions of the cranial base are shortened and the cranial base angle is increased (Moss, 1959). Craniofacial cartilages in the cloverleaf skull malformation associated with thanatophoric dysplasia have already been described in Section III,B.

In the Crouzon syndrome, the anterior and posterior portions of the cranial base are shorter than normal. The greatest shortening occurs in the region of the clivus. The sphenooccipital synchondrosis is radiographically patent in most children and adolescents with the Crouzon syndrome, although occasionally it may be closed. Fusion of the sphenopetrosal and petrooccipital synchondroses has been documented. The cranial-base angle is highly variable, but mean values do not vary significantly from normal. The cribriform plate has a lower position than normal. The morphology of the orbital region is markedly altered, the length of the orbit being reduced. The shortening is located primarily in the region between the greater wing of the sphenoid and the anterior wall of the temporal fossa, probably being caused by lack of forward sutural growth in the temporal region and in the cranial base (Kreiborg, 1981).

In the Apert syndrome, the cranial base is shorter and flatter than normal with extreme shortening of the clivus. The sphenooccipital synchondrosis has a decreased number of chondrocytes and hypertrophic cartilage cells, indicating diminished chondral growth activity. In some instances, premature bony fusion of the sphenooccipital synchondrosis has been noted. Marked resorption occurs on the cerbral surface of the cranial base and marked bony

apposition is found on its pharyngeal surface (Kreiborg *et al.*, 1976; Ousterhout and Melsen, 1982).

V. MISCELLANEOUS DISORDERS

Basilar impression is a skeletal anomaly in which the cranial base is flattened on the cervical spine. In primary basilar impression, a distinct developmental defect of the chondrocranium, anomalies such as occipitalization of the atlas and Klippel-Feil anomaly may be associated. Various other anomalies also may occur such as aqueductal stenosis, Arnold-Chiari malformation, syringobulbia, and syringomyelia. Some families with basilar impression are familial, an autosomal dominant mode of transmission being implicated. Basilar impression may also be associated with acroosteolysis, an autosomal dominant condition consisting of dissolution of the terminal phalanges, short stature, bizarrely shaped skull, and premature loss of teeth. Secondary basilar impression is characterized by an invagination at the base of the skull from bone-softening disorders such as Paget's disease, osteomalacia, hypoparathyroidism, osteogenesis imperfecta, Hurler syndrome, osteoporosis, and rickets (Paradis and Sax, 1972; Gorlin *et al.*, 1976).

In microcephaly, significant shortening of the cranial base occurs. A moderately positive correlation exists between the length of the cranial base and the head circumference (Babineau and Kronman, 1969).

In uncontrolled hydrocephaly, the cranial base is significantly lengthened in its anterior portion but not in its posterior portion. There is also flattening or inferior positioning of the squamous portion of the occipital bone (Forrester *et al.*, 1966).

In trisomy-21 syndrome the width and length of the cranial base are smaller than normal. The cranial fossae are shorter with the largest difference occurring in the posterior cranial fossa (Kisling, 1966).

The trichorhinophalangeal syndrome is characterized by cone-shaped epiphyses, sparse fine hair, bulbous nose with tented alae, and variable growth retardation. The most common form of the disorder follows an autosomal dominant mode of transmission (Gorlin *et al.*, 1976). The posterior cranial base is significantly shortened and deflected upward. This alteration can be attributed to a primary defect in endochondral growth. King and Frias (1979) showed a significant correlation between the cranial base abnormalities and shortening of the middle phalanx of the second finger, one of the most commonly and severely affected tubular bones in the trichorhinophalangeal syndrome.

Cleidocranial dysplasia is characterized by aplasia or hypoplasia of one or both clavicles, exaggerated development of the transverse diameter of the

cranium, and delayed ossification of the fontanels. The condition has autosomal dominant inheritance (Gorlin *et al.*, 1976). Cranial-base length may be reduced in some instances. Some patients also may have a smaller cranial-base angle (Davis, 1974).

The oral-facial-digital syndrome I consists of abnormally developed frenula, cleft tongue, hypoplasia of the nasal alar cartilages, medial pseudocleft of the upper lip, asymmetric cleft palate, and various malformations of the digits including clinodactyly, syndactyly, and brachydactyly. The condition has an X-linked dominant mode of inheritance, lethal in males (Gorlin *et al.*, 1976). In this condition, the cranial-base angle is dramatically increased in both children and adults, indicating no increased flexure of the angle with growth. The platybasic response appears to be a sphenoidal discrepancy in which the sella turcica is positioned downward and forward in relation to other midsagittal structures (Pitts, 1970).

The otopalatodigital syndrome is an X-linked recessive disorder consisting of a distinctive facial appearance with an overhanging brow, prominent supraorbital ridges, downslanting palpebral fissures, and ocular hypertelorism. Other features include conduction deafness, cleft palate, short stature, and generalized bone dysplasia (Gorlin *et al.*, 1976). Frontal and occipital bossing and thickening give the skull a mushroom-like appearance. The cranial-base angle is decreased and the clivus lies farther posterior than normal in relation to the cervical spine (Dudding *et al.*, 1967).

Ocular hypertelorism is etiologically and pathogenetically heterogeneous. The condition may occur with frontonasal encephalocele and with craniosynostosis syndromes such as Apert and Crouzon syndromes. These disorders have already been discussed. Widely spaced eyes may have other causes. Mann (1970) suggested that early ossification of the lesser sphenoidal wings could lock the eyes into their lateral fetal positions. In many cases of ocular hypertelorism, the increase in interorbital distance is accompanied by enlargement of the ethmoid labyrinth and prolapse of the cribriform plate (Fig. 16) (Converse, 1977). Some cases of ocular hypertelorism are associated with duplication of the nasal septum, and the crista galli may be widened or duplicated (Tessier, 1976). Moss (1965), in studying orofacial clefting and its association with ocular hypertelorism, suggested that dysplasia of the nasal capsule may be related to both the formation of clefts and widely set eyes at the same time.

Neurofibromatosis is an autosomal dominant hamartoneoplastic/malformation syndrome consisting of multiple neurofibromas, café-au-lait spots, skeletal anomalies, and other defects. The condition has protean manifestations (Gorlin *et al.*, 1976). On occasion, bony dysplasia of the sphenoid may occur, producing an orbital defect with pulsating exophthalmos (Figs. 17 and 18). Deformity of the sella turcica may also be present (Bruwer, 1955).

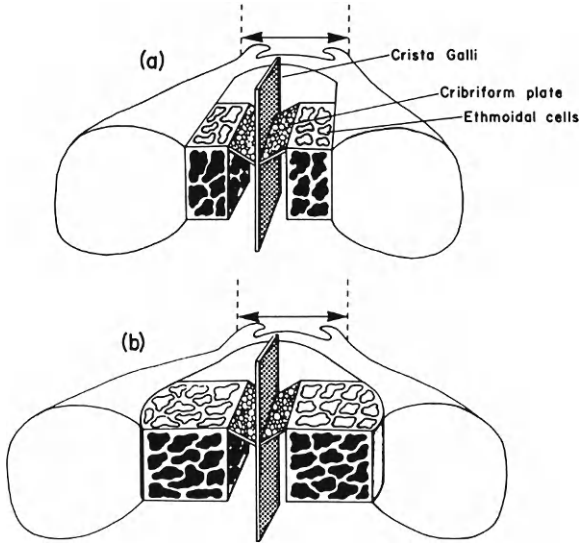

Fig. 16. (a) Diagramatic representation of normal interorbital anatomy. (b) Orbital hypertelorism with enlargement of ethmoid labyrinth and prolapse of the cribriform plate. Modified and redrawn from Converse (1977).

VI. CONCLUSION

A major problem exists today in the field of craniofacial anomalies because the two major kinds of workers—human dysmorphologists and experimental teratologists—tend to have little contact and tend not to appreciate the significance of each other's contribution to the subject. Experimental

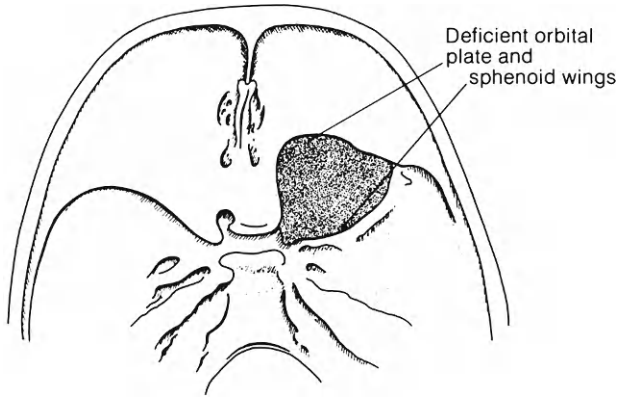

Fig. 17. Deficient orbital plate and sphenoid wings in neurofibromatosis. From Bruwer (1955).

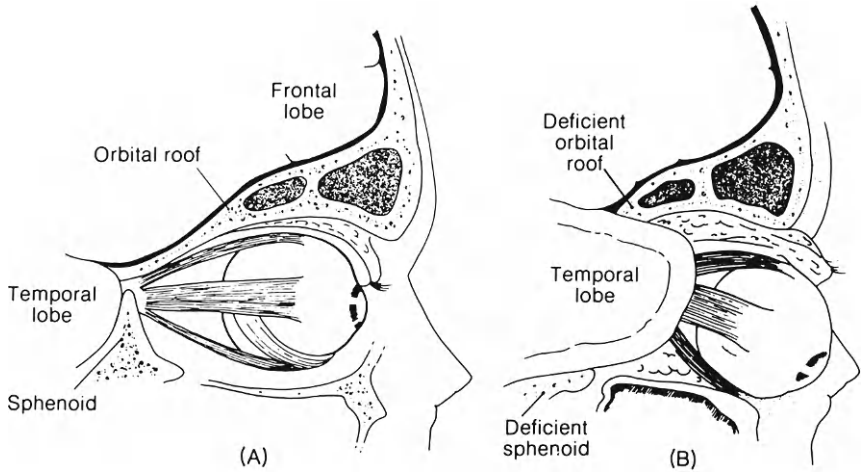

Fig. 18. (A) Normal anatomy. (B) Deficient orbital roof and sphenoid wing allowing prolapse of the temporal lobe with exophthalmos. From Bruwer (1955).

teratologists understand the *pathogenetic heterogeneity* of individual craniofacial anomalies, but they tend not to appreciate how *etiologically heterogeneous* and how variable such anomalies are at the human level. Thus they frequently select animal models that probably have little bearing on human problems. Studies of "achondroplastic" animals are a case in point. Closer collaboration with human dysmorphologists should result in better selection of animal models for study that have human relevance. Human dysmorphologists, for their part, have focused too much attention on the descriptive aspects of the subject, without paying sufficient attention to mechanisms. The future is ripe for collaborative endeavors.

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8

Immunology of Cartilage

Michael W. Elves

I. INTRODUCTION

From the point of view of the immunologist cartilage is, on the face of it, a dull and uninteresting tissue. From the early days of modern plastic surgery it was known that cartilage could be grafted between individuals for the reconstitution of cartilaginous defects, and would be resorbed only slowly if at all. There was little difference between results obtained using foreign (or allogeneic) cartilage and those using heterotopically transplanted autologous cartilage (Gibson and Davies, 1960; Gibson *et al.*, 1957). The assumption that cartilage enjoyed some kind of immunological privilege, which put it beyond the attention of the immune system, was the basis of the interest shown in the late 1960s in the possibility of using foreign articular cartilage grafts to restore damaged or degenerated joints (Pap and Krompecher, 1961; Laurence, 1969). Although such grafts did enjoy some initial success they invariably failed in the long term. Similar results were obtained in animal experiments (Campbell *et al.*, 1963; Chesterman, 1968). This experience with

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cartilage grafting promted a renewed interest in the nature of the immunological privileged state enjoyed by cartilage grafts in some situations. A review of this field will be given next.

Interest in the immunology of cartilage, and in particular articular cartilage, has also been stimulated as a result of the demonstration of the involvement of the immunological system in diseases such as rheumatoid arthritis, other inflammatory arthritides, and relapsing polychondritis, which involve cartilage damage. Indeed it has been claimed that chondrocyte-specific antigens both exist and may be targets for an autoimmune reaction (Bobechko, 1970, 1971). This area will be discussed further later.

II. THE IMMUNE RESPONSE

An immune response may ensue if a foreign material gains entry to the body. There are, however, two important requirements to be met, assuming that the host is competent to mount an immune response. First the material must have antigenic sites on it in order to stimulate the immune system (i.e., have *antigenicity*). Second, those antigenic sites must be accessible or "visible" to the immune system (i.e., be *immunogenic*). As will be seen later, it is important to distinguish between antigenicity and immunogenicity when considering cartilage. It is also important to distinguish between an *immune response* and an *inflammatory response.* In the latter cases the principally involved cells are the phagocytes of the body which cause the destruction of foreign materials, either inside or outside the cell, by means of hydrolytic enzymes which they produce and secrete. In the case of multiple exposures to the same foreign material, a new inflammatory response is initiated by each exposure and the response is similar to the last, or to a response against an unrelated foreign body. In the case of the immune response the macrophages may be involved, but the principal cells engaged are the lymphocytes and their relations. The effector mechanisms involved in the response are the antibodies produced by plasma cells, "killer" lymphocytes, and a group of molecules collectively called the *lymphokines.* All three mechanisms can bring about the demise of the foreign invader. The important features of the immune response are (*1*) its specificity (i.e., the response, and the antibodies produced by it, are directed only against the antigens of the foreign material and not against unrelated materials), and (*2*) the phenomenon of immunological memory. The latter ensures that should there be a second exposure to the same foreign material the immune response will be initiated earlier and will proceed at a brisker pace (this is the basis of immunization). In practice however, the reaction to an immunogenic foreign material is usually a mixed reaction having both inflammatory and immunological components.

In the present context it would be inappropriate to discuss in detail the intricacies of the immune responses; for these the reader is referred to one of the standard texts on the subject. Only a brief outline of the immune response will be given here.

The invading immunogenic material must come into contact with cells of the immune system in order to initiate the response and sensitize the host. This contact may be made in peripheral tissues where the antigen encounters circulatory and migrating lymphocytes or in the lymphoid tissue (i.e., spleen, lymph nodes, or Peyer's patches). In the case of some antigens there may be involvement of macrophages, which process and alter the antigen to put it into a more immunogenic form capable of stimulating the lymphocytes. The lymphocytes can be subdivided into different functional types although they may share the same morphology. The T lymphocytes are cells derived from the thymus which have a regulatory function in the humoral immune response (i.e., antibody production), and a central role in the cell-mediated immune (CMI) response. It is the latter that is of most importance in the reaction against grafts. The B lymphocytes are derived from the bone marrow and are the main precursors of antibody-producing cells.

The response to an antigen is complex and involves interaction between the different lymphocyte classes and subclasses. Some antigens may influence B cells directly and trigger them into antibody production, (i.e., T cell-independent antigens). Others trigger T cells which then interact with B cells which go on to antibody production. These are the so-called "T-helper" cells. Triggered T cells may themselves produce effector, or killer, T cells which are able to damage foreign grafts. They can also synthesize a variety of lymphokines. These are a group of nonantibody molecules that have various properties and can influence other cell types. Macrophage inhibition factor (MIF) will act upon macrophages and cause them to become immobilized (inhibited), and at the same time to become metabolically and phagocytically active (Dumonde *et al.*, 1969). Thus MIF is an important mediator of the inflammatory component of the reaction. Cytotoxic factor (CF) has the property of being able to kill other cells and cause tissue damage and has been implicated in the tissue damage found in the periodontal disease when there is an immune response to plaque antigen (Horton *et al.*, 1973).

A further subset of T cells are the suppressor cells (T_s) which interact with other T and B cells and exert a negative influence on them. These cells are thus important for the control and termination of the immune response. If they become defective or depleted the result will be a continuing immune response, and if this is against a "self" component of the body an autoimmune disease may result. A defect in T_s cells has been claimed in rheumatoid arthritis.

The immune response is a complex network of interaction between cells to

produce an effective and controllable defense mechanism. Essential features of the immune response are cell proliferation, usually in the lymphoid tissues, and T and B cell response to antigens by transformation into large lymphoid cells which undergo mitotic division. Hence the enlargement of lymphoid tissues involved in an immune response. Through this proliferative activity the number of cells involved in the immune reaction is increased. Further amplification of the response occurs through the agency of lymphokines which will recruit macrophages and also naive lymphocytes into the reaction. Thus although the immune response may be initiated by interaction of only a few lymphocytes with the antigen, it is quickly amplified to provide an effective defense mechanism and systemic state of immunity. This is in contrast to the inflammatory reaction which remains localized, rarely becoming systemic, and starting anew on each exposure.

An immune response will result in the generation of specific effectors, either antibodies or sensitized or killer lymphocytes, and these can be detected in the laboratory by the use of appropriate techniques. Only if such effectors are found can the presence of an immune response be inferred. It is important that this situation is appreciated because there have been instances, particularly in the early literature, where an immune reaction to a graft has been claimed on the basis of histopathological examination of transplanted tissue. Although the presence of lymphocytes or plasma cells in tissues around a graft may suggest an immune response, they give no indication of the specificity of the response (i.e., whether against the graft or a contaminating organism). Misleading conclusions have been drawn using such criteria.

While considering the effects of an immune response on cartilage, it is worthwhile to note that, in addition to the immunological effector mechanisms, tissue damage may also be caused by products of macrophage activation (i.e., the inflammatory component of the overall response against the tissue). Through the operation of lymphokines produced by stimulated lymphocytes it is inevitable that an ongoing inflammatory response will occur as a result of antigenic stimulation.

III. ANTIGENS IN CARTILAGE

An important question now needing an answer is whether or not cartilage, as a tissue, possesses antigenic sites. These would obviously be necessary as a trigger for an immune response against foreign, or even autologous cartilage. In the case of cartilage there are three components that require consideration as possible sources of antigenic material—the chondrocytes, collagen, and proteoglycan of the extracellular matrix.

A. Chrondrocytes

The cells of most tissues contain, as integral parts of their surface membranes, a variety of antigenic sites. These antigens may be (*1*) a unique characteristic of that tissue (i.e., *tissue-specific* antigens), (*2*) present on all cells of the body but unique to that individual (i.e., *individual-specific*), or (*3*) a common characteristic of all individuals of a given species (i.e., *species-specific*). Individual-specific antigens are of most importance in the context of tissue transplantation but tissue-specific antigens may be of importance in determining cellular differentiation, and may have some role in the development of autoimmune disease or of cancer.

There has been a suggestion, based on experimental studies in rabbits, that cartilage cells may have tissue-specific antigens which can give rise to autoimmune reactions under certain conditions.

Bobechko (1970) claimed that when articular cartilage of the knee joint was abraded, or if a slice of autologous cartilage was placed in a joint, a proliferative reaction occurred in what he claimed was the regional lymph node. The result of these procedures was destruction of the intact cartilage of the joint, which could be averted by removal of the popliteal lymph node. In other experiments the same author infected one knee joint with staphylococci and claimed that the subsequent damage to the cartilage of the knees could be prevented by removal of the popliteal node (Bobechko, 1971). This type of evidence for the existence of tissue specific autoantigens is very inadequate for a number of reasons. First, there was no demonstration of any *specific* immunological effector against a component of the cartilage. The proliferative reaction observed may well have been in response to subclinical infection introduced while the knee joint was open. Second, the immune response is essentially a systemic reaction and excision of a local lymph node draining a skin allograft has no significant effect upon graft rejection. This is because the next node in the chain will react. Third, as the present author has shown, the popliteal lymph node is not the principal draining node for the knee joint in quadrapeds (Elves, 1978).

Better, but as yet unconfirmed, evidence for the existence of chondrocyte-specific antigens, comes from the studies of Gertzbein and his colleagues (1976, 1977). They exposed isolated rat epiphyseal chondrocytes in tissue culture to lymphocytes from either the cartilage donor (syngeneic) or from an unrelated individual (allogeneic). They found that in both the syngeneic and allogeneic situations the lymphocytes were stimulated to transform and proliferate, indicating the recognition by the lymphocytes of antigens on the chondrocytes. When these workers mixed two sets of allogeneic lymphocytes in a normal mixed leukocyte reaction (MLR) (Bain *et al.*, 1964), the response was higher. However if syngeneic lymphocytes constituted the MLR then there was no reaction. Furthermore, this group found that

allogeneic kidney cells exposed to lymphocytes caused only a slight reaction. The positive MLR reflects recognition, by reactive lymphocytes in the culture, of histcompatibility (H) antigens of the major H system (HLA in Man; H-2 in the mouse; Ag-B (or H-1) in the rat). Antigenic disparities involving only minor H antigen systems do not give a positive MLR (Elves, 1967, 1968; Wilson, 1967; Wilson *et al.*, 1967). Furthermore, with the exception of epidermal cells (Cochrun *et al.*, 1971; Gillette *et al.*, 1972), other types of cells have failed to stimulate allogeneic lymphocytes in mixed cultures (Hardy and Ling, 1969; Schellekens and Eigsvoogel, 1970). It is surprising therefore that a positive reaction was obtained with syngeneic chrondrocytes. One possible explanation for this finding is that the lymphocytes are stimulated not by the chondrocytes but by the proteolytic enzymes used to isolate them. The isolated cells are rarely free of adherent matrix which may retain enzymes (Fincham, 1973). In an attempt to eliminate this possibility Gertzbein and colleagues (1977) used enzyme-treated lymphocytes in the MLR with negative response. Lymphocytes, however, have no coating of proteoglycan to retain the enzymes. Trypsin and other proteolytic enzymes are known to stimulate lymphocyte transformation *in vitro* (Mazzei *et al.*, 1966a,b).

Langer *et al.* (1972) also claimed to have demonstrated the existence of chondrocyte-specific antigens. They injected rats with enzymatically isolated syngeneic chondrocytes or implanted shavings of syngeneic cartilage, and then used the leukocyte migration inhibition (LMI) assay (David *et al.*, 1964) to demonstrate specific immunity. There are, however, a number of flaws in these experiments, the chief of which is, again, a failure to eliminate the enzymes as the immunogenic material. Enzyme would have been present both in the cell preparation used for immunization and also in the antigen preparation used in the assay. Fincham (1973) has demonstrated that chondrocytes isolated with collagenase, and well washed before being used to immune rabbits, elicited the production of anticollagenase antibodies. The use by Langer and his colleagues of enzyme-treated liver cells as a control was not correct because, like leucocytes, liver cells do not possess a proteoglycan coating.

The most persuasive evidence for the existence of chondrocyte-specific antigens comes from the work of Malseed and Heyner (1976). They used isolated chrondrocytes from bone rudiments of fetal rats to immunize either rabbits (heterogeneic) or an allogeneic strain of rats. Cytotoxic anti-chondrocyte antibodies were detectable in the sera of immunized animals. In the case of the rabbit, antibodies were directed against chondrocytes of both the original donor strain (Fischer rats) and an unrelated strain (DA rats), but only low activity was found against rat lymph node cells. This is somewhat surprising as this result would imply that chondrocytes carry species-specific antigens that are not present on rat lymphocytes. If one im-

munized rabbits with rat kidney cells, for example, then the resulting antibody will react with other rat cell types including lymphocytes. A further feature of the rabbit antibodies produced by these workers was that they did recognize antigens on rat erythrocytes. The alloantibodies they produced in DA rats had cytotoxic activity against both DA and Fischer chondrocytes but, again, did not react with lymphocytes from either strain. This result would imply that chondrocytes do not possess antigens of the major Ag-B antigen system. There is other evidence, to be discussed later, which indicates that this is not the case. Again immunization across an Ag-B barrier with tissues such as kidney homogenates will invariably produce anti-Ag-B antibodies which will react with lymphocytes. One possible explanation for this discrepancy is that the cells they used, being from fetal tissues, may not yet be expressing Ag-B antigens at their surface. Clearly these experiments require independent verification.

The presence on articular cartilage chondrocytes of antigens of the major H system has been demonstrated by the present author (Elves, 1974a). Cells isolated enzymatically from sheep articular cartilage were used in cytotoxic assays with antisera against various antigens of the major sheep H system. Lymphocytes from cartilage donor sheep were tested in parallel. There was a good correlation in the reaction with these antibodies between chondrocytes and lymphocytes. The only difference observed was the longer time required by the antibody to kill chondrocytes compared with that to kill lymphocytes. This was almost certainly due to the presence of adherent proteoglycan matrix around the cartilage cells which acted as an impediment to the antibody gaining access to the cell surface. Thorough removal of this remnant matrix, by further enzyme treatment, reduced the time required for serum to kill the cells.

It can therefore be concluded that cartilage cells possess species-specific antigens which may be relevant in the context of cartilage xenografts, and also antigens of the major H system. There is also some evidence to suggest that there may also be one or more cartilage-specific antigens on the chondrocyte.

B. Collagen

Collagen is the major structural protein of cartilage, forming as it does the framework of the extracellular matrix and conferring mechanical strength on the tissue (see Volume 1, Chapter 7). For a long time it was generally considered that collagen was not antigenic, and therefore could not elicit an immune response. Work in the last two decades has shown however, that this is in fact not the case. Collagen possesses a number of different antigenic sites, some of which are dependent on the three-dimensional configuration of the molecule whereas others are not.

The collagen molecule consists of three polypeptide chains (α chains) each of which is helically twisted on itself and is also twisted with the other two chains to form a triple helix. Thus the collagen molecule resembles a length of rope in its structure. There are at least five types of α chains each of which has glycine in every third position in the amino acid sequence. This glycine is responsible for the coiled configuration of the molecule. There are four major types of collagen to be found in tissues, each of which has its own α chain characteristics. In all but one case the three α chains of the molecule are identical, the exception being type I collagen, which is found in skin, bone, and tendon, and has two identical α chains [α 1(I)]$_2$, the third being different (α 2). Cartilage collagen is unique and is made up of three α1(II) chains (i.e., type II collagen). Although the greater length of the collagen molecule is in the form of the triple helix, there is at each end a short nonhelical region which is due to the lack of the glycine at every third position. The collagen molecule can be altered in the laboratory. Thus mild heat causes separation of the three α chains, which can be reassociated under certain conditions to regain the normal structure or to produce "artificial" collagens. Furthermore, treatment of the collagen with proteases will remove the nonhelical terminal sequences of the α chain. In terms of the amino acid sequences, it has been shown that those in the helical regions of the molecule are remarkably similar from species to species, whereas those in the terminal regions show interspecies variation. The structure of collagen is obviously more complex than has been outlined above, but the information given is sufficient to understand the immunological properties of the molecule.

Most of the early studies of collagen immunology were carried out using rather impure and ill-defined material and the results obtained were confusing (Kirrane and Glynn, 1968). Using better defined material, three major antigenic sites have been revealed. In the earliest studies it was found that rabbits would produce antibodies against bovine collagen, and these would not react with pepsin-treated collagen (Schmitt *et al.*, 1964; Davison *et al.*, 1967; Steffen *et al.*, 1968a; Pontz *et al.*, 1970). This suggested that the antigenic sites of the collagen lay in the terminal nonhelical regions. However, those who used other species to raise anticollagen antibodies obtained different results. Thus antibodies raised in mice (Nowack *et al.*, 1975), rats (Beil *et al.*, 1973), or chickens (Furthmayr *et al.*, 1972) would react with enzyme-treated collagen. Clearly, therefore, the species used to produce the antibodies is important in interpreting results.

A number of groups have used cyanogen bromide (CNBr) to obtain peptides from α chains of collagen molecules. CNBr peptides from both C- and N-terminal ends of the chain have now been shown to possess antigenic sites. It has been claimed that there are antigenic differences between different types of α chains in terms of their ability to stimulate an immune response

(α1 is less immunogenic than α2), and also that the α1 chain has antigenic sites only at the C-terminal end (Lindsley *et al.*, 1971). Another important feature of the nonhelical terminal antigenic determinations is that they show little interspecies cross-reactivity: they are thus species-specific (Pontz *et al.*, 1970; Timpl *et al.*, 1972).

The antibodies used in the aforementioned studies were raised in rabbits. When other species were used to produce the antibodies it became obvious that there were also antigenic sites present in the helical part of the chains (Furthmayr *et al.*, 1972; Beil *et al.*, 1973; Hahn and Timpl, 1973; Nowack *et al.*, 1975). It also became clear that the antigenic sites of the helical region were dependent upon the conformation of the molecule. Thus anti-collagen antibodies raised in rats against calf collagen would not react with α chains separated by denaturation. If the α chains were reassociated however, then the reactivity with the antibody was restored (Beil *et al.*, 1973). If the wrong α chains were used to produce artificial helices, then they would not react with the antibody (Hahn and Timpl, 1973; Hahn *et al.*, 1974). It can therefore be concluded that all three α chains are involved in the helical region antigenic site. Like the terminal region antigenic sites, the helical antigens are also species-specific and show little interspecies cross-reactivity.

There is a third group of antigenic sites on the collagen molecule which is situated on the central portion of the α chain but which does not depend on the other two α chains. Thus α chains from which the C- and N-terminal ends have been removed will still react with rabbit and anticollagen antibodies (Davison *et al.*, 1967; Steffen *et al.*, 1968a). To demonstrate the presence of these antigens it is essential to use denatured collagen, because helix formation masks them (Steffen *et al.*, 1967; Beil *et al.*, 1973). In contrast to the two antigen groups already mentioned, the central region α-chain antigens are not species-specific (Steffen *et al.*, 1968a, 1971a). Using CNBr peptides from the central parts of the α chain, evidence has been obtained which indicates that different α chains may vary in their immunogenicity (Pontz *et al.*, 1970; Timple *et al.*, 1971). However, further study of this aspect is required before the crossreaction pattern between α chains is clear (Hahn *et al.*, 1975).

Summarizing the situation, it may be concluded that collagen does possess at least three categories of antigenic sites: the sequential sites occurring on the terminal, nonhelical, regions; those on the central parts of the α chain; and the conformational sites on the intact triple helix. Collagen thus has the potential to arouse an immune response. Moreover, if collagen molecules are modified by acetylation or tyrosylation then they can become more immunogenic and develop new antigenic specificities (Jasin and Glynn, 1965).

A major question that can be posed is whether or not these antigenic sites on collagen are likely to be immunogenic in clinically relevant situations.

They will arouse both antibody production and development of cell-mediated immunity under artificial circumstances (Adelmann *et al.*, 1972; Adelmann and Kirrane, 1973; Beard *et al.*, 1978). There is also evidence that the nonconformational antigenic sites will trigger T lymphocytes in mice (Rassenwasser *et al.*, 1980). As these antigens are species-specific in the main, it is conceivable that cartilage transplantation across a species barrier may result in an anti-collagen immune response. However, it is worth pointing out that in the studies reviewed briefly earlier the immunization schedules have been very artificial and use of an adjuvant always employed. In the allogeneic transplantation situation it is unlikely that collagen in a cartilage graft will arouse specific immunity (Peacock and Petty, 1960). However when the immune response to collagen in subjects with an abnormal immune response is considered, the position may well be different as will be shown later.

C. Proteoglycans

The second major component of the extracellular matrix is the proteoglycans which occupy the interstices of the collagenous network. They are responsible for controlling the water content of the tissue and for conferring the properties of elasticity and resilience on cartilage. It is not possible to discuss at length here the structure of proteoglycan molecules (see Volume 1, Chapter 8), but there are a number of features that are of relevance in the context of their immunology. The basic unit of the proteoglycan molecule is a protein core of variable length along which are attached the glycosaminoglycans. The principal glycosaminoglycans found in cartilage are the chondroitin sulfates and keratan sulfate. The arrangement is such that the proteoglycan molecule resembles a test tube brush (Rosenberg, 1980). At the proximal end of the protein core is a special "link" protein. It is through the link protein that the proteoglycan molecules are attached to hyaluronic acid to give rise to long aggregates.

The earliest studies in this field tended to suggest that the glycosaminoglycan chondroitin sulfate was not antigenic (Glynn and Holborrow, 1952; Boake and Muir, 1955). There have been a number of studies of the antigenicity of proteoglycans obtained from cartilage by disruptive techniques. Usually bovine nasal cartilage has been used for this work and an adjuvant has been employed. Di Ferrante (1963), in his studies, immunized rabbits with material obtained in this way, and obtained antibody that reacted with a light fraction from a proteoglycan extract (PP-L). If the PP-L used as the antigen in the antibody-detecting system was first treated with hyaluronidase or papain, then it no longer reacted with the antibody (Di Ferrante, 1964). This group also showed that the antibodies they obtained did not cross-react with proteoglycans from other species. Other studies are not in agreement, in

that they showed that although treatment of the PP-L with trypsin abolished its reactivity with the antibody, treatment with hyaluronidase did not (White *et al.*, 1963a,b; Loewi, 1964; Loewi and Muir, 1965; Sandson *et al.*, 1966). Antibodies to proteoglycan have also been raised using material from pig (Sandson *et al.*, 1966), and human cartilage (White *et al.*, 1963a,b; Sandson *et al.*, 1966). Further studies have revealed that there is interspecies cross-reactivity of some anti-PP-L antibodies. Thus, antibovine PP-L antibodies have been found to cross-react with human PP-L and porcine PP-L (Di Ferrante and Pauling, 1964; Sandson *et al.*, 1966). Antibodies against porcine proteoglycan cross-reacted with proteoglycan from human, bovine, or guinea pig cartilage.

The situation is in fact more complicated because in addition to a species-common antigenic determinant on proteoglycan, other antigens have been revealed which are species-specific (Di Ferrante and Pauling 1964). Often pretreatment of the proteoglycan with hyaluronidase was necessary to reveal these other specificities (Loewi, 1964; Loewi and Muir, 1965; Sandson *et al.*, 1966). Most studies have indicated that these antigens are associated with the protein element of the proteoglycan and are sensitive to proteolytic enzyme digestion (Loewi, 1965; Barland *et al.*, 1966; Hirschmann and Dziewiatkowski, 1966; Sandson *et al.*, 1970).

More recent studies have been directed toward the more precise correlation between the antigenic sites and the molecular structure of the proteoglycan. Sandson and co-workers (1970) used proteolytic enzymes to prepare "doublets" consisting of two glycosaminoglycans joined by a short polypeptide sequence from the original core protein. They then removed the glycosaminoglycans using hyaluronidase. The remaining polypeptide was found to have the ability to absorb antibody against the species-common antigen. This location of the species-common determinant at the junction of the glycosaminoglycan and peptide sequence of the doublet has been confirmed (Baxter and Muir, 1972). The doublet preparation did not absorb activity against the species-specific determinant.

There is other evidence for the existence of antigenic determinants on the core protein. Baxter and Muir (1972) used proteoglycan-derived material from which the linkage region of the glycosaminoglycan chain was removed leaving the core polypeptide sequence intact. This material elicited the production by rabbits of antibodies against a species-common determinant. The antibody activity was removed by absorbtion with doublets. Biochemical studies have indicated the heterogeneity of core protein in the proteoglycan molecule (Tsiganos and Muir, 1969; Brandt *et al.*, 1970). Using mild processes and gel chromatography, material which was excluded from the gel gave rise to multiple precipitation lines when put against antiproteoglycan antibody in diffusion plates. Both species-common and species-specific an-

tigens were revealed following hyaluronidase digestion. Smaller molecular weight proteoglycans on the other hand possessed only the species-common determinant, and hyaluronidase treatment was unnecessary. Clearly, therefore, core-protein antigens must be masked in the larger proteoglycans by the glycosaminoglycans (Brandt *et al.*, 1970, 1973). Some evidence exists that suggests that the core protein to which the keratan sulfate chains are attached is antigenically distinct from that carrying chondroitin sulfate chains (Tsiganos and Muir, 1967; Brandt *et al.*, 1970; Wieslander and Heinguard, 1979; Christner *et al.*, 1980). Use of monoclonal antibodies to study core-protein structure has also indicated the existence of at least two antigenic determinants (Dorfman *et al.*, 1980).

The other proteoglycan component that has been examined in some detail is the glycoprotein link through which the proteoglycan units are attached to the hexuronic acid. There is clear evidence that when the link protein is isolated it can be shown to possess a species-specific antigenic determinant. These link protein antigens are not revealed in intact proteoglycans (Di Ferrante *et al.*, 1970, 1972; Keiser *et al.*, 1972; Wieslander and Heinguard, 1979). Keiser and Sandson (1974) examined the link protein of proteoglycan from human articular cartilage and have demonstrated three antigenic determinants. One was present in proteoglycan subunits and was species-common; the second was unique to link protein, but not to man as it was also present in bovine material; the third was unique to the link protein and was species-specific.

In summary therefore it may be said that proteoglycans of cartilage do possess antigenic determinants, but that in the intact structure these are probably masked to a large degree by the glycosaminoglycan. The antigenic determinants are species-specific in some instances and species-common in others. They seem, however, to be associated with the protein elements, (i.e., link and core protein). One interesting feature of these studies is that, although intact proteoglycans are used to immunize rabbits, in order to demonstrate the antigen–antibody interaction in the laboratory it has often been necessary to remove the glycosaminoglycans with hyaluronidase to reveal the antigenic sites. The implication from this is that, after injection *in vivo,* the proteoglycan is broken down before stimulating an immune response. If this is true then it follows that the proteoglycans are not immunogenic while they are intact in cartilage, but once damaged by enzymes they may become so. Studies of the localization of proteoglycan in cartilage slices using antiproteoglycan antibodies and immunofluorescence support this view. Thus, poor staining is seen in sections of intact cartilage matrix, but intense reactions are obtained if the sections are first treated with hyaluronidase (Loewi, 1965; Barland *et al.*, 1966).

Finally, the aforementioned studies have all been carried out using cartilage proteoglycans. Using antisera produced against this material a number of groups have shown cross-reactivity between the proteoglycan of cartilage with that in other tissues including basement membranes such as those of renal tubule glomerulae, tracheal mucosa, thyroid, aorta walls and endothelium of other vessels, the sarcolemma of muscles, heart valves, and intervertebral discs (Loewi, 1965; Pankovich and Korngold, 1967; Sandson *et al.*, 1968). Cross-reacting antigens are also found on certain microorganisms (Hamerman and Sandson, 1970). These observations could well be of importance in some disease states.

IV. IMMUNOGENICITY

From what has been outlined it is clear that the cellular component of cartilage, and the two principle constituents of the extracellular matrix of that tissue possess antigenic determinants that can be demonstrated in the laboratory. Clearly, there is evidence that the two structural components are able to elicit specific immune responses and hence, under the conditions of the experiments, must be regarded as immunogenic (i.e., able to stimulate the immune system to respond). However, it must be stressed that all of these experiments have involved *(1)* separation of the component in question from its normal anatomical relationships with other structures, *(2)* injection into a foreign species so that it is xenogeneic material, *(3)* use of a potent oil-based adjuvant containing mycobacteria (Freund's complete adjuvant). The situation is thus far removed from either that likely to exist in the allograft or autograft situation, or in the subject developing a connective tissue disease. It is under both of these conditions that we need to examine the ability of cartilage and its components to arouse an immune response.

A further point that is worth considering is the role of the irrelevant immune response in the development of immunity to cartilage. As will be discussed later, the matrix of cartilage protects its components both from the initiation of an immune reaction and from suffering the consequences of one. It will only serve this function as long as it remains intact. Should as immunoinflammatory reaction be triggered by a foreign material (e.g., a microorganism) near the cartilage, then it is possible that cartilage matrix degradation may result from the action of enzymes, and other factors, produced by the cells involved in that irrelevant reaction. This damage in turn releases antigenic components of the matrix which could then become immunogenic. There is evidence, as will be seen later, to indicate that both anticollagen and antiproteoglycan antibodies develop in certain disease states.

V. THE IMMUNE RESPONSE TO CARTILAGE GRAFTS

From the experience of plastic surgeons and evidence from animal experiments it is clear that cartilage, unlike most other normal tissues, does not undergo rejection; although over some months or years the graft may show evidence of resorption (Gillies, 1920). Studies using $^{35}SO_4$ uptake, as a marker of chondrocyte viability, have shown that the cells of the cartilage allograft are still viable and able to manufacture matrix proteoglycans after long periods in the host. This is true for grafts in humans (Gibson *et al.*, 1957; Gibson and Davies, 1960), and in other species (Craigmyle, 1958b; Pap and Krompecher, 1961; DePalma *et al.*, 1963; Stjernswärd, 1965). Often the allograft is indistinguishable from the cartilage iso- or autograft after some months in the host. Yet it is clear, as reviewed already, that cartilage has tissue antigens of the major H system as well as those associated with the matrix components.

There have been a number of studies aimed at detecting the presence of an immune reaction in recipients of cartilage grafts. Many workers have used techniques that cannot be regarded as indicative of a specific anticartilage immune response. Studies using histological assessment have shown quite clearly that cartilage allografts do arouse a cellular reaction (Loeb, 1926; Dupertius, 1941; Heyner, 1969; Langer and Gross, 1974). The cells involved in the reaction are very heterogeneous and include typical inflammatory cells (macrophages and polymorphs), lymphocytes, and plasma cells. The presence of the latter two cell types is suggestive of an immune response but cannot indicate its specificity. Although the cells may be reacting against the graft they are also possibly directed against contaminating organisms and other foreign materials. Lymphocytes are also cells that participate in general inflammatory reactions (Rebuck and Crowley, 1955; Cronkite *et al.*, 1960; Volkman, 1966; Wulff and Sparrejohn, 1966).

Another technique that has been applied is the measurement of increase in size of the regional lymph node. This is due to proliferation of lymphocytes that have been triggered by an antigen (Scothorne and MacGregor, 1955). Craigmyle (1958a) found that costal cartilage allografts caused no significant lymph node enlargement. However if a second graft of the same type as the first (i.e., "second set" graft) was put into the recipient then a reaction did occur and the node enlarged. Again, however, the specificity of the response cannot be inferred from this experiment. Craigmyle (1958c) has also used the second-set skin graft assay to investigate this problem. This is based upon the generation of specific immunological memory following introduction of immunogenic material; a second graft having antigens in common with the first graft will be rejected more rapidly. It was found that 28% of rabbits receiving diced costal-cartilage allografts rejected a subsequent

skin graft from the cartilage donor in an accelerated manner. Using mice, and the second-set skin graft assay, Peacock and co-workers (1960) were unable to detect any sensitization in recipients of allogeneic grafts of xiphisternum. However, Stjernswärd (1965), using similar grafts and mouse strains with incompatibility for the major H system (i.e., H-2), did obtain evidence of sensitization in 69% of recipient animals. If the donor/recipient combinations were identical for the H-2 system, but differed for minor, non-H-2, antigens, then sensitization again occured. However, in order to sensitize the recipient against the minor H-Y (i.e. male Y-linked) antigen multiple cartilage grafts were required. The xiphisternum graft is not entirely cartilage and has a fibrous perichondral envelope that lacks the dense matrix. It is feasible that this was the component of these grafts that stimulated the immune response. This would account for the fact that, although there was evidence of specific antigraft immunity, the chondrocytes were still viable 290 days later. Thus is would appear that although the afferent, or sensitization, arm of the immune response had been triggered by some component of the graft, the effector mechanisms generated were without any effect upon the graft. Stjernswärd (1965) has attempted to remove the perichondrium by stripping, or by serial passage through several allogeneic hosts prior to implantation into the test animal: Still the majority of animals were sensitized. However, in the case of the stripped grafts there is a strong possibility that the cartilage was damaged and chondrocytes exposed. Heyner (1973) repeated these experiments, using the same mouse strain combinations, and was unable to confirm the results.

Others have used the LMI assay, and claimed to have demonstrated specific sensitization of rats by cartilage allografts with incompatibility for the major Ag-B (H-1) antigen (Langer and Gross, 1974). These experiments were carried out using slices of articular cartilage. With this type of graft there are two possible reasons why exposed cellular antigens may have been present. First, as a result of opening chondrocyte lacunae during slicing and thus exposing cells, and second, there may have been bone and bone marrow elements included with the graft. Bone and bone marrow elements are highly immunogenic (Elves and Ford, 1974). When the cut, deep surface of the graft was sealed with plastic adhesive no immune reaction was detectable.

There have also been a number of attempts to demonstrate an antibody response to cartilage allografts; these too have given conflicting results. Stjernwärd (1965) claimed to have detected anti-H-2 hemagglutinating antibodies in the mice receiving grafts of xiphisternum. Heyner (1973), however, was unable to confirm this finding. Langer and Gross (1974), in their rat study, failed to detect development of cytotoxic anti-Ag-B antibodies. In sheep, however, the present author has been able to demonstrate cytotoxic antibodies in animals receiving cartilage allografts implanted in

defects in the knee joint (unpublished observation). The grafts in these animals were subject to wear as they formed part of the articulating surface. Furthermore, the antibodies did not appear until some months after grafting.

From what has been reviewed, it can be concluded that, in general, cartilage as an allograft is not as effective an immunogen as most other tissue grafts would be. There is clearly confusion about the exact degree of immunogenicity, but it must be emphasized that in most of these experiments the grafts have consisted of cartilage that has been quite severely damaged in the course of preparation. Under these circumstances it is inevitable that antigenic cellular material will be exposed; under normal *in vivo* conditions of course this will not occur, and the chondrocyte will remain buried and inaccessible. As has been briefly mentioned, even if the cartilage graft does elicit an immune response there remains the question of whether or not the effector mechanisms can actually cause destruction of the graft. This aspect will be further considered later.

If the antigenic barrier between graft and host is made larger, by crossing a species barrier, then the tissue graft (xenograft) is in most cases rejected more rapidly than in the case of an allograft and antibody against the graft is a very prominent feature of the reaction. Cartilage xenografts behave differently in that there is no rapid rejection, and the grafts will survive for long periods of time. They are however, resorbed more rapidly than allografts (Loeb and Harter, 1926; Craigmyle 1958b). These grafts also elicit a greater inflammatory reaction in the graft bed (Elves and Zervas, 1974). There is some evidence that the cartilage xenograft can elicit an immune response. Craigmyle (1958a) reported a proliferative reaction in the regional lymph node draining cartilage xenografts in rabbits. Again it must be stressed that this is not a measure of specific immunity. Using the second-set skin graft assay no evidence of immunity against the cellular antigens of the graft could be found in mice receiving cartilage xenografts (Peacock *et al.*, 1960). Elves and Zervas (1974) examined rats receiving xenografts of sheep articular cartilage for the presence of antisheep antibodies. They found reactions in only 3 of 17 recipients. Thus even in a xenogeneic system it would seem that the cellular elements of cartilage grafts are only very poorly immunogenic.

Little attention has been paid to possible immune reactions to the matrix components of cartilage grafts. In the xenogeneic situation there is the possibility of reaction against the species-specific antigens of collagen or proteoglycans. There have, however, been no attempts to detect effectors against these components. The more intense inflammatory reaction around xenografts may indicate that these components are stimulating a response. In the allograft situation there has been one attempt to seek antibodies against matrix proteoglycans of cartilage grafted into the knee joints of dogs; negative results were obtained (Seligman *et al.*, 1972).

In conclusion it may be said that in the case of intact cartilage grafted into allogeneic hosts the cells, and probably also the matrix components, fail to stimulate any significant specific immune response. This failure is not due to a lack of appropriate antigens by the graft, but is due to the shielding of the antigens from the immune system by the dense matrix of the tissue. This was a situation predicted in 1947 by Bacsich and Wyburn. Even in xenografts the matrix confers a high degree of protection, although in this case it may not be absolute. If, however, the matrix is removed from the cartilage cells, and these are then implanted into allogeneic hosts they are rejected (Heyner, 1969) and there is some evidence suggesting development of immunity (Langer and Gross, 1974).

VI. IMMUNOGENICITY OF CARTILAGE IN DISEASE STATES, ESPECIALLY RHEUMATOID ARTHRITIS

A. Autoimmunity

There has been considerable interest in the possibility that certain diseases of connective tissues, and in particular rheumatoid arthritis, may be a result of, or exacerbated by, an immune response to one or more components of the tissues involved. These are the so-called *autoimmune diseases,* and any immune response of significance to the pathogenesis of the disease must be directed against a "self" component. The cellular basis of autoimmunity is not clear at present. Early ideas on the subject centered around Burnett's "forbidden clone" hypothesis (Burnett, 1959). He postulated that during embryological development those lymphocytes that were programmed to react against self antigens were eliminated on contact with that antigen. By the time of birth, or shortly after, there were no self-reactive lymphocytes remaining except those recognizing self-antigens that were masked, or not in contact with the blood or lymph (e.g., thyroid antigens). Release of these antigens by tissue damage in later life may therefore lead to an immune reaction against that tissue. This hypothesis received considerable support from the demonstration that a state of immunological tolerance to a foreign antigen can be induced in rodents by exposure to the antigen in fetal life or soon after birth (Billingham *et al.,* 1956). It is realized now that this view of immunological self-tolerance is naive. The discovery of the various lymphocyte subsets with different functions (i.e., helper, suppressor, killer, antibody producer, etc.) has indicated the complexity of the immune response which, as mentioned above, is now seen as a complex series of cellular interactions. The immune response is thus the result of the interplay of a network of functional components with its built-in controls, both positive and negative. In

the context of autoimmunity it is likely that there is a breakdown in the normal immunoregulatory mechanism (Gershon, 1974). An important cell in this context is the suppressor cell which acts, as its name implies, upon other T and B lymphocytes in an inhibitory way (Zembala and Asherson, 1973; Baker, 1975). If these cells are removed from the animal by appropriate treatment then that animal will mount an enhanced antibody response to certain antigens; the inhibitory influence having been removed (Baker *et al.*, 1970). Certain laboratory rodents are prone to development of autoimmune diseases. Best known among these are the NZB and NZW mouse strains which may develop autoimmune hemolytic anemia or a disease resembling systemic lupus erythematous (SLE) as they age. Examination of these animals has demonstrated a progressive loss of suppressor cells in their immune system with age (Barthold *et al.*, 1974). If the susceptible animals are given spleen cells from young mice the incidence of disease decreases. If on the other hand, young NZB mice receive spleen cells from old animals they will develop the hemolytic anemia.

Thus, the current view is that autoaggressive immune reactivity is the result of disturbance in the balance of the various regulatory elements in the immune system. The cells involved in the response are probably qualitatively normal.

In rheumatoid arthritis there is evidence of immunological derangement. A majority of patients with this disease will have the altered immunoglobulin, rheumatoid factor (RF), in their serum. However, individuals with agammaglobulinemia, who have little or no humoral antibody response, can develop rheumatoid arthritis (Good *et al.*, 1957). By use of a variety of lymphocyte markers (Raff *et al.*, 1970; Jondal *et al.*, 1972; Wybran *et al.*, 1972), a number of defects have been found in the blood lymphocyte population of patients with rheumatoid arthritis. Thus, it has been claimed that B cells are increased in this disease (Papamichail *et al.*, 1971), but this is not the experience of all investigators (e.g., Mellbye *et al.*, 1972). However, the blood cell population may not reflect that in the periarticular tissues (Mellbye *et al.*, 1972). There is some evidence that the T-cell population is increased in synovial fluid in patients with rheumatoid arthritis (Vernon-Roberts *et al.*, 1974; van de Putte *et al.*, 1976). This alteration seemed to be specific to rheumatoid arthritis and was not seen in other inflammatory joint diseases (van de Putte *et al.*, 1976). There is some evidence to indicate a suppressor cell defect in both rheumatoid arthritis (Keystone *et al.*, 1980) and systemic lupus erythematous (Bresnihan and Jasin, 1977). It is possible therefore that rheumatoid arthritis is the result of an altered homeostatic mechanism in the patient rather than the alteration in the antigenic structure of joint components. One possibility that cannot be ruled out, however, is that the initiating antigen is an infecting organism that cross-reacts with an-

tigens in cartilage (Hamerman and Sandson, 1970), or the product of an immune response against an organism (Washburn *et al.*, 1980).

As one can see from the reviewed evidence, cartilage is a tissue that is only feebly immunogenic and in the intact situation is not immunogenic at all. If the tissue becomes damaged and the antigenic sites exposed the situation could well be altered. Tissue damage is a prominent feature of inflammatory joint disease, and so, leaving aside the question of an etiological role, the possibility of an immune reaction against cartilage components can be considered.

As far as the cellular component of cartilage is concerned, there is as yet no evidence in humans with joint disease of any immune reaction. The evidence for cartilage-specific antigens remains the rather dubious experimental studies referred to earlier. In the case of matrix components however, there is mounting evidence of immune reactivity.

B. Anticollagen Immunoreactivity

The analytical studies discussed above have indicated the existence of both species-common and species-specific antigens on collagen. There is no evidence of individual-specific antigens. Furthermore, in order to elicit the immune response, a xenogeneic system, together with a powerful adjuvant, was used. It is surprising therefore to find anticollagen immune effectors in patients with joint disease.

In the 1960s Steffen and his group showed the presence of antibodies against calf collagen in the serum of 30–40% of patients with rheumatoid arthritis (Steffen and Timpl, 1963; Steffen *et al.*, 1968b). They found that the antibodies were more frequently found in subjects with severe and rapidly progressive disease. Since this time there have been many confirmatory reports using calf (Kreigel *et al.*, 1970) and human native or denatured collagens as antigens (Steffen, 1972; Cracchiolo *et al.*, 1975). Similar antibodies were also found, but at low frequency (8–10%), in normal subjects and patients with traumatic joint damage and other connective tissue diseases (Michaeli and Fudenberg, 1971; Steffen *et al.*, 1973; Cracchiolo *et al.*, 1975). There seemed to be no correlation between the presence of anticollagen antibodies and serum levels of RF (Michaeli and Fudenberg, 1971; Steffen, 1972). Antibodies against collagen antigens were also detectable in the synovial fluid from joints affected by rheumatoid arthritis (Steffen *et al.*, 1971b; Cracchiolo *et al.*, 1975; Menzel *et al.*, 1976). They were also regularly found in patients with traumatic synovitis, although no such antibodies were detectable in the serum from these subjects (Cracchiolo *et al.*, 1975).

More recent studies have used collagen of the three major types as antigens. Andriopoulos and his colleagues (1976a,b) examined serum from 110

patients with rheumatoid arthritis and both serum and synovial fluid from another 24 patients with this condition. They found antibodies against the three major collagen types in these samples. (type I, 77% positive; type II, 71%; type III, 62%). In addition, antibodies against denatured type I collagen were found in 88% of cases, and against type II in 62%. In general, where comparisons were possible, antibody titres were higher in the joint fluid than in the serum. There is, however, some disagreement among different groups who have used a variety of techniques to detect antibody. Thus, using a radioimmune assay, Claque and his colleagues (1980) found that 42% of patients with rheumatoid arthritis had antibody in the serum against type II collagen, and 49% had anticollagen I antibodies. No antibodies were found in psoriatic arthritis, but in 30% of patients with ankylosing spondylitis there were both types of antibodies. In the anticollagen II-positive patients in the last group there was peripheral joint disease. Beard and co-workers (1979) used a hemagglutinating assay and found evidence of antibodies against denatured type II collagen, but not against native collagen, in sera from 30–40% of patients with rheumatoid arthritis. When a fluorometric assay was used, the incidence of positive reactions fell to 1 in 62 patients. Other studies from this group indicated a very low incidence (3%) of positive reactions against native type II collagen, without evidence of activity against type I or III collagens (Greenbury and Skingle, 1979; Beard *et al.*, 1980). There was some evidence of a correlation between presence of antibody and joint erosion. Others have found a high incidence of antibody against denatured type II collagen (74%), and to a lesser degree against native type I collagen (30%), in the synovial fluid of patients with rheumatoid arthritis. The antibody also cross-reacted with denatured type III collagen (Menzel *et al.*, 1978).

In juvenile rheumatoid arthritis one study has indicated a high incidence of antibodies against both type I (30.6%) and type II (31.8%) collagens (Steffen *et al.*, 1980). Only in those cases with type II collagen antibodies was there any correlation with severity of disease. In Still's disease the incidence of antibodies against type II collagen (33%) was higher than that of antibodies against type I collagen (13.8%); and in these cases there was a correlation between clinical severity and the presence of both, or either, type of antibody (Steffen *et al.*, 1980).

Another disease in which cartilage breakdown is a major feature is relapsing polychondritis. In one study of 15 patients, anti-type II collagen antibodies were found in 5 cases during the phase of disease activity and titres of antibody were correlated with severity of disease. No antibodies to other collagen types were detected (Foidart *et al.*, 1978). In this disease there is evidence of immunoglobulin and immune complex deposition in cartilage and particularly at the chondrofibrous junction (Valenzuela *et al.*, 1980). Immunoglobulin–collagen complexes have also been found in the synovial

fluid of patients with rheumatoid arthritis (Menzel *et al.*, 1976) and in their synovial fluid cells (Steffen *et al.*, 1974).

There have been a number of attempts to demonstrate cell-mediated immune reactivity against collagen. Trentham and his colleagues (1978) were able to show that lymphocytes from 74–78% of the rheumatoid arthritic patients studied were able to respond *in vitro* to type II and III collagens by lymphokine production; indicating that these patients had cellular immunity against these antigens. There was no reaction against type I collagen nor against denatured α chains. This finding was confined to rheumatoid arthritis, and lymphocytes from patients with other inflammatory or degenerative arthritides failed to give any reaction. Stuart and co-workers (1980) used a similar approach and were able to demonstrate sensitivity of lymphocytes from 50% of patients with rheumatoid arthritis to native collagens. There was an even higher incidence of sensitivity to α chains (90%). However sensitization against collagens and their α chains was also demonstrated in patients with gout and ankylosing spondylitis, but not in normal subjects or those with degenerative arthritis.

There is therefore evidence to suggest that, in the context of rheumatoid arthritis, collagen of the articular cartilage and periarticular soft tissues can elicit specific immunological responses of both the humoral and cell-mediated types. As this disease is associated with defects in the immunoregulatory mechanisms, the most likely explanation for this finding is a loss of suppressor cell activity, and hence an uncontrolled immne response against this self-component. However, there is also some suggestion, as was mentioned above, that antibody production against collagen may also occur in subjects with nonrheumatoid diseases or even in some normal individuals. The assumption that is made however, is that these individuals are immunologically normal. This may not be the case. The significance of these anticollagen immune reactions is difficult to resolve. It may be suggested that they are a secondary feature of rheumatoid arthritis in view of the fact that they are not in evidence in all patients with this disease. Furthermore, the correlation with other parameters of the disease process is by no means good. It is more likely that these immune responses are initiated as a result of exposure of the antigen due to progressive tissue damage. There is evidence for the presence of collagens in the synovial fluid arthritic joints (Cheung *et al.*, 1980). However, an immune response to collagen in an already damaged joint may well be an important factor in the progress of the disease and further joint damage; this aspect will be discussed next.

C. Antiproteoglycan Immunoreactivity

There is less information available in the case of autoimmune reactions to the proteoglycan component of cartilage than in the case of collagen. Initial attempts using crude proteoglycan extracts failed to detect any specific an-

tibodies in either the serum or synovial fluid from patients with rheumatoid arthritis (Herman *et al.,* 1973). However, using a more refined antigen preparation, later studies indicated the presence of antiproteoglycan antibodies in patients with either rheumatoid or osteoarthritis (Herman and Carpenter, 1975). This report still requires confirmation.

Herman and his group have also examined the cell-mediated immune response to proteoglycan in patients with arthritis. Using the lymphocyte stimulation test and crude proteoglycan as antigen, positive reactions were found in about one-third of the patients (11/32). All positive patients had demonstrable joint destruction, but active disease and destructive changes were also present in 75% of those patients giving a negative response. In no normal subject was a positive result obtained (Herman *et al.,* 1973). This group has also found that in 12 of 29 patients with rheumatoid arthritis the lymphocytes would react to proteoglycans by production of a cytotoxic lymphokine (Herman *et al.,* 1974). Once more all patients with positive reactions had active disease. Evidence of cell-mediated immunity against proteoglycans is not confined to rheumatoid arthritis. Thus, using the lymphocyte transformation test or lymphotoxin production, positive results have been obtained in patients with tophaceous gout, septic arthritis, psoriatic arthritis, and osteoarthritis (Herman *et al.,* 1973, 1974). The two tests did not give concordant results however; the lymphotoxin system gave the higher incidence of positive reaction.

There is evidence therefore, to indicate the possibility of immune reactivity against proteoglycan determinants in patients with joint disease. Rather than being of etiological significance however, these reactions are more likely to be initiated as a result of cartilage damage and exposure or alteration of antigenic determinants on the proteoglycan molecule. The antiproteoglycan immune responses are not specific to rheumatoid arthritis and, on presently available evidence, they occur in other nonrheumatoid arthroses. In relapsing polychondritis there have been a number of attempts to demonstrate antiproteoglycan antibodies but most have proved negative (Herman and Hess, 1971; Herman and Dennis, 1973; Rajapakse and Bywaters, 1974). In one report however, in which an immunofluorescence technique was used, antiproteoglycan immunoglobulin was found in two of three patients with this disease (Hughes *et al.,* 1972). Attempts to demonstrate cell-mediated reactions against cartilage proteoglycans in this disease have been more successful. Two groups have obtained evidence of sensitization using the lymphocyte transformation test and the LMI (Herman and Dennis, 1973; Rajapakse and Bywaters, 1974), and found some degree of correlation with clinical activity of the disease (Herman and Dennis, 1973).

In the case of joint disease there is evidence for the presence of pro-

teoglycans in the synovial fluid and phagocytic cells of the periarticular tissue (Sandson, 1967; Herman *et al.*, 1973). These molecules may well have had their antigenic sites exposed by the action upon them of proteolytic enzymes present in the joint (Sapolsky *et al.*, 1973).

VII. THE INFLUENCE OF IMMUNE RESPONSES ON CARTILAGE

From the foregoing it is clear that although cartilage is a tissue that possesses cell-surface antigens and matrix components with antigenic determinants on them, the tissue is only weakly immunogenic in a grafting context. Even when species barriers are transgressed there is only a feeble inconsistent immune reaction. In two situations the conditions may alter and immune responses occur. The first, when the cartilage becomes damaged by disease processes, has already been discussed. The second is the case of osteoarticular grafts.

Articular cartilage cannot be used alone as a graft to repair a diseased joint. This is because cartilage lacks sufficient healing capacity to form a union with the bone of the graft bed (Aichroth *et al.*, 1972). Thus the simplest form of osteoarticular allograft is the shell graft, which has a thin layer of subchrondral bone beneath the cartilage to ensure bony union with the graft bed (Aichroth *et al.*, 1972). In other forms of osteoarticular graft much larger amounts of bone are included. Under these circumstances the lack of immunogenicity of cartilage is of academic interest because the bony element will stimulate allograft immunity and production of relevant effectors (Elves, 1974b; Elves and Ford, 1974). The same will be true in the xenograft situation (Elves and Salama, 1974; Elves and Zervas, 1974). In the case of damage to cartilage, either through disease or surgical manipulation, the antigenic determinants of the cells or matrix components will be exposed and potentially accessible to the immune system. Thus a consideration of the effects of an established immune response on cartilage is important.

There are three groups of immunological effectors that require consideration in the present context. These are lymphocytes, antibodies, and lymphokines.

A. Lymphocytes

Although lymphocytes are responsible for initiating an immune response and also, in the case of killer cells, being tissue-destructive agents, they are not the only cells involved in the immunoinflammatory reaction. Through the effects of lymphokines other cell types such as macrophages and granulocytes will be drawn in. Clearly the lymphocytes themselves will not

have direct access to the cells, collagen or proteoglycans of the cartilage as the intact matrix would present an absolute barrier. If the cartilage becomes damaged then cellular access does become possible. Heyner (1969) showed that chondrocytes, stripped of their proteoglycan matrix, were rejected after implantation by an allogeneic host and lymphocytes were prominent in the cellular reaction to the implant. It is unlikely, therefore, that the lymphocytes have a direct role in rejection of cartilage even if they are sensitized by another graft component such as bone (Elves, 1976; Musculo *et al.*, 1976).

The cellular composition of the inflammatory tissue that surrounds the heterotopic foreign-cartilage graft includes lymphocytes but most prominent are the mononuclear phagocytic cells (Elves and Zervas, 1974). These cells are also prominent in the periarticular tissues of the inflamed joint. In the case of cartilage grafts these cells are associated with fibrillation of the cartilage at its interface with the inflammatory tissue. Although the macrophages are not directly involved in the immune reaction, they may be influenced by immunological mediators, such as lymphokines, or antigen–antibody complexes. Once activated, these cells will produce a variety of proteolytic enzymes, including collagenase, which will have an effect on the integrity of the cartilage (Denman, 1979). The role of lymphokines and immune complexes is discussed further later.

B. Antibodies

From the evidence reviewed above it is clear that antibodies may be formed against antigens of the major histocompatibility system, if one considers the transplantation situation, or against cartilage matrix components in some patients with autoimmune disease. The question now to be asked is, How relevant are these antibodies to cartilage damage? In animals receiving osteoarticular allografts it is clear that antibodies formed against the cellular elements of the graft are found in the synovial fluid in contact with the graft (Elves, 1973). In rheumatoid arthritis, antimatrix antibodies are detectable in the synovial fluid of some affected joints. However, immunoglobulins are relatively large molecules (MW above 120,000) and the molecular size limit for penetration of cartilage matrix has been calculated to be about 68,000 (Maroudas *et al.*, 1968). Therefore, it may be anticipated that antibodies would be largely excluded from cartilage matrix, and hence ineffective as mediators of cellular matrix damage. Experiments have been carried out in order to investigate this point, and these have clearly shown that immunoglobulins cannot penetrate far into cartilage (Lachmann *et al.*, 1969; Poole *et al.*, 1973). Cytotoxic antibodies against H-antigens have been shown to penetrate intact cartilage and kill chondrocytes but only very slowly (Elves, 1973). Using an organ culture system, Fell and her colleagues

(1966) were able to show that antibody against chicken red blood cells and complement could only kill the outermost perichondral cells of a chick cartilage explant. In these experiments some matrix damage was observed which was thought to be due to release of lysosomal enzymes from the cells (Dingle *et al.*, 1967). When the cartilage matrix is enzymatically depleted the situation is altered, and antibody will now penetrate the tissue (Lachmann *et al.*, Cooke *et al.*, 1972; Poole *et al.*, 1973). Fell and Barratt (1973), using pig articular cartilage in coculture with soft connective tissue, found that matrix depletion followed incubation with appropriate antibody. This did not occur in the absence of the connective tissue elements. Thus it must be concluded that antibody cannot penetrate beyond the surface layers of cartilage due to exclusion forces probably generated by the proteoglycan component. However, antibody, if appropriate, may cause some cell damage in the superficial cartilage or in periarticular soft tissues, and, as a result of released hydrolytic enzymes, matrix damage and depletion will occur. Under these altered circumstances antibody will penetrate the matrix-depleted areas of the cartilage and cause further damage.

Antibody against matrix or cellular components of cartilage may also have an indirect action in the joint. This would be due to the formation of complexes with the antigen, and then combination with complement. There is now evidence for the presence of immune complexes in the superficial layer of articular cartilage in rheumatoid arthritis and other inflammatory arthritides (Cooke *et al.*, 1975; Ohno and Cooke, 1978; Shiozawa *et al.*, 1980), and also in cartilage from animals with antigen-induced arthritis (Cooke and Jasin, 1972; Hollister and Mannick, 1974; Jasin and Cooke, 1978; Washburn *et al.*, 1980). The importance of these immune complexes is that, although they may cause no damage themselves, they will be chemotactic for inflammatory cells such as macrophages and polymorphonuclear leukocytes (Ugai *et al.*, 1979). These, on activation, will be active producers of tissue-degrading enzymes (Glant and Ol'ah 1980). The activation of the complement system by the antigen–antibody complex will itself involve the generation of proinflammatory mediators. There is also evidence that polymorphonuclear leukocytes will adhere to cartilage containing immune complexes, degranulate and phagocytose the immunoglobulin-containing material (Ugai *et al.*, 1979). The suggestion has been made that the immune complexes are related to pannus formation in rheumatoid arthritis, and the inflammatory tissue clears the complexes from the cartilage (Shiozawa *et al.*, 1980).

Thus it is possible that humoral antibody, gaining access to the joint space, may not be damaging in its own right, but may evoke and maintain a chronic inflammatory reaction through complex formation with the antigen. This would cause enzymatic degradation of the cartilage and lead to ongoing tissue damage.

C. Lymphokines

The third group of immunological effectors to be considered are the lymphokines. There are relatively small, nonimmunoglobulin molecules produced by lymphocytes after their activation by an antigen. There are a number of different lymphokines having a variety of effects, but their principal importance is that they play a major role in amplifying the immune response by recruitment of noncommitted lymphocytes, and also by involving proinflammatory cells such as macrophages. Other lymphokines may have direct effect, sometimes toxic, on other cell types (Maini *et al.*, 1979).

There are two ways in which lymphokines may play a casual role in cartilage damage in either the graft or the disease context. First, those which have stimulatory activities upon inflammatory cells [i.e., macrophage, inhibitory or activating, factors (MIF and MAF)], and leukocyte activating factors (LAF) will cause activation of the relevant cells at the site of the immunoinflammatory reaction (Herman *et al.*, 1977, 1980). There is evidence that cells in synovial tissue from rheumatoid joints may produce lymphokines (Stastny *et al.*, 1973; Maini *et al.*, 1979). This may therefore provide a further mechanism, in addition to activation by immune complexes, for the production of degradatative enzymes such as collagenases which could act upon cartilage matrix (Wahl *et al.*, 1975; Dayer *et al.*, 1977). MIF and LAF could therefore cause damage to cartilage indirectly, through the operation of inflammatory cells. The second possibility is that certain lymphokines may have a direct action upon the cells of the cartilage. The lymphokines are generally of sufficiently low molecular weight to permit their penetration through the cartilage matrix. Thus if lymphokines with cytotoxic activities are produced (Lawrence and Landy, 1969; Horton *et al.*, 1973), they could have a direct effect upon chondrocytes (Herman *et al.*, 1974).

Finally, mention should be made of a further lymphokine that, although not acting on cartilage itself, may nevertheless be of relevance in destructive inflammatory joint disease. This is osteoclast activating factor (OAF), which, as its name implies, stimulates osteoclasts to resorb bone (Horton *et al.*, 1972). There is evidence that OAF may be of relevance in periodontal disease, and it may be suggested that it could be of importance in the destructive bone changes found in rheumatoid arthritis.

VIII. CONCLUSION

Cartilage is a tissue that possesses antigenic determinants on both its sparse cellular population and on the two principal structural components of its matrix. In the case of the cells, however, the surface antigens are not, under normal conditions, accessible to the immunological apparatus. The cells are

protected to a large degree by the dense matrix in which they are buried. Although this matrix presents an almost complete barrier to the afferent arm of the immune response, the barrier is probably imperfect when the immune effectors, and in particular lymphokines, are considered. Thus the composite grafts of bone and cartilage are immunogenic, by virtue of the cellular component of the bone (i.e., marrow and/or endosteal cells), and in this circumstance the cartilage may be rejected and resorbed. In connective tissue diseases, and in particular rheumatoid arthritis, there is increasing evidence of immune reactivity against matrix components and especially collagen. The fact that evidence of antimatrix immunological effectors cannot be demonstrated in every patient with rheumatoid arthritis, and can be demonstrated in some nonrheumatoid joint diseases, suggests that those effectors are not primary etiological factors. Nevertheless, their presence will exacerbate damage in the affected joint and predispose it to chronic disease. Both cell-activating lymphokines and the presence of antigen-antibody complexes in the surface layer of articular cartilage must be important mediators of chronic inflammation and tissue destruction. In the case of the lymphokines particularly, the immune response by which they were elicited need not be directed against any joint component but may hve been stimulated by a third party antigen. There are reports of inflammatory arthritis developing in animals immunized with antigens such as mycoplasma or heterologous protein following introduction of the antigen into the joint (Dumonde and Glynn, 1962; Lowther *et al.*, 1978; Washburn *et al.*, 1980). Thus cartilage in a joint may be an innocent bystander damaged by an immune response to a foreign antigen. On the other hand it is possible to induce chronic inflammatory polyarthritis in rats and mice simply by immunizing them with type II collagen (Trentham *et al.*, 1977, 1980; Stuart *et al.*, 1979; Courtenay *et al.*, 1980; Morgan *et al.*, 1980). In this animal model, injection of the collagen into the joint is not necessary, and both humoral and cell-mediated immune responses to type II collagen are in evidence (Trentham *et al.*, 1980). However, it is important that type II collagen is used for the immunization: Immunization with other collagen types does not lead to development of the polyarthritis.

Finally it should be emphasized that the most potent cartilage-degrading mechanism, in both the transplantation and disease situation, is probably the secretion or liberation by macrophages and polymorphonuclear leukocytes of proteolytic enzymes that can degrade matrix components of the tissue. Although these cells are themselves not a part of the immune system they are, nevertheless, influenced and directed quite considerably by various products of the immune response. Not only are the lymphokines potent stimulating agents, but these inflammatory cells may also be triggered by immunoglobulins, via their Fc receptors, and various components of the

complement cascade (e.g., via C3b receptors). The contribution of these inflammatory cells is probably the major factor in cartilage destruction. The immune response, whether to an immunogenic cartilage component, a cross-reacting microorganism, or an unrelated antigen in the joint, may not have much direct effect upon intact cartilage but would provide a driving mechanism for other cells. Once the integrity of the cartilage breaks down, immune effectors would have a role to play in extending the damage and establishing a chronic reaction.

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9

Chondrogenesis in Regenerating Systems

Richard J. Goss

I. INTRODUCTION

Cartilage is unusual because it is rarely vascularized, never innervated, and devoid of fibroblasts. It is one of the few tissues in the body that is made up of only one kind of cell. Whether cartilage evolved before, during, or after bone is a matter for phylogenetic conjecture. In any case, it has persisted for two important reasons. One is its tough resilience, which makes it useful for articular surfaces and in other sites (e.g., auricular, costal, nasal) where a flexible skeleton is desirable. The other is its adaptation as a cartilaginous plate to regulate the elongation of bones.

Cartilage plays a prominent role in many regenerating systems, both epimorphic and tissue, if only because most appendages possess endochondral skeletons. *Epimorphic regeneration* is the replacement of amputated appendages, as opposed to *tissue regeneration,* which is the repair of individual tissues following their injury. The distinction between these two kinds of regeneration is important because of the different extents to which chondrogenesis occurs following the loss of cartilage.

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II. EPIMORPHIC VERSUS TISSUE REGENERATION

A. Epimorphic Regeneration

Epimorphic regeneration of an amputated body part is achieved by direct outgrowth from the stump (Goss, 1969). In lower invertebrates it is the mechanism by which whole fractions of the body may grow back, as in the restoration of bisected worms, some of which can reproduce whole organisms from fragments of themselves. Animals incapable of surviving bisection nevertheless retain the ability to replace amputated appendages, at least in some forms. The starfish can regenerate arms, the octopus replaces its tentacles, and arthropods grow back legs and claws. Among vertebrates, there are numerous examples of appendages that can regenerate. These include the fins, taste barbels, and gill filaments of teleost fishes, the limbs and jaws of some amphibians, and the tails of lampreys, amphibians, and lizards. Even mammals are not without occasional powers of regeneration, as represented by such diverse structures as deer antlers, which are replaced annually, and the ears of rabbits, cats, and bats, which can fill in holes punched through them.

In the aforementioned cases of epimorphic regeneration, amputation is followed by epidermal wound healing, usually involving the formation of a thickened cap of cells beneath which the blastema is destined to form. Meanwhile, the cells of mesodermal origin subjacent to the level of amputation undergo a loss of their specialized characteristics. This dedifferentiation yields a population of cells derived from a variety of tissues which can no longer be distinguished morphologically from one another. Migrating distally, they accumulate into a blastema beneath the wound epidermis and begin to differentiate again, first proximally and later at progressively more distal levels. Apical proliferation of blastema cells provides the cells needed for morphogenesis. As each new tissue develops, it does so in continuity with its counterpart in the stump. Morphogenesis continues until the missing structure is replaced and the cells of the apical zone of proliferation are used up. In many such appendages a neurotrophic influence is necessary for the blastema to form. This may be mediated by nerves in fins, barbels, and limbs, or by the spinal cord in tail regeneration.

Thus, for epimorphic regeneration to occur there must be an epidermal wound, a mesodermal source of blastema cells, and in some cases, sufficient innervation. Recent investigations have also suggested that appropriate information must be present in the stump for the blastema to embark upon successful morphogenesis.

B. Tissue Regeneration

Tissue regeneration shares certain processes with epimorphic regeneration. Both involve an injury that creates a morphological discontinuity. Both

undergo a loss of specialization in the cells destined to repair the damage. In both cases these cells proliferate and migrate to the wound site where they then redifferentiate into the appropriate types needed for repair.

Tissue regeneration comes in as many forms as there are tissues to regenerate (McMinn, 1969), which includes virtually everything except teeth. Some tissues may regenerate better than others, but in all cases it is important not to confuse tissue regeneration with various forms of compensatory growth (Goss, 1978). The latter phenomena do not require injury as an antecedent, but are triggered by increased functional demands. Physiological regeneration—the turnover of tissue components—is still another type of growth whereby synthesis and degradation at various levels of organization are normally in balance. Whether or not this represents a special case of the more general phenomenon of wound healing is a possibility worth serious consideration.

Among the more somatic tissues of the body (as distinguished from visceral ones), the healing of injuries in skin, muscle, tendons, bone, blood vessels, and nerves is especially relevant to epimorphic regeneration because these are the tissues normally present in appendages. Each one of them is capable of repair following injury, a repair more appropriately classified as wound healing than regeneration proper.

Skin heals wounds by the familiar immigration of epidermis over the underlying granulation tissue, the latter to become the scar which constitutes the regenerated dermis. Skeletal muscle is equally famous for its regenerative capacities following incision, crushing, mincing, ischemia, burns, or freezing. It does so by virtue of satellite cells which give rise to myoblasts capable of fusing into multinucleate fibers (discontinuous regeneration), or simply by sprouting from the severed ends of muscle fibers (continuous regeneration).

Severed tendons will complete their continuity by the accumulation of a mass of cells between the cut ends and subsequent synthesis of new collagen fibers to bridge the gap. Broken bones are repaired by a similar mechanism. A callus is formed out of cells derived from the nearby injured bone. Differentiation then follows the familiar sequence of chondrogenesis, osteogenesis, and remodeling. The repair of blood vessels can be achieved in a number of ways. Interrupted endothelium is resurfaced by the immigration of new cells from peripheral regions. Meanwhile the denuded intima undergoes a considerable thickening reminiscent of atherosclerotic plaque formation. The possible regeneration of entire cross sections of blood vessels, if this indeed occurs at all, has not been adequately investigated, although there is evidence for such regrowth between the cut ends of transected veins and arteries. More commonly, however, the reestablishment of vascular continuity is achieved by the sprouting of new capillaries and by

collateral vascularization. Finally, in the regeneration of peripheral nerves, distal fibers separated from their nerve cell bodies undergo Wallerian degeneration. Proximal stumps give rise to new sprouts that, if allowed to follow their old pathways, will regenerate toward their end organs as they become enveloped in new Schwann cells.

Thus, all of the tissues normally present in an appendage are themselves capable of limited regeneration, a regrowth more akin to wound healing than to epimorphic regeneration. It is worth noting, however, that the latter is not achieved by the additive regenerations of all of the tissues involved. It is conceivable that such a mode of appendage regeneration could have evolved, but the question is, why did it not? If each of the tissues in the cross section of a stump were to grow out on its own, they would at best have been able only to complete the continuity of the particular segments that were present in the stump itself. Each muscle, for example, might have regenerated as far distally as its particular insertion. Each skeletal element might have been expected to reconstitute only itself. Even if tissue regeneration were capable of reestablishing the morphological integrity of these individual muscles or skeletal elements, it is difficult to imagine how more distal muscles and bones might have been regenerated *de novo* without at least some remnants from which they could take their origins. Thus, the segmental nature of most appendages would seem to militate against their regeneration by means of exaggerated versions of tissue regeneration alone. This may explain why the blastema was invented in the first place.

C. Differences and Similarities

The differences between epimorphic regeneration and tissue regeneration outnumber their similarities. Although both involve trauma that interrupts their continuities, epimorphic regeneration requires an integumental injury which must be repaired by epidermal wound healing. The extent of dedifferentiation that takes place in epimorphic regeneration exceeds that occurring in tissue regeneration. Whereas the latter involves only cells of its own kind, dedifferentiation in epimorphic regeneration involves virtually all types of cells proximal to the level of amputation. Further, there is reason to believe that these dedifferentiated cells need not necessarily redifferentiate along their original pathways (See Chapter 1, this volume). Although metaplasia is not an easy phenomenon to prove, the regeneration of complete appendages from incomplete stumps suggests a potentially wide latitude of developmental prospects in dedifferentiated cells. Thus, a blastema is made up of indistinguishable cells from diverse origins, cells possibly endowed with capacities for differentiating into types different from their original forms. A blastema is very different from the masses of cells (e.g., calluses, granulation tissues) involved in tissue regeneration. Not

only are its cells derived from a greater spectrum of sources, but it represents a morphogenetic unit capable of differentiating into an integrated assemblage of parts, the more distal ones of which are not even represented in the amputation stump. Further, a blastema would not be a blastema without its epidermal covering. There is experimental evidence to suggest that this epidermis plays a significant role in promoting dedifferentiation of the underlying mesodermal cells, their accumulation into the blastema proper, and their coordinated proliferation and differentiation into the regenerate itself.

A blastema is not necessarily a homogenous mass of undifferentiated cells. The ones situated more apically constitute a growth zone where proliferation sustains continued elongation of the regenerate. More proximally, the cells undergo differentiation. This proximodistal polarity of the blastema and its subsequent regenerate is another distinguishing characteristic that sets it apart from tissue regeneration (in which the sequence of repair is not so closely bound up with polarity).

Finally, there is a utilitarian imperative that operates in epimorphic regeneration, but not in tissue regeneration. The best example of this is the neurotrophic influence without which certain vertebrate appendages (e.g., limbs, fins, and barbels) cannot regenerate. This neurotrophic influence is necessary for blastema formation and the proliferation of its cells. In contrast, nerves are not known to be required for tissue regeneration per se. In denervated limbs, for example, skin wounds can heal, fractured bones can mend, and even injured muscles can regenerate despite their atrophy. Thus, many epimorphic systems have evolved a conspicuous dependence upon nerves, a dependence *not* present in their individual tissue components. This neurotrophic dependence is perhaps most dramatically noted in the effects of denervation. In addition to the well-known inability of denervated appendages to regenerate following amputation, under certain circumstances they undergo total morphological disintegration after denervation. In the amphibian, amputated denervated limbs undergo extensive regression sometimes leading to their disappearance altogether unless regenerating nerves reverse the process (Thornton and Kraemer, 1951). This regression is believed to be the result of unchecked dedifferentiation such as would be expected to occur in the absence of blastema formation. Regression is less pronounced in adult limbs, presumably because their skeletons are bony rather than cartilaginous, and thereby more resistant to resorption. It is significant that regression occurs only under the influence of wound epidermis, the implication being that this is responsible for initiating mesodermal dedifferentiation, whether it is to culminate in blastema formation or devolve into regression.

In the catfish taste barbel a similar situation prevails, but with an

interesting difference (Kamrin and Singer, 1955). Here, regeneration is dependent upon adequate innervation, as in the amphibian limb. However, amputation of the barbel is not necessary to trigger its regression in the absence of nerves. Denervation of the otherwise intact barbel leads to histolysis at the tip, as a result of which the barbel gradually shortens until such time as regenerating nerves may reverse the process. In these appendages, therefore, the very morphological integrity of the structure is continuously dependent upon an adequate nerve supply. Although not explored experimentally, it is possible that the lengths of barbels are commensurate with the number of nerve fibers.

The fins of fishes, which also depend upon nerves to regenerate (Goss and Stagg, 1957), undergo partial regression in the absence of nerves, a regression limited to the interradial regions between the more resistant bony fin rays. However, if amputated fins are allowed to regenerate before being denervated, such regenerates will undergo more obvious regression in the absence of nerves (Schiff, 1979), presumably owing to their less mature state of differentiation. It is not known if the normal elongation of fins that occurs with overall body growth of the animal also depends upon nerves, but the similarity between ontogeny and regeneration of the fish fin suggests that neurotrophic influences may be operating in both situations. This is particularly interesting in comparison with the outgrowth of limb buds in higher vertebrates, a phenomenon that occurs prior to the ingrowth of nerves. Amphibian limbs, therefore, acquire their dependence upon neurotrophic influences secondarily, and their regeneration is thereby different from the original development of the limb.

There is another correlation that may be relevant. In amphibian limbs, elongation is not achieved by an apical growth zone once all of the parts have been formed. Enlargement occurs by internal expansion. In fishes, however, fins and barbels elongate by terminal growth, a potential that lasts throughout life. This suggests the possible persistence of apical growth zones in these appendages, a condition that could also involve the intimate association between epidermis and mesoderm which in amphibians is realized only in a healing wound. Thus the capacity of unamputated fins and barbels to regress following denervation may be correlated with the lifelong potential for growth in fishes. This condition necessitates the attenuation of dermis at the tips of appendages, thereby setting the stage for elongation when nerves are present, but regression when they are not.

III. FRACTURE HEALING

The capacity of bones to mend themselves following fracture has much in common with epimorphic regeneration: Substantial amounts of new tissue are produced, the cells are derived from the adjacent injured bone, and the

undifferentiated fracture callus is composed of seemingly dedifferentiated cells resembling those found in blastemas. However, fracture healing is not epimorphic regeneration. It does not depend upon epidermal wound healing. It does not involve dedifferentiation to the extent that occurs in amputated appendages. The fracture callus is in fact not a true blastema. Differentiation does not proceed in a proximodistal direction and fracture healing does not depend upon innervation. Nevertheless, the process by which bones heal their fractures is an impressive example of tissue regeneration, the mechanism of which involves cartilage formation to a significant degree.

As in the case of epimorphic regeneration, the histogenesis of cells in the fracture callus is an important consideration. Although the bone itself is the most obvious source, one cannot categorically rule out the possibility that surrounding soft tissues might participate in fracture callus formation. These alternatives have been explored in experiments taking advantage of the fact that X irradiation will inhibit fracture healing (Cooley and Goss, 1958). Doses of 3000 R effectively preclude the capacity of an exposed bone from healing fractures in the mouse. Further, the bones of mice can be transplanted ectopically (e.g., subcutaneously or intramuscularly) where they become revascularized and retain their capacity to heal fractures. By combining irradiation and transplantation, it is possible to explore experimentally whether the fracture callus is derived from the bone or the surrounding tissues. There are four possible combinations of conditions that can be set up using the mouse ulna as the test bone and the thigh as the transplantation site. Control unirradiated ulnae left *in situ,* or transplanted to the thigh, heal their fractures normally (Figs. 1 and 2). Ulnae exposed to 3000 R and grafted to similarly irradiated thighs fail to heal fractures. When an unirradiated ulna is grafted to an irradiated thigh, it is capable of healing subsequent fractures normally, a result consistent with the hypothesis that bone is the source of its own callus cells. Conversely, irradiated ulnae transplanted to unirradiated thigh muscle proved unable to repair fractures. This does not rule out the possibility that surrounding soft tissues might not passively become swept up in the healing process, but it proves that such tissues are neither sufficient nor necessary for fracture healing to occur.

It is well established that endochondral bones heal fractures by means of a cartilaginous callus. Dermal bones, if they do so at all, repair themselves by direct ossification (Pritchard, 1969). Although the cells of these two types of bones would appear to be programmed to differentiate into either cartilage or bone, respectively, there is experimental evidence that the pathway of differentiation may not be entirely determined by endogenous predispositions. The persistence of cartilage on articular surfaces suggests that chondrification might be favored by the mechanical movement of bones. Indeed, the artificial induction of chondrification in *(1)* cultured avian dermal bones sub-

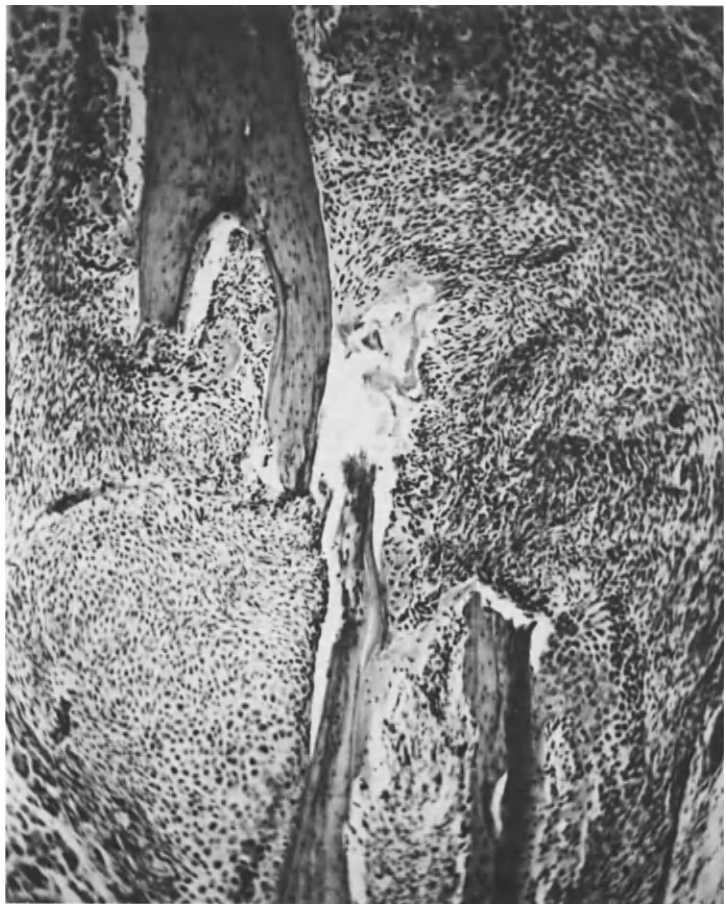

Fig. 1. Fracture callus in a mouse ulna 7 days after fracture. Chondrification is commencing adjacent to the shaft of the bone.

jected to repeated mechanical movements and *(2)* fractured dermal bones, is consistent with this hypothesis (Hall, 1967, 1968; Hall and Jacobson, 1975). Evidently the cells of dermal bone can be deflected from their normal pathways of osteogenesis by appropriate experimental manipulation. This raises the possibility that the cells of endochondral bone might be similarly subject to mechanical interventions. Studies of healing in bones that have not been completely broken, but injured by drill holes or saw cuts, have shown that under these circumstances the degree of chondrification in the fracture callus is significantly reduced (Leudtke and Angevine, 1950; Radden and Fullmer, 1969; Mindell *et al.,* 1971). This may be interpreted as a consequence of the more rigid immobilization that prevails when the bone is

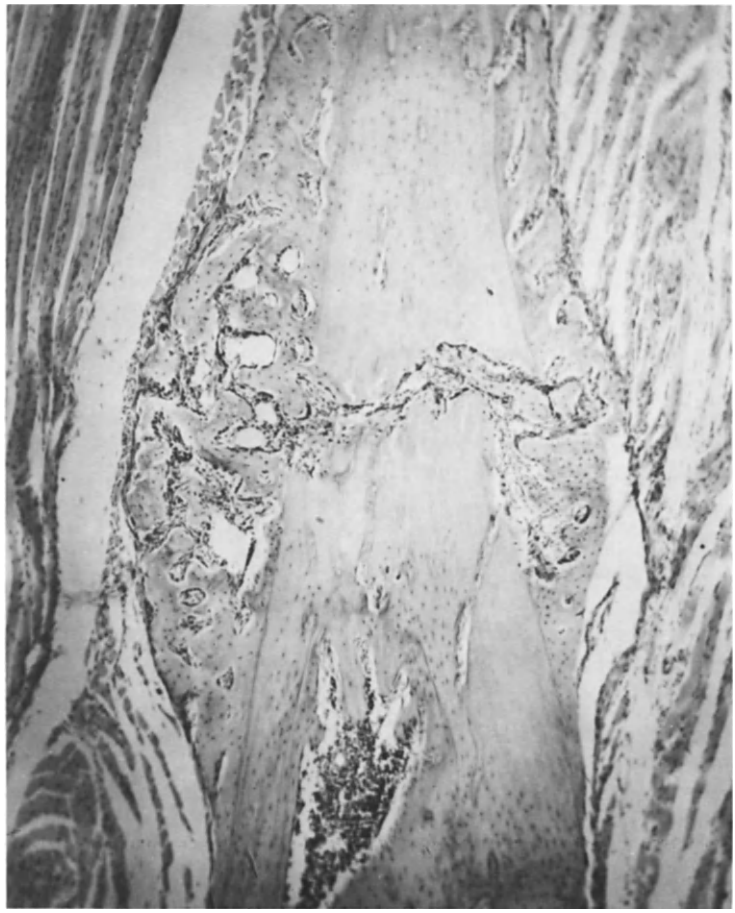

Fig. 2. After 14 days, the callus of the mouse ulna has consolidated into an ossified mass of bony trabeculae, which will subsequently undergo remodeling.

its own splint. Thus, the healing of drill holes and saw cuts tends to occur by direct ossification with little or no cartilage formation, despite the endochondral classification of the bones involved. Indeed, the rigid fixation of fractured tibiae in rabbits has been shown to favor ossification over chondrification in the callus (Lettin, 1968).

If cartilage formation in a fracture is causally related to its mobilization, then chondrification should be enhanced and ossification held in abeyance by excessive and repeated mobilization (Lindholm *et al.*, 1970). Experiments in which the fractured femur of the rat has been "refractured" by bending it on itself daily for 47 days have confirmed that under these circumstances the callus produces cartilage in excess, cartilage that may persist as long as the

treatment continues. Only when the bulk of the cartilaginous callus interferes with further mobilization does ossification supervene.

It is tempting to interpret the foregoing results in mechanical terms, but these must be translated into chemical events if cells are to respond. Mechanical mobilization would be expected to interfere with revascularization. This could lead to reductions in the delivery of oxygen or nutrients to the callus, or to the buildup of waste products. Although hypoxia may favor chondrification, the possible roles played by other factors remain in question.

IV. EPIMORPHIC REGENERATION

Cartilage plays a central role in the epimorphic regeneration of vertebrate appendages. It is present in the fins of the lungfish *(Protopterus),* the taste barbels of the catfish, and the gill filaments of teleosts, all of which rengenerate. In amphibians it is found in such regenerating structures as limbs, jaws, and urodele tails. It is absent in the tadpole tail. Cartilage also regenerates in place of the bony vertebrae of lizard tails. Among mammals, chondrogenesis occurs in the annual regrowth of antlers, the centripetal growth from the margins of holes cut through certain ears, such as those of the rabbit, and in regeneration of the nasal septum in the neonatal rodent. In these various systems it is useful to explore the histogenesis of regenerating cartilage, the factors influencing its morphogenesis, the possible dependence of chondrogenesis on neurotrophic influences, and in some cases the dependence of epimorphic regeneration on the presence of cartilage in the stump.

A. Taste Barbels

Cartilage plays its most unique and important role in the regeneration of the catfish taste barbel (Fig. 3). These curious structures, studded with epidermal taste buds, are adaptations for locating food in the murky depths. In some tropical species the length of such barbels may exceed that of the rest of the body. Located ventrally, laterally, or dorsally, their anatomical structure is relatively simple. (Fig. 4). They contain a cylindrical rod of cartilage enveloped in an unusually thick perichondrium. Running parallel to the cartilaginous rod is a conspicuous branch of the seventh cranial nerve. Its fibers supply the taste buds in the epidermis. Aside from cartilage, nerves, and skin, the taste barbel consists only of blood vessels and connective tissue. Except in their most proximal regions, barbels lack muscles and bone.

The amputated taste barbel regenerates rapidly and completely. The blastema that forms beneath the wound epidermis differentiates into an extension of the original cartilaginous rod (Fig. 5). The regrowth of nerves,

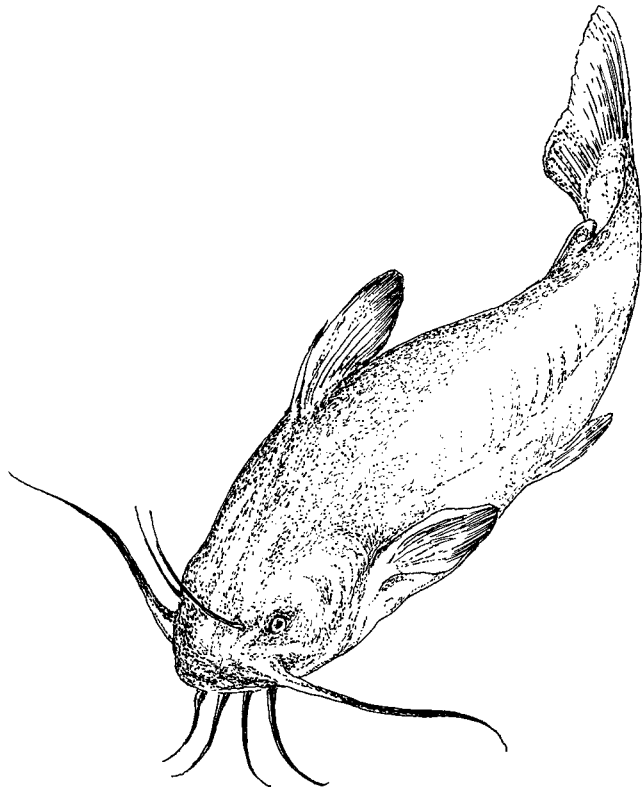

Fig. 3. The catfish, or bullhead *(Ameiurus nebulosus)*, possesses 4 pairs of taste barbels.

blood vessels, and epidermis keeps pace. New taste buds differentiate in the regenerate.

It has been found that if the cartilaginous rod is removed, the barbel will not regenerate following amputation (Goss, 1954). Removal of the rod can be achieved by making a small incision at the base of the appendage, transecting the rod, and extracting its distal portion from the barbel, much like pulling one's arm out of a sleeve. The rodless barbel survives by virtue of its intact blood supply and nerves, but fails to regenerate following amputation through the rodless portions (Fig. 6). The exact explanation for the failure of the rodless barbel to regenerate is not known. However, if the rod is removed, and either devitalized by immersion in boiling water, or exposed to high levels of X radiation, regeneration of the barbel still fails to occur following reinsertion of such cartilaginous rods (Goss, 1955). Therefore, it is not the mere physical presence of the rod that promotes regeneration.

Judging from the histology of regenerating barbels, the thickened

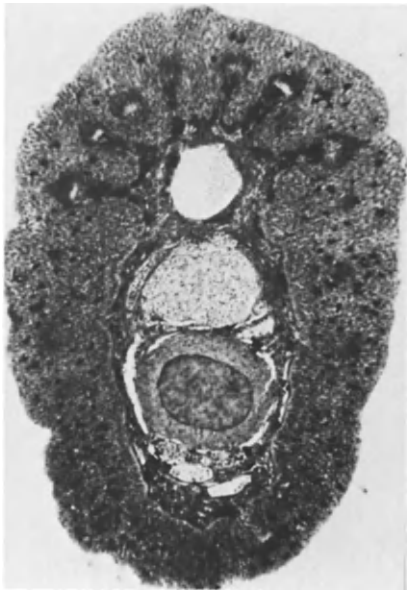

Fig. 4. Cross section through a catfish taste barbel showing the taste buds in the epidermis of the leading edge (top). Internally, there are blood vessels (top), nerves (middle), and a cartilaginous rod (bottom) enveloped in a thick perichondrium. Reproduced from Goss (1969) with the permission of the publisher.

perichondrium around the cartilaginous rod would appear to play an important role in blatema formation. The cells in the perichondrium immediately proximal to the level of amputation migrate distally, significantly depleting the perichondrium of cells in that region. There is reason to believe, therefore, that the perichondrium may be the principal if not exclusive source of blastema cells. If so, this would explain why the absence of the cartilaginous rod (including most of its perichondrium) would preclude regeneration of the barbel.

The production of accessory taste barbels also illustrates the importance of the cartilaginous rod in promoting regeneration (Goss, 1954). If an injury is inflicted on the epidermis alone, wound healing occurs in the absence of lateral regeneration. Injuries involving both the skin and nerves may elicit a very limited outgrowth lacking cartilage. However, if the cartilaginous rod is severed, along with the nerves and skin, while preserving the circulation to the distal parts of the barbel, a complete sidegrowth may occur if the proximal cartilaginous rod is displaced laterally enough to involve it in the regeneration process. When such sidegrowths include nerves, cartilage and skin, they may regenerate to considerable lengths commensurate with the level at which the operation was performed.

The role of the cartilaginous rod in barbel regeneration has been further explored to determine how long its presence is required (Goss, 1955). It is possible to amputate a barbel and then extract the rod from the stump at various intervals afterward. When the rod is removed 2–4 days following

Fig. 5. Eight-day regenerate of an amputated taste barbel showing the blastema off the end of the cartilaginous rod.

amputation, regeneration is still prevented. If the rod is removed after 6 or more days, however, regeneration occurs. This represents the approximate period of time required for a blastema to form.

Another approach has been to extirpate the cartilaginous rod for some distance proximal to the level of barbel amputation. Under these cir-

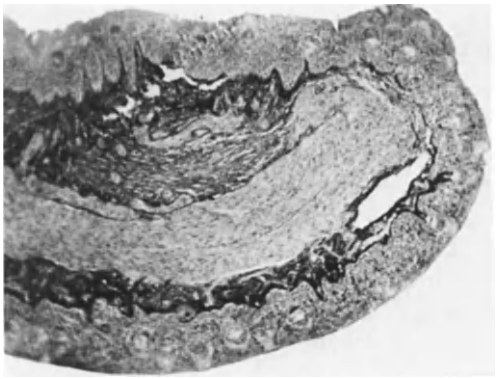

Fig. 6. Longitudinal section through a taste barbel from which the cartilaginous rod has been extracted. Although the nerve remains intact, no regeneration has occurred from the amputation stump.

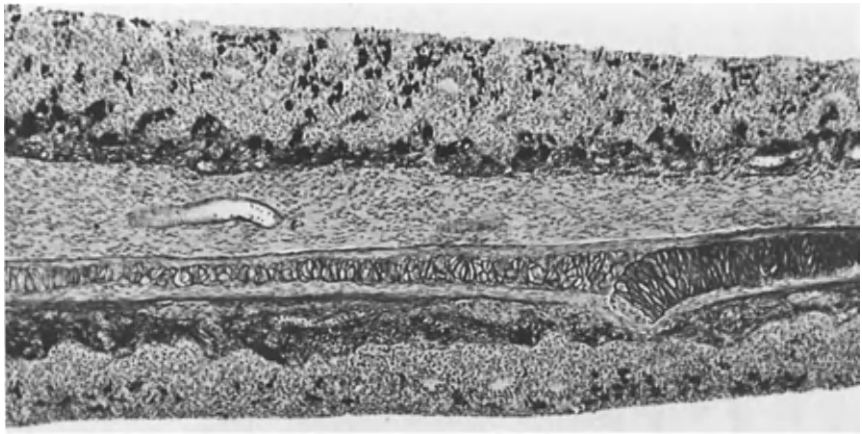

Fig. 7. Regeneration of the cartilaginous rod in a distoproximal direction in a taste barbel from which the proximal length of the rod had been removed 19 days earlier. Reproduced from Goss (1956b) with the permission of the publisher.

cumstances, the rod regenerates from its more proximal level, and the entire barbel regenerates once the rod reaches the level of amputation.

This experiment reveals another capacity of the cartilaginous rod, namely, its ability to regenerate considerable portions of itself in the absence of barbel regeneration (Goss, 1956b). If the rod is transected, or a segment removed, the intervening regions are soon filled with new cartilage. Similar results occur even in cartilaginous rods grafted to fins. Chondrogenesis proceeds from both the proximal and distal stumps after transection. Indeed, if considerable lengths of the cartilaginous rod are removed through a proximal incision of the barbel, the missing portion of the rod is replaced by new cartilage, even in a distoproximal direction (Fig. 7). This phenomenon amounts to a very exaggerated version of tissue regeneration. Since it takes place in the absence of nerves, epidermal wound healing, and the regrowth of the entire barbel, it is a remarkable example of cartilage regeneration unsurpassed in any other known vertebrate system.

Morphogenesis of the regenerated cartilaginous rod typically involves the differentiation of a cylindrical outgrowth similar to the original rod in the stump, with which it is in continuity. In order to learn if the morphological configuration of such regenerates derives from their counterparts in the stump, the regeneration of taste barbels containing more than one cartilaginous rod has been explored. This is achieved by removing the original cartilaginous rod and reinserting it, along with as many as three extra rods from other barbels, back into the original appendage. When amputated through the region containing supernumerary rods, regeneration occurs normally. Despite the existence of extra cartilage rods in the stump, only one rod

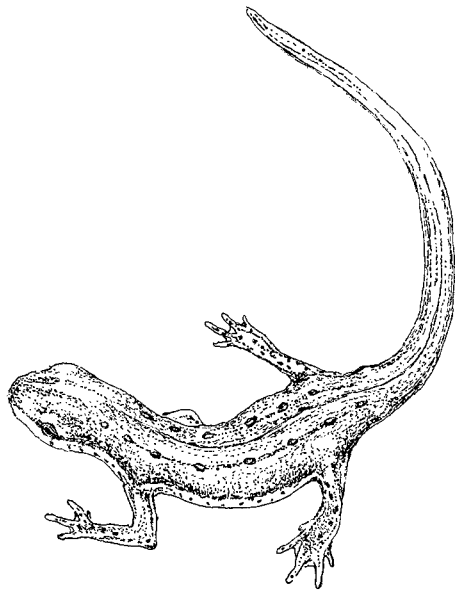

Fig. 8. The red-spotted newt *(Notophthalmus viridescens)* is capable of regenerating structures such as limbs, tail, jaw, snout, lens, retina, and intestines.

differentiates distal to the level of amputation. These results may be taken to indicate that the rods in the stump serve only as a source of blastema cells. They would appear to have no morphogenetic influence in the development of the blastema, the cells of which are endowed with the capacity to differentiate only into a single cartilaginous rod irrespective of the number of rods in the stump.

B. The Amphibian Jaw

The lower jaws of urodele amphibians (Fig. 8) will regenerate following transverse amputation (Fig. 9), provided that some of the mandible remains in the stump (Goss and Stagg, 1958b; Graver, 1973, 1974). The regenerates that are eventually produced are incomplete. They contain neither tongue nor branchial skeletal parts, and sometimes the outgrowths of the mandibles are abnormally short. Nevertheless, mandibular regenerates consist of cartilage and bone, and develop in association with the differentiation of a new row of teeth.

The mandible consists of three skeletal elements, the dentary and prearticular bones, surrounding Meckel's cartilage. The dentary is a dermal bone, whereas the prearticular is endochondral. This classification is consistent

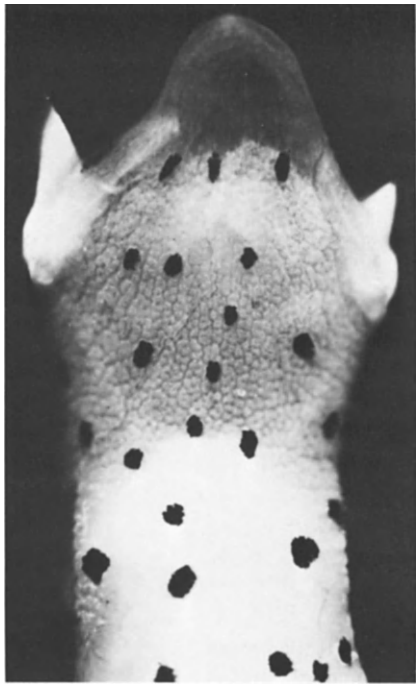

Fig. 9. Ventral view of a regenerated newt lower jaw 10 weeks after amputation of its distal half. (A piece of black paper has been inserted into the mouth to improve visibility of the regenerate.) Reproduced from Goss and Stagg (1958b) with the permission of the publisher.

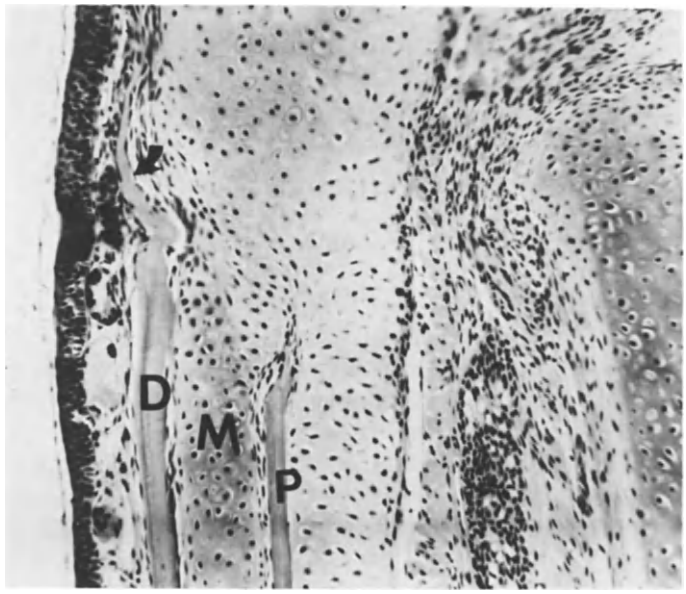

Fig. 10. Mandibular regenerate in a newt jaw 56 days after amputation. A cartilaginous extension has developed from the prearticular bone (P) of the mandible, fusing secondarily with Meckel's cartilage (M). The dentary bone (D) regenerates by direct ossification from its severed end (arrow).

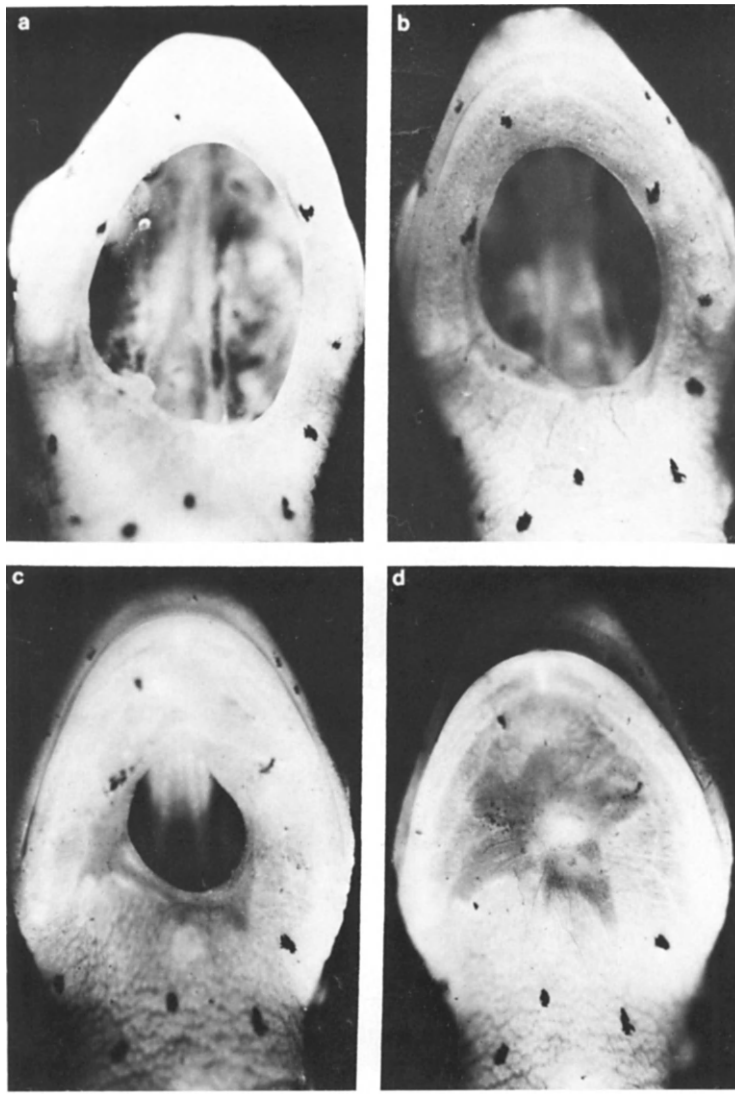

Fig. 11. Stages in the regeneration of the floor of the mouth 1(a), 2(b), 3(c), and 4(d) weeks after operation. Ingrowth occurs from around the margins of the aperture.

with their modes of regeneration. When the blastema forms off the ends of the mandibles, chondrification is first observed in association with the stump of the prearticular bone (Fig. 10). The mass of cartilage that forms off its end secondarily fuses with Meckel's cartilage. There is no histological evidence that Meckel's cartilage itself is capable of regeneration. The cartilaginous

Fig. 12. Chondrogenesis in the regenerated intermandibular region of a newt jaw has given rise to an isolated mass of cartilage (arrow) 10 weeks after excision of the floor of the mouth. Reproduced from Goss and Stagg (1958a) with the permission of the publisher.

outgrowths from the two mandibles eventually fuse at the midline to reconstitute the mandibular arch. Meanwhile, the dentary bone, in keeping with its dermal origin, regenerates by direct ossification. The remnants of the branchial bones in the lower jaw do not regenerate, but form short cartilaginous masses on their ends.

There is yet another way in which the lower jaw may express its regenerative potentials. This is in response to the removal of the floor of the mouth, while leaving the mandibles themselves intact (Goss and Stagg, 1958a). Following this operation, regeneration occurs from the inner margins of the mandibles to fill in the apertures by centripetal growth (Fig. 11). The blastema responsible for this regeneration is a circular one from which a new floor of the mouth is differentiated. Consequently, the original opening is progressively reduced in size until the final pinhole is obliterated by fusion of the converging margins of the regenerate. The tissue thus produced restores the floor of the mouth, but does not give rise to a new tongue or branchial skeletal parts. However, it does differentiate new muscle, and small salivary glands in the oral epidermis. Of particular interest, however, is the differentiation of cartilage in most of these regenerates (Fig. 12). This cartilage typically forms independently of any adjacent bone, and is located centrally in the regenerate. Although it is not bilaterally symmetrical, there is a tendency for it to approach this condition. The significance of this cartilage is not understood, (it is presumably ectopic as defined in Chapter 1) but one cannot escape the impression that it may be homologous with the parts of the branchial skeleton removed in the original operation, despite the fact that it is not in direct continuity with the stumps of these bones. The histogenesis of this cartilage remains to be determined.

C. Limb Regeneration

When a limb blastema begins to differentiate, chondrogenesis occurs in continuity with the bone stumps at the level of amputation. The question may be asked, whether the blastema cells are predisposed to form cartilage where the new skeletal elements should be, or are induced to do so by the subjacent bones. There is reason to believe that both alternatives are correct (Goss, 1956a). If the bones of the limb are extirpated prior to amputation through the boneless regions, subsequent regeneration results in the production of normal skeletal elements. These usually differentiate only distal to the level of amputation (Fig. 13), a phenomenon that reflects the inability of bones to be regenerated following their complete extirpation, except in association with epimorphic regeneration of the entire appendage. On the other hand, if an extra bone is transplanted to a limb, amputation through the level of the graft results in the differentiation of a cartilaginous regenerate of the extra bone, in addition to the production of normal skeletal elements in the regenerate. Thus, bones are not necessary for the regeneration of their counterparts by the blastema, but have the capacity to induce chondrogenesis when present. Clearly, there is a certain degree of metaplasia that occurs in regeneration whereby the remaining tissues are capable of compensating for the absence of others in the course of blastema formation and regeneration. The same situation prevails with respect to muscles, almost all of which can be removed from a limb stump without preventing the differentiation of normal musculature in the subsequent regenerate (Carlson, 1972).

Chondrogenesis in the regenerate is induced by healthy skeletal tissues in the stump (Goss, 1956a). If the grafted extra bone is first killed by immersion in boiling water, or if it is exposed to high levels of X radiation, no chondrogenesis occurs in the blastema with which it is in contact. If an extra bone is transplanted in such a position that its uninjured epiphysis is situated at the level of amputation, again no cartilage if formed off the end, presumably because the grafted bone was not amputated. It would seem likely, therefore, that the induction of cartilage in the blastema is initiated by osteoblasts that might otherwise have been involved in the production of a fracture callus. Transplantation of the mandible, or bones of the branchial skeleton, to the limb, followed by amputation through the level of the graft, results in the differentiation of cartilage off the ends of the skeletal transplants. Similarly, the transplantation of limb bones, or mandible, to the tail at the level of subsequent amputation results in the differentiation of cartlaginous regenerates in association with the regeneration of the tail (Fig. 14). Inert structures, such as glass rods, have no such effect.

The limbs of larval amphibians are distinguished from those of adults in

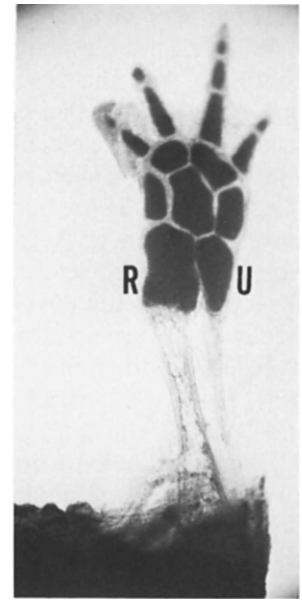

Fig. 13. Regenerated newt arm in which the cartilage has been stained with methylene blue. Prior to amputation the ulna was removed. However, ulnar regeneration (U), like that from the intact radius (R), has occurred only distal to the level of amputation. Reproduced from Goss (1956a) with the permission of the publisher.

that their skeletal elements are cartilaginous rather than bony. This may be correlated with the regenerative capacity of skeletal elements in amphibians. In adult newts, excised limb bones are never regenerated, even if they had previously been fractured in several places to induce cartilage formation, or if they were replaced by minced blastemas from forelimbs amputated 3 weeks previously.

In contrast to the situation in adults, some larval amphibians possess the capacity to regenerate their missing cartilaginous elements (Goss, 1958). Relatively few tadpoles of *Rana pipiens* exhibited an ability to regenerate missing tibiae from hind limbs capable of epimorphic regeneration at the lower leg level. In the larval urodele, *Ambystoma maculatum,* the ulnae may regenerate in over half the cases following their complete removal. It would appear that the tissue regeneration of skeletal elements takes place more readily in immature amphibians possessing cartilaginous skeletons than in mature ones with ossified bones.

In a sense, limb regenerates recapitulate the original development of the limb in embryonic and larval forms. Their possession of cartilaginous skeletal elements prior to maturation and ossification testifies to the equivalence of young regenerates with larval limbs. Accordingly, it would be interesting to learn if the cartilaginous skeletal elements of young limb regenerates on adult amphibians might be capable of being replaced following extirpation, as has been shown to be the case in larval limbs. When the ulnae were removed from forelimb regenerates in groups of adult newts at

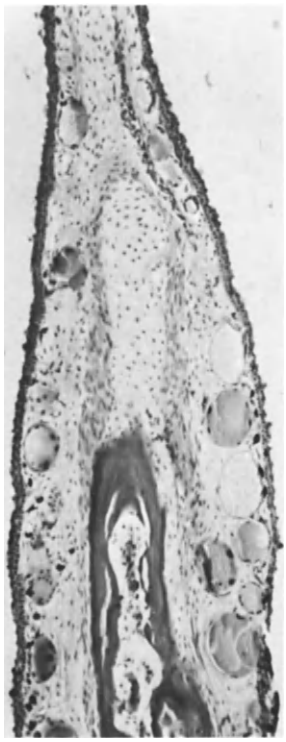

Fig. 14. Dorsal tail-fin regenerate 6 weeks after amputation through an implanted radius. Cartilaginous regeneration has occurred distal to the bone. Reproduced from Goss (1956a) with the permission of the publisher.

2-week intervals from 6 or 16 weeks following the original amputation, regeneration of the missing ulnae occurred in a higher percentage of cases in young regenerates than in older ones. The incidence dropped from about 90% in 6-week regenerates to 20–30% in 10- to 16-week regenerates. During the course of maturation of the regenerates studied in this experiment, considerable ossification of the skeletal elements occurred, a change with which the decreasing capacity for skeletal regeneration would seem to be correlated.

In view of the dependence of epimorphic regeneration on an adequate nerve supply to the limb, it would be interesting to determine if the regeneration of cartilaginous skeletal elements in larval limbs (or in the regenerates of adult ones) might also require innervation. Experiments have shown that denervation of these limbs followed by injury to their tissues may result in regression of the entire appendage (Thornton and Kraemer, 1951). Although this phenomenon was observed in some instances of denervated limbs deprived of their cartilaginous ulnae, in the remaining cases the incidence of skeletal regeneration was significantly less than in control limbs with intact innervation (Fig. 15a,b). This is reminiscent of the failure of extirpated

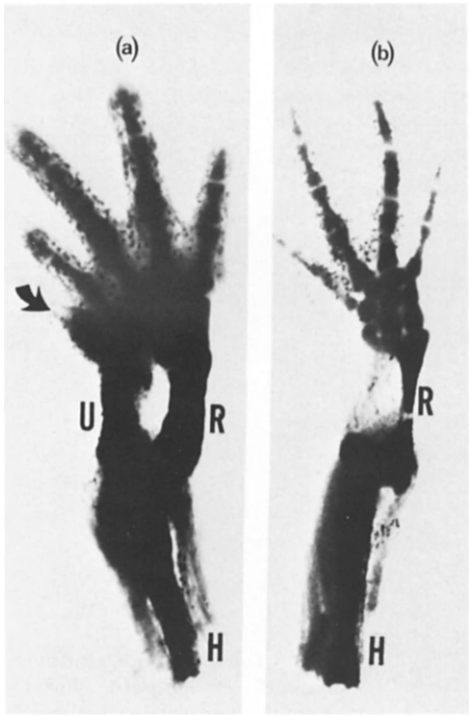

Fig. 15. (a) Right arm of larval *Ambystoma* from which the ulna had been extirpated 3 weeks previously. A new ulna (U) has been formed *in situ*, and a supernumerary digit (arrow) has regenerated. (b) Left arm of same animal which was denervated when the ulna was removed. After 3 weeks no regeneration of the ulna has occurred. H, Humerus; R, radius. Reproduced from Goss (1958) with the permission of the publisher.

segments of fin rays from the otherwise unamputated fins of teleost fishes to be regenerated following denervation (Goss and Stagg, 1957). It remains to be explained how such dependence on nerves in the regeneration of skeletal elements can be reconciled with the independence of other forms of tissue regeneration (including fracture healing) from neurotrophic influences.

Cartilage has been particularly useful in attempts to determine the histogenesis of tissues in the regenerate, and to explore metaplasia, that is, the possibility that cells derived from one kind of tissue in the stump might be capable of differentiating into another kind in the regenerate. Cartilage lends itself to this problem because of its cellular purity, uncontaminated by blood vessels, nerves, or fibroblasts. Therefore, when tritium-labeled car-

tilage is grafted into limbs there is little or no possibility that noncartilage cells may have been included. Thus, the subsequent appearance of labeled cells in noncartilaginous tissues of the regenerate would constitute positive evidence in favor of metaplasia. For obvious reasons, it is essential that cells be prelabeled if they are to be accurately identified in the regenerate.

There are two useful ways to label such cells. One is to encourage the incorporation of tritiated thymidine into the DNA of their nuclei. This can be conveniently achieved in the cartilage of regenerating limbs by taking advantage of the rapid rates of proliferation in the blastema, during which phase the animal can be injected repeatedly with tritiated thymidine (Oberpriller, 1967; Steen, 1968, 1970; Desha, 1974), confident that much of it will be incorporated into future chondrocytes. In this kind of experiment, however, there can be no guarantee that the tritiated thymidine will not be taken up into other cells in the body, including those of the limb to which the cartilage might be transplanted. Accordingly, it is necessary to make allografts of the cartilage to the limbs of unlabeled animals. Owing to its lack of vascularity, cartilage is one of those few tissues that is not readily rejected as allografts by the immunological responses of the host. Indeed, the genetic disparity between individual newts is not so great that foreign grafts are subject to prompt rejection. Allografts in these animals commonly survive up to several months, depending upon the genetic relationships between donor and host. Alternatively, it is possible to encourage the uptake of tritiated thymidine *in vitro* prior to grafting tissues back into the same animal.

Another way to identify cells is to take advantage of the experimental production of triploid axolotls, the nuclei of which have three nucleoli (Patrick and Briggs, 1964; Steen, 1968, 1970; Namenwirth, 1974). Thus, triploid tissues grafted into diploid limbs can be identified in subsequent regenerates. One advantage of triploid cells over those labeled with tritiated thymidine is that the label in the former cells is not diluted by subsequent mitotic activity.

In order to determine the fate of stump cells involved in limb regeneration, various labeled tissues (Oberpriller, 1967; Steen, 1968; Namenwirth, 1974), usually cartilage (Patrick and Briggs, 1964; Steen, 1968; Namenwirth, 1974), have been grafted to the arms of newts and axolotls, whereupon the limb is amputated through the transplant region. Labeled cartilage cells are readily traced to cartilage in the regenerate. Even chondrocytes from nonregenerating parts of the body (e.g., coracoid, scapula, visceral arch) may become incorporated into the limb cartilage of the regenerate (Steen, 1968). Nonlimb cartilage elements tend not to dedifferentiate to the extent that limb cartilage elements do, and the skeletal parts into which they are incorporated are often abnormal. Nevertheless, the evidence is clear that chondrocytes can redifferentiate into new chondrocytes during the process of regeneration. They are also traced to connective tissue, but almost never to skeletal muscle nuclei. The instances in which myonuclei in the regenerate have been found

to carry the label of grafted cartilage are too rare not to be suspected of being artifacts. The consensus of these experiments is that chondrocytes in the limb stump tend to remain as cartilage or connective cells in the regenerate.

In other experiments, labeled muscle has been grafted to the limb prior to amputation (Steen, 1968, 1973; Namenwirth, 1974). Because muscle is not a histologically pure tissue, it is perhaps not surprising that its label was subsequently found in the cells of cartilage, muscle, and dermis. Although these results are interesting in that they show that connective tissue cells can probably give rise to cartilage in the regenerate, the mixed nature of muscle tissue does not allow the conclusion that muscle cells per se can give rise to a tissue as different as cartilage.

In an attempt to resolve this dilemma, embryonic muscle has been grown in culture where it consists of a mixture of cells destined either to become connective tissue fibroblasts or muscle fibers (Steen, 1973). Although it is difficult to distinguish these two types of presumptive cells in early cultures, it is possible to clone them in order to obtain pure lines of cells. When such clones are labeled and transplanted into the amputated hind limbs of *Xenopus,* the subsequent regenerates contain connective tissue cells, myotubes, and chondrocytes bearing the original label. Analysis of the subsequent development of such clones *in vitro* indicates that these three types of tissues can be derived either from presumptive connective tissue fibroblasts or from muscle cells. These findings are compelling evidence that embryonic muscle cells can in fact give rise to cartilage in the process of regeneration. Although this does not necessarily involve dedifferentiation, it sheds light on the potentials for determination in certain cell types.

A less direct, though equally interesting, approach to the problem of histogenesis involves the exposure of limbs to regeneration-inhibiting doses of X rays followed by the transplantation to them of unirradiated tissues. The rationale for this experimental approach is to determine which individual tissues are capable of inducing regeneration from such irradiated limbs. It also permits analysis of the tissue types found in such regenerates, types derived perforce from the original transplants. Cartilage has commonly been used for such grafts. When it is derived from the limb itself, regenerates are invariably produced (albeit morphologically abnormal) that contain the usual spectrum of tissue types, that is, cartilage, muscle, blood vessels, nerve sheaths; and connective tissue (Stinson *et al.,* 1972; Namenwirth, 1974; Wallace *et al.,* 1974; Maden and Wallace, 1975). When the cartilage is from a part of the body not capable of regeneration (e.g., scapula), it may induce the production of cartilage at the level of amputation, but this is not organized into a limb regenerate (Eggert, 1966; Stinson *et al.,* 1972). When limb cartilage in such transplantations is labeled by virtue of being triploid, its cells are subsequently found in cartilage, perichondrium, connective tissue, dermis, and fibroblasts, but not in muscle cells (Namenwirth, 1974).

Other kinds of tissue transplants have also been found capable of giving rise to regenerates from otherwise nonregenerating X-rayed limbs. These include segments of branchial nerve, skin, and muscle, all of which mediate the production of histologically complete regenerates (Wallace and Wallace, 1973; Desha, 1974; Namenwirth, 1974). Transplants of epidermal sheets separated from their underlying dermis do not give rise to regenerates. The labeled epidermal cells remain as epidermis. Although there is some evidence that despite irradiation the cells of a limb stump may be incorporated into the regenerate (Desha, 1974), the transplantation of labeled grafts to irradiated limbs shows that skin (dermis) and muscle can give rise to virtually all types of mesodermal cells in the regenerates, including cartilage (Namenwirth, 1974). It has also been shown that when cartilage grafts are from genetically different axolotls, the regenerates they are responsible for producing are subsequently rejected, a process that involves all of the tissues in the new growth (Maden and Wallace, 1975). This evidence suggests that chondrocytes may in fact redifferentiate into a variety of cell types, although the possibility that chondrocytes can become myocytes remains as remote as ever.

D. Tail Regeneration

With the exception of most fishes, the tails of all cold-blooded vertebrates are capable of regeneration following amputation. Such tails typically are composed of spinal cord and its associated segmental ganglia, myotomes, and an axial skeleton. Skeletal support may consist of a notochord in the tails of cyclostomes and larval amphibians, and cartilaginous or bony vertebrae in the tails of urodele amphibians. In lizards an unsegmented cartilaginous tube replaces the vertebrae in the tail regenerate.

Tail regeneration in cyclostomes gives rise to a complete replica of the original, including spinal cord, segmental ganglia, myotomes, and notochord (Niazi, 1963). There is no cartilaginous or bony skeleton in such structures. In contrast, the similar tails of larval amphibians regenerate quite differently. In tadpoles, regeneration is not entirely complete. Although the spinal cord itself grows back, its segmentally arranged ganglia fail to develop. The notochord reconstitutes itself in the regenerate. Such tails, which are destined for resorption at metamorphosis, lack all capacity for chondrogenesis or osteogenesis. Unlike other vertebrate tails, regeneration in tadpoles does not depend upon the presence of the spinal cord, but the notochord is required (Morgan and Davis, 1902).

Urodele larvae have tails with notochords in younger forms, but segmental cartilaginous vertebrae in more mature ones. Ossification occurs after metamorphosis. At all ages, amputation is followed by the regeneration of a cartilaginous skeleton (Holtzer *et al.*, 1955). In the case of tails possessing notochords, this cartilage develops as a continuation of the notochord distal to the level of amputation. Transplantation experiments have shown that

such cartilage is induced by the ventral motor portions of the spinal cord (Holtzer, 1956). If the spinal cord is severed and deviated to the dorsal fin region, it will induce the development of a supernumerary tail from the surrounding tissues. Such accessory outgrowths are histologically complete in that they contain myotomes and cartilaginous vertebrae in the absence of the notochord. If segments of spinal cord are grafted elsewhere in the tail, amputation through the region of the transplant will result in the production of tail structures organized around the spinal cord regenerate distal to the level of amputation, structures that include muscle and cartilage, but not notochord. These findings may be taken to indicate that the cartilage induced by the spinal cord is derived not from the notochord, but from other tissues in the tail. Indeed, the notochord itself, even in the absence of chondrification induced by the spinal cord, is incapable of regeneration in larval urodeles following its transplantation to the dorsal fin, and subsequent amputation.

The tails of adult urodeles regenerate in much the same way as those in larval forms. In both cases, the spinal cord is necessary for regeneration to occur. Spinal cord regenerates are complete, including paired segmental ganglia. The bony vertebrae of the original tail are replaced by cartilaginous ones. Whether or not these are induced by the spinal cord, as is the case in larval forms, has not been tested experimentally. The number of such vertebrae, however, is a function of the level of amputation. Despite the metameric similarity between successive vertebrae, each one would appear to have its own identity in the sequence. Blastemas formed at posterior levels of amputation, when transplanted to more anterior stumps (Iten and Bryant, 1976), give rise to numbers of vertebrae commensurate with the more posterior level from which the blastemas were derived, although the anterior stump may be responsible for intercalary vertebrae development.

Not all tails regenerate exact replicas of the original, but the lizard tail (Fig. 16) is unique in this respect. As the only structure that reptiles are capable of regenerating, the tails of many lizards have acquired the capacity for autotomy, or self-amputation. This attribute they share with certain urodeles (e.g., *Plethodon*), a response triggered by mechanical trauma. Pinching the tail, for example, stimulates local muscular contractions that pull on the vertebrae to which they are attached so as to break the tail off along a preformed cleavage plane. The portion of the tail thus disposed of continues to wiggle, presumably long enough to distract predators while the lizard or salamander escapes. The relatively high percentage of individuals in a population with regenerated tails testifies to the importance of autotomy as a survival mechanism.

The stump of a lizard tail, whether lost by autotomy or surgical transection, becomes sealed with a scab. The protruding distal portion of the ex-

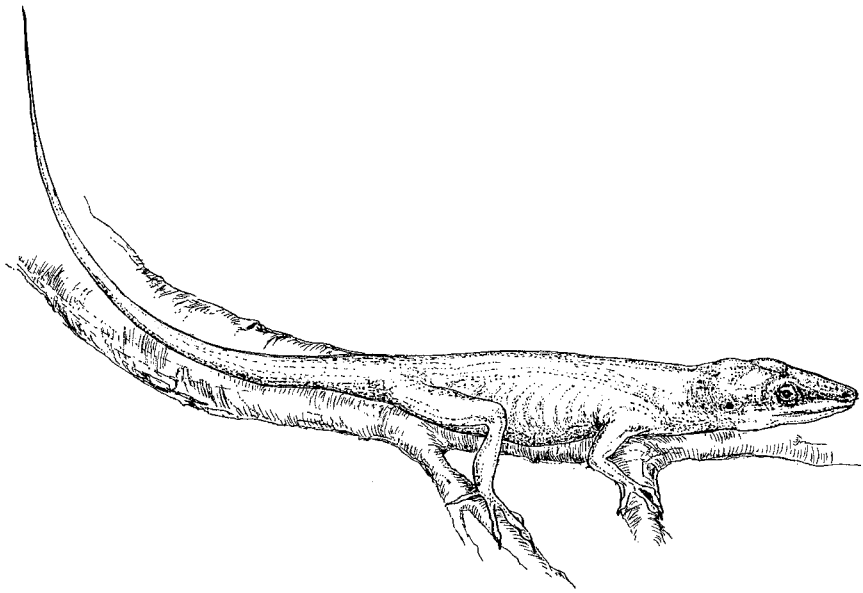

Fig. 16. *Anolis carolinensis,* whose tail can be autotomized and regenerated as an anatomically different structure.

posed vertebra typically becomes desiccated and is eventually ablated as the migrating wound epidermis separates the dead tissues from the living portions of the stump. Once the blastema has formed under the influence of the regenerating ependymal tube of the spinal cord (Simpson, 1964), chondrogenesis commences in association with the bony vertebrae in the stump. Instead of giving rise to segmental vertebrae, however, the lizard tail regenerate forms an unsegmented tapering cylinder of cartilage. This vertebral replacement remains open at its posterior end, and may be perforated here and there to allow the passage of blood vessels. At later stages, there may be limited ossification at proximal levels. The rest of the regenerate includes myotomes and an outer layer of skin, the scales of which tend to be smaller than those in the original tail, and not organized segmentally. The spinal cord regenerates as an ependymal tube surrounded by the outgrowth of nerve fibers from the stump. Neither ganglia nor new neurons differentiate. Clearly, the regenerated lizard tail represents an adaptation for survival. One can only conclude that the unusual nature of its new skeleton is a concession to energy conservation. Although the regenerate can itself grow back after reamputation, it is incapable of autotomy. Thus, it is not as good as the original, but is presumably good enough to serve whatever purpose tails are there for.

E. Regeneration in Mammals

In recent years a number of systems have been found in mammals that are capable of epimorphic regeneration (Goss, 1980). In some of these cases cartilage plays a significant role not only because it is an integral part of the structure to be regenerated, but also because its presence seems to be indispensable to certain inductive processes without which the potential for scar formation cannot be diverted into the pathways of blastema production.

Chondrogenesis occurs in several types of mammalian regenerating structures. It is involved in the annual regrowth of deer antlers, where it is a transient tissue subsequently converted to bone. In the external ears of certain mammals, chondrogenesis is responsible for the regeneration of the cartilaginous sheet in the replacement of missing tissue from around the margins of holes punched through their full thicknesses. Finally, a somewhat similar situation prevails in the regeneration of the nasal septum in infant animals. Parenthetically, there are other examples of mammalian regeneration which, although they do not involve chondrogenesis, are worth mentioning. These include the regrowth of tissues in the membranes of bat wings, and the reestablishment of continuity in perforated guinea pig tympanic membranes (McMinn, 1975).

1. Deer Antlers

As secondary sex characters, antlers are grown only by males (except in reindeer and caribou). When fully mature, antlers are composed of solid, dead bone. They are thus distinguished from the horns of other ungulates in which the hard component is derived from cornified epidermis. Antlers also differ from horns in two other important respects. They are usually branched, and they are dropped off each year to be replaced by new outgrowths. Horns are unbranched structures, except for the spur in the pronghorn antelope, a structure that is strictly epidermal and not reflected in the bony core of the horn. The pronghorn is another exception to the rule in that its horn sheaths are deciduous (as are deer antlers). However, the germinative layer of epidermis in these horns remains as a source of the new horn sheath grown each year after the mating season. Other horned animals add new material to their horns annually, accumulating a lifelong set of annual growth increments in which an accurate record of the animal's age is laid down.

When the old antlers are shed after the mating season, an event triggered by the decline in testosterone secretion (Wislocki *et al.*, 1947a), in turn responsive to changes in the environmental photoperiod (Goss *et al.*, 1974), the stump of the bony frontal pedicle from which the antler was detached remains as a raw wound (Fig. 17a). This is soon healed over by ingrowth of the

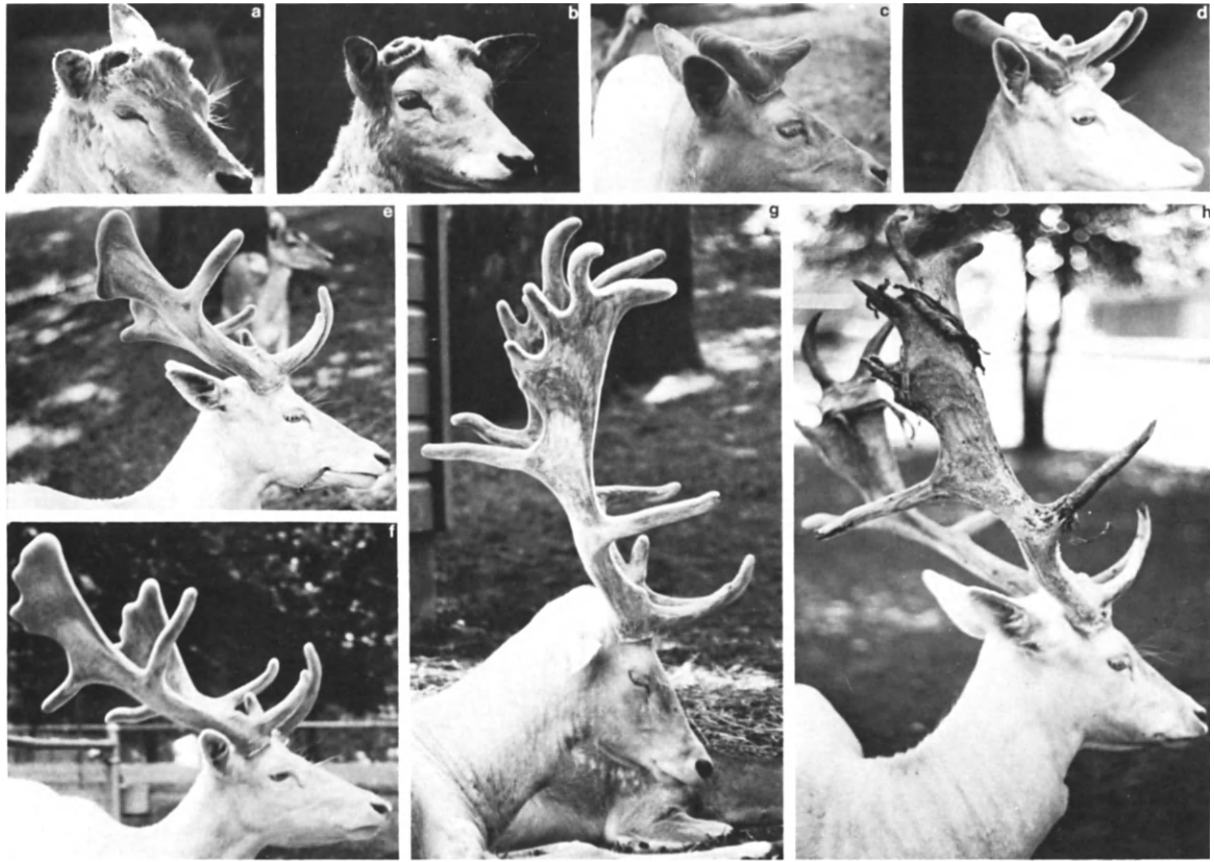

Fig. 17. Successive stages in the growth of antlers in a fallow deer. The old antlers were shed on 23 April (a) and subsequent photographs were taken after 12 (b), 30 (c), 43 (d), 70 (e), 87 (f), 106 (g), and 125 (h) days. The velvet was being shed on 26 August (h).

surrounding skin of the pedicle (Fig. 17b), skin which often becomes swollen even before the old antlers are shed. Within a few weeks the healed wound rounds up into an antler bud which, during the course of the spring and summer, elongates and branches into the familiar pattern of the final antler (Fig. 17c–h). The rate at which such antlers grow exceeds virtually every other known elongating system in the animal kingdom, reaching rates up to several centimeters per day at the steepest inflection of the growth curve (Goss, 1970). In the so-called Irish elk *(Megaceros),* extinct since the Pleistocene, antlers over 2 m in length were often produced, presumably in growing seasons no longer than those that prevail today. Thus, such antlers would have had to elongate at the *average* rate of 2 cm per day, but may have exceeded twice this rate if one allows for the fact that growth curves are seldom linear.

The antler bud consists of a mass of proliferating mesodermal tissues enveloped in a very special kind of epidermis, called "velvet." Antler velvet differs from the epidermis of the scalp from which it is derived in that its surface is shiny and its hairs protrude perpendicularly, providing a velvety texture to the growing antler. The hairs are produced from follicles that are differentiated *de novo* at the growing tip of the antler. They are accompanied by prominent sebaceous glands. Internally, the antler resembles a regeneration blastema. Its fibroblast-like cells proliferate at a high rate and are responsible for the elaboration of quantities of fibrous collagen. Antlers grow by terminal addition, and each branch is equipped with an apical growth zone which persists until the end of the summer when the full size of the antler has been attained. The cells left behind by the advancing growth zone undergo a sequence of changes, the first of which is chondrogenesis (Fig. 18). This cartilaginous zone lies just proximal to the growing tip. It is a very peculiar type of cartilage, so much so that some investigators have denied the true cartilaginous nature of this tissue, despite its undeniable histological resemblance to cartilage. The reason for the confusion lies in the fact that the cartilage in the growing antler is richly vascularized. The antler is supplied with arteries that carry blood in the velvet skin to the growing tips, whereas venous return is mostly down through the internal tissues of the antler. Thus, the apices of the antler possess numerous blood vessels that arch inward and downward through the more proximal tissues, including the cartilage.

Farther down, the matrix of the cartilage becomes calcified, and still more proximally ossification occurs (Fig. 19). Although this proces has the unmistakable characteristic of endochondral ossification, its details are not well understood. Some investigators have contended that, in the apparent absence of chondroclasts, the calcified cartilage becomes converted directly into bone (Wislocki *et al.,* 1947b). Others, however, have identified multinucleate chondroclasts involved in the replacement of cartilage by bone

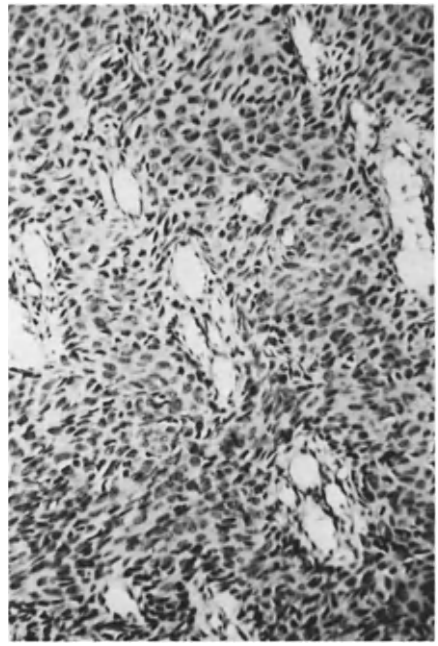

Fig. 18. Cartilage differentiates in a spongy configuration, traversed by numerous blood vessels, in the zone of chondrification in the growing tip of an antler of a sika deer.

(Banks, 1974). Despite evidence for the existence of chondroclasts, there is also reason to believe that calcified cartilaginous spicules may persist, on the surfaces of which new bone may be deposited. This bone is cancellous, consisting of a spongy network of trabeculae, particularly in the interior of the antler, but also at the ends of the tines once elongation has ceased (Fig. 20). Peripherally, the bone of the growing antler tends to be more compact, and toward the end of the growing season there is a wholesale deposition of mineral in the growing antler converting it into the hard solid bony structure possessed by the animal in the mating season.

This ossification of the mature antler leads to the constriction of the vascular channels through which blood is normally drained from the antler. The result is a general ischemia responsible for the death of the velvet skin which is then actively rubbed off by the deer (Fig. 17h). This occurs in late summer or early autumn in time for the mating season. Not surprisingly, it is triggered by rising levels of male hormone at that time of year.

The interesting process by which deer are able to replace their antlers each year raises some problems of interpretation. Nineteenth-century biologists

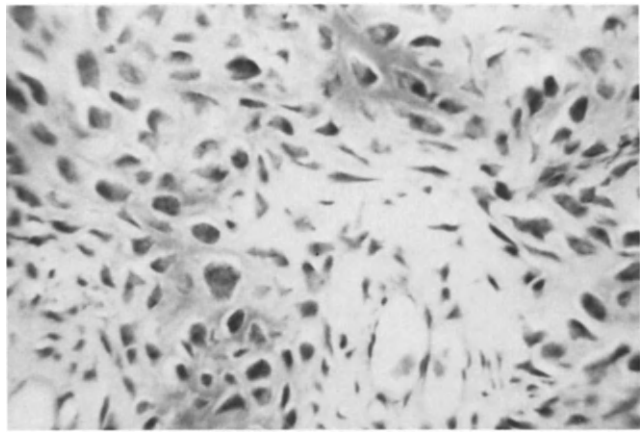

Fig. 19. Trabecular bone develops proximal to the cartilage, interspersed by vascular channels, in the growing sika deer antler.

wondered if the antler bud were an apophysis or an epiphysis. An apophysis is a direct outgrowth from the frontal bone. An epiphysis is a separate center of ossification that fuses secondarily with the skull. The so-called ossicone in the giraffe is an example of the latter situation, but no such structure has been identified in the Cervidae. The apparent presence of cartilage in the growing antler is indicative of an endochondral bone, but one that is produced originally from the periosteum of the frontal bone, which is dermal.

Unanswered questions about antlers abound, not the least of which relates to their histogenesis. Deletion and transplantation experiments have thus far failed to reveal which tissues in the pedicle give rise to the antler. Ingenious experiments by Hartwig and Schrudde (1974), however, have proven that in the fawn the first antlers to grow are derived from the periosteum of the frontal bone at the site where the antler pedicle is destined to develop. Removal of the overlying skin does not prevent subsequent antler development, whereas excision of the incipient pedicle protuberance from the frontal bone, or of its overlying periosteum, precludes further antler development (Goss *et al.*, 1964; Hartwig and Schrudde, 1974). Moreover, if the presumptive pedicle periosteum of a fawn is transplanted beneath the skin of the forehead or leg, ectopic pedicle outgrowths are induced, followed by the production of antler tissue distally (Hartwig and Schrudde, 1974). Thus, the pedicle periosteum is capable of inducing antler tissue from overlying skin, even on different parts of the body. These findings suggest that although the skin seems to be rather passively involved in the production of antlers, the mesodermal part of the antler is most probably derived from the periosteum of the frontal bone.

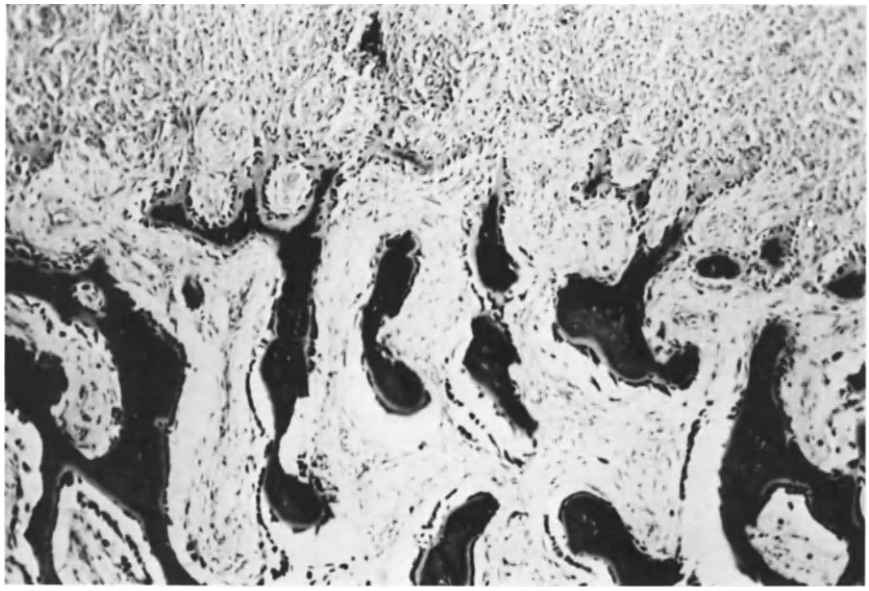

Fig. 20. When elongation of mature antlers ceases, the spongy bone at the growing tip becomes more densely mineralized as appositional ossification commences. Reproduced from Goss (1970) with the permission of the publisher.

2. Regeneration of Ear Tissue

If a hole is punched through the full thickness of a rabbit's ear, it is filled in with new tissue derived from the margins of the hole (Fig. 21.). Such ears are capable of obliterating holes up to 1.75 cm in diameter. Although the transverse amputation of the tip of a rabbit's ear does not induce its regeneration, V-shaped notches in the edge of the ear regenerate at their acute angles. Thus, this type of regeneration would seem to depend upon a concave margin, perhaps because its growth may be sustained by the focusing of proliferating tissues at the growing edge in order to maintain a critical density of proliferating cells.

The mechanism by which this kind of regeneration occurs is not unlike that previously described in other epimorphic regenerating appendages (Joseph and Dyson, 1966). The ear consists of a sheet of auricular cartilage sandwiched between the inner and outer layers of skin. Healing at the margin of a hole is accomplished by the migration of epidermis (Fig. 22) from either side until they meet in the middle. This occurs toward the end of the first postoperative week. The reason for the extended period of wound healing would seem to be attributed to the presence of the cartilaginous sheet, the exposed edge of which becomes desiccated and must in due course be lost with the scab. This ring of dead cartilage separates itself from the subjacent viable

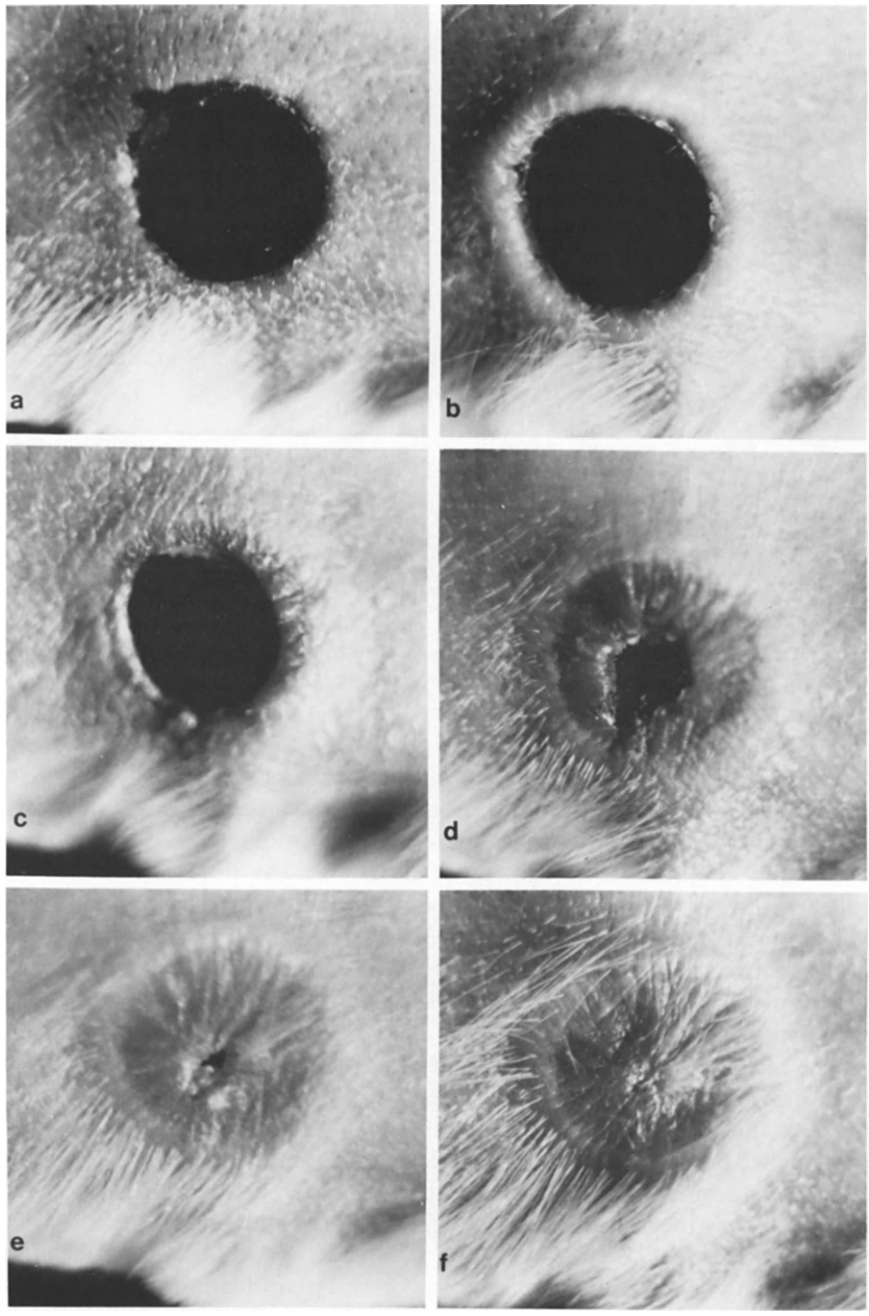

Fig. 21. Stages in the regeneration of tissue to fill in a 1-cm hole through a rabbit ear, photographed 1 day (a) and 1 (b), 3 (c), 4 (d), 5 (e), and 6 (f) weeks postoperatively.

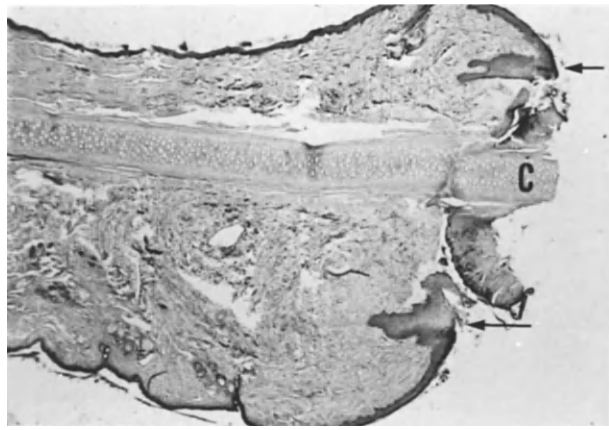

Fig. 22. Wound healing in a rabbit ear 7 days after a hole had been punched through its full thickness. The exposed cartilage (C) dies and is destined to be shed with the scab as migrating epidermis seals the margins of the hole. Note the conspicuous epidermal downgrowths (arrows) at the inner and outer corners of the wound.

tissues, making it possible for the migrating sheets of epidermis to fuse. Subsequently, a mass of apparently undifferentiated cells accumulates beneath the wound epidermis and off the end of the severed sheet of cartilage (Fig. 23). These cells resemble the blastemas seen in other regenerating systems. They are capable of extensive proliferation. Convergent growth of this peripheral blastema progressively reduces the size of the aperture while

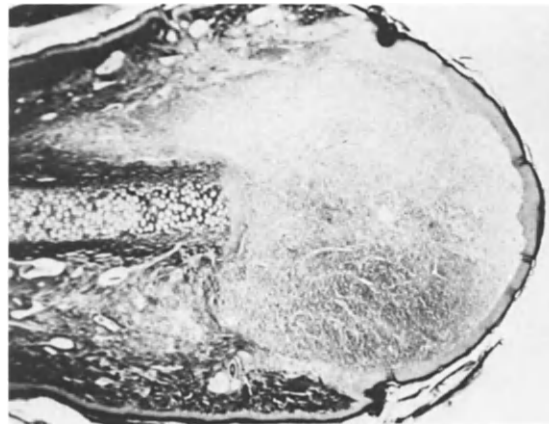

Fig. 23. Regenerating blastema at the margin of a hole punched through a rabbit ear 14 days earlier. Chondrification will begin at the severed end of the original cartilaginous sheet. (Courtesy of Dr. L. N. Grimes.)

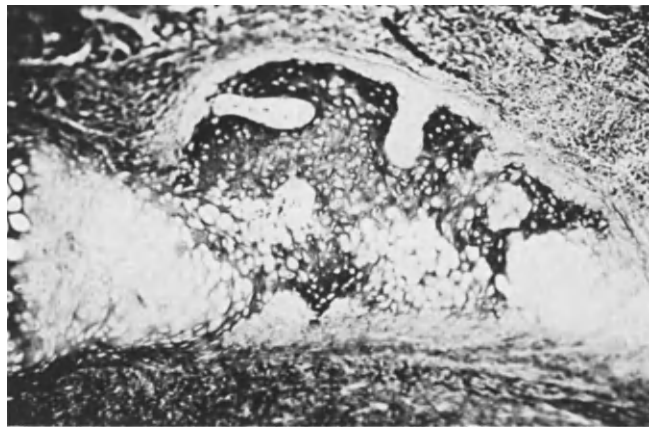

Fig. 24. Regenerated cartilage in continuity with its counterpart (extreme left) in the replaced portion of a rabbit ear. As sometimes happens, bone has differentiated near the periphery of the regenerated cartilaginous sheet. (Courtesy of Dr. L. N. Grimes.)

depositing new tissue around the margins of the hole. The cells left behind by the convergent growth zone then differentiate into new cartilge off the end of the old. In favorable instances, the regenerated tissue will bridge a 1-cm gap in 6 to 8 weeks (Goss and Grimes, 1972). A new sheet of cartilage normally reestablishes the continuity of the cartilaginous sheet of the ear. Sometimes, for unexplained reasons, ossification may occur circumferentially, just within the original edges of the wound (Fig. 24). Grimes (1974b) showed that if an extra sheet of cartilage is grafted to the ear, subsequent regeneration yields only a single cartilaginous sheet (Fig. 25).

Unlike the rabbit, the ears of practically all other mammals are incapable of regenerating tissue to fill in holes. In order to seek the explanation for this difference, the histological sequence of events following injury has been compared between the ears of rabbits, which do regenerate, and those of dogs and sheep, for example, which do not. In the latter cases, scar tissue occurs following the completion of epidermal wound healing. In the rabbit, a blastema is formed instead. It has been observed that during the course of epidermal migration in the rabbit ear, there is a conspicuous downgrowth (Fig. 22) of epidermal cells into the underlying tissues at both the inner and outer corners of the wound (Goss and Grimes, 1975). These downgrowths extend for a millimeter or so, and have not been observed to occur in nonregenerating ears. In the rabbit, they are located immediately next to the cut edge of the dermis, a position suggesting the possibility that they may somehow interfere with dermal regeneration per se, a process otherwise believed to be responsible for scar formation. These epidermal downgrowths

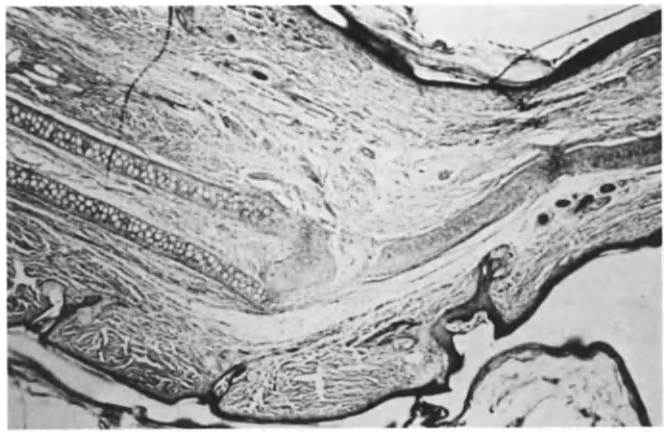

Fig. 25. Section through a regenerated rabbit ear in which two sheets of cartilage were present at the margins of the wound. Nevertheless, a single sheet of cartilage (right) differentiated in the regenerate. Reproduced from Grimes (1974b) with the permission of the author and publisher.

fail to occur when regeneration of the rabbit ear is inhibited by exposure to 3000-R X rays (Grimes, 1974a). They also fail to occur when the cartilaginous sheet of the ear has been removed prior to cutting a hole through the region lacking cartilage. This operation prevents regeneration (Fig. 26). Again, there is a correlation between the existence of epidermal downgrowths and the capacity of the ear to regenerate (for a further discussion see Volume 1 Chapter 5).

Although the cartilaginous sheet of the ear is itself regenerated in the course of replacing the full thickness of the ear, it is important to note that if part of the sheet of cartilage is removed from the otherwise intact ear, chondrogenesis fails to occur internally (Stoll and Furnas, 1970; Skoog *et al.*, 1972). Thus, cartilage is incapable of regenerating as a tissue, but can participate in epimorphic regeneration of the ear. The significance of this is important. It means that in order for cartilage to regenerate it must be in close association with an overlying epidermal wound. The latter is, of course, indispensable for the onset of epimorphic regeneration. The implication is that the epidermal downgrowths in the rabbit ear may be involved in some kind of inductive communication with the nearby edge of the cartilaginous sheet, an induction possibly significant for the promotion of regeneration in this system. Thus, cartilage is essential for the regeneration of rabbit ears, and the regeneration of the ear tissue is necessary for chondrogenesis to occur.

Rabbits are lagomorphs, an order of mammals which also includes hares and pikas, both of which are likewise able to regenerate ear tissue the way rabbits do. Pikas are short-eared lagomorphs that live in mountainous rock

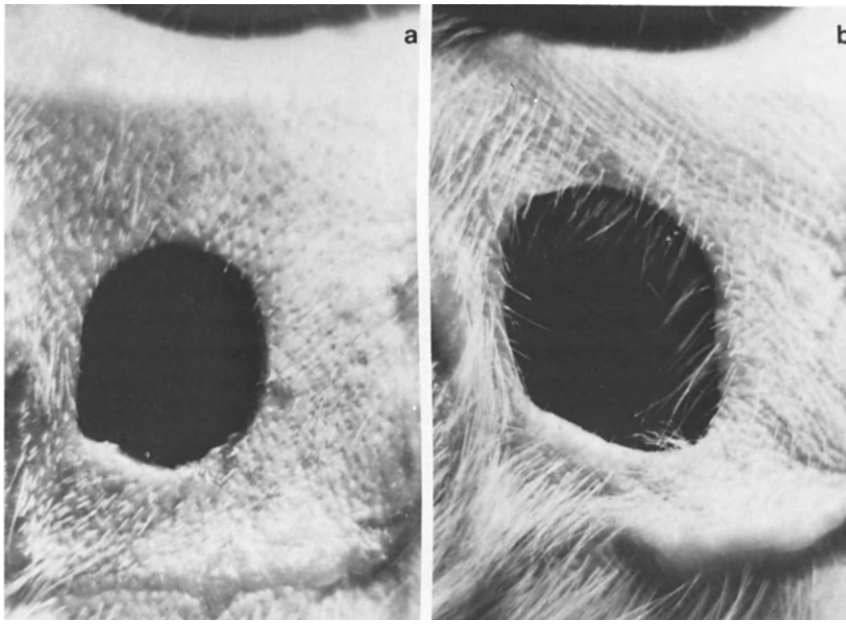

Fig. 26. (a) One-day-old hole punched through the part of a rabbit ear from which the cartilaginous sheet had been removed (note square outline of healed skin flap previously reflected to expose cartilage). (b) The same hole 6 weeks later to illustrate the complete absence of regeneration, and in fact the enlargement of the original aperture.

slides at high altitudes. The fact that their ears are capable of filling in holes may be taken to indicate that the regenerative ability of rabbit and hare ears cannot be explained by the relatively large sizes of these structures. Many other mammals have been tested for this ability, and found not to be able to regenerate. These include mice, rats, hamsters, gerbils, guinea pigs, opossums, dogs, sheep, and deer. Even the Patagonian cavy, a remarkably rabbit-like rodent from Argentina, is incapable of regenerating tissue to fill in holes punched through its long ears. Two other exceptional mammals have been found capable of regenerating tissue. One is the domestic cat, an animal that can readily grow new tissue to fill holes punched through its external ears, and that also completes the continuity of its cartilaginous sheet. Indeed, if the ear cartilage is removed prior to punching the hole, regeneration fails to occur, an attribute shared with the rabbit. The other exception is in bats, many of which fly by echolocation. Fruit bats, which navigate by night vision, are *unable* to regenerate holes punched through their ears. Insectivorous bats, however, and the fruit bats of Central and South America which fly be echolocation, *do* regenerate tissue to fill in holes punched in their external ears. Curiously, these regenerates lack cartilage. They consist

of the inner and outer layers of skin separated only by an intervening layer of connective tissue.

The regeneration of holes in bat ears provides two important perspectives to the problem of mammalian regeneration. One is that it is probably an adaptive trait, although it is not always obvious to what this regenerative ability has adapted. Second, the inability of bat ears to regenerate the cartilaginous sheet suggests that the capacity for ear regeneration, which presumably evolved independently in different orders of mammals, did not necessarily adopt identical mechanisms of development.

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10

*Bioelectricity and Cartilage**

Brian K. Hall

I. INTRODUCTION

The clinical and economic value of any method(s) that will speed growth, repair, or maintenance of skeletal tissues should not be underestimated. In the United States alone each year 25,000 children are born with legs of unequal length: ideal treatment—stimulation of skeletal growth. Two million individuals fracture their limbs each year; 100,000 of them fail to repair but instead become persistent nonunions or form false joints: ideal treatment—stimulate repair of skeletal tissues. Twenty million individuals (one-tenth of the population) suffer from osteoporosis: ideal treatment—improved maintenance of skeletal tissues. The financial cost, both medical and in lost productivity, may run as high as 10–15 billion U.S. dollars per year. Add to this list individuals born with congenital pseudarthroses and those who lose

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portions of digits or limbs through amputation, and the implications for enhanced repair become enormous.

The columns of cells in the cartilaginous growth plates of the epiphyses of long bones parallel the longitudinal polarization of the bone, a polarization based in the piezoelectric properties of collagen (Shamos and Lavine, 1967; Athenstaedt, 1974). This is one type of bioelectricity that could influence the behavior of cartilage. Cartilage is predominantly a hydrated gel of polyanionic glycosaminoglycans. Hence its extracellular matrix has a net negative charge and would attract positively charged ions, molecules and cells—another bioelectrical phenomenon affecting cartilage. The growth plate is electronegative with respect to the underlying bony metaphysis and the deep layers of articular cartilage are electronegative with respect to more superficial layers. As will be discussed, such electronegativity is correlated with growth, repair, and regeneration in a number of biological systems, including cartilage.

Electrical potentials can be recorded from mechanically loaded cartilages. They range from -100 to 400 μV in tracheal cartilage, all the way up to -1 to 4 mV in articular cartilage (Bassett and Pawluk, 1972). The electrical potentials of loaded bone (-100 to 200 μV) fall into the lower end of this range. It is feasible that mechanical effects on cartilage could be transduced via altered electrical potentials.

The naturally occurring electronegativity of cartilage can be augmented by applying an electrical or electromagnetic current to cartilage *in vivo* or by maintaining cartilage in a field *in vitro*. This chapter reviews the response of cartilage to such applied electrical or electromagnetic fields.

The literature on bioelectricity is vast and growing. Spadaro (1977) lists two publications appearing in 1967, 30 in 1974, and the number doubling every 2 years. Perforce, I shall have to omit much of this literature. I will not deal with the aspects of bioelectricity that apply to bone as a tissue. Piezoelectric properties of bone, electrical stimulation of bone growth and repair, and the response of osteoblasts to electrical fields *in vitro* will not be treated. I will deal with some of the clinical studies on repair of fractures in man, even though the data is usually of a type (radiographic) and taken at a time (the end of the reparative process) that effects on cartilage have to be inferred rather than directly observed. Both *in vivo* and *in vitro* studies will be covered.

Types of electrical stimulation used on the skeleton are numerous; ac/dc currents, pulsed/nonpulsed, direct/indirect, high/low voltage, high/low amperage, constant/intermittent, electrical/electromagnetic, etc. For details on the electrical parameters used in particular studies the reader should consult the original articles. Such details are very important when comparing

one study with another but economy of space and the generalist nature of the audience precludes providing all the details here.

Although a number of reviews have been published on bioelectricity, none have specifically addressed cartilage. Some discuss bone (Bassett, 1965, 1968; Eriksson, 1976; Marino and Becker, 1977; Spadaro, 1977; Connolly, 1981), some the repair of fractures (Becker, 1972a, 1974; Brighton, 1980), and some electromagnetic fields (Bassett, 1978, 1981). Others are more general (Becker, 1972b, 1974), reporting proceedings of conferences or providing broad overviews (Liboff and Rinaldi, 1974; Brighton *et al.*, 1979). Even the popular press (*New York* magazine) has produced a recent and lengthy two part article (Weymouth, 1980a,b).

II. CARTILAGE IN LIMB REGENERATION

A. Historical Note

Anyone with an interest in links between electricity and regeneration would have to go back to the seventeenth or eighteenth centuries to find the first reported studies by scientists such as Galvani and Spallanzini. More modern interest was rekindled by the observations that electrical currents affect regeneration of whole flatworms and of amphibian limbs (for reviews, see Rose, 1974; Borgens *et al.*, 1979a). When allowed to regenerate in a 60-cycle alternating current, the flatworm, *Dugesia tigrinum,* shows faster growth and differentiation as well as altered polarity of the regenerating tissues. These effects are maximized in conditions of electronegativity (Marsh and Beams, 1952; Marsh, 1968; Smith, 1970).

Within the vertebrates, electrical stimulation of regeneration of appendages containing cartilage has been described in both amphibians and mammals.

B. Amphibians

Regeneration of amphibian limbs, with special reference to cartilage is reviewed by Goss (see Chapter 9 in this volume for an overview of all the stages and processes involved). I will only consider the possible role of bioelectrical factors in regeneration up to the cartilaginous stage. Such regeneration involves covering the wound stump with epidermis, ingrowth of nerves, dedifferentiation of cells at the stump to form a blastema of undifferentiated cells, and redifferentiation of cartilage and other tissues from the blastema.

All salamanders (urodeles) are capable of regenerating whole appendages but *most* frogs (anurans) are not. Contrary to popular misconception, species in *some* genera of frogs (discoglossids, pipids, hylids) are quite

capable of regenerating appendages. In others (ranids) regeneration is rare but only in bufonids has regeneration *never* been observed (Scadding, 1981). The crucial role of nerves for regeneration in salamanders (and in those frogs which regenerate?) and their deficiency in ranid and bufonid frogs is thought to be one of the major reasons for differences in regenerative ability (Goss, 1969, and Chapter 9, this volume; Rose, 1974).

In 1961, Becker showed that surface electrical potentials, recorded after amputation of an appendage, differed significantly between regenerating salamanders and nonregenerating frogs (*Rana pipiens, R. catesbiana*). The salamander stump became strongly electronegative (− 30 mV) 15 to 20 days after amputation. At that stage the frog limb stumps were electropositive (+ 10 mV: nonregenerating limbs are slightly electronegative). The stumps of these amputated limbs were electrically polarized, but in opposite directions. In salamanders, distal tips of the stumps were electronegative and the proximal shoulders electropositive. This polarity was reversed in the frog limbs. If the distal stump has to be electronegative for dedifferentiation, blastema formation, and regeneration of cartilage to occur, then clearly frogs would fail to regenerate by virtue of their electropositive limb stumps.

Bodemer (1964) sought to determine whether an applied electrical current could initiate regeneration in *Rana pipiens* and *R. catesbiana*. He amputated their forearms, exposed the second spinal nerve (which innervates the forearm) and placed them across bipolar silver electrodes for two 12-sec stimulations per minute for 20 min. The current delivered was 0.3 V. Such electrically stimulated frogs regenerated small cone-shaped structures, 5–10 mm long. Some of these contained cartilage and muscle and some had organized the cartilage into digits. Such regeneration was never seen in controls. Thus, direct electrical stimulation of the nerves innervating a limb provided a sufficient stimulus to evoke regeneration from species that normally do not regenerate. Electrical stimulation of nerves may act by allowing them to release a neurochemical agent (Goss, 1969) and therefore act by a different mechanism from that which operates when nonneuronal tissues are electrically stimulated.

Smith (1967) was the first to implant electrodes into frog's forearms to generate an electrical field at the amputation stump. Whether the stump was electropositive or electronegative, a regeneration blastema and cartilage formed in at least 50% and, in some trials, as many as 80% of the amputated animals. Clearly, the application of an electrical field is a sufficient stimulus for cartilage to regenerate. Smith (1970) has reviewed his work in the context of electrical fields in other regenerating systems. The position of the electrode in the stump, although making little difference to the number of animals differentiating cartilage, materially affected the number which went

on to form at least one digit (Smith, 1974). Although none of the animals that had electrodes placed preaxially in the stump went on to form digits 40–50% of all animals formed cartilage. Borgens *et al.* (1979a) have shown that peak electrical currents occur in the postaxial region of the stump. The signal for the differentiation of cartilage and that for subsequent morphogenesis and pattern formation of the differentiated cartilage are evidently not the same, differing either in strength or in type.

Borgens and his colleagues (see, for example, Borgens *et al.*, 1979a,b) have further pursued the role of bioelectricity in salamander limb regeneration by showing that substantial currents of the order of 50–100 $\mu A/cm^2$ flow out from the amputation stump. Although nerves play a crucial role in amphibian limb regeneration (see above) they argue that nerves are not the source of the electrical fields within amputated limbs. Denervation, which prevents regeneration, does not affect the electrical potentials. They demonstrate convincingly that (*1*) these electrical currents are Na^{2+}-dependent, (*2*) that they reside in a Na^{2+} pump within the skin, (*3*) that the electrical currents generated within the skin of frogs are comparable to those in salamanders, and (*4*) that in frogs this current is lost by leakage into the substantial lymph spaces which frogs, but not salamanders, have under the skin.

Given the known inductive role of epithelia in allowing dedifferentiation and blastema formation to occur (Goss, 1982; Hall 1982; Volume 2, Chapter 5), the localization of the electrical field in the skin is of considerable interest. When the skin is removed from a salamander limb before amputation, both the electrical potentials and the ability to regenerate cartilage are lost (Oberpriller, 1968; Borgens *et al.*, 1979a). The possibility that the epithelium's inductive ability resides within its bioelectrical properties is obviously worth investigating. Na^{2+} has been shown to be an effective inducer of primary embryonic induction in amphibians.

Smith's (1974) study on frogs showed that electrical fields, although sufficient to induce cartilage to regenerate, only stimulated subsequent morphogenesis and pattern formation when the electrodes were positioned postaxially within the limb stump. This regionalization could be based on (*1*) the epithelium (postaxial epithelium inductively active, preaxial epithelium not), (*2*) the mesenchyme of the stump (e.g., postaxial tissues differ from preaxial), or (*3*) on both, reflecting, for example, differential contact between epithelium and underlying tissues in different regions of the stump. Consistent with (*1*) is the localization of electrical activity in the skin described above and Slack's (1980) observation that pre- and postaxial epithelia on salamander limbs are not morphogenetically equivalent. Preaxial epithelium when grafted onto postaxial tissues yields a normal regenerate after amputation. Postaxial epithelium grafted onto preaxial tissues yields a

double postaxial regenerate. Ability to differentiate cartilage is unaffected, clearly establishing a morphogenetic role for epithelium separate from its role in inducing differentiation. Consistent with (*2*) is the concept of non-equivalence developed by Lewis and Wolpert (1976). Postaxial cartilages, no matter from what proximodistal level within the limb, share an affinity with one another which is expressed in their morphogenetic properties. Preaxial cartilages would have a different set of morphogenetic properties even though histologically, cytologically, histochemically, and biochemically they are identical to postaxial cartilages. This is the explanation used for the differing morphologies of radius and ulna which lie side by side in the limb. Consistent with (*3*) are our studies (Hall, 1982c; R. J. Van Exan and B. K. Hall, unpublished observations) which show that epithelia on different faces of the embryonic mandibular arch have differing inductive abilities, partly expressed because of differential contact between mesenchymal and epithelial cells in various regions of the arch. The peak electrical currents in the postaxial regions of salamander amputation stumps could mediate these processes by localizing inductive activity within postaxial epithelium and/or by ordering cell migration and contact with the epithelium.

C. Mammals

A discussion of electrically enhanced repair of *fractured* long bones is excluded from this section but can be found in Section III.

1. Regenerating Rat Forelimbs

Several attempts have been made to initiate regeneration of completely amputated forelimbs in the rat using electrical fields applied to the stumps.

Becker (1972c) and Becker and Spadaro (1972) modified the electrodes used by Smith (1967) on frogs and implanted bimetallic (platinum–silver) electrodes delivering 3–6 nA current into the stumps of the amputated forelimbs of 21-day-old male sprague dawley rats. The animals were examined histologically seven days later. In the best cases, blastema formation, longitudinal bone growth, and a new cartilaginous epiphyseal growth plate were all seen. Controls bridged the stump with new subperiosteal bone but showed none of the cartilage or longitudinal growth exhibited by the electrically stimulated animals.

More recent studies raise a note of caution in attributing the above results to electrical stimulation alone. Libbin *et al.* (1979) obtained electrodes from Dr. Becker to standardize conditions and implanted them into amputated rat forelimbs. Cartilage and bone formed *whether or not* current was flowing through the electrodes. Less bone did form in the absence of electrical current. These authors attributed at least some of the regnerated cartilage and bone to mechanical stimulation of stump tissues by the physical presence

of the electrodes (although mechanical stimulation can be transduced electrically—see Section I).

In an earlier paper this same group had shown the suturing muscle over the stump provided a mechanical environment sufficient to enable cartilage and bone to regenerate (Person *et al.*, 1979). If the muscle provided a source of chondrogenic cells rather than a particular mechanical environment, then the cartilage formed would really be ectopic (see Chapter 1, this volume). Its intimate association with the skeletal tissues of the stump perhaps argues against it being ectopic.

Suturing muscle across the stump, implanting electrodes, or passing 3–6 nA currents through implanted electrodes all yield qualitatively similar regenerates. Although the mechanism eludes us, these experiments give promise for future studies on mammalian limb regeneration. Perhaps regeneration of the amputated tips of the digits as occurs in children under 3 years of age could be induced in older individuals. So far only skeletal and myogenic tissues have been induced to regenerate in the rat. Complete regeneration would also require regeneration of a nerve and blood supply.

2. *Regenerating Deer Antlers*

A further example of mammalian regeneration involving cartilage and possibly associated with bioelectricity is the regeneration of antlers in male deer. Goss, who has done much of the work on antler regeneration, has reviewed the topic in the preceding chapter and both he and Hall (1978) discuss the peculiar skeletal tissues in the antler. One of these tissues, now known to be cartilage (Beresford, 1981, regards it as a secondary cartilage), has little extracellular matrix, is highly vascularized and is replaced by bone in an atypical pattern of endochondral ossification—the cartilage is not removed before bone is laid down. Although unusual in comparison to typical human cartilage, the range of cartilages is now known to be considerable (see Volume 1, Chapters 1 and 2).

Lake *et al.* (1979) have described electrical potentials on the surfaces of antlers of the Rocky Mountain mule deer (*Odocoileus hemionus*) that correlate with longitudinal antler growth. There is an electrical potential gradient along the antler—the tip is electronegative (-11 mV at peak antler growth in mid-July) and the proximal end electropositive ($+0.6$ mV). You will recall a similar gradient along the amputated amphibian limb (Section II,B), and the association of electronegativity with growth mentioned in the Introduction. The electrical potential at the tip of the antler changes during the growing season (April to September) and is positively correlated with the antler's linear growth rate (Table I). That the tissue of the growing antler is sensitive to these changing electrical potentials was confirmed by applying current to growing antlers and observing alterations in their growth rate

TABLE I

Growth Rate and Surface Electrical Potentials of Antlers of the Rocky Mountain Mule Deer[a]

	April–May	June–July	August–September
Growth rate (mm/day)[b]	3.4 ± 0.4	4.0 ± 0.3	0.9 ± 0.2
Surface potential (mV)[b,c]	−4.9 ± 0.7	−8.2 ± 1.4	−0.1 ± 0.6

[a] Based on data in Lake *et al.* (1979).
[b] $\overline{X}$ ± SEM, based on at least 8 measurements over a 2-month period.
[c] As measured at the tip of the antler.

(Lake *et al.*, 1978; Bubenik *et al.*, 1982). Whether it is cartilage that is responding and if so how (increased formation, calcification, etc.) has not been determined. How this phenomenon relates to the photoperiodically cued, testosterone-induced regeneration of antlers (see Chapter 9, this volume) is also unknown.

3. Regenerating Rabbit Ear Cartilage

Goss (Chapter 9, this volume) and Hall (Volume 2, Chapter 5) discuss regeneration of the cartilage in the rabbit ear. Chang and Snellen (1982) recorded surface electric potentials from such regenerating ears, in which the temporal course and magnitude (−20 mV) were very similar to those described from amphibian limbs. Nonregenerating ears showed the smallest changes in surface potentials. No further studies of this system are known to me.

III. CARTILAGE IN REPAIR OF FRACTURES

It is not my intention in this section to deal with all apsects of the repair of fractures nor to deal with all studies where electrical or electromagnetic fields have been used to enhance repair. I will only cover those studies that pertain to electrically mediated chondrogenesis in the repair of fractures. Studies on repair of dermal skull bones or of adult long bones where cartilage is not involved will be excluded. *In vitro* studies are covered in Section VI. Because histological or biochemical analyses cannot readily be carried out on repairing fractures in man, some inferences will have to be made from clinical studies concerning the cartilaginous phase and the effect of electricity upon it.

A. Amphibians

Becker and Murray (1967, 1970) and Harrington and Becker (1973) have reported a remarkable series of experiments which they claim provide the mechanism for formation of cartilage during repair of fractured long bones in the frogs *Rana pipiens* and *R. clamitans*.

Midshaft fractures of the tibiofibularis (the fused tibia and fibula) heal within 3 weeks. The blastema that forms is said to arise largely from the hematoma at the fracture site and only partly from cells of the activated periosteum. Before fracture, the periosteum and the bony surface of the tibiofibularis are both slightly electronegative (−1 mV). Immediately after fracture, the periosteum becomes strongly electronegative (−7 mV) and the bony surface electropositive (+2 mV). Combining this observation with the hematogenous origin of the blastema, Becker and Murray set out to observe the response of nucleated frog red blood cells to electrical fields applied *in vitro*. After considerable experimentation they found that a nonuniform field pattern with currents of 300–700 pA, a current density of 1pA/mm^2, and a voltage drop across single cells of 0.3 mV induced a dramatic series of morphological and biochemical changes in the red blood cells (see Figs. 9,11, 17, and 18 in Becker and Murray, 1970). The red blood cells lost their hemoglobin, underwent nuclear expansion, accumulated RNA, and began to synthesize proteins, shown by electrophoresis to be different from those produced by unstimulated cells. These morphological changes were inhibited by puromycin (4 μg/ml) and actinomycin (18 μg/ml) and were not accompanied by DNA synthesis (as evidenced by [^{3}H]thymidine autoradiography, Harrington and Becker, 1973). These changes were not seen in either leukocytes or epithelial cells exposed to electric fields.

Clearly, frog red blood cells are modified morphologically and biochemically after exposure to an electric field. The changes are sufficiently dramatic to be classed as a dedifferentiation, although Harrington and Becker (1973) following Hay (1966) felt that reinitiation of DNA synthesis and mitosis was required to call the changes a dedifferentiation. However, it is not de- but redifferentiation that requires DNA synthesis.

I have no quarrel with the results reported above. Clearly the changes are dramatic and well documented. However, Becker and Murray (1970) go on to conclude that these cells "subsequently redifferentiate as fibrocartilage cells responsible for the initial fracture callus." Unfortunately, they have no *direct* experimental evidence for this statement. They do have the three independent observations that (*1*) electrical activity changes at the fracture site, (*2*) the blastema appears to arise from cells of the hematoma, and (*3*) red blood cells dedifferentiate when cultured in an electric field. It is tempting to put the three observations together so that electrical changes after a fracture induced dedifferentiation of red blood cells, which subse-

quently redifferentiate into callus and form cartilage, and it may be so, but the critical experiment observing dedifferentiated red blood cells redifferentiating into chondrocytes still has to be done.

B. Laboratory Mammals

The rabbit tibia and fibula have served as models for electrically enhanced repair of fractures in mammals. The surface of the tibia carries an electrical potential that varies in polarity along its length. The proximal epiphysis is electronegative (−3.7 mV), the midshaft electrically neutral (isoelectric) and the distal shaft electropositive (+3 mV, Friedenberg and Smith, 1969). Thirty minutes following a complete fracture of the tibia, whether fractured distally or proximally, the entire tibial surface becomes electronegative. Thus the association of electronegativity seen in growth, regeneration, and in fractured frog long bones is also seen in the fractured rabbit tibia. [Lopez-Duran Stern and Yageya (1980) report that the fractured rat tibia is electropositive.]

A 20-μA current applied via a cathode situated in the fracture site accelerates repair by stimulating formation of both cartilage and bone (Friedenberg and Brighton, 1974). A low-voltage (15 V), capacitively coupled electrical field both produces electrical potentials in bone and accelerates chondrogenesis in fractured bones (Brighton *et al.*, 1981a,b). Lavine *et al.* (1969, 1971) also demonstrated that a 2- to 4-μA direct current accelerated repair of fractured rabbit femora, but one cannot directly assess its effect on the cartilaginous stages of repair from the data presented. The *presumption* is that if repair is accelerated, the preceding cartilaginous phase must have been either speeded up or more extensive.

In the *absence* of a fracture, a 20-μA current administered into the medullary cavity causes excessive deposition of endosteal, intramedullary bone but cartilage is not formed (Friedenberg *et al.*, 1974). Yasuda (1974) reported a contrary finding. Direct current to the medullary cavity for three weeks stimulated chondrogenesis, provided that currents of 10–100 μA were used. Below 10 or above 100 μA cartilage was not found. Furthermore, he found that the direction of cartilage growth could be controlled by varying the position of the electrodes. Deposition of cartilage began at the cathode and spread toward the anode. Cathode placement can be used to control the site at which chondrogenesis will commence. Friedenberg *et al.* may, in fact, have delivered a lower current than recorded, explaining the discrepancy between these two studies.

Electrical currents have also been applied to the femora of adult mice by implanting electrodes (Crelin and Deuker, 1970). Although not a fracture, this injury was repaired by callus and new bone formation. Both mice given

100 μA of direct current and those with no current flowing through the electrodes formed periosteal cartilage, apparently by modulation of progenitor cells from the marrow. Mitotic activity was not observed. As will be discussed later, electrical currents do not stimulate the cellularity of the fracture callus, even though mitotic activity can be stimulated *in vitro* (see Section VI).

Dogs have also been used as experimental animals ever since Bassett *et al.* (1964) observed enhanced cellularity of bone in the vicinity of implanted electrodes. Levy (1974) used a pulsed electrical field (firing rate 0.7 Hz, peak current amplitude of 500 μA, peak voltage of 1.4 V and pulse width of 5 msec) both to initiate chondrogenesis from the periostea of intact canine femora and to unite fractured femora. His impression of the histological data was that cartilage was undergoing metaplasia to bone, a possibility discussed in more detail in Hall (1972, 1978) and Beresford (1981).

Pulsed electromagnetic fields have also been used to stimulate repair of fractured canine long bones. Bassett *et al.* (1974a,b) applied such a field to fractured beagle fibulae for 28 days. Coils placed over the hindlimbs were used to apply one of two electromagnetic fields. One was a 1-msec duration pulse, repeating at 1 Hz with a peak voltage of 2 mV/cm^2. The second was a 150-μsec pulse at 65 Hz, with a peak voltage of 20 mV/cm^2. Repair of the fractures was enhanced under both sets of conditions. Large amounts of hyaline and fibrocartilage but little endochondral ossification was seen in fractures exposed to the first pulse field. Little cartilage was present in animals given the second field and what was present was being invaded by blood vessels and replaced by endochondral bone. Such endochondral ossification was not seen in controls. A similar sequence of events, with bony bridging of the fracture, was seen in fractured rat radii exposed to pulsed electromagnetic fields as described by Bassett *et al.* (1981c, 1982). Figures 6 and 7 in Bassett *et al.* (1982) illustrate typical results obtained. Pulsed electromagnetic fields also accelerate repair of the fractured rabbit fibula (Iannacone *et al.*, 1981), as measured by stiffness of the fracture.

Bassett *et al.* (1981b) quote Muller *et al.* (1968) as showing that rigid internal fixation applied to persistent nonunions in dogs promoted calcification, vascularization, and replacement of callus cartilage. Pulsed electromagnetic fields have similar effects (Shim, 1981). Bassett *et al.* (1979a) observations on Ca^{2+} kinetics of chondrocytes maintained in pulsed electromagnetic fields *in vitro* (see Section VI,B) affirm that such fields act by enhancing calcification of callus cartilage. In this respect they are unlike electrical fields which stimulate osteogenesis (Bassett, 1981). Little mitotic activity is seen at the fracture site (Bassett *et al.*, 1974a,b; and see Crelin and Deuker (1970) in their study on repair of the murine femur). Improved repair is *not* because of

stimulation of mitotic activity at the fracture gap (if anything there are fewer cells in electrically or electromagnetically stimulated fractures than in controls) but reflects enhanced calcification of the cartilage that forms.

C. Clinical Studies

In the United States each year 100,000 fractures fail to heal, leaving those individuals with persistent nonunions. Some develop a false joint or pseudarthrosis, a condition which also occurs as a congenital anomaly. Electrical and electromagnetic fields have both been used since the early 1970s to heal such persistent nonunions and pseudarthroses. [Actually, the use of electrical currents to treat nonunions and pseudarthroses goes back to 1812 and 1850 respectively (Bassett *et al.*, 1974a).] Modern clinical applications take advantage of the paramaters found effective in healing fractures in laboratory mammals.

The first reported healing of a nonunion in the modern era was a nonunion in the middle malleolus of the ankle of a 51-year-old woman (Friedenberg *et al.*, 1971). This nonunion had persisted unhealed for 14 months before an electrical implant of the type shown in Fig. 3.8 in Brighton (1980) was implanted with the cathode protruding into the fracture gap. Continuous application of a constant current of 10 μA for 9 weeks resulted in bone completely bridging the gap and healing the nonunion (see Fig. 3.7 in Brighton, 1980). Subsequent clinical trials have shown that 20 μA for 12 weeks, delivered through 4 cathodes, effectively heals 80–85% of the 300 patients treated up to 1980 (Brighton, 1980).

Clinically, healing is defined by uniform density of bone bridging the gap as seen in X rays taken before and after the treatment. Mobility of the bone is lost. Whether these nonunions heal by forming a cartilaginous callus or by direct stimulation of osteogenesis cannot be determined just from before and after X rays. Lavine *et al.* (1972, 1974) used electrical stimulation to heal a congenital pseudarthrosis of the tibia in a 14-year-old male and a persistent nonunion in a female suffering from neurofibromatosis. They examined biopsy specimens of callus cells with transmission electron microscopy 4 months after continuous application of a 3.5-μA current (together with a bone graft in the second case). Before treatment, the gap contained fibroblasts embedded in large amounts of collagen. Four months later, cells with abundant membrane-bound vesicles, atypical mitochondria, vacuoles, and active rough endoplasmic reticula were seen. No indication of chondroblastic cells was given (they would be expected to arise earlier) but data from the laboratory mammals (Section III,B) suggest that chondrogenesis would be expected.

Spadaro (1977) summarized 595 cases of application of electrical stimulation to a variety of skeletal situations in man. These included nonunions,

congenital pseudarthroses, delayed unions, spinal fusions, and fresh fractures of both long bones and jaw and utilized a variety of electrical treatments—currents between 0.1 and 100 μA, dc and pulsed dc currents, alternating currents and 0- to 60-Hz electromagnetic fields. An astonishing 95% of these report positive results. For a recent summary and discussion of work to date using electrical implants see Brighton *et al.* (1979).

In addition to electrical stimulation, pulsed electromagnetic fields have also been used to enhance repair of fractures in man. The application of such fields by Bassett *et al.* (1974a,b) and by Iannacone *et al.* (1981) to enhance repair of fractures in dogs and rabbits was described above. Calcification and vascularization of cartilage and its replacement by bone were all accelerated. The advantage of such treatment is that the field can be applied without surgery because the coils are placed externally on the limbs. Electrical stimulation is most effective when the electrodes are placed into the fracture gap. Noninvasive application of electromagnetic fields and implanting electrodes are the two current clinical methodologies for enhancing repair of fractures in man.

The first clinical application of pulsed electromagnetic fields in repair of pseudarthroses and nonunions was by Bassett *et al.* (1977). A single coil producing a pulse width of 300 μsec at a peak current of 10 $\mu A/cm^2$ was applied to 29 patients. Success, as defined radiographically and clinically, was 70% (85% when two coils were used). Success in nonunions of the tibia in adults is now 96% (Bassett *et al.*, 1978, 1979b). The clinical data has been reviewed by Bassett (1978) and by Bassett *et al.* (1981a,b). Although it does not allow a discussion of the effect of pulsed electromagnetic fields on chondrogenesis, the data on laboratory mammals (see Section III,B and Bassett, 1981) and the *in vitro* studies (see Section VI,B) show that pulsed electromagnetic fields enhance calcification of callus cartilage. A preliminary report indicates that they may also be used to repair damage due to avascular necrosis (Bassett *et al.*, 1981d).

IV. ARTICULAR CARTILAGE

Given that erosion and inability to repair articular cartilage are major drawbacks to successful treatment of degenerative joint diseases (see Chapter 4, this volume) any treatment that can augment the repair process or slow degenerative changes is eagerly awaited. Baker *et al.* (1974a) applied electrical stimulation to rabbit femoral condyles in an attempt to enhance their repair. Four-millimeter defects, extending to the subchondral bone, were made into the articular cartilage. Control defects were left empty. The experimental group received bimetallic electrodes. Forty-eight hours after implantation, currents of 3 nA and interelectrode potentials of 40–70 mV

were recorded. The rabbits were left with active electrodes for up to 9 weeks and their condyles then examined by light, transmission, and electron microscopy. Controls showed repair by fibrous tissue and fibrocartilage, derived from mesenchymal cells of the subchondral marrow. In 70% of the experimental animals, hyaline articular cartilage was laid down, often in sufficient amounts to completely fill in the original defect. Thus electrical stimulation alters the type of cartilage deposited in the defects. The hyaline cartilage evidently arose by proliferation of chondroblasts at the margins of the defects. Hyaline chondrogenesis was even further augmented by the use of battery operated devices delivering direct currents between 15 and 500 mV (Baker *et al.*, 1974b). I am unaware of any attempts to use electrical stimulation to slow degenerative changes in articular cartilage but given that degeneration only starts after mitotic activity has slowed or stopped, that repair is associated with reinitiation of mitotic activity (this section, and Hall, 1978), and that electrical stimulation can enhance division of chondrocytes *in vitro* (see Section VI,B), such a study is well worth the long time that would be required to carry it out.

V. OXYGEN TENSION

Applied electrical fields may act directly on cartilage cells. This would imply that the cell membrane has the capability of sensing and responding to alterations in its electrical microenvironment. Evidence in support of this possibility will be presented in Section VI. Alternatively, electrical fields may alter some component of the cells' microenvironment, therefore acting indirectly to alter cellular activity. One possible candidate for indirect action is local O_2 tension surrounding the cell.

Bassett and Herrmann (1961) showed very convincingly that cells derived from embryonic chick tibiae formed bone if cultured in an atmosphere of 35% O_2, but formed cartilage when cultured in 5% O_2. As all other culture conditions were constant, they concluded that low oxygen tension favors chondrogenesis from cells capable of forming either bone or cartilage. This conclusion, which is supported by various other studies (Hall, 1970), may be correlated with the known low O_2 tension measured in cartilage *in vivo* (tensions as low as 2–3 mm Hg) and the general anaerobic metabolism of cartilage as a tissue. Oxygen consumption can be as low as 0.1 μM O_2 per g wet weight per hour in articular cartilage. This may be compared with rabbit kidney where the comparable value is 96 μM (see Volume 1, Chapter 9 for a further discussion). Conditions of low O_2 tension would favor both formation and maintenance of cartilage at the expense of other tissues (such as bone) which require a vascularized, highly oxygenated environment. If elec-

trical stimulation lowers O_2 tensions in, for example, a fracture, then cartilage rather than bone would be expected to bridge the gap.

Brighton and Friedenberg (1974) and Brighton *et al.* (1975) evaluated O_2 tension and pH in the vicinity of electrodes set up in an O_2 consumption chamber. Currents of 10, 20, or 100 μA were passed through saline or tissue culture media for 5 min. The cathodes consumed molecular O_2 so that O_2 tension around them was lowered. Consumption of O_2 was inversely proportional to current applied being 2.34 pmol/μA/sec at 10 μA and 1.59 pmol/μA/sec at 100 μA current. pH, on the other hand, rose near the cathode, the rise equalling 0.04 pH units/min at 100 μA current. Lowering of O_2 tension, if it occurs when electrodes are implanted *in vivo,* would favor both differentiation of cartilage from progenitor cells and maintenance of preexisting cartilage. However the increased alkalinity, especially as it overcomes the buffering capacity of culture media (at least at the high currents used) might work against any O_2 effect. Changes in local O_2 tension, as a possible mediator of electrical effects *in vitro* or *in vivo,* need to be investigated further.

VI. *IN VITRO* STUDIES

A. Synthetic Activity of Skeletal Cells

The application of electrical fields to cells, tissues, or organs maintained *in vitro* goes back to the 1960s. Bassett and Herrmann (1968) reported in abstract form that synthesis of collagen by fibroblasts cultured in an electrostatic field increased by 100% over synthetic rates measured in control cultures. Solutions of native collagen respond to electrical currents above 2.6 V by undergoing polymerization, as measured by formation of crossbands. Polymerization is inversely proportional to the voltage applied (Marino and Becker, 1970). Thus both intra- and extracellular events can be modified by electrical fields.

Kamrin (1974) cultured human diploid lung fibroblasts (WI-38) on demineralized bone matrix (a known inducer of cartilage when implanted *in vivo* or cultured with competent cells; see Volume 2, Chapters 1 and 5) in an electrical field of 3.5 μA at 1 sine-wave cycle/sec. Many of the fibroblasts, especially those in direct contact with the demineralized bone matrix, became multinucleated and accumulated inclusions. His conclusion, based on histochemical analysis of the eroding bone matrix, was that the fibroblasts had been transformed into osteoclast-like cells. This is surprising in light of the more recent evidence that osteoclasts are derived from blood-borne monocytic cells (Hall, 1975). Presumably, the transformed fibroblasts were only osteoclast-like (e.g., they lacked ruffled borders) and were func-

tioning as phagocytes, as do fibroblasts within the periodontal ligament. Kamrin presumed that the fibroblasts' response to the applied electrical field was mediated by alterations in permeability and transport across their membranes, which in turn activated quiescent genes within them. The possibility that platinum ions from the electrodes might have had toxic effects on the cells has been eliminated by Marino *et al.* (1974).

Transmembrane mediation of electrical stimulation is certainly conceivable for cells of the osteoblastic series. Osteoblasts *in vitro* have a resting membrane potential of −20 mV and possess an active metabolic pump (Schusterman *et al.*, 1974). The surface potential recorded on intact bones has been shown by Friedenberg *et al.* (1973) to be the summation of these cell potentials. Osteoblasts are coupled to one another being able to transmit both ions and molecules the size of parathyroid hormone (Sheridan, 1974; Jeansonne *et al.*, 1979). The membrane potential of osteoclasts can be modified by both parathyroid hormone and by calcitonin (Mears, 1969). Fibroblasts, whose *in vitro* resting membrane potential of −16.5 mV is similar to that of osteoblasts, can vary that potential with changes in culture conditions—sparse or dense cell density, culture on plastic or collagen, etc. (Bard and Wright, 1974). They can also be oriented *in vitro* by exposure to electrical fields (1 mA current) but neoplastic cells, with disturbed surface charges, do not respond (Katzberg, 1974). Modification of the normal resting potential of their membranes is a possible way for cells to respond to electrical currents and fields. Analysis of the situation in cartilage, where the cells are enclosed in a negatively charged extracellular matrix, will obviously be more difficult, although isolated sternal chondrocytes increase glycosaminoglycan and decrease collagen production when cultured in 10–1000 nA/mm^2 fields (Rich *et al.*, 1981).

Cell membrane-bound enzymes could also modulate electrical currents. Osteosarcoma cells show elevations in the cell membrane based enzyme adenylate cyclase and in cyclic AMP when cultured in a pulsed electrical field (Facklam and Hassler, 1981). Chondrocytes, isolated from their extracellular matrices, show similar behavior (see Section B,1).

This series of studies shows that synthetic activity of skeletal and connective tissue cells may be modified under the influence of electrical fields applied *in vitro* and that changes in membrane potential and/or in membrane associated enzymes could mediate the electrical fields into biosynthetic events.

B. Growth of Cartilaginous Long Bones

There is now quite a respectable body of data which shows (*1*) that growth of embryonic cartilaginous long bones can be accelerated when they are

cultured in an electrical field, and (*2*) that the mechanism of that acceleration of growth is increased division of the chondroblasts.

1. Embryonic Chick Tibiae

a. Cultured in Electrical Fields. Several laboratories have investigated growth and related synthetic activity of embryonic chick tibiae, organ cultured in electrical fields. Watson *et al.* (1975) appear to have been the first although as early as 1972, Norton (1972) and Norton and Moore (1972) had examined the response of membrane bones to electrical fields applied *in vitro* and Norton (1974) used electrical stimulation to accelerate the growth of long bones in adult birds, where enhanced osteogenesis was correlated with electronegativity (Norton and Kramer, 1974).

Watson *et al.* (1975) cultured tibiae from 8- and 9-day-old embryos for 9 days in the presence of electrical fields. At 9 days of incubation the tibia is largely cartilaginous, although some subperiosteal bone is present (osteogenesis having commenced at 7½ days) and the perichondrium over the midshaft has already been transformed into a periosteum. [Scott-Savage and Hall (1979) provide a summary of the maturational events in tibial development.] Watson *et al.* used either constant or pulsed (1 pulse/sec) electrical fields of 1,000 V/cm^2 and found (*1*) that there was no difference in the overall histology of tibiae cultured in electrical fields compared with controls, and (*2*) enhanced growth of the tibiae cultured in the pulsed but not in the constant fields. After 9 days culture the tibiae in the constant electrical field were only 1% (n.s.) longer than controls whereas those cultured in the pulsed field were 12% longer ($p < 0.5$). Whether this linear growth was at the expense of growth in width (i.e., merely reflecting redistribution of major growth paramaters with no net increase in tibial mass) was not studied. The mechanism for the accelerated linear growth (e.g., enhanced proliferation and/or deposition of extracellular matrix, accelerated replacement of cartilage by bone, etc.) was also not studied.

A group at the University of Connecticut has also used the tibia of the embryonic chick as a model. Norton *et al.* (1976, 1977), Rodan *et al.* (1978), and Bourret and Rodan (1979) use tibiae from older (16-day) embryos by which age much of the shaft consists of bone with the remaining cartilage organized into distinct resting, proliferative, and hypertrophic zones. They used a 5-Hz oscillating electrical field of 1166 V/cm^2, that is, comparable to that used by Watson *et al.* above. Whole tibiae maintained in these fields for short periods of time show a 21% decrease in cyclic AMP levels, the concentration falling from 15.8 to 13.0 pmol/tibia. This response was only observed when the long axes of the tibiae were oriented parallel to the electrical field leading them to speculate that (*1*) tibiae contain receptors sensitive to electrical

fields, (*2*) the receptors are oriented along the long axis of the bone, and (*3*) reduction in cyclic AMP levels is the first step in the tibias' response to electrical stimulation.

The above results may be compared with the similar reduction in cyclic AMP levels observed when tibial chondroblasts are exposed to short durations (15 min) of physiological levels (60 g/cm^2) of hydrostatic pressure (Bourret and Rodan, 1979), a similarity which argues for a similar cellular mechanism of action of mechanical and electrical factors (see discussion in Rodan *et al.*, 1978).

Cyclic AMP, along with Ca^{2+}, has been postulated as a modulator of mitotic activity (McMahon, 1974). Do electrical fields that modulate cyclic AMP levels influence mitotic activity? This question has been investigated by Norton *et al.* (1977), Rodan *et al.* (1978), and Bourret and Rodan (1979) using chondroblasts isolated from the proliferative zones of tibiae from 16-day-old embryos. They find that electrical fields stimulate (*1*) [^{3}H]thymidine incorporation into DNA, (*2*) DNA synthesis, and (*3*) the number of cells moving out of G_1 and G_2 and into the S phase of the cell cycle. Exposure to the electrical field for 15 min increases [^{3}H]thymidine incorporation by 27% over control values. Exposure for 6 hr increases incorporation by 50–60%. Osteoblasts, but neither skin fibroblasts nor lymphocytes, show similar increases, arguing for some localization of this response to skeletal cells (Rodan *et al.*, 1978). Therefore both cyclic AMP levels and rates of mitotic activity are influenced by electrical fields.

Rodan *et al.* (1978) then used the drugs tetracaine, tetrodotoxin and vermapamil to study the possible involvement of Na^+ and Ca^{2+} in the stimulation of mitosis. All three drugs reduced the electrical field-stimulated increase in [^{3}H]thymidine incorporation into chondroblasts *in vitro*. [^{3}H]thymidine incorporation was not altered by changes in medium Ca^{2+} concentration ranging from 10^{-3} to 10^{-8} *M*. These authors interpret their results as follows: (*1*) electrical fields of 1166 V/cm^2 induced membrane depolarization because of an influx of Na^+ into chondroblasts of the proliferative zone, (*2*) intracellular Ca^{2+} levels rise because of release of stored Ca^{2+}, (*3*) adenylate cyclase levels drop in response to the altered intracellular Ca^{2+} concentrations, (*4*) levels of cyclic AMP decline because of the reduction in adenylate cyclase, (*5*) DNA synthesis is initiated, and (*6*) mitosis begins as cells move from G_1 and G_2 into S. Such a mechanism could clearly explain the increased growth of embryonic tibiae cultured in electrical fields and is consistent with the known action of Ca^{2+} on cyclic AMP and of the latter on mitosis.

Elevated intracellular Ca^{2+} concentration inhibits adenylate cyclase in isolated membrane preparations from chondroblasts of the proliferative zone but has no effect on adenylate cyclase from membranes isolated from

hypertrophic chondrocytes (Rodan *et al.*, 1977). This break in the chain may explain why mitosis ceases when chondrocytes undergo hypertrophy.

b. Cultured in Pulsed Electromagnetic Fields. Fitton-Jackson and Bassett (1980), Fitton-Jackson and Farndale (1981), and Fitton-Jackson *et al.* (1981) have examined the action of pulsed electromagnetic fields on tibiae from 13½-to 14-day-old embryonic chicks maintained in organ culture for 8 days. Although still determining optimal field strengths and duration of exposure, some interesting results have already emerged. Initial field strengths varied between 2 and 30 gauss. Continuous exposure to such fields for 7 days severely impaired cellular morphology. Therefore the fields were applied for 3 hr on/9 hr off, 6 hr on/6 hr off, or 9 hr on/3 hr off. Either a single pulse repeating at 72 Hz or a pulse train with pulses of 5 msec duration, at 67-msec intervals, repeating at 15 Hz, was used. These fields were chosen to mimic the wave forms used clinically in treatment of pseudarthroses and nonunions respectively [see Bassett (1978) and Fitton-Jackson and Bassett (1980) for details of the wave forms and Section III,C for the clinical application].

After 8 days culture under these conditions experimental and control tibiae had similar gross morphologies and weights. Uptake of [^{3}H]thymidine was depressed in both bone and cartilage after prolonged (9 hr on) exposure to either single-pulse or pulse-train fields. Shorter exposures did not affect incorporation into cartilage. The depression of [^{3}H]thymidine incorporation is surprising for prolonged exposure to the fields depressed cyclic AMP levels to between 25 and 50% of control values. You will recall, from the studies reported earlier, that electrical fields depress cyclic AMP levels but *enhance* [^{3}H]thymidine incorporation. Those electrical fields were only applied for 15 min. Perhaps very short-term exposure of cartilage to electromagnetic fields would enhance chondroblast proliferation, but at the present time exposure to an electrical field is the preferred means of stimulating cartilage cells to divide.

Hydroxyproline and hexosamine levels, as measures of collagen and glycosaminoglycans respectively, were determined. The data for the cartilaginous portions of the tibiae are presented in Table II. Hydroxyproline content increased after the 9-hr on/3-hr off pulse-train cycle but was either decreased or unchanged under all other conditions. Total hexosamine was consistently and dramatically depressed under all conditions (Table II). Whether this depression indicates a detrimental effect on the cartilage (e.g., rapid degradation of extracellular matrix) or enhanced replacement of cartilage by bone (reflecting, for example, enhanced calcification of cartilage—see later) remains to be worked out. The increased collagen content is consistent with the latter possibility.

^{45}Ca incorporation was very low in the cartilaginous portions of the tibiae and was unaffected by either of the pulsed electromagnetic fields. However,

TABLE II

Hydroxyproline and Hexosamine Contents of Cartilage from Tibiae Cultured in Pulsed Electromagnetic Fields[a,b]

Field type[c]	Hydroxyproline (%)	Hexosamine (%)
Single pulse		
3/9	88	65
6/6	102	63
9/3	87	66
Pulse train		
3/9	95	79
6/6	108	81
9/3	138	72

[a] Based on data in Fitton-Jackson and Bassett (1980).

[b] Tibiae from 13½- to 14-day-old embryonic chicks were cultured for 8 days. Results are presented as percentage of control values.

[c] Duration of repetitive cycles of pulsed electromagnetic fields for the last 7 days of culture are shown as hours pulse on/hours pulse off (12-hour period). For details of wave form, see text.

the Ca^{2+} content of isolated chondroblasts and chondrocytes from tibiae of slightly younger (12-day) embryos can be modified by pulsed electromagnetic fields (Bassett *et al.*, 1979a). The effect is complex, for a pulse train of 5-msec pulses, repeating at 5 Hz *increases* ^{45}Ca incorporation whereas 325-μsec wide single pulses, repeating at 66 or 74 Hz, *depress* ^{45}Ca incorporation (Fig. 1). Whether the difference in behavior between cartilage in organ culture and isolated chondrocytes reflects the damping effect of the extracellular matrix in the organ rudiment or differences in field characteristics has to be determined. Callus cartilage, which shows enhanced calcification when a pulsed electromagnetic field is applied, has little extracellular matrix (see Fig. 6 in Bassett *et al.*, 1982), and so may be behaving more like the isolated chondrocytes *in vitro* than like the tibial rudiments. The clinical data certainly should prompt workers to undertake more *in vitro* studies, especially to examine the response of callus cartilage *in vitro*. Archer and Ratcliffe (1981) have confirmed the findings of Fitton-Jackson and her colleagues, again using cartilage from embryonic chicks.

2. *Neonatal Rat Tibiae*

Although chondrogenesis has not been studied, some data available on osteogenesis establish methods that could be applied to cartilage.

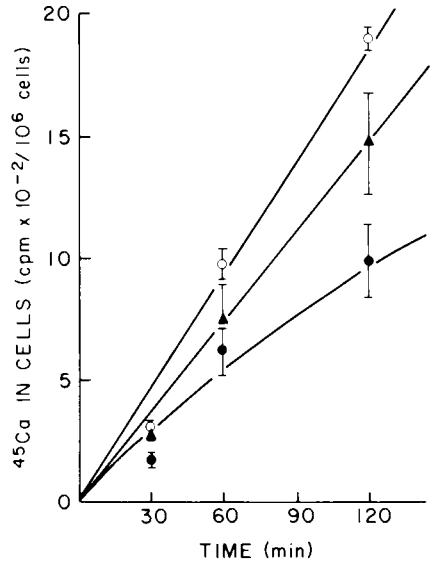

Fig. 1. ^{45}Ca incorporation by chondrocytes from 12-day-old embryonic chicks maintained *in vitro (a)* in the absence of electromagnetic stimulation (control, ▲), *(b)* after exposure to a single-pulse electromagnetic field (325-usec wide, repeating at 66 or 74 Hz), which *depresses* ^{45}Ca incorporation (●), or *(c)*, after exposure to a pulse-burst electromagentic field (5-msec pulses, repeating at 5 Hz) which *increases* ^{45}Ca incorporation (○). Reproduced from Bassett *et al.* 1979a) with the permission of the author and the publisher.

Treharne *et al.* (1980) cultured tibiae from twenty one-day-old sprague dawley rats for 8 days either in direct currents (5, 10, or 20 μA) or in pulsating currents (22 or 44 μA at 1 cycle/sec). The pulsating currents were chosen to deliver the same charge as the 10- and 20-μA direct currents. Even with this similarity, more bone accumulated in tibiae exposed to direct than pulsating currents. The authors interpreted the increased bone mass as evidence of stimulation of osteogenesis. However it may well be that bone resorption was retarded or that the total volume of bone present was, in fact, the same in treated and control tibiae for they appear not to have quantified total bone present but rather only to have measured random histological sections. Any conclusion on stimulation of osteogenesis is premature, although intramembraneous osteogenesis certainly can be stimulated by application of an electrical field *in vitro* (Norton and Moore, 1972). Application of electrical fields to chondrogenesis in mammalian long bones *in vitro* needs to be studied.

3. Neonatal Rat Ribs

Brighton and colleagues (1976) have studied the *in vitro* response of the epiphyseal growth plate of costochondral junctions of fifth to tenth ribs from 21-day-old sprague dawley rats. Exposure for 10 days to electrical fields between 500 and 3000 V/cm showed 1500 V/cm to be optimal for growth, ^{45}Ca, ^{35}S and [^{3}H]thymidine incorporation into the epiphyseal cartilaginous portions of these ribs. These paramaters were increased by 30–40% over control values (Table III). It is of some interest that 1500 V/cm is only slightly higher than that shown by Norton and Watson and their col-

TABLE III

Effects of Electromagnetic Fields on Rat Costochondral Cartilages Maintained *in Vitro*[a,b]

Parameter	Field Strength (V/cm)					
	500	1000	1500	2000	2500	3000
Length	100	124	134	142	80	70
Ca^{45} incorporation	108	95	134	89	106	98
S^{35} incorporation	121	138	128	87	91	118
[^{3}H]thymidine incorporation	100	98	144	102	86	86

[a] Based on data in Brighton *et al.* (1976).

[b] Results are presented as percentage of control values as measured after 10 days continuous *in vitro* exposure to the fields.

leagues as sufficient to stimulate mitosis and growth of embryonic chick tibiae *in vitro* (see Section VI,B,1).

Unlike recent work using electromagnetic fields, optimal strengths for these high voltage electrical fields are now established. Nevertheless, work on field strength continues. Brighton and Wisneski (1981) and Mooar *et al.* (1981) used the rat costochondral junction to show that 24-hour exposure to a low-voltage (10-V) capacitively coupled field significantly stimulated [^{3}H]thymidine incorporation into DNA. Stimulation of DNA synthesis and mitosis is now routinely obtained with electrical fields applied to cartilage(s) *in vitro.*

4. *Fetal Mouse Palatal Cartilage*

Lieb *et al.* (1980) exposed palatal shelves from 14-day-old fetal mice to a pulse of high frequency, electromagnetic radiation before placing the shelves into culture for 24 hours. Two dramatic results ensued. (*1*) The palatal epithelium was lost from the shelves, and (*2*) cartilage formed within the palatal mesenchyme. The appearance of cartilage after just 24 hr suggests that the cells were already primed to chondrify when removed from the fetuses (see Tyler and Koch, 1977). Lieb *et al.* attributed chondrogenesis to influx of Ca^{2+} into the mesenchymal cells but the possibility that the electromagnetic radiation released an epithelially based induction ought to be considered as an alternative (see Hall, 1982, and Volume 2, Chapter 5 for discussions of epithelial induction of cartilage). Although use of electromagnetic *radiation* does not parallel use of electromagnetic *fields,* the former may be a useful was of enhancing chondrogenesis

VII. DEMINERALIZED BONE MATRIX-INDUCED CARTILAGE

It has been very well established by the work of Urist (Chapter 1, Volume 2) and colleagues that demineralized bone matrix implanted *in vivo* induces local cells of the host to accumulate within the matrix, to proliferate and to undergo chondrogenesis. Although such demineralized matrices are usually referred to as inducers of *bone,* it is in fact cartilage that is first induced to form, the bone arising by subsequent replacement of the cartilage. Cartilage is induced as a temporary tissue and bone as a permanent ossicle. Inductive activity appears to reside within a protein extractable from the bone matrix (Urist, 1980). However, several authors have examined the possibility that surface electrical charge, on or within the matrix, might play a role in the inductive process.

Given the known stimulation associated with the negative electrode in electrically induced osteo- and chondrogenesis, de Groot (1973) argued on theoretical grounds that if the bone matrix protein was positively charged, electronegativity (whether applied or naturally occuring as in calcified bone) would concentrate the protein, hence promoting induction. Such an electrochemical interaction could occur in the absence of vital cells, as is the case in demineralized bone matrix.

Marino and Becker (1974) subjected bone matrix to *prolonged* acid treatment, a treatment which abolished its ability to induce bone but had no effect on the piezoelectric constant of the implanted matrix. They concluded that surface piezoelectricity was not the basis for the induction. On the other hand, Reddi and Huggins (1974) obtained data indicating that surface charge on powdered bone matrix was important in mediating its inductive properties. Evans blue, an electronegative dye, suppressed the ability of host fibroblasts to infiltrate matrix, a necessary first step for their accumulation and subsequent chondrogenesis. This inhibition was reversed using the polycationic quaternary ammonium base, hexadimethrine. So although piezoelectric properties are not important, surface charge evidently is.

VIII. DISCUSSION AND SUMMARY

How does one summarize the diversity of data available on bioelectricity and cartilage? What generalizations can be made?

It is clear that naturally occuring electrical potentials can be recorded from cartilages and their constituent cells and that cartilages (e.g., epiphyseal growth plate, articular) exhibit electrical polarization with the actively growing sites being the more electronegative.

Mechanical deformation of cartilage alters its inherent electrical activity so that it becomes more electronegative. One generalization is that *physical forces may be transduced into electrical activity to which cartilage can respond.* This association of electronegativity with growth and deformation also accompanies repair and regeneration of cartilage. A second generalization is that *electronegativity is associated with growth, morphogenesis, reparative, and regenerative processes in cartilage.*

In addition to possessing natural bioelectricity, cartilage can respond to electrical currents. This is true whether the currents are applied *in vivo* by implanting electrodes, or *in vitro* by culturing cartilage in electrical fields. *In vitro,* the response involves proliferation and increases synthesis and deposition of extracellular matrix products. *In vivo,* the response can lead to regeneration of cartilage in amputated appendages or to repair of fractures by cartilage. *Cartilage can respond to the application of electrical currents.* Paradoxically, given the results of *in vitro* studies, little division of chondroblasts is seen during electrically stimulated repair of fractures. A close study of the action of electrical fields on the production of callus cells in all stages of repair needs to be made.

Cartilage can also respond to *electromagnetic fields.* Such fields do not stimulate proliferation (they may even inhibit it) but do stimulate collagen synthesis and have a profound effect on calcification of cartilage. Whether electrical and electromagnetic fields act via common mechanisms has yet to be determined. In sites where strength is required, as in treating nonunions and pseudarthroses, electromagnetic fields have proven very successful. Electrical fields are better suited to situations, (such as regeneration of appendages, or repair of articular cartilage) where proliferation, dedifferentiation, and/or redifferentiation of cells is required.

Electrical fields can significantly alter the synthetic activity of cartilage cells and promote the formation of hyaline cartilage at the expense of fibrocartilage or fibrous tissue. Why then do fresh fractures not respond to electrical stimulation as readily as nonunions do?

Ranid frogs, which do not regenerate amputated appendages, can be induced to undergo partial regeneration when electrodes are implanted into the amputation stump. Whether such electrical stimulation acts by overcoming the normal electropositivity of the frog amputation stump, by preventing dissipation of the skin current into the lymph, or by releasing an epithelial inducer of cartilage, remains to be determined. Postaxial placement of the electrode is critical for regeneration to the digit stage, implying a morphogenetic role for electrical stimulation. It is clear that in salamanders the skin plays a vital role in regeneration by providing an electrical field required for dedifferentiation, blastema formation, and subsequent regeneration of cartilage.

Rats can be induced to partially regenerate their amputated forelimbs. This can be achieved by suturing muscle over the stump, by implanting inactive electrodes or by passing current through implanted electrodes. Such experiments have brought to light a hidden potential for regeneration in the rat but do not clarify the role of electrical stimulation in that regeneration. This is an exciting area for future work on bioelectricity.

Is the action that electrical and electromagnetic fields exert on cartilage direct or indirect? Data from culture of isolated chondrocytes provide strong evidence for *direct* action of electrical fields on chondroblast cell membranes mediated by influx of Na^{2+}. Subsequent elevation of intracellular Ca^{2+} and changes in adenylate cyclase and cyclic AMP levels, stimulate DNA synthesis. Whether extracellular matrix in intact cartilage modifies this response has to be determined. Electromagnetic fields act by stimulating Ca^{2+} incorporation but evidently not by stimulating DNA synthesis. This either represents the early stage of the art in electromagnetic field studies or a fundamental difference in mode of action of electrical and electromagnetic fields.

Electrical fields may also act *indirectly* by modifying local O_2 tension and pH levels which, in turn, would have considerable effect upon a relatively anaerobic tissue with the low oxygen consumption of cartilage. Further analysis of these direct and indirect effects of electricity on cartilage is a major challenge for future workers in this field. The recent formation of the "Bioelectrical Repair and Growth Society," which had its first meeting in November, 1981 and its second in September, 1982, is one sign of the maturing of the study of bioelectricity and cartilage.

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