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Preface

As an addition to the European postgraduate training system for young neurosurgeons we began to publish in 1974 this series devoted to Advances and Technical Standards in Neurosurgery which was later sponsored by the European Association of Neurosurgical Societies.

The fact that the English language is well on the way to becoming the international medium at European scientific conferences is a great asset in terms of mutual understanding. Therefore we have decided to publish all contributions in English, regardless of the native language of the authors.

All contributions are submitted to the entire editorial board before publication of any volume.

Our series is not intended to compete with the publications of original scientific papers in other neurosurgical journals. Our intention is, rather, to present fields of neurosurgery and related areas in which important recent advances have been made. The contributions are written by specialists in the given fields and constitute the first part of each volume.

In the second part of each volume, we publish detailed descriptions of standard operative procedures, furnished by experienced clinicians; in these articles the authors describe the techniques they employ and explain the advantages, difficulties and risks involved in the various procedures. This part is intended primarily to assist young neurosurgeons in their post-graduate training. However, we are convinced that it will also be useful to experienced, fully trained neurosurgeons.

The descriptions of standard operative procedures are a novel feature of our series. We intend that this section should make available the findings of European neurosurgeons, published perhaps in less familiar languages, to neurosurgeons beyond the boundaries of the authors countries and of Europe. We will however from time to time bring to the notice of our European colleagues, operative procedures from colleagues in the United States and Japan, who have developed techniques which may now be regarded as standard. Our aim throughout is to promote contacts among neurosurgeons in Europe and throughout the world neurosurgical community in general.

We hope therefore that surgeons not only in Europe, but throughout the world will profit by this series of Advances and Technical Standards in Neurosurgery.

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A. Advances

Prostaglandins, Thromboxane, Leukotrienes and the Cerebral Circulation in Health and Disease

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Arachidonic acid is ubiquitously distributed throughout the body and its derivatives form an extraordinary and bewildering array of compounds of widely differing properties. It is most unwise to generalize about how a given cell or tissue will utilize each pathway. This system responds to both physiological and pathological stimuli but the respective roles of the various products can be very difficult to unravel as will become all too apparent. The recent British Medical Bulletin (1983) provides an excellent overview of the subject. Von Euler (1936) created the term "prostaglandin" to christen the depressor, smooth muscle-stimulating acidic lipid that he and Goldblatt had demonstrated in human seminal plasma. Bergstrom began to determine the structure of this group of compounds in 1947 with publication in the early 1960s (for review, see Bergstrom *et al.* 1968). Following the isolation and description of the effects of the primary and relatively stable prostaglandins ($PGF_2\alpha$, PGE_1, PGE_2, PGD_2, etc.) came the discovery that prostaglandin synthesis was inhibited by the non-steroidal anti-inflammatory agents such as indomethacin and aspirin (Vane 1971). Certain discrepancies became apparent in the period 1970–1976 between the effects of prostaglandin synthesis inhibition and the effects of the known endogenous prostaglandins. Then, in rapid succession, came the description of the short-lived derivatives of arachidonic acid metabolism, including rabbit aorta-contracting substance (Palmer *et al.* 1973), the cyclic endoperoxides, PGG_2 and PGH_2, and thromboxane A_2 and its inactive metabolite thromboxane B_2 (Samuelsson *et al.* 1978, for review). Many tissues, particularly platelets, have the capacity to generate thromboxane A_2, which is very potent both at inducing platelet aggregation and in contracting smooth muscle. In 1976 came the description of the physiological antago-

nist to thromboxane A_2. Vane's group demonstrated that microsomes from arterial walls enzymatically transform PGG_2 and PGH_2 to an unstable product that relaxes arterial strips and prevents platelet aggregation-prostacyclin or PGI_2 (Moncada and Vane 1978, for review). Finally, the alternative lipoxygenase pathway for arachidonate metabolism has been explored recently and the whole family of so-called leukotrienes and lipoxins identified (Samuelsson and Hammerstrom 1980, Piper 1981, Serhan *et al.* 1984).

What is the current state of knowledge regarding the role of prostaglandins and other derivatives of arachidonic acid (eicosanoids) in the normal control of the cerebral circulation? Do these compounds have major roles in acute cerebrovascular disease—either in a damaging or in a protective capacity? Are the therapeutic regimes designed to alter the prostanoids in the brain based on a firm foundation, or are they meddlesome and likely to put patients at risk? This chapter updates our original reviews (Pickard 1981, Pickard and Walker 1984).

The Biochemistry of Arachidonic Acid Metabolism

Arachidonic acid is a twenty carbon polyunsaturated fatty acid with four double bonds (C20 : 4, eicosatetraenoic acid) found ubiquitously in phospholipids of cell membranes. It is obtained from the diet and also from dietary linoleic acid, an 18-C fatty acid which is converted by mammals to dihomo-γ-linolenic acid and arachidonic acid by chain elongation and desaturation. Arachidonic acid is oxidized enzymically to a large number of products which include the prostaglandins, thromboxanes, prostacyclin, the leukotrienes (some of which were known collectively as "SRSA"—slow reacting substance of anaphylaxis), and a variety of hydroperoxy- and hydroxy-derivatives (see Fig. 1) (Granstrom *et al.* 1982, Nelson *et al.* 1982, Moncada 1983). These are referred to collectively as "eicosanoids". Many of them have been demonstrated to have pharmacological properties in vivo and in vitro—these cover a very wide spectrum of activities. Although a number of other C20 polyunsaturated fatty acids (PUFAs) can be converted to prostaglandins (PGs), for example C20 : 5, C20 : 3, arachidonic acid is the commonest substrate, mainly because of its greater abundance.

The biosynthetic pathway from arachidonic acid to *prostaglandins* proceeds by incorporation of molecular oxygen to form an unstable endoperoxide intermediate PGG_2 (Figs. 1 and 2 a). The hydroperoxy group (OOH) at C15 is then converted rapidly to a hydroxyl group (OH) forming PGH_2, another unstable endoperoxide. A single enzyme, cyclooxygenase, catalyses both these reactions. PGH_2 is susceptible to numerous enzymic

and chemical transformations. Reduction converts it to $PGF_2\alpha$. Two other products, PGE_2 and PGD_2, may be formed non-enzymically under non-reductive conditions, or enzymically. Glutathione is an essential co-factor for the PGE_2 isomerase. PGE_2, $PGF_2\alpha$, and PGD_2, differ structurally only in the substituent groups of their 5-membered ring. They are frequently

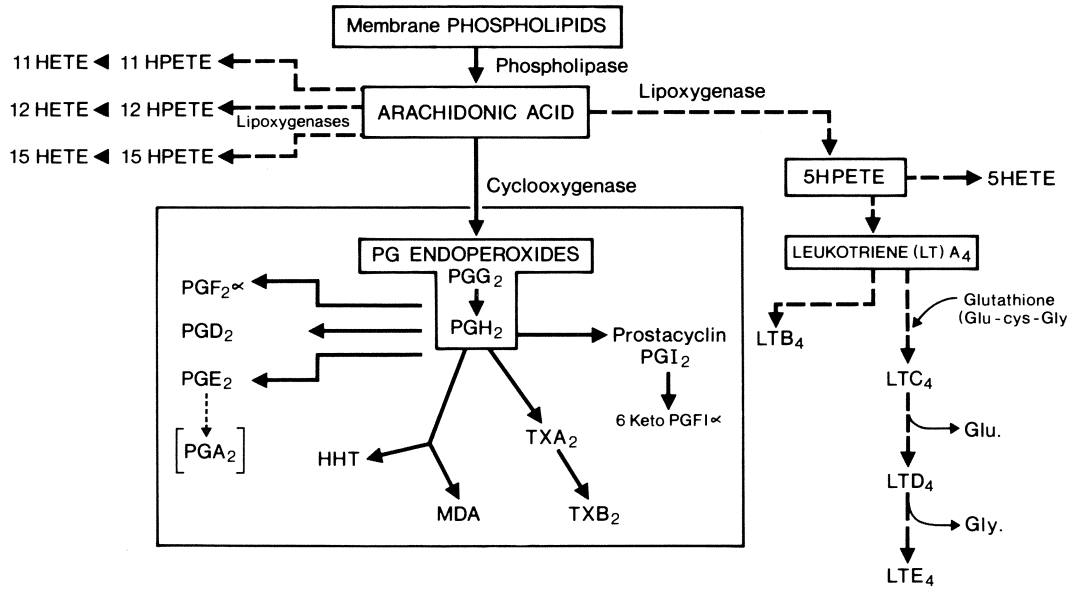

Fig. 1. Metabolism of arachidonic acid. ——→ cyclooxygenase pathway and products. — — — → lipoxygenase pathways and products. *PG* prostaglandin, *LT* leukotriene, *TX* thromboxane, *HPETE* hydroperoxy eicosatetraenoic acid, *HETE* hydroxy eicosatetraenoic acid, *HHT* 12 hydroxyheptadecatrienoic acid (17 carbon), *MDA* malondialdehyde (3 carbon)

referred to as the "Primary Prostaglandins". PGH_2 may be cleaved into a 17C fragment, 12 hydroxyheptadecatrienoic acid (HHT) and a 3C fragment, malondialdehyde (MDA). Alternatively PGH_2 may be converted to Thromboxane A_2 (TXA_2) or the bicyclic derivative prostacyclin (PGI_2; formerly PGX), by the enzymes Thromboxane synthetase and prostacyclin synthetase respectively. These are now known to be extremely important products of the pathway. Both are unstable. TXA_2 has a half life of 30 seconds, undergoing spontaneous conversion to a stable inactive metabolite, TXB_2. Prostacyclin has a half life of around 3 minutes in aqueous solution at PH 7.6, and is converted to a more stable hydrolysis compound 6ketoPGF1α (6oxoPGF1α). TXA_2 is a potent aggregator of platelets and

has a profound contractile effect on a variety of smooth muscles. Prostacyclin is the most potent natural inhibitor of platelet aggregation known, and at higher concentrations inhibits platelet adhesion. It is also a potent vasodilator of all vascular beds studied, including the cerebral

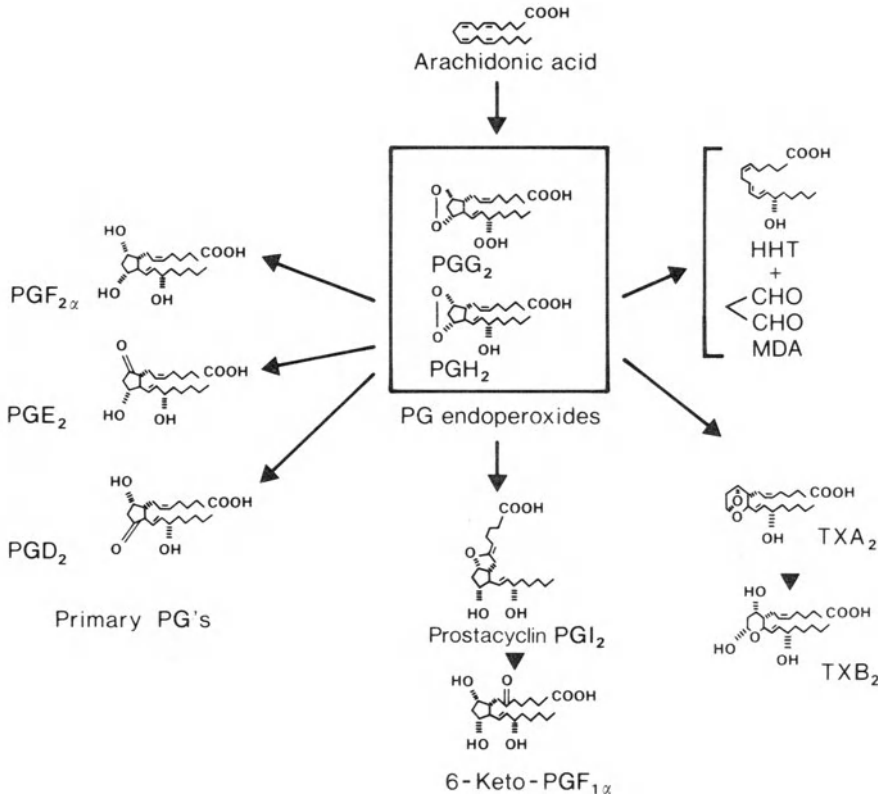

Fig. 2a. Some metabolites of arachidonic acid produced by the cyclooxygenase pathway (see Fig. 1 for key)

circulation (Moncada 1983). In the vascular system prostacyclin is produced mainly in the vessel walls, and TXA_2 in platelets. Although it has been demonstrated that arteries could utilize PG endoperoxides (PGG_2 and PGH_2) formed by platelets for prostacyclin synthesis (Bunting *et al.* 1983) this hypothesis has been challenged. Some believe that platelets do *not* normally provide endoperoxides for vascular prostacyclin production (Needleman *et al.* 1979, Granstrom *et al.* 1982).

If, instead of being acted upon by cyclooxygenase, arachidonic acid is oxidized by a group of enzymes known as *lipoxygenases,* a range of non-

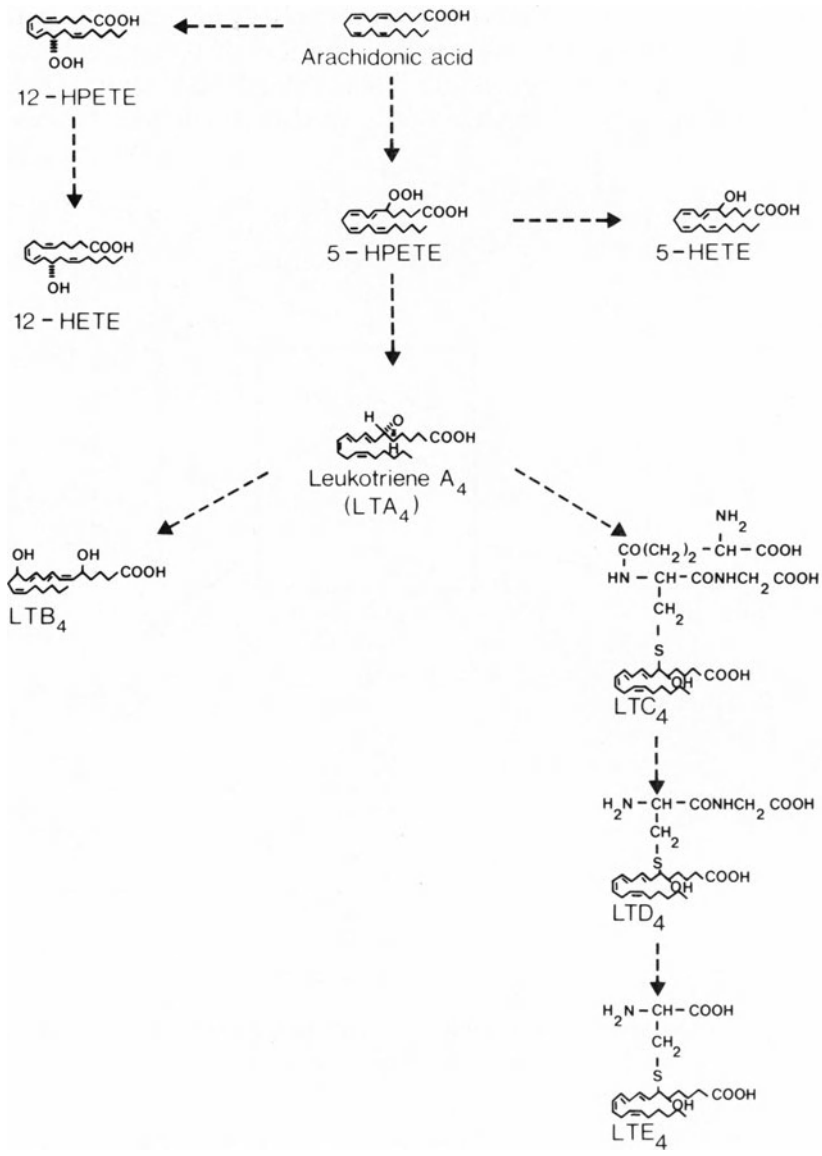

Fig. 2 b. Some metabolites of arachidonic acid produced by lipoxygenase pathways (see Fig. 1 for key)

cyclic hydroperoxy eicosatetraenoic acid derivatives (HPETEs) is formed (Figs. 1 and 2 b). These are named according to the site of the hydroperoxy (OOH) group within the molecule—viz 5-, 11-, 12-, or 15-HPETE. The hydroperoxy groups are readily reduced to hydroxyl (OH) groups, giving the corresponding hydroxy eicosatetraenoic acids (HETEs) viz 5-, 11-, 12-,

or 15-HETE. There is some difference among the tissues as to which HPETE/HETE's are formed. Thus 12 HETE is the major HETE product of platelets, whereas macrophages produce 5- and 15-HETE, and polymorphonuclear leukocytes (PMNs) predominantly 5-HETE. HETEs have potent chemoattractant effects on PMNs. *5-HPETE* is known to be an extremely important eicosanoid: PMNs and basophils were shown to convert this further into a 5–6 epoxide intermediate known as Leukotriene (LT) A4. This is readily metabolized further in two ways: one to give a derivative with two hydroxyl groups (5, 12, dihydroxy eicosatetraenoic acid), LTB4; the other to form a series of 5-hydroxy-6-thioether derivatives by the addition of glutathione (a tripeptide, glutamic acid: cysteine: glycine) through its cysteine residue to C6, producing LTC4. Sequential removal first of the glutamic acid residue from the side chain, and then of the glycine leaves LTD4, and LTE4 respectively (Figs. 1 and 2 b). LTB4 is an extremely potent chemotactic factor and seems to function as an extracellular mediator which attracts leukocytes to areas of inflammation. Recent work indicates that *SRSA* (*slow reacting substance of anaphylaxis*), a reactive substance with potent action on smooth muscle of the bronchial tree, is a mixture generally of LTC4, LTD4, and in rats small amounts of LTE4. SRSA has been implicated in asthma, and the roles of the leukotrienes in this condition are under intensive investigation (Piper 1983). It has been shown that macrophages also synthesize leukotrienes (Bonney and Humes 1984), and release of LTs from some other tissues has been demonstrated, although generally in small amount. A related series of leukotrienes has also been described in which 15-HPETE is used as precursor instead of 5-HPETE (Radmark *et al.* 1982).

Recently Serhan *et al.* (1984) have isolated a further series of oxygenated derivatives formed from arachidonic acid in human leukocytes, formed apparently through the interaction of multiple, distinct, lipoxygenase pathways. These compounds ("trihydroxytetraenes") have four conjugated double bonds and three hydroxyl groups. This group has proposed the trivial names *lipoxin A and lipoxin B* for the first two compounds of the series to be characterized. When added to human neutrophils, lipoxin A stimulated superoxide anion generation and degranulation without provoking substantial aggregation.

Metabolism of Eicosanoids

Preformed eicosanoids are not stored within cells for secretion. Eicosanoids are synthesized and released extremely rapidly in response to a stimulus to the cell. They have very short half lives in the body, and it is now widely believed that these are locally acting agents with a very restricted

territory of activity. Claims that prostacyclin is a circulating hormone have been repudiated (Blair *et al.* 1982). Outside the Central Nervous System, primary PGs of the E and F series are rapidly dehydrogenated and reduced to give the corresponding 13,14-dihydro-15-keto products. A single pass through the lungs converts more than 90% of Primary PGs to the inactive metabolites. Further metabolism yields tetranor carboxylic acid derivatives which are excreted in urine. The major urinary metabolite of TXB_2 in man is 2, 3, dinor TXB_2 (Granstrom *et al.* 1982). In addition to spontaneous conversion to 6ketoPGF$_1\alpha$, prostacyclin is metabolized enzymically. Two circulating metabolites have been identified as 2,3-dinor-6, 15-diketo-13, 14-dihydro-20 carboxyl PGF1α and 2,3-dinor-13, 14-dihydro-6, 15-diketoPGF1α (Rosenkranz *et al.* 1981). The importance of enzymic inactivation in man is under investigation. The metabolism of leukotrienes has not been elucidated fully yet.

Brain has been found to have little or no capacity to metabolize the primary PGs (Nakano *et al.* 1972), and the importance of *enzymic* inactivation of prostacyclin in brain is unknown. TXA_2 is inactivated *non*-enzymically to TXB_2, and prostacyclin to 6ketoPGF1α as elsewhere. It is believed that PGs are normally removed from the CNS by facilitated carrier—mediated PG transport across the choroidal and extrachoroidal regions of the blood brain barrier (Bito *et al.* 1976a). This has been demonstrated for PGE2 and PGF2α (Bito *et al.* 1976b), and (preliminary report only) for TXB_2, prostacyclin, and 6ketoPGF1α (di Benedetto and Bito 1980). However there is some evidence that the role of transport processes for TXB_2 may differ from that for $PGF_2\alpha$ (Galli *et al.* 1980). Some PG's may be removed additionally during "bulk flow" of CSF across the arachnoid villi.

Basic Physiology of Eicosanoid Synthesis and Secretion

Many aspects are still poorly understood. How is synthesis initiated? Precursor arachidonic acid does not exist free within cells but it is esterified with membrane phospholipids from which it has to be released. This is considered to be the rate-limiting step for eicosanoid synthesis, and it is achieved enzymically by phospholipases. Phospholipase A_2 is distributed universally and it removes arachidonic acid directly from phospholipids. Phospholipase C is of greater importance in platelets (Bonney and Humes 1984). It removes inositol phosphate from phosphatidyl inositol, leaving diacylglycerol. A second enzyme, diacyl glycerol lipase, then releases arachidonic acid from this (Fig. 3). The importance of phospholipase C in brain is unknown. Events at the surface cell membranes seem to trigger arachidonic acid release, whether this is trauma, chemical damage, "membrane perturbation" during phagocytosis, a hormonal or immunological stimulus, or in the brain, ischaemia. In platelets the arachidonic acid

liberating enzymes phospholipase A_2 and diglyceride lipase were associated with intracellular rather than surface membranes (Carey *et al.* 1984 b). How are the phospholipases activated? In porcine endothelial cells arachidonate release was dependent on influx of extracellular calcium and subsequent

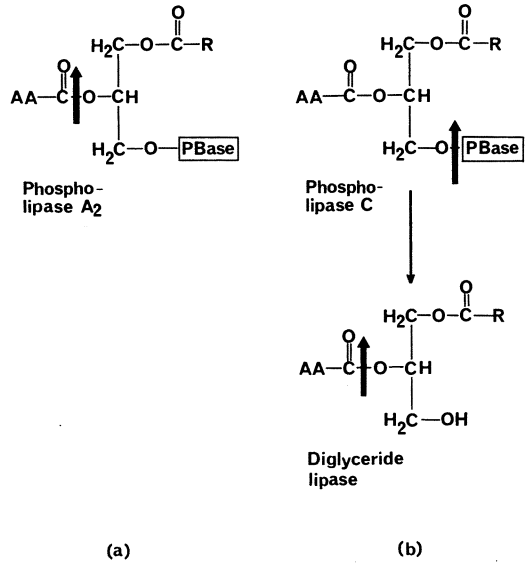

Fig. 3. Release of arachidonic acid from phospholipids a) by phospholipase A_2, b) by combined actions of phospholipase c and diglyceride lipase.

AA—c— = bound arachidonic acid

R—c— = bound long chain fatty acid

P Base = a phosphorylated base *i.e.* phosphatidyl-inositol, -choline, -ethanolamine, or -serine

calcium-calmodulin activation of phospholipase. The calmodulin binder trifluoperazine inhibited activation. The response to hormone stimulation (bradykinin) was transient, probably reflecting a subsequent fall in intracellular calcium to basal levels by calcium—calmodulin activation of calcium—ATPase ("calcium pump") (Whorton *et al.* 1984). In platelets too, phospholipase A_2 was calcium and calmodulin dependent, and intracellular membrane vesicles showed an ATP-dependent sequestration of calcium which was inhibited by calmodulin antagonists (Carey *et al.* 1984 b).

Moskowitz *et al.* (1983) demonstrated that phospholipase A_2 in the presence of Ca^{++} was stimulated by calmodulin and $PGF_2\alpha$, and inhibited by PGE_2, cAMP and cGMP in the presence and absence of calmodulin. They concluded that Phospholipase A2 is a finely regulated enzyme controlled directly by a host of key intracellular regulators.

Involvement of *inositide* metabolism in the transduction mechanism from receptor activation to control of cellular processes such as contraction

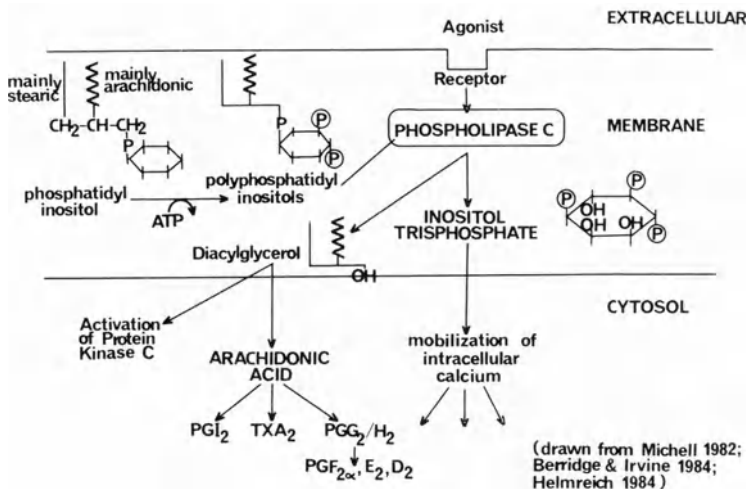

Fig. 4. Inositide metabolism initiated by binding of an agonist to a cell membrane receptor

and secretion, was discovered by the Hokins (1953), who showed that the incorporation of ^{32}P into phospholipids in pancreas was stimulated by acetylcholine. Michell (1975) found that increased phosphatidylinositol turnover is usually correlated with activation of those receptors that are linked to Ca^{++} mobilization, and that increased phospholipid turnover might be the cause and not the effect of increased cellular calcium. The earliest detectable event which is calcium-independent, is an increase in the concentration of inositol trisphosphate. The breakdown of polyphosphatidylinositols precedes that of phosphatidylinositol itself. Inositol trisphosphate then mobilizes intracellular calcium from the endoplasmic reticulum (Fig. 4) (Michell 1982, Berridge and Irvine 1984, Helmreich 1984).

What Determines the Nature of the Eicosanoids Produced by Individual Cells?

Once released, it is believed that the unesterified arachidonic acid could serve as substrate for oxygenation by both cyclooxygenase and lipoxygenase pathways. However the cellular location of the enzymes is different.

Whereas 5-lipoxygenase is a soluble enzyme, cyclooxygenase has been reported to localize in the endoplasmic reticulum (Bonney and Humes 1984), and in platelets it is in membranes of vesicles associated with the dense tubular system (Carey *et al.* 1984 b). With the single exception of the soluble enzyme PGD isomerase, enzymes that further metabolize cyclooxygenase derivatives are also associated with intracellular membranes of the endoplasmic reticulum (Carey *et al.* 1984 a and b, Darte and Beaufay 1984, Jonas *et al.* 1984). PGE_2 isomerase is not associated with surface membranes (Darte and Beaufay 1984). There is evidence that microtubules in some cells play a crucial role in PG synthesis (Jonas *et al.* 1984). Thus it seems as if several distinct cellular organelles have an integrated action in eicosanoid synthesis, but it is not known how this is coordinated.

What is known about the factors which determine which eicosanoids are synthesized by cells of different tissues? Can this pattern be modified under different conditions? Clearly the enzyme complement of cells is important. Arachidonic acid and phospholipase A_2 are present ubiquitously in the body. With the exception of red blood cells (which lack cyclooxygenase activity, Harris *et al.* 1979), cyclooxygenase and the lipoxygenases are also widely distributed. However enzymes which process their products further may show differential distribution. For example platelets are very rich in TX synthetase (Granstrom *et al.* 1982) and produce only negligible amounts of prostacyclin. The vascular endothelium however is rich in prostacyclin synthetase, and prostacyclin is the main product of arachidonic acid in all vascular tissues tested so far (Moncada 1983). In polymorphonuclear leucocytes arachidonic acid is metabolized predominantly by lipoxygenase pathways and leukotriene end-products are released (Vanderhoek and Bailey 1984). However many cells can produce a spectrum of eicosanoids. Availability of enzyme co-factors may be one determinant. Reduced glutathione is a co-factor of PGE isomerase. It was shown recently that macrophages depleted of glutathione have a decreased capacity to synthesize PGE_2 (Darte and Beaufay 1984). Enzymes may show different sensitivities to substrate or product inhibition in vivo: PGE_2 isomerase seems to be relatively sensitive to inhibition by high substrate concentrations of arachidonic acid, so that PGE_2 production might decrease at the expense of other PGs (Flower *et al.* 1973). Oxygen tension might be important, although the evidence for this is slender so far. Rodrigues and Gerritsen (1984) observed that prostacyclin synthesis was decreased (reversibly) during incubation of microvessels from rabbit cerebral cortex in an oxygen-free atmosphere so that the ratio of prostacyclin/PGE_2 was lower than in the presence of oxygen. Recent work has highlighted the importance of interaction between cells in modulating PG production. There have been several reports that conditioned media obtained from adherent monocytes stimulate production of PGE_2 many fold by connective tissue cells

(fibroblasts, synovial cells, chondrocytes) (reviewed Pickard *et al.* 1984, Jonas *et al.* 1984). Similarly culture supernatants of Conconavalin A stimulated lymphocytes led to a long-lasting and continuous release of PGE_2 by macrophages for more than forty-eight hours, presumably in response to a lymphokine (Jonas *et al.* 1984).

The explanations for all these different responses are unknown, but clearly a better understanding of factors modulating eicosanoid production is of fundamental importance.

Pharmacological Inhibitors of Arachidonic Acid Release and Metabolism

It is now possible to inhibit the metabolism of arachidonic acid at many sites in the biochemical pathways. Some of the agents are too toxic for use in vivo.

Inhibition of Phospholipase Activity

Anti-inflammatory corticosteroids reduce PG production in vivo but not in vitro. They induce the synthesis and/or release of two proteins: "macrocortin", MW 15,000, first isolated from perfused guinea pig lungs, and "lipomodulin", MW 40,000, isolated from rabbit neutrophils. These have been reported to inhibit phospholipase A_2 (Blackwell and Flower 1983), but definite proof is still lacking (Bonney and Humes 1984). It is not known whether similar proteins are induced within the CNS. Mepacrine, chlorpromazine, and halothane are all reported to inhibit the enzyme (Blackwell and Flower 1983).

Inhibition of Cyclooxygenase

In 1971 Vane and others reported that non-steroidal anti-inflammatory drugs (NSAIDs), notably aspirin and indomethacin, inhibited PG synthesis (Moncada and Vane 1979). It has been shown that these and most other NSAIDs inhibit cyclooxygenase activity. Aspirin acetylates a lysine residue in the active site of the enzyme. Inhibition by indomethacin is by non-covalent interaction with the enzyme, probably not at the active site. Ibuprofen competes reversibly with substrate arachidonic acid for binding with enzyme (Flower 1974, Higgs and Vane 1983—Reviews). NSAIDs also inhibit PG metabolizing enzymes (Samuelsson *et al.* 1978). Many NSAIDs have actions in addition to those on the arachidonic acid cascade, and not all their effects in vivo should be attributed uncritically to their actions on eicosanoid production. Indomethacin has been shown to have actions on a number of enzyme systems, although generally at higher concentrations than those which inhibit cyclooxygenase (Flower 1974). It inhibits phosphodiesterase in some tissues, and has been reported to inhibit cyclic AMP-

dependent protein kinase. At high concentrations (0.5–1 mM) it has effects on ion fluxes in various human and guinea pig tissues. Low doses of indomethacin stimulate, and high doses inhibit, PG transport across the choroid plexus (Pickard 1981, Review). Inhibition of arachidonic acid metabolism by cyclooxygenase may be associated with an increase in products from lipoxygenase activity: concentrations of indomethacin which completely inhibited cyclooxygenase, increased production of 12HPETE or 5HPETE in platelets or leukocytes respectively (Higgs and Vane 1983). Sometimes the inhibitors have unpredictable actions: ibuprofen, a cyclooxygenase inhibitor, also inhibited 5-lipoxygenase in human PMN leukocytes, but selectively activated 15-lipoxygenase (Vanderhoek and Bailey 1984).

Inhibition of Lipoxygenase

Acetylenic analogues of arachidonic acid inhibit lipoxygenase activity by competing with the natural substrate. One is ETYA (5-, 8-, 11-, 14-eicosatetraynoic acid). NDGA (nordihydroguaiaretic acid) is an unrelated compound which inhibits lipoxygenase by an unknown mechanism. Although ETYA has been reported to be a specific inhibitor of 5-lipoxygenase, neither this nor NDGA inhibits lipoxygenases selectively—both are also weak inhibitors of cyclooxygenase (Higgs and Vane 1983). BW 755C is a pyrazole derivative which inhibits both lipoxygenase and cyclooxygenase preventing synthesis of PGs, hydroxy acids, and leukotrienes. FPL55712 is an antagonist of Leukotriene activity.

Inhibition of Thromboxane A_2 and Prostacyclin Synthesis

Imidazole and 1-substituted imidazole derivatives have been reported to be selective inhibitors of *TX synthesis* in human platelets, although some with longer alkyl substituent groups may also inhibit cyclooxygenase. "OKY1555" is a pyridine derivative which was shown to be a very potent and selective inhibitor of TX synthetase. "OKY1581", a related compound, has been used extensively in experimental studies. A number of synthetic PGH_2 analogues are also potent inhibitors. Dipyridamole, used as an antiaggregant because it inhibits platelet 3′5′cAMP phosphodiesterase, has been shown to inhibit TX production, but at high concentrations (Granstrom *et al.* 1982, Review).

Tranylcypromine has been used experimentally as a weak inhibitor of *prostacyclin synthetase,* although it also inhibits other enzyme systems (Moncada and Vane 1979).

Use of Aspirin to Prevent Thrombosis

Because aspirin binds irreversibly with cyclooxygenase it inhibits the enzyme in platelets for their entire lifespan, since platelets are unable to

synthesize new protein. Vascular endothelial cells overcome the effects of aspirin inhibition more quickly because they can synthesize proteins and generate new enzyme. Because platelet cyclooxygenase is more sensitive to inhibition than that in the vascular lining, treatment with very low doses of aspirin should prevent thrombosis (Moncada 1983). There have been many clinical trials of the use of aspirin for this purpose, using varied dosage regimens. The results have been generally disappointing and the problems of finding an optimal dose for all patients seem insurmountable.

Reactive Oxygen Species, Oxygen-Free Radicals, Lipid Peroxides, and Their Interaction with Arachidonic Acid Metabolism

In many of the pathological conditions of the brain in which eicosanoid production is increased, it has been found or speculated that there is production of oxygen-free radicals, and that lipid peroxidation occurs. The chemistry of these species is considered briefly, since their effects on brain structure and function may be additive to those of eicosanoids. Moreover both interact with the arachidonic acid cascade and can modify eicosanoid production.

Reactive Oxygen Intermediates and Oxygen-Free Radicals

Ground state molecular oxygen ($^{\bullet}O—O^{\bullet}$) has two unpaired electrons each located in a different π orbital, and each having the same spin quantum number. In order to oxidize another atom by accepting a pair of electrons from it, both new electrons must be of parallel spin so as to fit into the vacant spaces of the π orbitals. Because of this spin restriction, oxygen is unreactive towards organic molecules. The reactivity of oxygen can be increased in a number of ways (Halliwell and Gutteridge 1984 a). One is by interaction with enzymes (oxidases) which very often contain transitional metals capable of donating and accepting single electrons. Another is by excitation in the presence of certain biological pigments—(retinal is one)—so that one of the unpaired electrons of the oxygen is moved within the molecule. The product, singlet oxygen, $O_2{}^1Ag$, is an exceptionally reactive and oxidizing form of oxygen. In mammals it is formed in the lens and retina of the eye.

It is the third mechanism which may be relevant in cerebral ischaemia. In some circumstances the oxygen molecule can undergo reduction by single electron steps, generating a series of reactive intermediates which include superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical $OH^{\bullet}$. O_2^- and $OH^{\bullet}$ have an unpaired electron and are therefore called "oxygen-free radicals" (Dormandy 1983). $OH^{\bullet}$ is formed from H_2O_2 in the presence of ferrous iron (Fe^{++}) or copper (Cu^+).

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^{\bullet} + OH^-.$$

$OH^{\bullet}$ is also generated when superoxide radical and hydrogen peroxide or other peroxide interact (Haber-Weiss reaction):

$$O_2^- + H_2O_2 \rightarrow OH^{\bullet} + OH^- + O_2.$$

Again this is catalysed by Fe^{++}.

These reactive oxygen intermediates are unstable and highly reactive chemical species, and have a half life of microseconds. $OH^{\bullet}$ radicals in particular are extremely reactive and will "attack" and damage many cellular constituents including sugars, amino acids, nucleic acids and phospholipids (Halliwell and Gutteridge 1984 a). Reactive oxygen intermediates are produced by stimulated macrophages and polymorphonuclear leukocytes (Segal *et al.* 1983), as a result of oxidative detoxification processes using the microsomal P450 system (Dormandy 1983), and during the conversion of prostaglandin G_2 to PGH_2 by the peroxidase action of cyclooxygenase (Deby *et al.* 1984). It has been proposed that reactive oxygen intermediates are generated also as a result of severe incomplete cerebral ischaemia. This may occur *during* ischaemia as a result of disturbances in the electron transport chain, or in response to a sudden increase in tissue oxygen during the *reperfusion* period (Rehncrona 1984, Review). Normally the cell is well protected against these damaging intermediates by a number of endogenous antioxidants which include α tocopherol (vitamin E), and the enzymes catalase, glutathione peroxidase, and superoxide dismutase. However if excessive amounts are produced, as for example in ischaemic or inflamed tissues, this protection is overwhelmed. The radicals then cause damage to cell proteins, and to cell membranes by "attacking" the polyunsaturated fatty acids (PUFAs) of the phospholipids.

Lipid Peroxidation

Exposure of cell membranes to oxygen radicals stimulates the process of lipid peroxidation (Halliwell and Gutteridge 1984 b), which proceeds through a free radical mediated chain reaction as follows (Slater 1984): The oxygen radical abstracts a hydrogen atom from membrane bound PUFA:

$$PUFA(H) + R^{\bullet} \xrightarrow{\text{oxygen-free radical}} PUFA^{\bullet} + RH$$

$PUFA^{\bullet}$ takes up oxygen, to become a lipid peroxy-free radical, in which double-bond re-arrangements have resulted in production of a conjugated diene ($R-CH=CH-CH=CH-R_1$) with characteristic UV absorption around 233 nm

$$PUFA^{\bullet} + O_2 \rightarrow \underset{\text{lipid peroxy-free radical}}{PUFAO_2^{\bullet}}$$

The lipid peroxy-free radical can then abstract a hydrogen atom from a neighbouring PUFA substrate molecule to become a lipid hydroperoxide, and propagate the chain reaction:

$$\text{PUFAO}_2^{\bullet} + \text{PUFA(H)} \rightarrow \underset{\text{lipid hydroperoxide}}{\text{PUFAO}_2\text{H}} + \text{PUFA}^{\bullet}$$

Lipid hydroperoxides break down, especially in the presence of transitional metals, to produce a variety of products including malondialdehyde (MDA). Lipid peroxidation is often monitored by measuring MDA or conjugated dienes. It should be remembered however that another source of MDA is the reaction (probably a bimolecular reaction) (Granstrom *et al.* 1982) in which PGH_2 is converted by TX synthetase to HHT + TXA_2 + MDA (Figs. 1 and 2 a). Lipid peroxidation disturbs membrane functions, and the hydrocarbons and aldehydes produced from their further degradation may cause more cell damage (Halliwell and Gutteridge 1984 b). The brain contains a high concentration of PUFAs, and an increased formation of lipid hydroperoxides has been implicated in the development of senescence and cellular damage (Kaplan and Ansari 1984), as well as in ischaemic and traumatic brain damage (Demopoulos *et al.* 1984). However the evidence that free radical mechanisms have a primary importance for the development of irreversible brain damage in vivo is insubstantial (Rehncrona 1984) (see below). It has been suggested that radical-induced peroxidation is more often the result than the cause of cell damage (Halliwell and Gutteridge 1984 b).

Effects of Oxygen-Free Radicals and Lipid Hydroperoxides on Arachidonic Acid Metabolism

In some experimental models, generation of oxygen-free radicals stimulates PG production. For example xanthine oxidase-derived oxygen radicals caused a 30-fold stimulation of TXB_2 generation in isolated perfused rabbit lungs, and increased the mean arterial perfusion pressure. Both effects were prevented by catalase. "Membrane perturbation" as a result of free radical attack on membrane lipids may have been responsible (Tate *et al.* 1984). Activated neutrophils induced a time- and dose-dependent release of PG6ketoF1α from human and bovine endothelial cell monolayers which was reduced significantly by catalase but not superoxide dismutase. Catalase similarly inhibited 6ketoPGF1α release from endothelium by a hydroperoxide-generating system. The phospholipase A_2 inhibitor mepacrine also attenuated the response, suggesting that hydrogen peroxide might act by triggering endothelial phospholipase activation (Harlan and Callahan 1984).

There is evidence that the prevailing concentration of lipid hydroperoxides is of critical importance in prostaglandin biosynthesis (Warso and

Lands 1983). Lipid peroxides in low concentration (10–100 nmol/l) *activate* cyclooxygenase, thereby promoting PG synthesis. Hemler *et al.* (1979) found that straight chain hydroperoxides were most effective, and that the efficiency of activation was directly proportional to chain length. PGG2 the hydroperoxy product of cyclooxygenase is also an activator, explaining the autoacceleration described for in vitro incubations. The continued presence of hydroperoxides is required for the enzyme activation (Warso and Lands 1983).

On the other hand, at higher concentrations, lipid hydroperoxides can *inhibit* some of the enzymes of PG synthesis. Cyclooxygenase is inhibited at concentrations of the order of 15 μmol/l. 15-hydroperoxy arachidonic acid (15HPAA) at concentrations exceeding around 1.5 μmol/l is a selective and potent inhibitor of prostacyclin production by vessel wall microsomes and fresh vascular tissue, and other fatty acid peroxides behave similarly (Moncada 1983). High concentrations of lipid peroxides are present in atherosclerotic plaques. Prostacyclin production by atherosclerotic arterial tissue has been shown to be significantly lower than by normal tissue, although there was no difference between arteries with early and advanced atherosclerosis. Low density lipoproteins also inhibit prostacyclin synthesis, and these too contain a high concentration of lipid peroxides. Animals fed on vitamin E deficient diets had increased aortic lipid peroxide levels associated with decreased prostacyclin in vitro. Since prostacyclin is the most potent endogenous inhibitor of platelet aggregation known, a decreased vascular production may play a role in the thrombotic events of atherosclerosis (Moncada 1983). PGE_2 isomerase seems to be susceptible too to peroxide inhibition (Darte and Beaufay 1984).

Methodological Problems in the Analysis of Eicosanoid Production in Brain

In measuring eicosanoids in brain tissues the objectives are to find out as accurately as possible which of these compounds are produced in vivo within individual brain compartments, and how much of each is produced during defined physiological and pathological conditions. These appear straight forward, but there are numerous methodological problems. Poor methodology undoubtedly accounts for much of the present confusion of ideas concerning the roles of eicosanoids in brain.

Analytical Problems

The six most commonly used analytical techniques are biological assays, radioimmunoassays, radiochromatography after incubation of tissues in vitro with ^{14}C arachidonic acid, gas chromatography/mass spectrometry (GC-MS), gas chromatography with electron capture detection and recently, high performance liquid chromatography (HPLC). The last is used

particularly for analysis of the leukotrienes, and lipoxygenase products. Unless it is used with radioactive detection, or coupled with radioimmunoassays, this method is relatively insensitive for prostanoids at present. Radioimmunoassays (RIAs) are probably used most often. These are sensitive, but are only possible if a stable standard is available in pure form, and an antibody that is selective for the compound being measured. Considering the large number of eicosanoids known now, there are good assays available for very few of them and this lack of assays is a major problem impeding current research (Report of Group for standardization of methods in Icosanoid Research 1984). Non-specificity of RIAs is a problem, and has led to gross overestimation of the amounts of certain prostanoids in biological fluids. This applies particularly to assays for 6ketoPGFIα. In reported studies using RIA as well as GC-MS, mean plasma concentrations have ranged from 115 to 400 pg/ml, yet Rosenkranz and Frölich (1984) have calculated that the true normal value must be less than 22 pg/ml, and using very sensitive techniques, two groups have reported plasma levels below 10 pg/ml (Siess and Dray 1981, Blair *et al.* 1982). Reported plasma concentrations much greater than this are likely to be erroneous. Methods using radiochromatography after pre-incubation of tissues with 14C arachidonic acid in vitro are insensitive, and perhaps more worrying, make the assumption that the exogenous substrate is incorporated into the same phospholipid pool as that from which endogenous arachidonic acid is released for eicosanoid synthesis. This may not be so. If the pools are different, the prostanoid products may also differ, and the findings will be misleading. Some have used radiolabelled PGH_2 as a substrate, but this may be converted non-enzymically to PGE2 (Rodrigues and Gerritsen 1984).

Each RIA will detect only one eicosanoid, and chromatographic techniques detect only a handful of products, yet the number of eicosanoids known is very large. Moreover lipoxygenase enzymes will act on endogenous PUFAs other than arachidonic acid to produce a host of products (Rigaud 1984). A very real worry is that the compounds that we can measure well, may be of much lesser importance pathophysiologically than compounds which current technology "ignores"—our hypotheses may be based on hopelessly inadequate information.

Eicosanoids Present in vivo

The determination of the amounts of arachidonate derivatives present in different compartments of the brain in vivo is extraordinarily difficult (Pickard and Walker 1984a). Ischaemia leads to a rapid accumulation of free fatty acids, including arachidonic acid, in neural tissue (Bazan 1971). Bosisio *et al.* (1976) showed that with cerebral ischaemia, there is a release of free arachidonic acid which is rapidly converted to prostaglandins,

including $PGF_2\alpha$. Even mild tissue damage precipitates eicosanoid synthesis. Post-decapitation ischaemia (Galli *et al.* 1980) and tissue trauma during sample preparation, probably account for the very high levels of prostaglandins which have been reported in animal tissues post-mortem, and trauma for those in human surgical specimens (Pickard and Walker 1984 b). These values bear no relationship to production in vivo. Much of the PGD_2 in rat brain homogenates probably results from in vitro manipulation (Gerozissis *et al.* 1983). PGA_2 and PGB_2, are dehydration products of PGE_2 formed easily by treatment of PGE_2 with acid or base respectively. It seems likely that much of the PGA_2 detected in tissues and enzyme incubations is formed non-enzymically during extraction and isolation procedures. Since there is no unequivocal evidence that PGA_2 is formed enzymically in vivo, it is difficult to assign a definite role to A (or B) type PGs at present (Flower 1974). Several ways have been tried to avoid these problems of tissue preparation. For small laboratory animals such as the mouse or rat, the use of focused microwave irradiation to sacrifice the animal probably avoids most of the artefact (Anton *et al.* 1983). The brain is "fixed" within a fraction of a second with heat inactivation and the enzymes are denatured (Schneider *et al.* 1982). Using this method Bosisio *et al.* (1976) found levels of prostaglandins of around 50 pg/gram of fresh tissue. Anton *et al.* (1983) found that basal levels of PGE and TXB_2 were fivefold less in the brains of mice killed by microwave irradiation than in those killed by decapitation, and were similar to those of animals given indomethacin thirty minutes before sacrifice. From their data, post-mortem anoxia causes a greater synthesis of TXB_2 than PGE_2. However despite its apparent usefulness, the technique has not been fully validated yet.

An alternative method has been the "kill perfusion" technique which aims to "freeze" PG synthesis biochemically by two minutes of perfusion of the upper half of the body (of gerbils) with indomethacin/saline (10 μg/ml) before removing the brain and dissecting it in the same solution (Bhakoo *et al.* 1984). Rapid freezing of brain or brain tissue is a further method, but there is a delay before tissue in the centre of the sample freezes, and it is not possible to measure eicosanoids in individual compartments. The majority of investigators have not tried to assess levels present in vivo, but have looked at the "capacity" of brain tissue slices to produce prostanoids during standardized conditions of incubation in vitro. Again this is problematic. The conditions of incubation have seldom been optimized in the manner usual for enzyme analyses. Different sensitivities of enzymes of the arachidonic acid cascade to substrate and product concentrations, deficiency of essential co-factors (particularly glutathione for PGE isomerase), non-enzymic conversion of PGH_2 to PGE_2 or PGD_2 (Rodrigues and Gerritsen 1984), the presence of lipid hydroperoxides from traumatized brain, the presence of metal contaminants in buffers, and many other

factors, will influence prostanoid biosynthesis. It seems unwise to place much emphasis on the ratios of individual prostanoids produced during in vitro incubations. An alternative approach is to measure PGs in CSF, since this bathes the brain surface, can be removed atraumatically without disturbing brain function, and is a technique applicable to man. It is uncertain which brain compartment (parenchyma or vasculature) makes the greatest contribution to CSF PG levels. Much of the 6ketoPGF1α probably derives from the choroid plexus. CSF PG concentrations also are the net result of PG entry into and removal from the subarachnoid space. Studies in which prostanoids were measured in brain superfusates in experimental animals have given unrealistically high concentrations (in nanogram amounts), and in a recent study in sheep in our own laboratory (unpublished), values during basal conditions were clearly too high, and probably resulted from trauma during perfusion.

Cerebrovascular Synthesis of Eicosanoids

Cerebral tissues of several species have been shown to synthesize eicosanoids in vitro. Among the products identified have been $PGF_2\alpha$, PGE_2, PGD_2, TXB_2 and 6ketoPGF$_1\alpha$ (reviewed Pickard 1981, Wolfe 1982, Walker *et al.* 1983). However in view of the methodological problems of analysing arachidonic acid metabolites in brain, the relevance of these observations to the in vivo situation is uncertain. Using focused microwave irradiation to sacrifice the animals to minimize post-mortem synthesis, PGE_2 and $PGF_2\alpha$ have been demonstrated in rat brain (Bosisio *et al.* 1976), and PGE_2 and TXB_2 in mouse brain (Anton *et al.* 1983).

The demonstration by Pickard *et al.* in 1975 of the production of a prostaglandin-like substance by the bovine middle cerebral artery using bioassay, was the first to show that cerebral prostaglandin synthesis cannot be considered as one homogeneous system—cerebral arteries alone could synthesize large quantities of prostaglandins. Prostacyclin is formed by vascular tissues from all tissues tested so far, and is the main metabolic product of arachidonic acid in isolated vascular tissue (Moncada and Vane 1979). Boullin *et al.* (1979) demonstrated generation of prostacyclin by human cerebral arteries from exogenous precursor. Abdel Halim *et al.* (1980) found that 6ketoPGF$_1\alpha$ was the dominant prostanoid produced by fresh human cerebral blood vessels in vitro, levels being 5–10-fold higher than $PGF_2\alpha$, the second most abundant. Since the absolute levels of 6ketoPGF$_1\alpha$ in brain blood vessels were much higher than those of grey and white matter, they suggested that prostaglandin formation was orientated towards prostacyclin in the vascular tissue of brain, and toward $PGF_2\alpha$ in the non-vascular. This is supported by work in this laboratory in which cell preparations from gliomas, shown to have little contamination with

vascular endothelial cells, produced $PGF_2\alpha$, PGE_2, and TXB_2 in short-term culture, but very little 6keto$PGF_1\alpha$ (Cooper *et al.* 1984).

Systemic blood vessels from humans and various animal species have been found to produce a range of eicosanoids in vitro. Most have found that 6keto$PGF_1\alpha$ is the major product, followed by PGE_2 and $PGF_2\alpha$, and some have observed TXB_2 production (Tuvemo *et al.* 1976, Neri Serneri *et al.* 1983, Siess *et al.* 1981). Neri Serneri *et al.* reported an active lipoxygenase-dependent pathway in human arteries, and Piper *et al.* (1983) demonstrated generation of a leukotriene-like substance from porcine vascular tissue, with greatest amounts being formed in the arterial adventitia. All layers of the arterial wall may produce eicosanoids. Moncada *et al.* (1977) demonstrated that although the ability to produce prostacyclin was greatest at the intimal surface of the rabbit aorta, the smooth muscle layer also produced a substantial amount. Medial smooth muscle cells in tissue culture produce significant quantities of prostacyclin together with PGE_2 and $PGF_2\alpha$ (Baenziger *et al.* 1979, Larrue *et al.* 1982), and a small amount of 15HPETE and malondialdehyde when incubated with arachidonic acid (Morisaki *et al.* 1984). Under basal conditions of perfusion of rabbit carotid arteries in vitro, intraluminal perfusate contained 6keto$PGF_1\alpha$ in highest concentration, followed by PGE_2, $PGF_2\alpha$, and small amounts of TXB_2. High concentrations of 6keto$PGF_1\alpha$ were released extra-arterially from cells of the outer arterial wall, followed by $PGE_2 > PGF_2\alpha > TXB_2$ (Pickard *et al.* 1984).

Cerebral blood vessels similarly produce a range of eicosanoids. The spectrum may differ according to the type of blood vessel, but methodological problems have obscured the picture. In vitro, dissected basilar arteries and choroid plexus synthesized PGE_2 and 6keto$PGF_1\alpha$, followed by $PGF_2\alpha$ and small amounts of TXB_2 (dog, Walker *et al.* 1983; rabbit, Walker *et al.*, unpublished). These prostanoids as well as PGD_2 were produced by bovine cerebral arteries in vitro (Hagen *et al.* 1979). Abdel Halim *et al.* (1980) and Goehlert *et al.* (1981) reported production of 6keto$PGF_1\alpha$ by the choroid plexus of small animals, and this may be the origin of some of the 6keto$PGF_1\alpha$ in CSF. The findings with isolated cerebral microvessels have been conflicting. In an early paper Gerritsen *et al.* (1979) reported that PGE_2 was the major product formed by isolated bovine cerebral microvessels in vitro, with only a small amount of 6keto$PGF_1\alpha$, but this was using PGH_2 as a precursor which is readily converted non-enzymically to PGE_2. When larger amounts of tissue were incubated, 6keto$PGF_1\alpha$ was predominant (Gerritsen *et al.* 1980). More recently this group reported that rabbit cerebral microvessels, using endogenous arachidonic acid as precursor, produced 6keto$PGF_1\alpha$ in largest amount in vitro, followed by PGE_2. TXB_2 was below the sensitivity of their assay (10–20 pg/tube) (Rodrigues and Gerritsen 1984). Gecse *et al.* (1982) found that brain capillaries from rats

and guinea pigs produced predominantly PGE_2 and PGD_2 with little 6ketoPGF$_1\alpha$. However Brown *et al.* 1984 observed that isolated cerebral microvessels from rats synthesized 6ketoPGF$_1\alpha$, and that production was increased markedly by the calcium ionophore A23187.

Actions of Eicosanoids at a Cellular Level

There are still many unanswered questions. This brief review covers only those actions relevant to platelet function and to vascular responses.

Actions of Prostanoids in Platelet Aggregation (1) TXA_2
(reviewed by Granstrom *et al.* 1982)

When a blood vessel is injured collagen in subendothelial tissues is exposed, and platelets in the blood adhere to it and aggregate (*primary* wave of aggregation). Binding of collagen to a receptor on the platelet surface activates a phospholipase, liberating arachidonic acid within the platelets. This in turn is metabolized to TXA_2 and to HHT, malondialdehyde, and 12HPETE, see Figs. 1 and 2). TXA_2 then a) stimulates the release of platelet constituents from the dense granules (*platelet release reaction*). These include ADP, a potent platelet aggregator, serotonin (which causes blood vessel constriction locally), Ca^{++}, and platelet factors which promote blood clotting; and b) through its own ability to induce platelet aggregation, it participates in *extension* of the aggregate (*second wave of aggregation*). As more platelets aggregate, they are stimulated in turn to produce TXA_2, and the process of aggregation "cascades". TXA_2 is not essential therefore for the *initiation* of aggregation, but is produced by aggregating platelets. Its actions are to initiate the platelet release reaction and to participate in propagating aggregation in the second wave. Patients with a deficiency of platelet cyclooxygenase bruise easily and have a mild bleeding disorder.

The mechanisms of action of TXA_2 on platelets are not understood clearly, but most evidence today supports the hypothesis that TXA_2 interacts with intracellular calcium for contractile processes (Granstrom *et al.* 1982). Although TX has been hypothesized to act as a calcium ionophore to transport calcium from the platelet dense tubular system to the cytoplasm, Carey *et al.* (1984 a) were unable to demonstrate this under "steady state" conditions. Rink and Hallam (1984) showed that a stable TXA_2 analogue raised platelet intracellular calcium from a basal level of near 100 nm towards 1 μm in 4–5 seconds and the increase seems to have been attributable to calcium influx, internal release accounting for less than a 2-fold release of intracellular calcium. Thrombin, Platelet aggregating factor (PAF), and ADP also elevated platelet calcium mainly by calcium influx, but were not dependent on TXA_2 generation for this action. These

authors consider that TXA_2 reinforces rather than mediates the effects of these agonists.

2. Prostacyclin. Prostacyclin inhibits platelet aggregation and the release reaction. This involves binding to a specific membrane receptor, and a subsequent increase in cyclic AMP. In this respect prostacyclin is 10 times more potent than PGD_2 and 30 times more potent than PGE_1. An increase in platelet cyclic AMP has been reported to inhibit arachidonic acid release from phospholipids, to inhibit cyclooxygenase and to have a direct inhibitory action on the contractile mechanism of the release reaction (Granstrom *et al.* 1982).

Mechanism of the Effects of Eicosanoids on Vascular Smooth Muscle

In vitro, eicosanoids are produced by all layers of the arterial wall (see above). Endogenous production of prostaglandins by smooth muscle is related partly to tone, for example gut, umbilical artery, ductus arteriosus and coronary artery (Eckenfels and Vane 1972, Tuvemo and Wide 1973, Coceani *et al.* 1975, Kalsner 1975). Clinically this has led to the successful use of indomethacin for the treatment of patent ductus arteriosus. To what extent do changes in smooth muscle contraction modulate endogenous production of eicosanoids by the smooth muscle cells? Does generation of eicosanoids within the arterial wall either *mediate* or *modulate* the actions of other vasoactive agents on arterial smooth muscle? Clearly these questions are interlinked.

There is now considerable evidence that the application to sensitive tissues of vasoactive peptides (Bradykinin, angiotension II, vasopressin), prostaglandins, and less consistently monoamines (noradrenaline; 5-hydroxytryptamine) stimulates eicosanoid production, and that blockade of such production alters the qualitative and quantitative nature of the effects of the agonist on the tissue (Alexander and Gimbrone 1976, Schrauwen *et al.* 1979, Mullane and Moncada 1980, Pilipi and Poyser 1981, Hassid and Williams 1983). Although direct measurement of cerebrovascular eicosanoid production has not been reported yet in response to peptides, cyclooxygenase inhibitors attenuate the cerebrovascular dilation produced by bradykinin, vasoactive intestinal polypeptide and angiotension II (Toda 1977, Toda and Miyazaki 1981, Wei *et al.* 1980) and potentiate the contractile effects of $PGF_2\alpha$, PGE_2, PGD_2, noradrenaline and haemorrhagic cerebrospinal fluid (Toda and Miyazaki 1978, Toda 1982, Brandt *et al.* 1981 a).

Dawson and Irvine (1978) noted that in many of those tissues in which physiological stimuli caused activation of phosphatidyl-inositol turnover, the same stimuli caused an increase in prostaglandin release. Such activation of phosphatidyl-inositol turnover and augmented production of

PGE_2 and 6keto$PGF_1\alpha$ has been found in smooth muscle (taenia coli, aorta, and in tissue culture; Coburn *et al.* 1980, Coburn 1983) in association with inhibition of the sodium pump with ouabain or low potassium solutions. The augmented PGE_2 release was not related to the cytosolic free calcium, to release of known neurotransmitters, or to changes in the surface membrane potential or to muscle tension. PGE_2 release in potassium-free media was entirely dependent on the presence of calcium in the bathing media and could be increased by raising calcium from 2.5–10 mmol/l (Coburn 1983).

It seems possible that eicosanoids may *modulate* the activity of other vasoactive agents, perhaps by modulating calcium influx and release (Pickard 1981). Koltai *et al.* (1984), from studies of $PGF_2\alpha$-induced contractions of isolated dog coeliac and basilar arteries, suggested that $PGF_2\alpha$ whilst producing contraction, also increased the formation and release of *relaxant* cyclooxygenase products, thereby building up a "braking system" which tended to attenuate contraction. Inhibition of cyclooxygenase would remove this brake. Hassid and Williams (1983) observing that two vasoconstrictors, vasopressin and Angiotension II, produced a dose dependent increase in prostacyclin release from rat mesenteric arteries, suggested that prostacyclin and other vasodilator prostanoids released from vascular smooth muscle may buffer peptide-induced vasoconstriction. This might account for the observation that indomethacin potentiated the ability of vasopressin to increase peripheral vascular resistance in humans (Glänzer *et al.* 1982). Förstermann *et al.* (1984) proposed that the major prostanoid regulating the tone of rabbit *coeliac* artery may be prostacyclin and that of the *femoral* artery may be PGE_2 (which they found to be a femoral artery vasoconstrictor).

Kadar and Sunahara (1969) were the first to demonstrate that the effects of exogenous PGE_1, $PGF_1\alpha$ and $PGF_2\alpha$ on spontaneous contractions of canine mesenteric arteries and veins were enhanced in low potassium concentrations, and that the effects of PGE_1 and $PGF_1\alpha$ but not $PGF_2\alpha$ were abolished by ouabain pre-treatment. Small increases in extracellular potassium concentration will produce relaxation of a variety of smooth muscles including cerebral arteries particularly following a prior period of sodium loading in a potassium-free medium (Toda 1976, Lockette *et al.* 1980). Interpretation of this potassium-induced relaxation as an index of sodium pump activity is not straightforward (Pickard and Perry 1984). However, in cerebral arteries from a variety of species including man (Toda 1976) and rat tail artery (Lockette *et al.* 1980), $PGF_2\alpha$ in both studies and PGE_2 in the rat tail artery significantly enhanced the magnitude of potassium-induced relaxation. The cyclo-oxygenase inhibitors indomethacin and meclofenamate reduced the magnitude of potassium-induced relaxation by more than 30% and $PGF_2\alpha$ was able to reverse the inhibition

of potassium relaxation by meclofenamate. Lockette *et al.* suggested that prostaglandins induce vascular smooth muscle relaxation by stimulation of the sodium pump and that endogenous prostaglandins normally potentiate potassium relaxation. This effect of the relatively stable prostaglandins may be mediated by activation of adenylate cyclase and stimulation of the electrogenic sodium pump by cyclic AMP (Somlyo *et al.* 1972). There is considerable evidence that the effects of various eicosanoids including prostacyclin may be mediated, at least in part, by changes in adenylate cyclase (Whittle and Moncada 1983 for review).

There is a paucity of published information on the effects of various prostaglandins particularly prostacyclin and TXA_2 on membrane potential and the various ion conductances. This is not surprising given the very short half-lives of prostacyclin and TXA_2. The use of a stable analogue such as Carbocyclic TXA_2 is not without its problems (Whittle and Moncada 1983). Such an analogue must exhibit the same profile of activity as the biologically important compound and although Carbocyclic TXA_2 has potent vasoconstrictor properties it fails to aggregate platelets. PGH_2 produces a slight transient contraction of cerebral arteries followed by a relaxation which may be the result of conversion to prostacyclin; the stable analogue of PGH_2, U-44069 produces only a maintained contraction of rabbit aorta (Toda 1980, Loutzenhiser and van Breemen 1981).

Many of the effects of eicosanoids are calcium dependent and their structure has stimulated speculation that they may act as calcium ionophores (Horton 1969, Harris *et al.* 1979, Kirtland and Baum 1972). With reference to excitation-contraction coupling in smooth muscle, some workers have found that prostaglandin-induced smooth muscle contraction is dependent on extracellular calcium (Smith *et al.* 1981, Godfraind and Miller 1982) whereas others have suggested that such a contraction is mediated by a release of calcium from intracellular stores (McNamara *et al.* 1980, Wheeler and Weiss 1980, Loutzenhiser and van Breeman 1981). There is a disparity in cerebral arteries between the quantitative effects of calcium-free medium and a calcium antagonist such as nifedipine or Verapamil, on the contraction produced by $PGF_2\alpha$ and carbocyclic TXA_2 which has been elegantly investigated by Uski *et al.* in Lund. In cerebral arteries from various species, calcium antagonists are much more effective than calcium-free medium in suppressing the contractile effect of $PGF_2\alpha$ and carbocyclic TXA_2 (Brandt 1981, Towart and Perzborn 1981, Toda 1982, Uski 1984). Uski's explanation is that calcium-free medium in the absence of a high concentration of chelating agent such as EGTA does not remove all the extracellular-bound calcium which is still in a form that can be used by membrane calcium channels. However, such channels are blocked by calcium antagonists, hence the disparity. Although the contraction of cerebral arteries is much more susceptible to calcium depletion and to

calcium antagonists than peripheral arteries, implying a greater dependence on extracellular calcium (Allen *et al.* 1979, Shimizu *et al.* 1980, Brandt *et al.* 1981 b), the effects of both $PGF_2\alpha$ and carbocyclic TXA_2 are associated with release of calcium from *intracellular* storage sites, particularly during the early phase of the contraction (Toda 1982, Uski 1984).

Eicosanoids as Candidates for the "Endothelium-Derived Relaxing Factor"

Acetyl choline relaxes isolated arteries by an unknown mechanism initiated in the endothelium (Furchgott and Zawadzki 1980). This may involve release of a relaxing mediator from the endothelium, or an action at myoendothelial junctions (Boeynaems and Galand 1983). Several other agents including ATP, thrombin, substance P, vasoactive intestinal peptide (VIP) and histamine also require an intact endothelium to express full dilator effects in vitro (Davies and Williams 1984). High affinity binding sites for prostacyclin have been described in arterial smooth muscle cells (pig aorta) (Rücker and Schrör 1983). One μm acetyl choline increased production of PG6keto$F_1\alpha$ by rabbit aortic rings significantly, and this effect was blocked by atropine and by removal of the endothelial cells. However the amount of acetyl choline needed to stimulate prostacyclin production was greater than that to relax vascular smooth muscle (0.01 to 1 μm), and the relaxing effect of acetylcholine was maintained in the presence of indomethacin. Moreover rabbit aorta is one artery not relaxed by prostacyclin. It seems unlikely that prostacyclin or any other prostanoid is the relaxing mediator for acetyl choline (Boeynaems and Galand 1983), or for VIP or histamine (Davies and Williams 1984). However the vasodilator effect of VIP on cat pial arterioles but not porcine cerebral arteries was completely inhibited by indomethacin, 3 mg/kg, administered intravenously (Wei *et al.* 1980, Winquist *et al.* 1982) and for VIP at least, the findings are conflicting. Because arterial relaxation (rat) to VIP, acetyl choline and histamine was not blocked by the cyclooxygenase inhibitor indomethacin, but was by ETYA (eicosatetraynoic acid) an inhibitor of lipoxygenase and cyclooxygenase activity, a lipoxygenase product has been implicated indirectly (Davies and Williams 1984). However this seems unlikely from studies of Gordon and Martin (1983), and some other candidate must be sought.

Actions of Eicosanoids on Cerebral Arterial Contraction in vitro and in vivo

The effects of arachidonic acid and its derivatives on cerebral arteries and pial arterioles has been studied extensively. The findings are summarized in Table 1. A consensus emerges when the extensive literature summarized in Table 1 is reviewed; discrepancies remain however. These may reflect a variable vulnerability to trauma of the endothelium and its

Table 1. *Effect of Arachidonic Acid and Its Derivatives on Cerebral Arteries and Pial Arterioles*

Species	*In vivo*	*In vitro*	AA	Prostaglandin							TXA_2	TX analogue	6-keto $PGF_{1\alpha}$	Reference
				G_2	H_2	G_2/H_2 analogue	$F_{2\alpha}$	E_2	D_2	I_2				
Man		+					C	C						Toda and Miyazaki 1978
		+			C					R	C			Boullin *et al.* 1979
		+					C			R				Brandt 1981
		+				C	C			R/C		C		Uski *et al.* 1981, 1983
		+				C	C	C			C	C		Forster and Whalley 1981
		+								R/C				Paul *et al.* 1982, 1983
Baboon		+			C					R	C			Boullin *et al.* 1979
	Ang.									R				Jarman *et al.* 1979
		+			Biphasic[a]					R/C[b]				Jarman *et al.* 1979
Dog		+					C							Allen *et al.* 1974
	Ang.		C				C	C						White *et al.* 1975
		+					C	C						Toda and Miyazaki 1978
		+								R/C[c]				Chapleau and White 1979
		+	C											Chapleau *et al.* 1980
		+			Biphasic[d]				C/R[g]	R			0	Toda 1980, 1982
	Pial									R				Branan *et al.* 1979
	Pial						C							Yamamoto *et al.* 1972
Cat	Pial						C							Welch *et al.* 1974
	Basilar						C							Kapp *et al.* 1976
	Pial		R	R				R	R	R				Ellis *et al.* 1979, Kontos *et al.* 1980
	Pial									R			0	Pickard *et al.* 1980
	Pial		R											Wei *et al.* 1980
		+				C	C			C		C		Uski *et al.* 1981, 1983, 1984
		+	C											Hardebo *et al.* 1981
	Pial						0							Ellis *et al.* 1983
	Pial		R											Busija and Heistad 1983
Mouse	Pial						C							Rosenblum 1975
	Pial		C/R[e]											Rosenblum 1981
Cow		+			C		C				C			Ellis *et al.* 1977
Rat		+					C			R/C[f]				Uski *et al.* 1981
	Pial						C							Ellis *et al.* 1983
Rabbit	Pial		R											Busija 1983
		+					0							Uski 1984

[a] Biphasic response in fresh arteries, contraction in stored arteries. [b] Relaxation at less than 10^{-7} M; contraction at 10^{-6} M. [c] Relaxation up to 10^{-7} M; contraction at 10^{-5} M. [d] Late relaxation blocked by 15 HPAA and tranylcypromine. [e] Transient contraction followed by relaxation. [f] Relaxation or contraction depends on the agent stimulating basal tone. [g] Contraction at rest; relaxation of $PGF_{2\alpha}$ induced contraction.

Abbreviations: AA, arachidonic acid; Ang., angiography; PG., prostaglandin; PGI_2, prostacyclin; TX, thromboxane; C, contraction; R, relaxation; 0, no effect (modified from Pickard 1981).

basal production of prostacyclin in the different preparations used. In retrospect, it is now clear that either basal production of such endogenous metabolites must be measured throughout the experiment or their production must be inhibited and a known concentration of metabolite(s) substituted. The problem of endothelial integrity in vitro can no longer be ignored, and histological controls are necessary (Furchgott and Zawadzki 1980, Lee 1981). It should also be remembered that arachidonic acid itself can induce such endothelial cell damage (Kontos *et al.* 1980, Ingerman-Wojenski *et al.* 1981). This endothelial phenomenon may explain the different results reported in canine cerebral arteries. Toda and Miyazaki (1978) found that aspirin and indomethacin potentiated the contractile effects of $PGF_2\alpha$ and PGE_2 whereas Chapleau *et al.* (1980) found that aspirin had no effect and meclofenamate/indomethacin in high concentrations inhibited the contractile effect of $PGF_2\alpha$. Indomethacin (3 μmol/l), whilst not influencing basal tone significantly, potentiated the $PGF_2\alpha$-provoked contraction in all arteries, but those from diabetic animals to a greater extent (Koltai *et al.* 1984). One μmol/l of $PGF_2\alpha$ induced significantly higher tone in isolated basilar arteries from Alloxan-diabetic dogs than normal dogs. Thromboxane is the most potent vasoconstrictor of cerebral vessels known. Carbocyclic TXA_2, a TXA_2 analogue, contracted guinea pig basilar artery at concentrations as low as 2.8×10^{-10} M, and had 100 times the potency of $PGF_2\alpha$ (Fujiwara and Kuriyama 1984).

Except in the cat PGE_2 has been found to contract cerebral arteries in vitro and in vivo. In the cat in vivo (closed cranial window) dilatation of pial arterioles occurred in response to topically applied PGE_2 (and also PGD_2, PGG_2, and prostacyclin; Ellis *et al.* 1983). This may reflect a species variation of the cat. Whereas in cats 10^{-7} to 10^{-5} M $PGF_2\alpha$ had no effect on large (> 100 μm) or small (< 100 μm) pial arterioles in vivo (closed cranial window), in rats $PGF_2\alpha$ induced a dose dependent contraction. Kapp *et al.* (1976) however reported constriction of transorally exposed cat basilar arteries to 0.1 to 1 μg/ml $PGF_2\alpha$, and Welch *et al.* (1974) of cat pial arterioles, and Andersson *et al.* (1983) of cat middle cerebral artery to 2.5 μm $PGF_2\alpha$. Similarly, Handa *et al.* (1974) and Peterson *et al.* (1975) found that both $PGF_2\alpha$ and PGE_2 constricted the cat basilar artery in vivo with topical application.

In most other vascular beds in the body, PGE_2 has a vasodilator action. However PGE_2 contracts human umbilical arteries (Horton 1979), and was found to potentiate adrenaline-induced contraction of rabbit *femoral* arterial strips markedly, whilst it inhibited adrenaline-induced contractions of rabbit *coeliac* strips markedly (Förstermann *et al.* 1984). It is not unusual for vasoactive drugs to possess both the properties of vascular contraction and relaxation (Berkowitz *et al.* 1984).

It is essential when describing the effect of say, prostacyclin, to define the

species, type of cerebral artery, dose, level of endogenous prostaglandin synthesis, and whether the effect is superimposed on resting tension or tension provided by stimulation of potential-operated or receptor-operated membrane channels (amine, peptide, or another prostanoid) (McCulloch and Edvinsson 1984 for review). For example in feline vessels with low resting tension, prostacyclin had a *contractile* effect that reached a maximum of 132% (basilar artery) and 23% (middle cerebral artery) of the potassium-induced (127 mM) contraction. In potassium-contracted feline vessels, prostacyclin caused a further contraction. When these vessels were contracted by $PGF_2\alpha$, prostacyclin induced *relaxation* which was most marked in the middle cerebral artery. Prostacyclin consistently relaxed the middle cerebral artery contracted by the prostaglandin endoperoxide analogue U-44069, whereas the basilar artery was almost unaffected. In human pial arteries with low resting tension, prostacyclin had no effects in concentrations below 10^{-6} M, whereas higher concentrations induced contractions. In potassium-contracted preparations, prostacyclin in concentrations below 10^{-6} M produced relaxation; in higher concentrations further contraction was induced. Human pial arteries contracted by $PGF_2\alpha$, U-44069, noradrenaline, or 5-hydroxytryptamine, consistently relaxed in response to prostacyclin (less than 10^{-6} M) Uski *et al.* 1983). In human pial arteries, the relaxation-mediating prostanoid receptor appears to be a prostacyclin—sensitive type (Lumley *et al.* 1982, Town *et al.* 1982, Uski *et al.* 1984). The mechanism of the contractile effect of prostacyclin at high doses (Chapleau and White 1979, Jarman *et al.* 1979, Paul *et al.* 1982, Uski *et al.* 1983) in the human basilar artery has been postulated to be via the release of a cyclooxygenase product (not TXA_2) which subsequently acts on the contractile TX receptor in the tissue (Paul *et al.* 1983). Hence the effects of various arachidonate derivatives are interdependent.

Von Holst *et al.* (1982) found that LTC_4 and LTD_4 neither contracted nor relaxed superfused human cerebral artery strips. In contrast Tagari *et al.* (1983) suggested that synthetic LTD_4 contracts the isolated human basilar artery and provokes both cerebral vasoconstriction and, on occasion, a biphasic response in the rat as detected by angiography. The leukotriene antagonist FPL 55712 had direct cerebrovascular effects and did not prevent LTD_4-induced contractions. Rosenblum (1985) found that LTB_4, C_4 and D_4 produced dose dependent constrictions of mouse pial arteries (up to 20% at 4×10^{-7} M)-LTC_4 constrictions were antagonized by FPL-55712. 15 HPAA contracts the isolated canine basilar artery both by inhibiting prostacyclin synthetase and stimulating endogenous lipoxygenase (Koide *et al.* 1982).

Arterial Smooth Muscle Receptors for Eicosanoids

The paucity of specific prostanoid receptor antagonists and agonists, coupled with the very short half-lives of TXA_2 and prostacyclin, has

impeded characterization of prostanoid receptors, both contraction—and relaxation—mediating. Based on studies of the relative potency of prostanoids and the use of antagonists, at least three types of contraction-mediating receptors have been suggested: TX sensitive, $PGF_2\alpha$ sensitive and PGE-sensitive receptors (Kennedy *et al.* 1982, Uski and Andersson 1984 for review). PGE-sensitive receptors may be divided into two subtypes: those blocked by SC19220, and those which are not blocked by this drug. A TX sensitive receptor has been described in both human and feline cerebral arteries based on rank order of potency (man: U46619 $\cong$ U44069 > PGB_2 > $PGF_2\alpha$ > $PGE_2 \cong PGD_2 \cong PGF1\alpha$ > TXB_2)—U46619 being a TXA_2 agonist and U44069 a PG endoperoxide analogue (Uski and Andersson 1984, Uski *et al.* 1984, Forster and Whalley 1981, Toda 1982). The TX antagonist EP045 blocked these contractions (Paul *et al.* 1983).

Cerebral Blood Flow—a Role for Endogenous Prostacyclin?

Has the cerebrovascular synthesis of prostacyclin a role in the modulation of cerebrovascular reactivity or does it act solely in its capacity to repel intracerebral platelet deposition and aggregation?

In the baboon anaesthetized with phencyclidine, nitrous oxide, and oxygen, indomethacin (either 10 mg/kg i.v., or 0.04–0.2 mg/kg/min by intracarotid infusion) reduces cerebral blood flow (CBF) at normocapnia by some 38% and severely impairs the response to hypercapnia (Fig. 5) (Pickard and MacKenzie 1973). Indomethacin has no effect at hypocapnia. There is no significant change in either cerebral oxygen consumption ($CMRO_2$), mean arterial blood pressure, or mean cerebral perfusion pressure and no constriction of the major inflow tract arteries such as the internal carotid or middle or anterior cerebral arteries. The effect of indomethacin on CBF is very rapid: sagittal pressure starts to decrease within 1 minute in man and baboon, and cerebral venous outflow decreases within 15 seconds in the rat (Sicuteri *et al.* 1965, Pickard and MacKenzie 1973, Dahlgren *et al.* 1981, Pickard 1981). The speed of this response indicates that indomethacin exerts its effect on a compartment in rapid equilibrium with blood, that is, the vessel wall (Dahlgren *et al.* 1981). It also indicates an extremely rapid turnover of the relevant arachidonate metabolite. There is a close correspondence between the dose-response curve for reduction in CBF and for inhibition of prostaglandin synthesis in the rat. Following cessation of an intracarotid infusion of 60 minutes of indomethacin, the effects on CBF resolve after some 2 hours in the baboon. *In vitro,* when indomethacin is removed from the tissue by washing, its effect is reversed within minutes—it is quite erroneous to assume that indomethacin produces irreversible inhibition of prostaglandin synthesis (Manku and Horrobin 1976, Hornstra *et al.* 1979). Furthermore, the pharmacodynamics of indomethacin vary considerably between species (Hucker *et al.* 1966).

Many other workers have now confirmed these cerebrovascular properties of indomethacin in other species, including man (Table 2). Methodological factors may explain the discrepancies in the literature. Dawson and Dalessio (1968) employed an inhalation ^{133}xenon technique for the measurement of human CBF but made no correction for either arterial recirculation of ^{133}xenon or for the slow extracerebral component of the washout curves. The depression of cerebrovascular reactivity by barbiturate

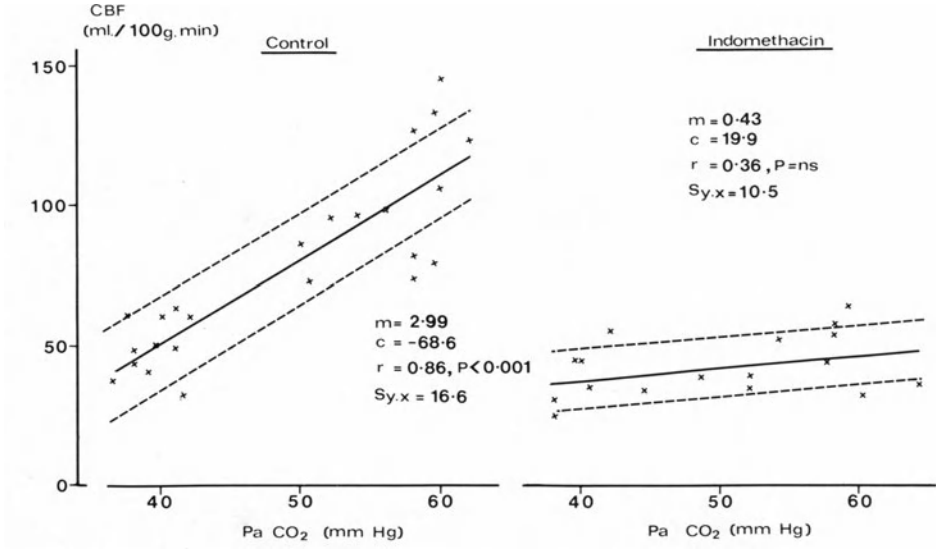

Fig. 5. Effect of indomethacin on the response of CBF to CO_2 in the anaesthetized baboon (from Pickard 1981, with permission)

anaesthesia (Fujishima *et al.* 1971, Grubb *et al.* 1974, Dahlgren and Siesjo 1981), together with the semiquantitative heat-clearance method employed, might explain the failure of Cuypers *et al.* (1978) to observe any cerebrovascular effects with indomethacin. Only a rigorous technique for the estimation of cerebral tissue perfusion will suffice to reveal further depression with indomethacin (Dahlgren and Siesjo 1981). Furthermore, Cuypers *et al.* dissolved their indomethacin in alcohol and saline, so that the equivalent of 0.4–1.8 ml of 96% ethanol was injected with the indomethacin. Recently Busija and Heistad (1983) in the cat, and Busija (1983) in the rabbit, using the microsphere technique, have not found a significant change in CBF either at normocapnia or hypercapnia with indomethacin. This may represent either a species difference or the effects of microspheres on the cerebral circulation, but neither may be the whole explanation. Bill (1979), Hierton (1981), Quintana *et al.* (1983), and Shigeno *et al.* (1983,

Table 2. *Effects of Indomethacin on Cerebral Blood Flow*

Species	Acute decrease	No change
Baboon	Pickard and MacKenzie 1973 Pickard *et al.* 1977 b, 1980 d Branston *et al.* 1981 McCalden *et al.* 1984	
Man	Amano and Meyer 1981 Wennmalm *et al.* 1981 Okabe *et al.* 1983 Eriksson *et al.* 1983 Pickles *et al.* 1984	Dawson and Dalessio 1968
Rat	Sakabe and Siesjo 1979 Francois-Dainville *et al.* 1980 Pickard *et al.* 1981 Dahlgren *et al.* 1981 McCulloch *et al.* 1982 Pappius and Wolfe 1983 Quintana *et al.* 1983	
Rabbit	Bill 1979 Hierton 1981 Pinard 1983	Cuypers *et al.* 1978 Busija 1983
Gerbil	Crockard *et al.* 1981	
Dog	Ruszczewski and Herbaczynska-Cedro 1978	Jackson *et al.* 1983
Puppy	Ment *et al.* 1983	
Cat	Vlahov 1976 Gabrielyan *et al.* 1979 Shigeno *et al.* 1983	Busija and Heistad 1983
Goat	Hoffman *et al.* 1982	

1985) also used the microsphere technique in the rabbit, cat and rat, and have also shown that indomethacin causes significant reduction in CBF. Furthermore, Pinard *et al.* (1983), have shown that indomethacin does cause a rapid decrease in CBF in awake rabbits and reduces the effect of maintained hypercapnia but not transient changes in arterial carbon dioxide tension. The microsphere technique is prone to methodological problems (Andersen *et al.* 1983, Consigny *et al.* 1982, Rosenberg *et al.* 1983).

Ruszczewski and Herbaczynka-Cedro (1978), concluded that indo-

methacin has no effect on the CBF CO_2 response, but close scrutiny of their results suggests the opposite. They used a combination of hexobarbitone, urethane, and chloralose to anaesthetize their dogs. CBF was estimated with the intracarotid xenon method without, apparently, resection of the scalp and temporalis muscle. This method is fraught with difficulties in this species because of inadequate separation of the intracranial from the extracranial circulation (Jennett *et al.* 1976). Normocapnic CBF was reduced from 22 to 11 ml/100 g/min by indomethacin (2 mg/kg, i.v.) and the CBF CO_2 response was halved (from 0.53 to 0.25 ml/100 g/min/mm Hg—our calculations on their data). These low values of CBF and CO_2 reactivity are a reflection of the anaesthetic regimen. In the conscious rat, indomethacin slightly reduces the animal's mobility but has no other obvious behavioural effects (Dahlgren 1981, McCulloch *et al.* 1982). The application of the (C^{14}) iodoantipyrine technique in the rat has revealed that indomethacin administration results in reduction in cerebral tissue perfusion in every region of the brain examined by between 30 and 50% from vehicle-injected control levels. Furthermore, the administration of indomethacin (0.3–30 mg/kg i.v.) does not alter significantly the rate of glucose utilization [using Sokoloff's (^{14}C) deoxyglucose technique] in any of the 38 discrete regions of the central nervous system that were examined (McCulloch *et al.* 1982). Furthermore, Siesjo's group has been unable to find any change in cerebral redox state with indomethacin despite the large reduction in CBF (Dahlgren 1981).

Indomethacin's vasoconstrictor effect is restricted to the parenchymal vessels—indomethacin either has no effect or relaxes pial vessels *in vivo* and the larger cerebral arteries *in vitro* (Vlahov and Betz 1974, Wei *et al.* 1980, Pickard *et al.* 1976, Chapleau *et al.* 1980) except in man where Brandt (1981) has found that some fresh cerebral arteries contract slowly with indomethacin. Cyclo-oxygenase inhibitors potentiate the contractile effects on cerebral arteries of various prostanoids and some other agonists in some species including man (Brandt 1981, Toda and Miyazaki 1978, Toda 1982) but not contractions induced by noradrenaline, serotonin, histamine and UTP nor the relaxations involved by acetylcholine, histamine, isoproterenol, ATP or adenosine (Hardebo *et al.* 1981). The response of pial arteries do not always provide a reliable guide to the effects of an agent on cerebral tissue perfusion (Edvinsson and MacKenzie 1977, Acar and Pickard 1980). This inability to use an isolated cerebral artery as a reproducible model of the cerebrovascular effects of indomethacin is a considerable handicap to exploring the detailed cellular mechanisms involved (for review of the pharmacology and other actions of indomethacin—see Pickard 1981, Pickard and Walker 1984).

Continuous treatment with indomethacin in man (0.8 mg/kg tds) does not reduce resting CBF although depression of the response to carbon

dioxide persists (Wennmalm *et al.* 1983, Eriksson *et al.* 1983). In contrast, following two days' pretreatment with oral indomethacin (100 mg/day), Pickles *et al.* (1984) found that indomethacin still results in a significant reduction in resting CBF but the reactivity to carbon dioxide was about normal. In the rat, Pappius and Wolfe (1983) found that CBF increased after 24 hours of indomethacin treatment following an acute decrease in CBF. It appears that the cerebral circulation may adapt to the chronic inhibition of prostaglandin synthesis much as the neonatal lung is able to do (Lock *et al.* 1980). The impairment of the CBF CO_2 response by indomethacin is singularly specific. Indomethacin has much less effect on any other aspect of cerebrovascular reactivity yet examined except, possibly, on reactive hyperaemia. However, not all these responses have yet been adequately explored.

In the conscious rat, there is a highly significant correlation between the rate of glucose utilization and blood flow in different cerebral structures. The relationship between blood flow and glucose utilization is fundamentally altered following indomethacin (McCulloch *et al.* 1982). However, there is still coupling between the two parameters, and the hierarchy of blood flow levels present in normal animals (that is, greatest in primary auditory areas, lowest in regions of white matter) is maintained following indomethacin. Whether this is indicative of the incompleteness of inhibition of prostaglandin synthesis (80%) or the involvement of other mechanisms in the coupling of flow and glucose use remains to be elucidated.

Indomethacin has no effect on the autoregulatory curve, including its lower limit to haemorrhagic hypotension and its upper limit to angiotensin-induced hypertension, in either the anaesthetized baboon or gerbil (Pickard *et al.* 1977 b, Crockard *et al.* 1982). An intracarotid infusion of indomethacin was used in the baboon to ensure that a falling plasma level of indomethacin did not complicate interpretation of the protracted experiments required to study autoregulation. The only change in the autoregulatory curve was that indomethacin significantly reduced the absolute value of CBF at all levels of mean arterial pressure but not the over-all pattern of response. The effects of indomethacin on the transient cerebrovascular responses to changes in cerebral perfusion pressure, as opposed to steady-state changes, have not yet been examined. In the kidney, it was suggested originally that autoregulation was impaired by indomethacin. The consensus view now is that steady-state autoregulation is unaffected but that transient responses may be impaired by indomethacin (Herbaczynska-Cedro and Vane 1973, Venuto *et al.* 1975, Beilin and Bhattacharya 1977). However, Shohami and Sidi (1984) have found that the level of 6keto$PGF_1\alpha$ (the metabolite of prostacyclin) doubles when arterial blood pressure is reduced from 120 to 80 mm Hg but does not increase further with more severe hypotension.

Other conditions such as carotid ligation and mechanically or serotonin-induced carotid artery spasm that impair the CBF CO_2 response also abolish or radically alter the cerebrovascular responses to hypoxia and changing perfusion pressure (Jennett *et al.* 1976). There is no angiographic evidence to suggest that indomethacin provokes any occlusion or spasm of either the internal carotid artery or the major intracranial arteries. Although indomethacin has no effect on the CBF response to *severe* hypoxia or hypoglycaemia (Sakabe and Siesjo 1979, Nilsson *et al.* 1981), it does impair the response to *moderate* hypoxia in the baboon (McCalden *et al.* 1984). Vlahov (1976) found that indomethacin (10 mg/kg i.v.) impairs the reactive hyperaemia provoked by the clamping of both carotid arteries in the cat for 1 minute. Reperfusion of the brain, after episodes of brief ischaemia, results in a large accumulation of arachidonic acid metabolites in brain tissue (vide supra). As in vascular smooth muscle, enough oxygen has to be present for conversion of the arachidonic acid, released from phospholipids during ischaemia, by cyclo-oxygenase (Kalsner 1977). Re-establishment of blood flow restores tissue oxygen sufficiently. Functional activation of the brain may be considered as either "physiological" or "pathological". Electrical stimulation of the nose of the rat presumably involves activation of both somatosensory and pain pathways. Nilsson *et al.* (1981 b) found that CBF, as estimated by the (^{14}C) iodoantipyrine autoradiographic technique, increased to 150% or more in frontal, sensorimotor, and parietal cortex and thalamus, and to about 100–130% in other structures. Indomethacin reduced baseline CBF to about 50%. From this level the percentage increase induced by electrical nasal stimulation was of the same magnitude as in untreated animals, but the pattern of specific somatosensory activation was abolished.

The effect of indomethacin on the cerebrovascular response to bicuculline-induced seizures is as complex as the response itself. As a generalization, in the selectively vulnerable areas where changes in local cerebral glucose utilization eventually greatly exceed the increase in local CBF, indomethacin has no effect. Where the coupling between local cerebral glucose utilization and local CBF remains intact, as in the cerebellum, indomethacin abolishes the increase in CBF (Ingvar *et al.* 1981, Ingvar and Siesjo 1981).

Whereas prostaglandins may exert an inhibitory control on extracranial sympathetic nerves (Hedqvist 1970), cervical sympathectomy has no effect on the cerebrovascular response to indomethacin either in the rat or rabbit (El Bouchi *et al.* 1983), nor does indomethacin affect the effect of sympathetic stimulation on CBF (Beausang-Linder 1982, Busija 1985). Transmural stimulation of isolated middle cerebral arteries produced contractions that were markedly antagonized by prazosin, an α_1-adrenoceptor blocking agent, but the contractions were not influenced by

indomethacin. The cortical cerebral vasodilatation produced in the rat by apomorphine, dexamphetamine, and immobilization stress were antagonized by indomethacin pretreatment.

In the dog and cat, indomethacin attenuates the cerebrovascular effects of bradykinin, vasoactive intestinal polypeptide and Angiotension II (Toda 1977, Toda and Miyazaki 1981, Wei *et al.* 1980). However in porcine cerebral arteries, the relaxation provoked by VIP was not affected by indomethacin (Winqvist *et al.* 1982). The effect of angiotensin II on pial arterioles in the cat displays remarkable tachyphylaxis (Acar and Pickard 1980). In the kidney, Aiken and Vane (1973) demonstrated that such tachyphylaxis is caused by intrarenal prostaglandin release. In the dog, angiotensin relaxes isolated cerebral arteries when contracted with $PGF_2\alpha$ (Toda and Miyazaki 1981). This angiotensin II-induced relaxation is blocked by both aspirin and indomethacin and by inhibitors of prostacyclin synthetase (15-hydroperoxyarachidonic acid and tranylcypromine). If this observation can be extended to other species, it may explain the paradox that angiotensin II is a very potent constrictor of pial arterioles but has little effect on CBF even after disruption of the blood-brain barrier (Pickard *et al.* 1977 c, Acar and Pickard 1978, Wei *et al.* 1978, Edvinsson *et al.* 1979). Spatz *et al.* (1983) reported an intriguing association between the presence of 5-hydroxytryptamine (5-HT) both in nerve endings and the endothelial cells of cerebral micro-vessels and in prostaglandin receptors on the endothelial cells. Indomethacin reduced the concentration of endothelial 5-HT, and this effect was reversed by the addition of prostacyclin. The chemical structure of indomethacin contains an indole ring and is similar to 5-HT. This similarity led Sicuteri (1965) to assess indomethacin's efficacy in the treatment of migraine and he noted the acute effects of indomethacin on CSF pressure. It is most unlikely however that indomethacin is acting as a partial agonist to 5-HT receptors: it has none of the contractile properties of 5-HT on cerebral or pial arteries nor does it depress cerebral metabolism (for example: Harper and MacKenzie 1977 a, b). Cerebral depletion of 5-HT produced by 5, 7, DHT does not reduce cerebrovascular reactivity to carbon dioxide (Dahlgren *et al.* 1981 b) and may slightly increase it (Itakura *et al.* 1985).

Is the acute reduction in CBF produced by indomethacin in humans, primates, rat, and gerbil a result of inhibition of prostaglandin synthesis in some compartment within the brain? If endogenous arachidonic derivatives play more than a permissive role in control of cerebrovascular reactivity, then it should be possible to demonstrate that the changes in concentration of the relevant eicosanoid within the appropriate compartment within the brain should change with, for example, hypercapnia. The problem is how to estimate such eicosanoid levels in the appropriate compartment. Severe insults such as cerebral ischaemia of varying duration do result in the release

of free arachidonic acid in the brain and subsequent generation of various prostaglandins (vide supra). However, more subtle insults such as changes in arterial blood gases have not been shown consistently to produce such changes. Two approaches have been adopted: first, the measurement of cerebral arterial venous differences in prostaglandin concentrations, and second, the changes in prostaglandin levels in whole cortex.

Ruszczewski and Herbaczynska-Cedro (1978) were unable to show any effect of changes in arterial carbon dioxide tension in the dog on the level of endogenous prostaglandin-like substances continuously bioassayed in sagittal sinus blood. An increased release of such substances occurred during hypoxia, cerebral ischaemia, and embolism. Indomethacin abolished these effects. McCalden *et al.* (1984) and Dux *et al.* (1981) demonstrated that the cerebral venous concentration of 6-oxo-$PGF_1\alpha$ (the prostacyclin metabolite) increased considerably during hypoxia with an increase during hypercapnia that did not reach statistical significance. However, in humans, Wennmalm *et al.* (1983) could find no evidence for the detectable release of arachidonic acid or prostacyclin metabolites (6-oxo-$PGF_1\alpha$; 6,15-diketo-13,14-dihydro-$PGF_1\alpha$) from the brain either in the basal state or during inhalation of carbon dioxide. The levels of 6-oxo-$PGF_1\alpha$ in this study were seldom above the threshold for detection (25 pg/ml). In contrast, in the study by Uyama *et al.* (1983), where internal carotid arterial and internal jugular venous concentrations of 6-oxo-$PGF_1\alpha$ were measured in five patients with mild cerebral thrombotic infarction, the levels were about 50 pg/ml with no evidence for cerebral production and no evidence for a change with hypercapnia.

Jackson *et al.* (1983), using radioactive microspheres to measure CBF in barbiturate-anaesthetized dogs, reported that indomethacin (10 mg/kg i.v.) had little effect on cerebral vascular resistance during hypercapnia despite reduction in the total brain secretion (A-V differences) of 6keto$PGF_1\alpha$. 6keto$PGF_1\alpha$ secretion did not increase with hypercapnia. The basal cerebral blood flow was very low (20 ml/100 g/min) and the authors waited 60 minutes before assessing the effects of a bolus administration of drug. The basal arterial and venous levels of 6keto$PGF_1\alpha$ were high and no extraction procedure was used prior to radioimmunoassay. Clearly there is fundamental disagreement between these studies that needs to be resolved and that almost certainly stems from analytical problems. Recently, using a sensitive and specific assay, Blair *et al.* (1982) have shown that the concentration of 6-keto-$PGF_1\alpha$ in venous blood of normal healthy individuals is extremely low (around 3 pg/ml). If these authors are correct, the much higher concentrations recorded in these studies probably reflect non-specific assays. There may well be a "dilution" problem of detecting alterations in the amount of this metabolite released by the brain, given the high rate of blood flow through the brain. Increased jugular levels of

prostanoids have been demonstrated, however, under some circumstances (Forstermann *et al.* 1981, Roberts *et al.* 1975).

The second approach has been to measure the levels of prostaglandins in whole cerebral cortex directly (Ellis *et al.* 1982). Following measurements of pial artery diameter through a cranial window, the window was removed and approximately 1 g brain tissue was scooped out with a spatula, immediately placed on a flat piece of dry ice, and smashed in the frozen state by hitting the tissue with another piece of dry ice. Using this procedure the authors found that brain tissue could be removed and frozen to a 1 mm thick wafer within eight seconds after starting removal. However, the control levels of PGE_2 and $PGF_2\alpha$ seem high, being of the order 170 to 190 pg/g wet weight, respectively, possibly because of mechanical and ischaemic damage to the tissue during the procedure. In Ellis's study, hypocapnia produced no change in the cortical levels of prostaglandins E_2, $F_2\alpha$, and 6-keto-$F_1\alpha$, whereas hypercapnia produced some reduction in prostaglandins E_2 and $F_2\alpha$ but not 6-keto-$F_1\alpha$. Hypoxia was associated with 45% reduction in E_2 with no change in $F_2\alpha$ or 6-keto-$PGF_1\alpha$. Quite apart from the problem of trying to detect changes in the levels of prostaglandins within the vascular compartment, this study did not utilize blockers of prostaglandin transport out of the central nervous system. If the rate of removal of the various prostaglandins is sufficiently rapid, then no changes in cortical levels might be seen under these circumstances. Much more work needs to be done to clear up this problem. Intravenous indomethacin causes a marked decrease in the prostacyclin metabolite in cisternal cerebrospinal fluid (CSF) in the dog within 10 to 30 minutes (Walker *et al.* 1983).

We have undertaken some preliminary investigations in five anaesthetized sheep to assess the value of the ventriculo-cisternal perfusion technique in studies to elucidate the role of PG's in CBF. Mock CSF was perfused through cannulae placed in one lateral ventricle and the cisterna magna. PGs were measured in timed collections of perfusate. CBF was measured by ^{133}Xe clearance in two sheep. Hypercapnia increased CBF 40% above basal value. Whilst maintaining hypercapnia, a bolus of 10 mg/kg of indomethacin injected into the femoral vein reduced CBF acutely by 53% of the hypercapnic value. In response to hypercapnia, an increase in PGs released into the perfusate occurred in all five animals, but the responses varied considerably. All the measured prostanoids (E_2, $F_2\alpha$, $6KF_1\alpha$, TXB_2) increased apparently non-selectively. In response to indomethacin (three animals) an early and marked decrease in all the perfusate PGs occurred despite continuing hypercapnia. By 80 minutes, all PGs had fallen to 2–8% of the pre-indomethacin values.

Whilst the results of this pilot study are compatible with the notion that PGs have a role in the control of CBF, this sheep model was found to be unsatisfactory—first because the local trauma of perfusion undoubtedly

increased PG concentrations in the perfusate to values much greater than those found in CSF from non-traumatized sheep; secondly because it was felt that the perfusion model was too slow and insensitive to detect rapid changes in vascular release of prostanoids; and thirdly, because the changes monitored are likely to reflect release of PGs from the outer layers of the blood vessels, and give no idea of the changes occurring in production in the inner arterial layers.

Another approach is to examine the effects of various inhibitors of cyclooxygenase and compare their effects on cerebral prostaglandin synthesis *in vivo* with their effects on cerebrovascular reactivity. When the various non-steroidal anti-inflammatory agents are compared, as the IC_{50} for prostaglandin synthetase decreases, so the degree of plasma protein binding increases, and solubility in an acceptable buffer for *in vivo* work becomes more difficult, particularly for cerebrovascular studies. For example, meclofenamic acid is up to threefold more effective than indomethacin in inhibiting prostaglandin synthesis *in vitro,* but 99.8% of it binds to plasma protein (Flower and Vane 1974, Ceserani *et al.* 1977). Transfer across the blood brain barrier would be minimal. Furthermore, it is only soluble at high alkaline pH.

Unfortunately, there is still no good data in the literature concerning *in vivo* inhibition of cerebral prostaglandin synthesis within the different brain compartments by a variety of cyclooxygenase inhibitors. Without such biochemical data it is not possible to elucidate the cerebrovascular effects of various cyclooxygenase inhibitors. The mechanisms of cellular uptake and subcellular distribution of these agents differ (Blanchard *et al.* 1979). Many studies refer to pathological situations where blood-brain barrier function is impaired. The widely quoted study of Abdel-Halim *et al.* (1978) refers to brain levels of prostaglandins obtained following decapitation and homogenization of the brain, and the levels in these circumstances are much higher than those found using microwave irradiation in the same species by Bosisio *et al.* (1976).

In humans, acute administration of sodium salicylate (1 g i.v., plasma level 0.9–1.6 mmol/l) reduced the CBF CO_2 response by 26%, with no significant reduction in CBF at normocapnia (Pickard *et al.* 1977 d). Acute administration of aspirin (45 mg/kg) failed to affect CBF (Eriksson *et al.* 1983) as does chronic administration of this drug (Amano *et al.* 1981). However, in the rat, lysine acetylsalicylate did reduce CBF between 22 and 31%, as determined by the microsphere technique (Quintana *et al.* 1983). The cerebrovascular effects of some of the newer cyclooxygenase inhibitors have also been tested (Hierton 1981). Scrupulous monitoring of systemic parameters (blood pressure, blood gases, body temperature) and cerebral oxygen metabolism are essential if changes in CBF are to be evaluated, as the study of salicylate vividly illustrated. In humans, naproxen (4 mg/kg bd for two consecutive weeks) had no significant effect on resting CBF,

cerebral oxygen consumption, or cerebrovascular reactivity to hypercapnia (Eriksson *et al.* 1983). In the anaesthetized rat, piroxicam had no effect on normocapnic or hypercapnic CBF; diclofenac significantly reduced CBF during normocapnia in 2 out of 25 structures by 11 and 20% and during hypercapnia in 6 out of 25 brain structures by 14 to 32%; lysine acetylsalicylate significantly reduced normocapnic CBF in 5 structures (15–30%) and 4 structures during hypercapnia (20–37%). The structures affected were different in the two groups (Hougaard *et al.* 1983, Wieloch *et al.* 1983). In the goat, both indomethacin and ibuprofen reduced CBF as measured with an electromagnetic flowmeter on the internal maxillary artery, but they did not reduce the effect of hypercapnia (Hoffman *et al.* 1982). The original paradoxical observation of Crockard *et al.* (1982) that 1-N-butylimidazole, a potent inhibitor of thromboxane synthetase, mimics the effects of indomethacin on the cerebral circulation in the gerbil has been confirmed by Sofeir *et al.* (1983) in the rabbit, where a large dose of 25 mg/kg i.v. totally blocked CO_2 reactivity but had no effect on resting CBF. Of considerable importance is the recent finding by El Bouchi *et al.* (1985) that intraventricular administration of diclofenac does reduce cerebral blood flow at rest and antagonizes the cerebral hyperaemia provoked by immobilization stress.

In order to interpret these interesting findings, it will be necessary to determine the effects *in vivo* of these compounds on intracranial prostaglandin synthesis. It may not be valid to assume that these drugs will affect cerebral tissues in the ways that have been demonstrated for more readily accessible extracranial tissues. Moreover, it will be necessary to determine whether blockade of one part of the arachidonic cascade may have secondary effects elsewhere in the cascade, perhaps to produce unsuspected increases in vasoactive eicosanoids. Furthermore, indomethacin may inhibit one form of brain cyclooxygenase more than another (Lysz *et al.* 1982). The observation that the vasoconstrictor activity of indomethacin is not always shared with other cyclooxygenase inhibitors has been made in other tissues, including the mesenteric and renal circulations of the rabbit and dog (Andersson *et al.* 1983), Feigen *et al.* 1981, Hierton 1981). This suggests that the vascular effects of indomethacin might be mediated via a mechanism different from that of prostaglandin synthesis inhibition. However, no other effect has yet been demonstrated that would explain the selective effects of indomethacin on cerebral parenchymal vessels (Pickard 1981, Pickard and Walker 1984).

Effects of Prostaglandins and Prostacyclin on Cerebral Blood Flow and Metabolism

There is a relative paucity of data documenting the effects of arachidonate derivatives on CBF as opposed to pial arterial calibre or *in vitro*

studies of cerebral arteries (see Pickard 1981 for detailed review). $PGF_2\alpha$ reduced both cerebral blood flow and oxygen consumption (Pickard, MacDonell *et al.* 1977 a, Eidelman *et al.* 1983). These effects of $PGF_2\alpha$ are potentiated in hypercholesterolaemic animals and this phenomenon may be associated with depressed vascular wall production of prostacyclin (Eidelman *et al.* 1983, Dembinska-Kiec *et al.* 1977).

There has been considerable controversy over the cerebrovascular effects of prostaglandins of the E series. The explanation lies in the exquisite sensitivity of temporalis muscle blood flow to PGE_2: it increases by 500% at 10^{-7} g/kg/min (Pickard *et al.* 1977 a). Only those investigations where there is the possibility of extracranial contamination has the suggestion been made that PGE_1 or PGE_2 might be vasodilatory (Denton *et al.* 1972, Pelofsky *et al.* 1972, Nakano *et al.* 1973). In addition, alcoholic solutions of the PGE series must be avoided (Yamamoto *et al.* 1972). Intracarotid infusion of PGE_2 has similar but larger effects than $PGF_2\alpha$ on CBF and cerebral oxygen consumption. The dose response curves for the two effects are very similar (Pickard *et al.* 1977 a). The intracarotid doses used for these studies are rather high, but these stable prostaglandins penetrate very poorly into the brain (Hansson and Samuelsson 1965, Green *et al.* 1967, Holmes and Horton 1968).

In the anaesthetized baboon the intracarotid infusion of large doses of prostacyclin (5×10^{-6} g/kg/min) not only increases CBF by up to 71% but also partly reverses the effect of indomethacin at hypercapnia (Pickard *et al.* 1980). These changes occur despite systemic hypotension and a severe tachycardia. Where arterial blood pressure is returned to near normal with angiotensin during an infusion of prostacyclin and indomethacin, CBF is further increased. In dogs, the intravenous infusion of a high dose of prostacyclin (3×10^{-6}g/min) reduced mean arterial blood pressure below the lower limit of autoregulation and provoked tachyarrhythmias but CBF fell by less than would be expected from the degree of hypotension (Boarini *et al.* 1984). In hyper-cholesterolaemic monkeys, greater concentrations of prostacyclin are required to produce the same cerebral vasodilator effect (Eidelman *et al.* 1983). In man, only much lower doses of prostacyclin (4–5×10^{-9} g/kg/min) are tolerated (Pickles and O'Grady 1982) and such doses produced an 8–9% reduction in CBF which is within the error of the methods (Brown and Pickles 1982, Cook *et al.* 1983). Not surprisingly, this low concentration of prostacyclin did not reverse cerebrovascular constriction provoked by indomethacin although CBF did increase by 10% (Pickles *et al.* 1984). Left atrial infusions of prostacyclin increased cerebral blood flow to the cortex of dogs, estimated by the microsphere technique (Einzig *et al.* 1980).

The intracarotid infusion of a thromboxane A_2 generating system

(thrombin and a suspension of platelets) reduces CBF and precipitates a stroke-like syndrome in the majority of rabbits (Shimamoto 1977, Asano *et al.* 1978).

Acute Cerebral Ischaemia: Background

The following sequence of events has been suggested to occur with cerebral ischaemia (Siesjo 1981, Raichle 1983): ischaemia leads to ATP depletion and thereafter a rise in intracellular calcium derived both from intracellular release of sequestered calcium, calcium influx and impaired calcium extrusion. This rise in free intracellular calcium activates microsomal and mitochondrial phospholipases. Phospholipid catabolism continues throughout the ischaemic period with progressive accumulation of free fatty acids. In the absence of any tissue oxygen, cycloxygenase activity is halted but, with even slight reperfusion and a tissue PO_2 of 12 torr, C20 PUFAs, particularly arachidonic acid, are processed down the casade. In addition lipid peroxidation from generated free radicals may augment the damage (Moncada 1983).

The evidence at present for a role for free radicals in post-ischaemic events is slender. The consensus of evidence as reviewed by Siesjo (1981) was that although brain tissue has a considerable capacity for lipid peroxidation, analysis of whole brain tissue revealed no evidence that this occurred *in vivo* with either complete or incomplete ischaemia. However, Siesjo added the caveat that if free-radical changes were circumscribed they would be difficult to detect by whole tissue analysis. Recently, Watson *et al.* (1983), using conjugated diene detection, have shown that lipid peroxidation does occur but only in focal areas in the brain following reversible global ischaemia. The level of endogenous antioxidants (α-tocopherol and reduced ubiquinones) fell during 30 minutes of ischaemia and declined even further with recirculation (Yoshida *et al.* 1982). This type of phenomen would explain the paradox (Hossmann and Kleihues 1973) that experimentally, animals subjected to 60 minutes of ischaemia do worse when a trickle of blood remains. Kontos *et al.* (1980) observed that direct application of arachidonic acid or PGG_2 to the surface of cat brain induced cerebral arteriolar damage that could be prevented by free-radical scavangers.

The pathophysiology of various types of cerebral ischaemia are very different and it is important to recognize that neuropathological quantification of ischaemic cell change and infarct size is the most reliable end point against which to judge therapeutic efficacy. The exact duration of ischaemia and pattern of reperfusion is critical to interpretation—there is now considerable evidence that, for example, the development of post-ischaemic oedema depends upon the severity of cerebral ischaemia (Iannotti and Hoff 1983, Avery *et al.* 1984, Bell *et al.* 1985). The interaction between

indomethacin and ischaemic changes in adenylate cyclase depends upon the duration and severity of ischaemia plus the duration of reperfusion (Taylor *et al.* 1984).

Global Cerebral Ischemia

Both Spagnuolo *et al.* (1979) and Gaudet and Levine (1979,1980) examined the effects of bilateral common carotid artery occlusion in the gerbil and demonstrated that during ischaemia the levels of PGD_2, $PGF_2\alpha$, TXB_2, HETE, and 6-keto-$F_1\alpha$ in the brain did not change during occlusion for up to 2 hours. In the second study, there was a small fall in the level of PGE_2 between 1 and 2 hours after occlusion. With reperfusion, there was an early and dramatic increase in brain levels of PGD_2, PGE_2, $PGF_2\alpha$, 6-keto-$F_1\alpha$, and thromboxane B_2. When measured at 5 minutes of reperfusion, prostaglandin levels were higher in brains whose arteries had been occluded for 5 minutes than in those occluded for 15 or 30 minutes. After occlusion for 5 to 30 minutes, prostaglandin levels did not return to normal until 105 minutes after reperfusion had begun. Since brain tissue has little capacity to metabolize prostaglandins, this slow return of prostaglandins to normal probably represents vascular washout and diffusion into CSF. Indomethacin and aspirin, but not dexamethasone, inhibited this increase. These authors also examined the effects on locomotor activity of these various manipulations following 5 minutes of occlusion and subsequent reperfusion. There was an initial latent period of inactivity from 0 to 15 minutes in both occluded groups, but with longer periods of reperfusion the indomethacin-treated gerbils became much more active than the untreated group. However the behaviour of the indomethacin-treated animals could not be considered normal, since they exhibited sustained hyperactivity.

Crockard's group have also studied ischaemia in a gerbil model (Crockard, Bhakoo *et al.* 1982, Bhakoo *et al.* 1984 a, b). They quantified brain prostaglandins during bilateral carotid ligation, and found increases in $PGF_2\alpha$ in all areas of ischaemia (findings at variance with those of Spagnuolo *et al.* and Gaudet and Levine) but no increase in PGE_2. TXB_2 was not demonstrable in their preparation. The increase in $PGF_2\alpha$ was reduced by pre-treatment with indomethacin (3 mg/kg) but not by dexamethasone. There was a small residual blood flow in these animals, which were monitored carefully. It is conceivable that in Gaudet and Levine's study the animals may have been more hypotensive—physiological data is not provided—and thereby rendered more ischaemic. Alternatively, the difference may reflect the use of intraarterial indomethacin at the time of sacrifice to try to "biochemically freeze" the brain prostaglandins. This technique's effectiveness may vary with regional CBF. The gerbil is also very prone to fits which may not always be clinically apparent (Crockard, personal communication; Picozzi *et al.* 1985). Cerebral PG synthesis is

stimulated by fits. Within 15 minutes of restoration of flow, a massive increase in $PGF_2\alpha$ occurred with a peak at 2 hours. Unlike Gaudet and Levine's study, Crockard's group found that PGE_2 did not increase during the first 30 minutes of recirculation but then increased progressively to ten times the basal level. The time-course of cytotoxic oedema and increased $PGF_2\alpha$ were very similar whilst that of vasogenic oedema related more closely to the increase in PGE_2. During 60 minutes of ischaemia in their gerbils, Bhakoo *et al.* (1984) found that all FFAs increased non-selectively with no evidence that a specific phospholipid was involved, confirming previous work (vide supra). This result indicates the non-specific action of hydrolytic lipases. A short sharp increase in all FFAs occurred during the first five minutes of reperfusion, but with a relatively greater increase in unsaturated fatty acids than saturated. Levels then decreased gradually up to three hours of reperfusion. Since reperfusion led to an increase rather than to a decrease in unsaturated FAs this group argue that free radical peroxidation is unlikely to account for the changes in this model. However, Enseleit *et al.* (1984) observed only relatively small changes in phospholipid up to 240 minutes of ischaemia in the gerbil. Phosphatidyl inositol and phosphatidic acid levels were significantly decreased following 15–45 minutes of ischaemia. All but phosphatidyl serine increased during 60 minutes of reperfusion after ischaemia for one hour.

Recently, Moskowitz *et al.* (1984) have shown that 6-sulfidopeptide-containing leukotriene-like immunoreactivity is synthesized in gerbil forebrains after bilateral common carotid occlusion and reperfusion. No detectable activity was found in brain regions remote from the zone of ischaemia (forebrain) nor were cerebral arteries a major source of such leukotriene-like biosynthesis.

A study by Shohami *et al.* (1982) in rats demonstrates the importance of careful documentation of events for the interpretation of changes in prostanoid production. Both carotid arteries were occluded and the blood pressure reduced to 50 mm Hg. Following either 5 or 15 minutes of ischaemia, recirculation was achieved by removal of the clamps and rapid return of blood pressure to normal. Brains were frozen *in situ*. Hypotension alone resulted in an increase in the prostacyclin metabolite 6-keto-$PGF_1\alpha$. PGE_2 accumulated during the first 5 minutes of ischaemia, and its level declined at 15 minutes and returned to control levels at 30 minutes of recirculation. TXB_2 increased during the whole time course of the experiment, and at the end of the post-ischaemic period its level was five times higher than control. Treatment with indomethacin (4 mg/kg i.v.) prior to ischaemia reduced the levels of these products and shortened the recovery time of electrical cortical activity after 15 minutes of ischaemia. It is of interest that the pattern of reduction effected by indomethacin in the various arachidonic products differed, and TXB_2 was reduced the most.

This led the authors to suggest that inhibition of TX synthesis may have improved the post-ischaemic reflow. In a series of papers, Hallenbeck and colleagues have examined the effect of manipulation of arachidonic acid metabolism on microvascular reperfusion of the brain following 35 minutes of complete CSF compression ischaemia followed by 30 minutes of recirculation in the dog, and also the effect of possible circulating platelet aggregates (Furlow and Hallenbeck 1978, Hallenbeck 1977, Hallenbeck and Bradley 1977, Hallenbeck and Furlow 1979). First, circulation of the animal's blood through glass-walled filters for one hour prior to induction of ischaemia significantly enhanced general reflow and prevented focal zones of impaired perfusion following the ischaemic insult. Intravenous infusion of indomethacin (1.5 or 4 mg/kg) one hour before ischaemia also eliminated the circulatory defects. Indomethacin or prostacyclin (30–180 ng/kg/min) infused alone after ischaemia had no beneficial effect, whereas significant enhancement of post-ischaemic reperfusion occurred in animals receiving the combination of indomethacin and prostacyclin after ischaemia. When blood was taken from the various series of animals and passed through columns to assess platelet retention, indomethacin prior to ischaemia and prostacyclin post-ischaemia both inhibited such platelet retention, whereas the indomethacin given 5 minutes after the end of the ischaemic period had no such effect. This suggests that if a cyclooxygenase product is implicated in impairment of post-ischaemic reperfusion, then it is involved very early following total ischaemia, as is platelet activation. The results of this experiment support the proposed role for TXA_2 in early ischaemic events. This type of compression ischaemia is a severe insult and obviously, with an eye to potential therapy in humans, it is important to consider more relevant models. Indomethacin (10 mg/kg) reduced the incidence of perfusion defects of the "no-reflow" type after 15 minutes of complete ischaemia (CSF compression) and 5 minutes reperfusion in the rat (Kågström *et al.* 1983).

Hossmann's group have examined the effects of prostacyclin administration during the post-ischaemic hypoperfusion period following one hour of complete ischaemia (Kerckhoff *et al.* 1983). They found no improvement in blood flow or reversal of the disturbed CO_2 reactivity nor any change in post-ischaemic blood coagulation. Sofeir *et al.* (1983) have examined the effects of large doses of a thromboxane synthetase inhibitor (imidazole, 25 mg/kg) in rabbits submitted to 5 minutes of global ischaemia achieved by clamping both common carotid arteries after prior permanent ligation of both vertebral and subclavian arteries. In this model, removal of the carotid clamps results in a hyperaemic phase followed by a hypoperfusion period.

Indomethacin, given 45 minutes before induction of ischaemia induced in rabbits by a combination of vascular occlusion and systemic hypoten-

sion, improved cortical reperfusion but did not influence cortical Po_2 or electrocorticographic activity. Post-ischaemic treatment had no effect (Boulu *et al.* 1981).

Regional (Incomplete) Ischaemia

Following unilateral common carotid artery occlusion in the gerbil, Gaudet and Levine (1980) found that the levels of PGD_2, $PGF_2\alpha$, and the prostacyclin metabolite 6-keto-$PGF_1\alpha$ increased in both hemispheres of the symptomatic animals but not of asymptomatic animals. The presence of fits did not appear to further increase the levels of PGs significantly, but fits may not be clinically obvious (Crockard, personal communication). The largest increase in prostaglandin levels was seen in the non-occluded hemisphere, and the levels were greatest at 15 minutes following occlusion. Levels had fallen significantly by 2 and again by 6 hours after occlusion. These increases in the levels of $PGF_2\alpha$ and PGD_2 were inhibited by prior administration of indomethacin, whereas the more moderate increases in 6-keto-$PGF_1\alpha$ were not significantly inhibited by indomethacin. Indomethacin had no effect on the decrease in specific gravity (*i.e.*, oedema formation) found after 6 hours in the occluded hemisphere. The mechanism of the contralateral increase in levels of prostaglandins following unilateral occlusion is not immediately obvious. This contralateral increase in arachidonic acid release and prostaglandin formation was not seen following a freezing injury by Wolfe and Pappius (1983).

The information concerning intracranial prostaglandin production after ischaemic events in humans is fragmentary. The precise pathological diagnosis is not always given, nor is the time of collection of the sample with respect to clinical condition. Carasso *et al.* (1977) reported that the level of PGE_2 in lumbar CSF is increased following stroke and noted that the more severe the neurological deficit the higher the levels. Others observed increases in $PGF_2\alpha$ levels. $PGF_2\alpha$ increased some 10-fold within 10 days of onset of symptoms and declined to near normal within one month (Egg *et al.* 1978). In the incomplete ischaemia model of Kågström *et al.* (1983: rat—bilateral carotid occlusion plus blood pressure of 50 mmHg for 15 minutes with 5 minutes reperfusion), indomethacin (10 mg/kg) increased flow to the severely ischaemic region but reduced flow to the mildly ischaemic zone.

Following middle cerebral occlusion in the cat, the pial arteries overlying the ischaemic core initially dilate but then constrict, whereas the pial arteries supplying the surrounding penumbra remain dilated. Using the microsphere technique, Shigeno *et al.* (1983) found that in animals during middle cerebral occlusion for 2 hours, indomethacin (4 mg/kg) did not further reduce flow in the ischaemic core but did significantly reduce flow in the surrounding penumbra. Following reperfusion in indomethacin-treated animals, hyperaemia was increased considerably and the hypoperfusion

phase abolished in the ischaemic core. The hyperaemic phase was prolonged. Indomethacin did not affect flow in the penumbra after reperfusion. The complexity of the situation is revealed by the studies of Awad *et al.* (1983 a, b). The middle cerebral artery of cats was occluded for six hours and animals received no therapy or an intracarotid infusion of prostacyclin in buffered saline (100 ng/kg · min) with or without an intravenous injection of indomethacin (4 mg/kg). Using intracarotid xenon-^{133}, a technique of questionable validity in this species, regional CBF decreased markedly in all animals on middle cerebral artery (MCA) occlusion; CBF then improved progressively in the untreated animals but did not in animals treated with prostacyclin. Electroencephalogram (EEG) changes, oedema, areas of fluorescein extravasation, and infarct size were not significantly different in the various groups. Extravasation of Evans blue dye was reduced by prostacyclin. Mean arterial pressure was stable in the animals receiving prostacyclin, whereas in untreated animals there was progressive hypertension during the 6 hours of occlusion. The authors suggest that the systemic haemodynamic effects of prostacyclin in the presence of impaired autoregulation may compromise regional CBF in the ischaemic zone and offset any direct beneficial effects. Indomethacin did not modify these effects of prostacyclin. During experimental ischaemic stroke, prostacyclin had no significant effect on either blood flow or water content (Hossmann 1982). Dempsey *et al.* (1985) found that indomethacin (4 mg/kg intraperitoneally twice daily pretreatment) would reduce ischaemic oedema in both core and penumbra and improve cerebral perfusion but only in cats (MCA model) anaesthetized with pentobarbital, not with ketamine.

The doses of prostacyclin used both by Hallenbeck and colleagues and Awad and colleagues are approximately 20 times the level that could be tolerated by awake humans without side-effects. Gryglewski *et al.* (1983) have recently reported the effects of prostacyclin in 10 patients admitted between 1 and 5 days after the onset of symptoms from carotid or intracranial artery occlusion. A dose of 2.5 to 5 ng/kg · min i.v. was given in 6-hour courses (4–10) over 1 to 2.5 days. The authors describe a dramatic regression of neurological symptoms: 6 patients were left without any deficit, 3 with minor residual deficit, and 1 patient died.

Martin *et al.* (1985) have completed a double-blind controlled trial of intermittent infusions of prostacyclin (5 ng/kg · min for 6 hours—infusions over 65 hours) in 32 patients with acute cerebral infarction. There were no significant differences in neurological score or disability status between the two groups. Neither of these contradictory studies is of sufficient size to be statistically capable of revealing anything but the grossest difference.

Tamura *et al.* (1979) have found that an imidazole derivative (Y9179) reduced the size of cerebral infarction produced by unilateral MCA occlusion in the cat, as determined at one week. However, in another study

in cats (Moufarrij 1984), the administration of the thromboxane synthetase inhibitor UK38485 had no beneficial effects on various indices of ischaemia. Starting immediately after MCA occlusion, the drug was given during four hours of ischaemia and then during two hours of recirculation. TXB_2 production was reduced by up to 96%. When the drug was given before MCA occlusion, cerebral infarction tended to be more *extensive* possibly due to redirection of TXA_2 precursors into lipoxygenase products which may propagate tissue damage. It is of considerable interest that the size of cerebral infarct was less in cats fed fishoil than in controls (Black *et al.* 1979). Koltai *et al.* (1984) have reported the fascinating observation that macrocortin—derived from rat peritoneal cells exposed to dexamethasone—reduces the mortality following bilateral common carotid ligation in this species.

Microembolism

Intracarotid platelet emboli may be produced by a variety of techniques including application of direct current (DC) through the carotid artery, partial constriction of a carotid artery, or infusion of sodium arachidonate or ADP into the carotid artery. Recurring reduction of carotid artery flow induced by partial constriction of the carotid artery in a dog is eliminated by TX synthetase inhibitors, prostacyclin, and acetylsalicylate, but increased by indomethacin and tranylcypromine, a prostacyclin synthetase inhibitor (Uchida *et al.* 1981). In rats, prior administration of indomethacin (5 mg/kg i.p.) and intravenous infusion of prostacyclin (200 ng/kg/min for 40 minutes) prevented cerebral ischaemia and infarction provoked by the electrogenic platelet thrombus technique (Dougherty *et al.* 1982). Pretreatment with the thromboxane synthetase inhibitor OKY1581 similarly ameliorated the effects of a sodium arachidonate injection into the internal carotid artery of the non-heparinized rat on cerebral energy metabolism and quantitative EEG activity (Fredriksson 1983) unlike aspirin which has no effect (Furlow and Bass 1976). In the rabbit, aspirin is effective (Fieschi *et al.* 1977). Similarly, ciloprost, a prostacyclin derivative, reduced the degree of Evans blue extravasation into the brain following sodium arachidonate injection into the carotid artery of the hypertensive rat (Borzeix and Cahn 1982). Accumulation of indium—labelled platelets in injured rabbit carotid artery and aorta is reduced by thromboxane synthetase inhibition (Hall *et al.* 1982, Randall and Wilding 1983). Clearly these results are of great relevance to the management of transient cerebral ischaemia in humans, where trials of aspirin therapy have been blighted by failure to be precise about the pathological process and by the dose of aspirin to be used. As prostacyclin has potent platelet antiaggregatory activity and sodium arachidonate induces platelet aggregation via the thromboxane pathway,

these results are really not surprising, but of concern are the rather high, and hence hypotensive doses of prostacyclin required. It is clearly important to know the dose-response curve of the effect of prostacyclin and also to know how effective it would be if given after induction of platelet aggregation. In contrast to Fredriksson's finding with OKY1581 (30 mg/kg i.v.), Rosenblum and El-Sabban (1983) found that prior administration of this agent, in doses from 10 to 300 mg/kg intraperitoneally to mice one hour before their pial vessels were injured by a combination of light from a filtered mercury lamp and intravascular sodium fluorescein, had no effect on intravascular platelet aggregation and arterial dilation produced. At the highest dose of OKY1581, enhanced aggregation appeared to occur. As the authors themselves point out, these findings cannot be used to deny that thromboxane synthetase inhibitors might have a place in therapy for conditions involving platelet aggregation, as the result may depend on species, vascular bed, or the method used to induce aggregation. In some pathological situations, intracerebral platelet deposition may help to reduce the extent of a lesion. One example is vasogenic oedema around a cold injury to the cerebral cortex—administration of antiplatelet drugs results in increased vasogenic oedema with improved filling of the microcirculation (Segawa and Patterson 1981). Rosenblum and El-Sabban (1978) gave tranylcypromine to mice and demonstrated one hour later that intravascular platelet aggregation was enhanced in the cerebral microcirculation but not in the mesenteric circulation. However, subsequent assay of 6-keto$PGF_1\alpha$ revealed only 25% reduction in the brain level and 50% reduction in the mesentery (Ellis *et al.* 1982).

Hallenbeck and co-workers (1982) used a different model of cerebral microembolization in dogs. They gave repeated intracarotid injections of small volumes of air titrated to maintain suppression of the cortical sensory evoked response for one hour. Various combinations of PGI_2, indomethacin, and heparin were administered, and their effect on recovery was monitored. Only the combination of all three agents produced a statistically significant augmentation of the return of the evoked response amplitude. This is a severe type of injury, and the pathology of microembolism is not like that of regional ischaemia: there is early multifocal disturbance of the blood-brain barrier with formation of vasogenic oedema, leading to ischaemia and subsequently cytotoxic oedema (Hossmann 1982). This is the reverse of the sequence of events with regional/complete ischaemia.

Miscellaneous Models

An increase in prostaglandin concentration occurs in the cat brain a few minutes following experimental fluid percussion injury (Ellis *et al.* 1981), and there is an associated doubling of phospholipase C activity in cerebral

cortical homogenates (Wei *et al.* 1982). The combination of PGI_2, indomethacin, and heparin improves neurological recovery after spinal trauma in cats (Hallenbeck *et al.* 1983).

Neither the increase in CBF during hypoglycaemia induced with insulin nor the reduction in flow in the post-hypoglycaemic period in the rat are mediated by mechanisms related to prostaglandin metabolism as reflected by the blockade with indomethacin (Nilsson *et al.* 1981).

The effect of asphyxia was assessed by Allen *et al.* (1982) in two-day-old guinea pigs. Asphyxiation in a nitrogen atmosphere for either 3 minutes or 3 minutes 40 seconds did not alter brain $PGF_2\alpha$ levels, but after 3 minutes 40 seconds of asphyxia, brain PGE_2 levels were significantly elevated above control. When asphyxia was followed by resuscitation with 95% oxygen and 5% CO_2, brain $PGF_2\alpha$ levels rose significantly while PGE_2 levels were not different from control values. Pre-treatment of animals with indomethacin lowered brain levels of both prostaglandins in controls, and prevented any asphyxiation or resuscitation-induced rise in brain prostaglandin levels. Times to primary apnoea and last gasp in asphyxiated guinea pigs were not affected by indomethacin. There is some analogy between these results and those of Gaudet and Levine with their bilateral carotid occlusion model in the gerbil, where there was no change in brain $PGF_2\alpha$ levels during ischaemia but a very significant rise with reperfusion. Nikolov and Milanova (1982) found in adult cats that both intravenous and intracarotid infusion of $PGF_2\alpha$ (10 μg/kg/min) significantly reduced the time to extinction of the electrocorticogram during asphyxic anoxia, and aso increased the time for its reappearance with resuscitation. $PGF_2\alpha$ has been reported to inhibit neuronal activity, as well as being a cerebral vasoconstrictor. Although prostacyclin had no effect in this asphyxic anoxia preparation, when infused in doses of 250 ng/kg/min i.v. it produced an improvement in the hypoxia-impaired EEG activity (Nikolov *et al.* 1982). Large systemic and intraventricular doses of PGI_2 induced a dose-dependent prolongation of survival time of mice subjected to hypoxic and anoxic hypoxia (Nikolov *et al.* 1984). However, inhibitors of phospholipase A_2 and of the arachidonate cascade also had antihypoxic effects (Nikolov 1984, Nikolov and Koburova 1984). Parallel assay of the various arachidonate derivatives will be required to advance this model.

An increase in CBF particularly to the germinal matrix may be part of the sequence of events that leads to intraventricular haemorrhage in premature babies. It is thus of considerable interest that Ment *et al.* (1982, 1983) reported recently that they were able to induce intraventricular haemorrhage in newborn beagle pups by a combination of haemorrhagic hypotension and subsequently volume re-expansion. Indomethacin pre-treatment (3 mg/kg) significantly reduced the incidence of such intraventricular haemorrhage. Using the (^{14}C)iodoantipyrine CBF technique, they

demonstrated that indomethacin reduced flow prior to the insult, and prevented the increases in CBF to the germinal matrix seen with volume re-expansion in the saline-pre-treated pups. Supplementation with the antioxidant vitamin E has been reported to reduce the incidence of intraventricular but not subependymal haemorrhage in babies under 32 weeks' gestation (Chiswick *et al.* 1983).

Cerebral Oedema

Can products of either the lipoxygenase or cyclooxygenase pathways be implicated in various forms of cerebral oedema, particularly in ischaemic oedema? There is considerable evidence in peripheral tissues that products of both the cyclooxygenase and lipoxygenase pathways may be involved in the formation of tissue oedema (Piper 1981, Williams 1979, Williams *et al.* 1983). In particular, slow-reacting substance A (LTC_4 and D_4) may enhance permeability of capillaries, but this only results in formation of oedema in the presence of a vasodilator, which may be another eicosanoid. In addition there is synergism between the various eicosanoids and complement derived peptides. Chan and Fishman (1978) demonstrated that polyunsaturated fatty acids (PUFA), including arachidonate and linoleate, induced swelling of isolated slices of rat brain cortex with a reduction in extracellular space (cellular oedema). This effect was not blocked by cyclooxygenase inhibitors such as aspirin or indomethacin, nor could it be reproduced either by autooxidation products of arachidonic acid or by hydroxy and hydroperoxy fatty acid products of the lipoxygenase reaction with linoleate. However, the effects of arachidonic acid could be mimicked with a similar dose-response curve by an ionic detergent, sodium dodecyl sulphate. *In vivo,* these same PUFA induced extracellular swelling. The *in vitro* swelling is accompanied by increases in superoxide free radicals, membrane lipid peroxidation, and malondialdehyde (Chan and Fishman 1980). Depletion of an endogenous antioxidant (vitamin E) increases the degree of water and sodium accumulation in brain after release of extradural compression in the rat (Yoshida *et al.* 1983). Aritaki *et al.* found similarly that the direct intracerebral injection of 160 μg arachidonic acid provoked a reduction in specific gravity in the brain locally. This effect could be inhibited by indomethacin, contrary to the findings of Chan and Fishman in isolated brain slices. Black (1985) has described how intracerebral injection of arachidonic acid (3.06 M) in rat produced local dye extravasation that was blocked by BW 755 C. Equally high doses of arachidonic acid were required to "open the blood-brain barrier" of cat pial arterioles after local administration but this effect was not mimicked by $LTC_4/D_4/E_4$ nor was it blocked by either indomethacin or BW 755 C (Unterberg, Baethmann, and Wahl 1985, personal communication).

Both free PUFA and superoxide radicals inhibit brain (Na^+, K^+)-

ATPase *in vitro* (see Chan *et al.* 1983 for review). Following MCA occlusion in the rat, Gotoh *et al.* (1983) have found that the isolated microvessel (Na^+, K^+)-ATPase activity was reduced in both hemispheres 24 hours later, in association with enhanced eicosanoid synthetic capacity. Indomethacin has no effect on the development of vasogenic cerebral oedema in the cat as produced by a cold lesion (Pappius and Wolfe 1976, Pickard 1980). However, Pappius and Wolfe (1983) have shown that, in the traumatized hemisphere and to a lesser extent in the contralateral hemisphere, unilateral focal freezing or heat lesions markedly depressed local cerebral glucose utilization. Such metabolic depression is maximal three days after the lesion. Both dexamethasone and cyclooxygenase inhibitors such as indomethacin and ibuprofen significantly ameliorated the effects of such trauma on local cerebral glucose utilization in the rat brain, independent of effects on cerebral oedema. Arachidonic acid release from membrane phospholipids was markedly increased in the lesioned area one minute after injury, but this release was not affected by either dexamethasone or indomethacin treatment. There was a sharp increase in $PGF_{2\alpha}$, PGE_2 and PGD_2, which was blocked by indomethacin but not by dexamethasone. Malondialdehyde formation as determined by the thiobarbituric acid reaction was unaffected by dexamethasone treatment. This effect on local cerebral glucose utilization can also be ameliorated by 5-HT depletion within the brain. To what extent this effect relates either to platelet or cortical depletion of serotonin remains to be explored.

With reference to ischaemic oedema, it is clearly important to distinguish between the changes occurring during complete ischaemia, following recovery and during incomplete ischaemia: arachidonic acid is released during complete ischaemia but is not converted to various eicosanoids until the circulation is partially re-established and oxygen is available for the biochemical reactions. With carotid occlusion in the gerbil and middle cerebral artery occlusion in the baboon, oedema increases with falling cerebral blood flow until at no flow in the gerbil there is no oedema formation at all. In the gerbil both dexamethasone and indomethacin pretreatment reduced oedema formation but only at flows of 5 to 7 ml/100 g/min. However, indomethacin and not dexamethasone reduced the rise in brain levels of $PGF_2\alpha$ following 1 hour of ischaemia. On reperfusion, as noted previously, there was an early rise in $PGF_2\alpha$ in brain which paralleled the changes in cytotoxic oedema (Bhakoo *et al.* 1984). Brain PGE_2 levels rose more slowly and mirrored the phase of vasogenic oedema. Bhakoo *et al.* (1984) suggest that FFAs released from damaged membranes may cause cytotoxic oedema in adjacent surviving cells and that FFA metabolites derived from the vascular compartment may contribute to blood-brain barrier damage and the extravasation of protein-rich fluid into the cerebral extracellular space. Clearly these findings are in apparent

contradiction to those of Chan and Fishman and of Wolfe's group and more work is required to resolve the disparity.

Gaudet and Levine (1979) found that dexamethasone did not reduce prostaglandin production during hyperaemia after release of carotid occlusion in the gerbil, but they also did not find that indomethacin changed specific gravity in their unilateral carotid occlusion gerbil preparation (Gaudet and Levine 1980). Clearly this evidence suggests either that prostaglandins are not involved in ischaemic oedema formation or that dexamethasone and indomethacin have very different mechanisms of action. Corticosteroids have a complicated range of actions on the arachidonic acid cascade, quite apart from their ability to inhibit phospholipase A_2 activity. They inhibit cyclooxygenase activity and enhance the activity of enzymes that metabolize prostaglandins, raise the activity of an endogenous cyclooxygenase inhibitor in serum, and accelerate the clearance of arachidonic acid (see Cranston *et al.* 1983 for references). Measurement of whole brain prostaglandin levels is a rather crude technique that says little about what is happening in individual compartments within the brain (vide supra). In the baboon, however, Harris *et al.* (1982) found that indomethacin did not reduce but actually increased ischaemic oedema in the MCA occlusion model. These water increases were significant for blood flows greater than 5 ml/100 g/min. However in the cat MCA model, Asano *et al.* (1984) suggested that indomethacin would reduce the oedema occurring on reperfusion but it had *no* effect on water accumulation during ischaemia.

Indomethacin alone at low doses in the gerbil (Iannotti *et al.* 1981) and at high dosage in the cat (Pickard 1980) produces an increase in brain water content without any prior induction of ischaemia. This increase in water content was not found in the baboon's normal hemisphere (Harris *et al.* 1982). One possibility for the increase in brain water, albeit species-dependent, is that free arachidonic acid may be shunted down the lipoxygenase pathway in the presence of indomethacin with the formation of leukotrienes (Higgs and Flower 1981). Harris *et al.* (1983) have examined this possibility. They found that the intracarotid infusion of LTC_4 without MCA occlusion in the baboon was associated with an insignificant change in water content. With MCA occlusion, a much greater accumulation of oedema occurred than in untreated animals. The antagonist of LTC_4 and D_4 (FPL 55712) had no effect on the degree of ischaemic oedema in this preparation, which is to some extent evidence against the notion that endogenous leukotriene formation has a part to play in the formation of ischaemic oedema. These authors have not yet reported the effects of combining this antagonist with indomethacin. FPL 55712 is not a particularly specific antagonist (Tagari *et al.* 1983).

An alternative strategy is to partly substitute eicosapentaenoic acid

(EPA) for arachidonic acid. EPA is a polyunsaturated fatty acid like arachidonic acid but has a higher degree of unsaturation and gives rise to PGI_3 and TXA_3. PGI_3, like PGI_2, inhibits platelet aggregation whereas TXA_3, unlike TXA_2, is a weak platelet aggregator (Bunting *et al.* 1983 for review). Phospholipids in the cell membrane of platelets from Greenland Eskimos contain 8% EPA compared with 5% in Danes. Eskimos have a low incidence of acute myocardial infarction, low blood cholesterol levels and an increased tendency to bleed (Dyerberg and Bang 1979). This delayed haemostasis may not necessarily reflect reduced platelet aggregability but impaired local vascular contractility after local injury (Begent *et al.* 1984). Dietary supplements of menhaden oil (17% EPA) reduced post-ischaemic hypoperfusion and brain water accumulation in gerbils subjected to bilateral common carotid ligation for 15 minutes with reperfusion (Black *et al.* 1984 a). However, intravenous EPA given immediately prior to occlusion and continued thereafter did not reduce brain water accumulation but did reduce post-ischaemic hypoperfusion (Black *et al.* 1984 b). Hence, reduction of ischaemic brain oedema by EPA may require incorporation of EPA into cell membranes which can only be achieved by long-term dietary supplements of EPA. Alternatively, other PUFAs may be involved: intravenous linoleic acid reduced brain water accumulation leaving CBF unaffected. The selective thromboxane synthetase inhibitor (U-63557A) had no effects alone in Black's gerbil model but potentiated the effects of intravenous EPA on CBF and not on oedema. U-63557A produced a small increase in brain levels of 6KetoPGF$_1\alpha$ (Black 1984 c).

Finally, Johansson (1981) has found that indomethacin pre-treatment significantly reduced protein extravasation into the brain after induction of hypertension with adrenaline but not after bicuculline seizures. This protective effect of indomethacin in adrenaline-induced hypertension is probably related to the vasoconstrictor effect of the drug. In bicuculline seizures, such an effect may be overruled by the metabolically induced cerebral vasodilation.

Cerebral Vasospasm

How far can excessive production of TXA_2, PGF_{2a}, or PGE_2, or defective synthesis of prostacyclin explain the cerebrovascular changes following subarachnoid haemorrhage (SAH)? The phenomenon of cerebral vasospasm and its paradoxes have been extensively reviewed (Wilkins 1980), and the various arguments are now becoming rather well honed. Cerebral arterial constriction visible on angiography in humans is most commonly seen following subarachnoid haemorrhage, but is also provoked by head injury and by meningitis. Any hypothesis that claims to account for delayed cerebral vasospasm following subarachnoid haemorrhage in humans must explain the following well-known observations. Such vaso-

spasm is seldom seen prior to 3 days after the bleed but becomes maximal around the end of the first week and declines thereafter. Its relationship to cerebral ischaemia and neurological deficit is capricious, a phenomenon that may reflect the ability of the individual cerebral circulation to accommodate to narrowed vessels; tissue ischaemia may only result when a further stress is applied such as a period of hypotension. The time course of cerebral vasospasm coincides with the risk of precipitating cerebral ischaemia with surgical intervention to clip the aneurysm. Early computed tomography (CT) scanning has revealed that the probability that any given artery will constrict increases with the thickness of the periarterial haematoma, but the relationship is not a simple one. Hence there is some relationship to one or more blood products, but human cerebral vasospasm is refractory to any known pharmacological blocker or synthesis inhibitor. Cerebral vasospasm may be the end result of interplay between the changing properties of the cerebral endothelium and vascular smooth muscle and the evolving pattern of vasoactive agents to which they are exposed. Vasospasm seems to reflect an exaggerated contractile response to vasoactive agents released when clot and arterial wall are in close apposition.

There remains considerable controversy in the literature as to how far these phenomena are reproducible, particularly in short-term experiments. Recently, new animal models have been developed in which delayed vasospasm may be achieved with features claimed to resemble those of the human condition (Espinosa *et al.* 1982, Liszczak *et al.* 1983, Peerless *et al.* 1982, Svendgaard *et al.* 1983). In all of these, sequential injections of blood are given with the objective of compartmentalizing and creating progressive entrapment of blood around the arteries.

In a dog model, inflammatory cells were present in the walls of arteries that had been spastic *in vivo,* and the adventitia was packed with red cells. Some medial smooth muscle cells appeared damaged (Liszczak *et al.* 1983). In patients dying after 4 weeks following SAH, Conway and McDonald (1972) noted increased blood pigments in arteries that had been spastic in life, and there was marked subintimal thickening by fibroblastic tissue. Hughes and Schianchi (1978) reported that in 12 patients with vasospasm who died within 3 weeks of SAH, inflammatory cells were present in the adventitia. In the tunica media, some smooth muscle cells appeared necrotic and there were numerous "macrophage-like" cells. Concentric subintimal thickening was the most striking abnormality in patients who died after 3 weeks, but it is not accepted universally that such changes are specific to cerebral vasospasm. A consensus is emerging that simple instillation of blood into the basal cisterns by a single injection does not cause histological change, except for round-cell infiltration of the outer layers of the artery; nor does it produce delayed vasospasm reliably (Smith *et al.* 1983).

Clotted blood releases an array of cerebral vasoconstrictor substances,

including 5-HT, TXA_2 and uridine triphosphate (UTP). Most of these, as well as fresh whole blood, platelets, and platelet extracts, have been shown to induce early acute vasospasm when injected intracisternally, or washed topically onto exposed basilar arteries. Although "platelet products" have been proposed as candidates for provoking cerebral vasospasm, most of these compounds are released immediately, and the characteristic feature of vasospasm is its delayed onset. Osaka *et al.* (1980) comment that platelets in SAH blood clot are devoid of 5-HT. White *et al.* (1980) observed that injection of thrombin into the cisterna magna of dogs induced spasm that was slower in onset but had a more sustained action than platelets (*i.e.,* longer than the 3 hours of their experiment). Since thrombin is released from fibrin during clot lysis (White *et al.* 1980), this is one blood clot derivative that is generated at the time of maximal vasospasticity. Prolonged incubation of clotted blood has been shown to result in the production of derivatives of haemoglobin that in high concentrations are vasoconstrictors (Wilkins 1980 for review). Haemoglobin stimulates PG synthetase and antagonises the inhibition of PG synthesis by aspirin and indomethacin. Haemoglobin also blocks non-adrenergic, non-cholinergic inhibitory neurotransmission in vascular smooth muscle including cerebral arteries, thereby potentiating neurogenic vasoconstriction (Bowman and Gillespie 1983, Lee 1984). Fibrin degradation products are vasoconstrictors and are another possibility (Forster and Whalley 1981).

The prostaglandin hypothesis of cerebral vasospasm arose in the early 1970s from the observation that $PGF_2\alpha$ causes vasoconstriction of cerebral vessels (White and Hagen 1982 for review). Both clotted blood and various intracranial tissues could act as sources for these vasoactive lipids. In particular, TXA_2 synthesis by aggregating platelets causes intense constriction of cerebral arteries *in vitro*. However, such synthesis occurs immediately and is unlikely to explain delayed vasoconstriction (which does not appear for some 3 days), particularly since TXA_2 has an extremely short half life (about 30 seconds). The simplistic form of the prostaglandin hypothesis supposed that prostaglandins, derived either from the brain itself or from the surrounding blood clot, precipitated contraction. The delayed contraction might be explained if release of other substances such as various biogenic amines, thrombin, or haemoglobin derivatives secondarily stimulated synthesis of prostaglandins in surrounding tissues or in the vessel wall itself (Pickard *et al.* 1975). For example, injection of thrombin into the subarachnoid space provokes constriction of the cerebral arteries in the dog, associated with a rise in the CSF levels of $PGF_2\alpha$ and PGE_2 (Hagen *et al.* 1977). Thrombin-induced contraction of isolated canine cerebral arteries is sustained and can be antagonized with both prostacyclin and the cyclooxygenase inhibitor meclofenamic acid (White *et al.* 1980). Hence, White has suggested that one

possible mechanism for delayed vasospasm is the release of thrombin from fibrin as the clot resolves, with subsequent stimulation of prostaglandin synthesis. This insult might be further compounded by cerebral ischaemia precipitating further eicosanoid synthesis, and subsequent potentiation of the effects of vasoactive amines.

There is considerable conflict between reports of the effect of cyclo-oxygenase inhibition and haemoglobin-induced contractions of cerebral arteries. Tanishima (1980) found that aspirin (10^{-5} M) had no effect on Hb contractions whereas Linder and Alksne (1978) found that high and non-specific doses of aspirin (17×10^{-3} M) inhibited whole blood contractions in dog cerebral arteries. Okamoto *et al.* (1984) found that a more selective dose of aspirin (5×10^{-5} M) attenuated haemolysate contractions of dog basilar arteries. Furthermore, studies on rat stomach strips exposed to superfusate of dog cerebral arteries showed release of PG-like substance by the haemolysate application. Indomethacin (10^{-6} g/ml) relaxed isolated bovine middle cerebral arteries and did not affect the response to fresh rabbit blood. However indomethacin significantly increased relaxation of the strips, after the blood had been washed away (Pickard *et al.* 1975). Indomethacin (10^{-5} M) considerably reduced the haemolysate-induced contraction of Guinea-pig basilar arteries (Fujiwara *et al.* 1984). OXY 1581 (TX synthetase inhibitor) had no effect. In contrast, Brandt *et al.* (1982) found that the contractile effect of haemorrhagic CSF was augmented by indomethacin. The whole blood contractions of rabbit cerebral arteries were potentiated by 20 mM aspirin and inhibited by dipyridamole (Linder 1983). Some of these differences may reflect species differences and the use of different cyclo-oxygenase inhibitors of different specificities.

At sites of cerebral vasospasm that are mechanically induced, white thrombi can be seen forming on the disrupted endothelial surface. Some of the small cerebral infarcts in patients dying of SAH, noted originally by Crompton (1964), might be consistent with platelet embolization from such thrombi. Such intravascular platelet aggregation might generate further TXA_2 release, leading to further cerebral arterial contraction and possibly endothelial disruption. With discovery of prostacyclin came the concept that part of the sequence of events precipitating cerebral vasospasm might be defective synthesis by the endothelium of prostacyclin. Atherosclerotic and hypertensive patients are at greater risk from cerebral vasospasm following aneurysmal rupture, and it might be relevant that, with experimental atherosclerosis, the fall in prostacyclin production occurs before any detectable anatomical damage (Boullin 1980, Dembinska-Kiec *et al.* 1977).

Few measurements have been made of intracranial prostanoids after subarachnoid haemorrhage. There are early reports of increased levels of $PGF_2\alpha$ in CSF following subarachnoid haemorrhage, and more recently two groups of workers have suggested that cerebral arterial generation of

prostacyclin *in vitro* is impaired following SAH (Maeda *et al.* 1981, Sasaki *et al.* 1981 a). Our own group has obtained quantitative data on changes in CSF prostaglandins and TXB_2 following SAH in both humans and dog and has compared the results with the *in vitro* production of these same eicosanoids by cerebral cortex, choroid plexus, and cerebral arteries from the dog (Walker *et al.* 1983). SAH results in a marked increase in CSF levels of $PGF_2\alpha$, PGE_2, and 6-keto-$PGF_1\alpha$ in both species, with a proportionately greater increase in PGE_2. In lumbar CSF collected from 5 patients 6 to 9 days after SAH, all prostanoids were increased, but compared with other metabolites, TXB_2 levels were relatively low. However a much higher value of TXB_2 was recorded in a sample collected within 24 hours of the bleed. Further unpublished work has shown that the prostaglandin concentrations in cisternal and lumbar CSF are high after SAH, but samples from ventricular CSF from patients at a similar stage have lower concentrations, indicating that prostaglandins are added to the CSF as it perfuses the subarachnoid space. The *in vitro* production of these prostaglandins and of TXB_2 by dog cerebral cortex and choroid plexi was not affected by SAH, but limited data suggested enhancement of PGE_2 synthesis by the larger cerebral arteries. The combined concentrations of the two cerebral vasoconstrictors $PGF_2\alpha$ and PGE_2 were around 2 nmol/l in cisternal CSF at 3 days and 0.5 nmol/l at 7 days following SAH in the dog, and around 3 nmol/l in lumbar CSF at 1 week in humans. These levels are not sufficient to cause significant contraction of cerebral arteries either from humans or dog *in vitro*. The spectrum of eicosanoids seen in CSF after subarachnoid haemorrhage does not fit with a simple contribution by clotted blood, particularly as the level of TXB_2 was so low. Ventricular samples from patients with hydrocephalus without SAH generally have very low PG levels (V. Walker and J. D. Pickard, unpublished observations), and from this it can be deduced that delayed clearance of the prostanoids from the subarachnoid space by an arachnoid granulation block is unlikely to have been the primary cause of the changes noted. The finding of enhanced PGE_2 synthesis by isolated cerebral arteries following SAH (Maeda *et al.* 1981, Walker *et al.* 1983) might reflect an abnormal arterial response to SAH. Consistent with these findings, the *in vitro* contractile reactivity of cerebral arteries taken from dogs is altered by subarachnoid haemorrhage—the response to amines is enhanced, but the response to pH changes and to sodium loading is depressed (Pickard and Perry 1984). We have examined the pattern of intraarterial and extraarterial prostaglandin production by perfused common carotid arterial segments *in vitro* taken both from control animals and from rabbits in which the arteries have been surrounded with a blood clot enclosed in a polyvinyl chloride cuff for one week. Histologically the adventitia of these latter vessels was infiltrated with red cells and inflammatory cells, particularly macrophages (Pickard *et al.* 1984). In these

studies, the late (third and fourth hours of perfusion) extraarterial release of PGE_2 was considerably increased from arteries that had been surrounded by a blood clot and sheath for 7 days previously. Significant increases were also observed for $PGF_2\alpha$ and TXB_2, but the levels attained were considerably lower than those of PGE_2. Such enhanced PGE_2 production may represent direct synthesis by inflammatory cells, particularly activated macrophages (Goetzl 1981), or may be the response of the arterial smooth muscle to the presence of such macrophages. On recognition of a phagocytic stimulus, both neutrophils and macrophages experience a respiratory burst characterized by increased oxygen consumption and release of highly reactive oxygen-free radicals and lysosomal proteases into the external environment (Fantone and Ward 1982). Stimulus-elicited macrophages produce more metabolites than the resident population, and activation of macrophages leads to a marked increase in production (Fantone and Ward 1982). Theoretically there are at least three ways in which macrophage products might stimulate PGE_2 production by adjacent arterial cells: a) lysis of clot by released proteases would increase the local concentration of thrombin, with the consequences described by White and Hagen (1982); b) oxygen-free radicals, by causing lipid peroxidation, may damage muscle cell membranes and trigger prostaglandin production—PGE_2 is a major product of arterial smooth muscle cells in tissue culture (Larrue *et al.* 1981); or c) macrophages may release a peptide that stimulates PGE_2 production by adjacent cells, as has been proposed for a hydronephrotic kidney model in rabbits (Okegawa *et al.* 1983). In proposing a role for inflammatory cells in vasospasm, it is of interest that the timing of spasm closely parallels the clearance of red cells from the CSF: the major route of red-cell removal is lysis with subsequent phagocytosis (Laurent 1980, Osaka *et al.* 1980). However, there remains considerable controversy over whether early removal of subarachnoid blood actually prevents the development of delayed vasospasm. Certainly, the results of early surgery in the hands of exponents such as Ljunggren *et al.* (1985) are to commended but controlled trials remain to be performed.

TXA_2 is released in large amounts during platelet aggregation and is a potent cerebral vasoconstrictor. Using our *in vitro* carotid artery perfusion model. we have shown that blood allowed to clot around the artery released TXB_2, which diffused out into the perfusing buffer. Similarly, when fresh blood was added to human CSF *in vitro* (to give approximately 300,000 red cells/mm^3 CSF, as in a moderately severe subarachnoid haemorrhage), and incubated for 72 hours at 37 °C, TXB_2 was produced in approximately 10 times the quantity of the other three prostanoids (Walker *et al.* 1983). In lumbar CSF collected from a patient within 24 hours of a bleed, TXB_2 was present in high concentration, whereas the concentration of TXB_2 was relatively low in lumbar CSF samples collected 5 days or more after SAH in

patients, and in cisternal CSF at 3 to 7 days following SAH in dogs (*vide supra*). White and Robertson (1983) have shown that a series of non-steroidal anti-inflammatory drugs (piroxicam, meclofenamate, ibuprofen, and aspirin) given intravenously to anaesthetized dogs 30 minutes before and 3 hours after the intracisternal injection of blood reduced the occurrence of vasospasm and behavioural change associated with such an injection as assessed up to 24 hours. However, prostacyclin infused via the vertebral artery failed to affect the vasospasm present 24 hours after intrathecal blood. Quintana *et al.* (1982) found that prostacyclin (50 ng/kg/min) inhibits the immediate vasoconstriction produced by hourly application of oxyhaemoglobin to the exposed basilar artery of the cat. Fukumori *et al.* (1983) showed that intravenous prostacyclin (25–75 ng/kg/min) or indomethacin (4 mg/kg) or both had no effect on angiographic delayed vasospasm produced *in vitro* in the canine basilar artery by cisternal blood. These *in vivo* findings are in contrast to the well described relaxant effect of prostacyclin on haemorrhagic CSF contraction of cerebral arteries (Brandt *et al.* 1982). In contrast, Chan *et al.* (1984) found that angiographic delayed vasospasm in rabbits was attenuated by prostacyclin and carbacyclin but with no change in CBF. OKY 1581 (TX synthetase inhibitor) and nutra lipid (precursor of EPA) given just after SAH both abolished angiographic vasospasm at 3 days and increased cerebral blood flow. Chyatte *et al.* (1983) found that ibuprofen reduced vasospasm in dogs after two SAH's. *In vitro* contractility was depressed in cerebral arteries from these dogs and ibuprofen was reported to improve such contractility and prevent myonecrosis. Sasaki *et al.* (1982) have demonstrated that the thromboxane synthetase inhibitor OKY 1581, administered immediately after the injection of subarachnoid blood into the dog and continued until sacrifice 4 days later, considerably reduced the degree of spasm during that period of time. However Fukumori *et al.* (1984) found that angiographic vasospasm was not reversed nor mean regional CBF increased significantly after intracisternal injection of fresh blood into dogs by infusing two TXA_2 synthetase inhibitors intravenously for 2 hours. In a pilot study in patients following SAH, OKY 1581 produced a suggestive but statistically insignificant improvement in post-operative vasospasm, ischaemic symptoms and outcome (Tani *et al.* 1984). The tentative conclusion must be that platelet-derived factors, including TXA_2, are important in the early hours after a bleed but contribute little to the maintenance of delayed vasospasm. Acute liberation of large amounts of platelet spasmogens as a result of rebleed may precipitate or exacerbate spasm in arteries whose walls have been "sensitized" by a bleed some days earlier.

Finally it has been suggested that lipid peroxides formed by free-radical reactions initiated by hypertension, trauma, or clot lysis, damage the vascular endothelium and act as specific inhibitors of prostacyclin syn-

thetase in the vessel wall (Kontos *et al.* 1980, Sasaki *et al.* 1979, 1981 b). Kontos and co-workers have shown that acute hypertension, induced either by experimental brain injury or by the intravenous administration of vasoconstrictor agents, causes discrete destructive lesion in the endothelial lining of the cat pial arterioles. After the hypertensive episode, these arterioles display sustained vasodilation and reduced responsiveness to hypercapnia and a change in arterial blood pressure. All these abnormalities are reduced by treatment with cyclooxygenase inhibitors and by topical application of free-radical scavengers to the brain's surface. Topical application of arachidonic acid and PGG_2 to the brain's surface induces similar cerebral arterial damage and vasodilation that is inhibited by scavengers of oxygen-free radicals. Kontos suggests that the mechanism of the arterial abnormalities from acute hypertension involves a sudden increase in prostaglandin synthesis that leads to generation of oxygen-free radicals. In patients with subarachnoid haemorrhage and cerebral vasospasm, there is significant elevation of lipid peroxides in the CSF, but not to levels which would account for cerebral arterial constriction (Asano *et al.* 1980). Unlike cat pial arterioles *in vivo,* arachidonic acid produces vasoconstriction of the basilar artery of the dog *in vitro* and *in vivo,* mouse pial arterioles *in vivo,* and of cat MCA *in vitro* (Table 1). Sasaki *et al.* (1981 b) have shown that the injection of 15-hydroperoxyarachidonic acid into the subarachnoid space of dogs produced long-lasting cerebral vasoconstriction, possibly due to inhibition of prostacyclin synthesis within the vessel wall. As discussed previously, there is considerable controversy over whether ultrastructural changes can be seen within the cerebral arterial wall of canine vessels within 1 week of a single injection of blood into the cisterna magna. However, Sasaki *et al.* (1982) described changes in the endothelium and tunica media 3 days after subarachnoid haemorrhage and found that the thromboxane synthetase inhibitor OKY1581, whilst reducing the incidence of vasospasm, had no effect on the ultrastructural changes. They suggest that the vasoconstriction and ultrastructural changes are independent and that OKY1581 blocks the former and not the latter: OKY1581 has no radical scavenging action.

An antioxidant (AVS) reduced the incidence of chronic, angiographic vasospasm in the dog (Asano *et al.* 1984). However, Wellum *et al.* (1982) and Okamoto *et al.* (1984) found that haemoglobin-induced vasoconstriction cannot be blocked by superoxide dismutase or other agents known to react with superoxide-generated products.

In summary, there are marked abnormalities in intracranial eicosanoid metabolism following subarachnoid haemorrhage, but more refined techniques will be required to define the changes occurring within various compartments and their overall significance. Furthermore, there is a paucity of well-controlled clinical trials which are sufficiently large for

statistical validity along the lines of those recently reported for antifibrinolytic therapy (Vermeulen *et al.* 1984—479 patients) and for dipyridamole (Shaw *et al.* 1985—677 patients). Neither study showed any difference in overall outcome with drug therapy: tranexamic acid increased the infarction rate whilst reducing the rebleed rate. Given the interest in calcium antagonists for the prevention of cerebral ischaemia after SAH (Allen *et al.* 1983) it may be relevant that verapamil inhibits platelet aggregation and produces a dose dependent fall in TXB_2 production during clotting (Mehta *et al.* 1983, Uotila and Dahl 1984).

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B. Technical Standards

Selective Amygdalo-Hippocampectomy* Operative Anatomy and Surgical Technique

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With 16 partly colored Figures

Contents

1. Introduction

The development in Zurich of selective amygdalo-hippocampectomy as a means of treating certain forms of epilepsy which were not necessarily related to gross structural lesions came about as a result of two main influences.

The first related to the surgical techniques previously developed here for

* Amygdalo-hippocampectomy indicates unilateral surgical excision of the amygdaloid body, hippocampal body and parahippocampus extending from the hippocampal fimbriae to the collateral sulcus.

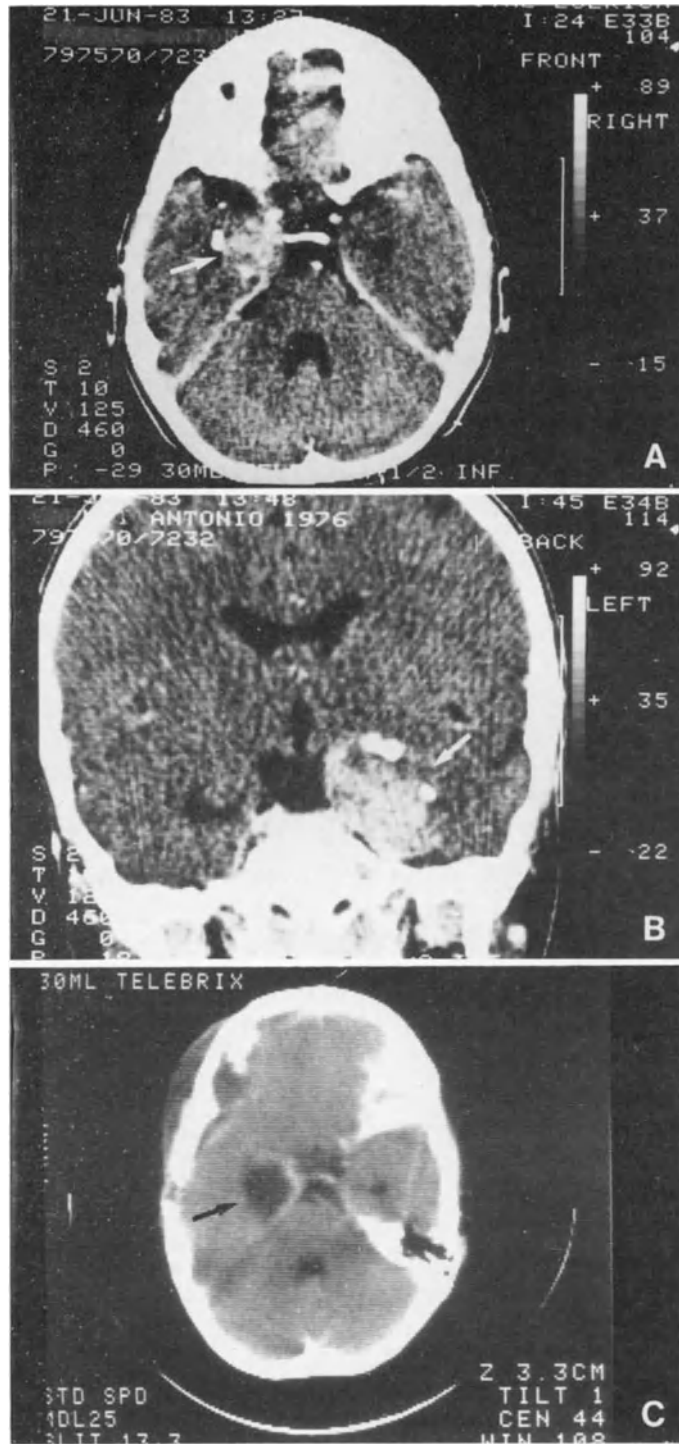

Fig. 1. A) Ganglioglioma of the left amygdala seen on CT (arrow) causing typical temporal epilepsia to a 7-year-old male patient. B) AP view of the ganglioglioma of the left amygdala (arrow). C) Postoperative CT demonstrates the tumour bed and removed amygdaloid body (arrow)

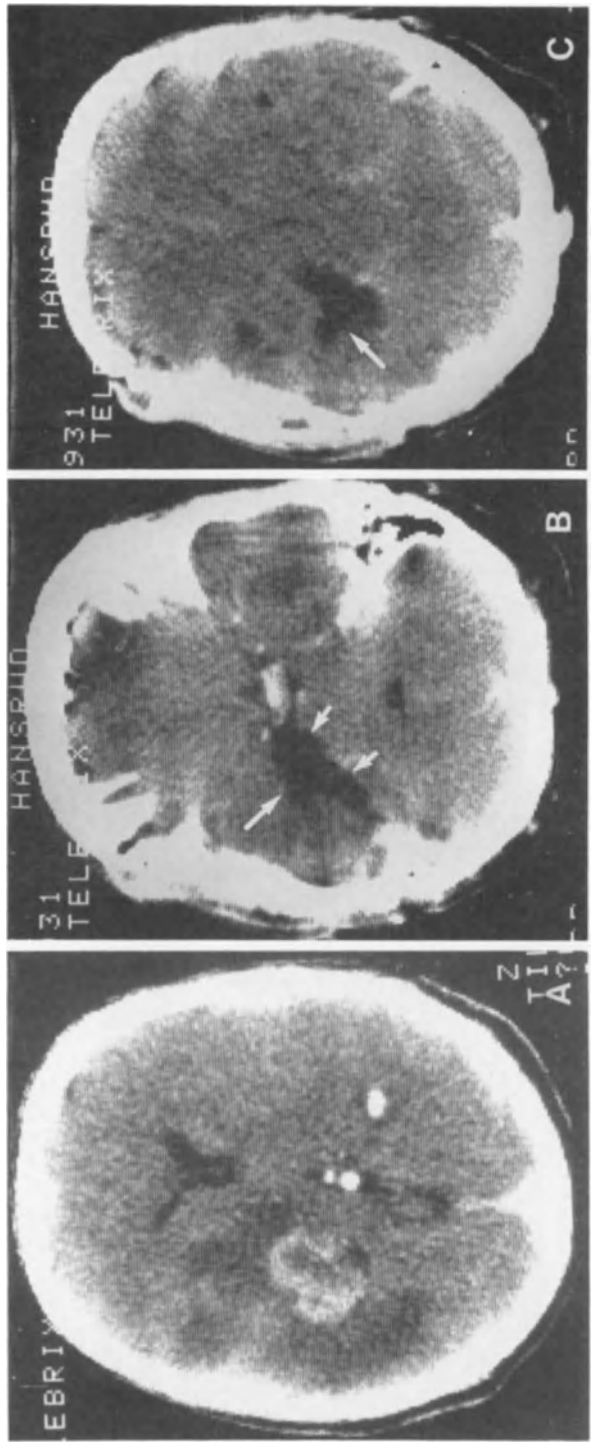

Fig. 2. A) Astrocytoma of the left medial temporal lobe with large hypodense areas around the tumour. Fifty-one-year-old male patient. B) Postoperative CT demonstrates the removal of the tumour and resultant amygdalo-hippocampectomy (arrows). C) Extension of the removal in supero-posterior area (arrow)

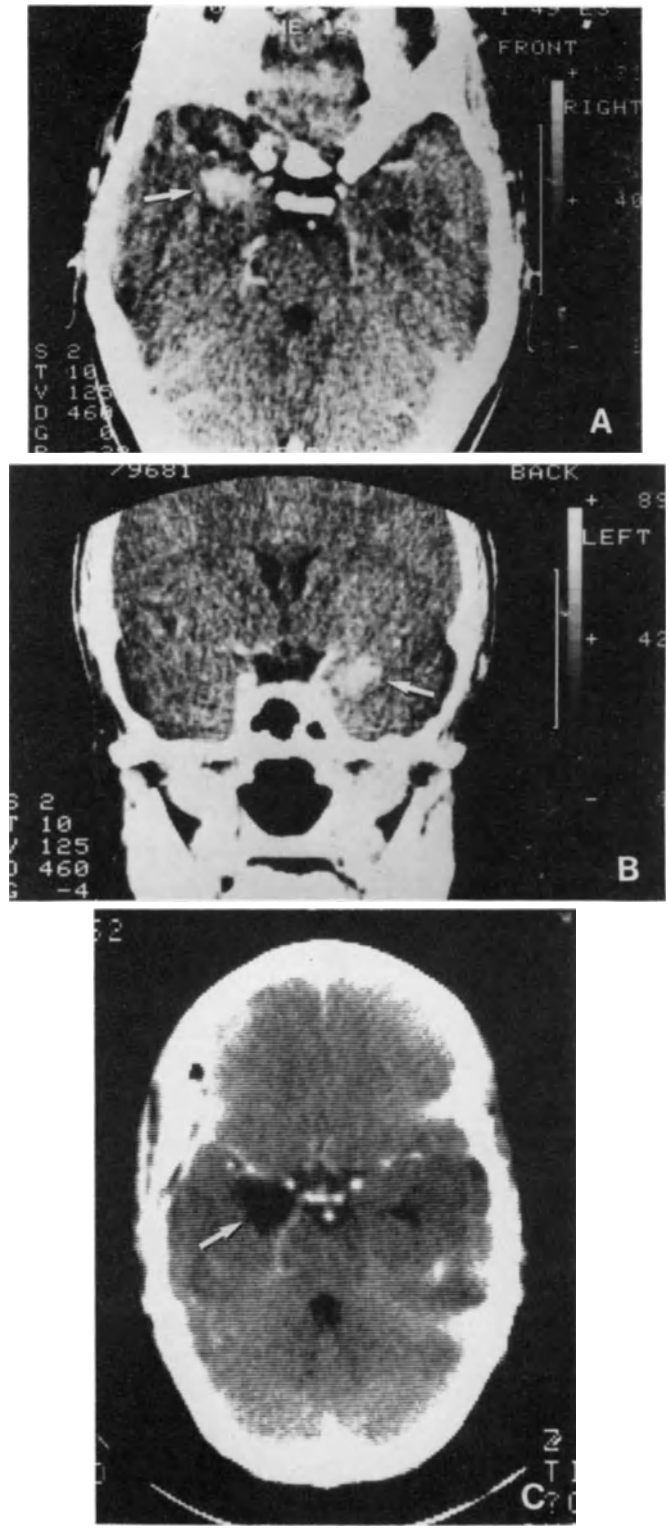

Fig. 3. A) Appearance of an unruptured AVM of the left amygdala (arrow) in a 22-year-old female patient with limbic seizures. B) AP view (arrow). C) Post-operative CT demonstrates removal of the AVM and selective amygdalo-hippocampectomy (arrow)

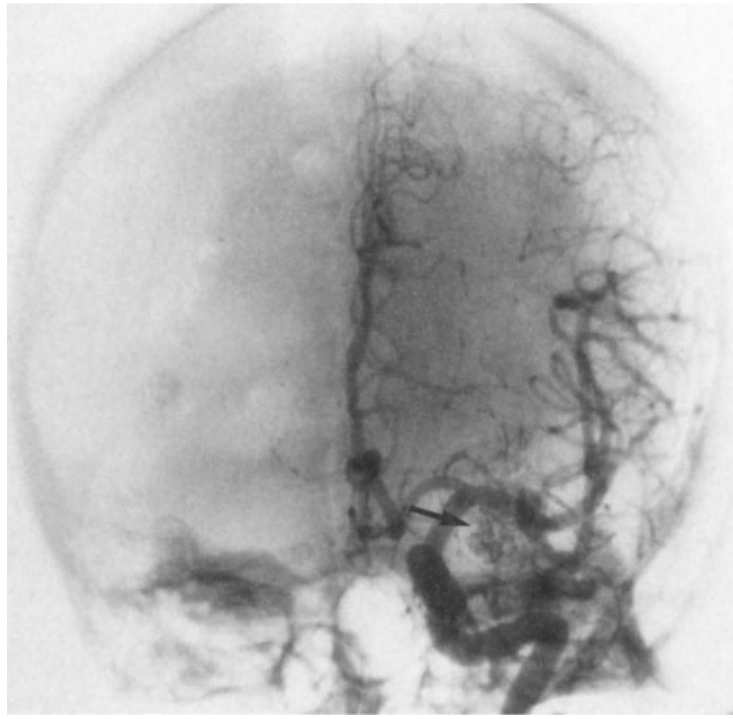

Fig. 3 D. Left carotid angiography visualizing the AVM (arrow)

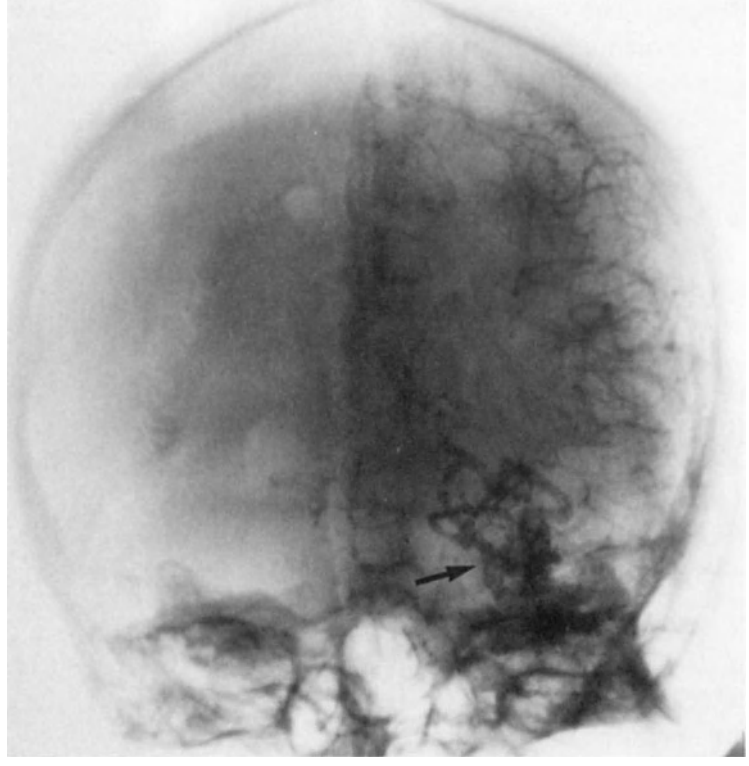

Fig. 3 E. The venous phase of the angiogram and AVM (arrow)

tumour and AVM resection in these areas. We had previously found that gangliogliomas, ganglioneuromas, astrocytomas and arteriovenous malformations confined to the mediobasal temporal lobe could be effectively extirpated without recourse to lobectomy. Procedures such as removal of sphenopetroclival meningiomas helped to promote the more complex operations through the development of optimal means of opening the Sylvian fissure.

Secondly, we had noted that local removal of mediobasal temporal tumours and AVMs had been attended by fewer post-operative fits if the amygdala and parahippocampus were resected simultaneously. Dr. Wieser pursued and developed this observation and line of thought and proposed the selective procedure as an alternative treatment to lobectomy in certain fully investigated and well defined cases of refractory idiopathic mesiobasal limbic epilepsy[13]. In such cases, the investigative procedures have become so complex that the surgeon must accept a secondary role and must be guided by his neurophysiological colleagues as to which patients are suitable candidates for surgery.

The removal of the amygdala and parahippocampus may be performed by various approaches, usually the transcortical (Niemeyer 1958) or via the anterior portion of the superior temporal sulcus or the subtemporal approach. The transcortical and sulcal approaches produce unavoidable cortical injury. The subtemporal lateral approach is feasible but requires excessive retraction. In our experience, many nontumoural temporal epilepsy patients were found to have herniated parahippocampal gyri, thus compounding the difficulties of the subtemporal approach.

The development of the pterional craniotomy and its various refinements for aneurysm surgery, provides familiarity in exposing the proximal Sylvian fissure just above the limen insulae. The trans-Sylvian route was readily adapted for exploration of the mediobasal superior temporal lobe with approach to the amygdala and parahippocampus. However, it must be emphasized that this approach is extremely confined, the incision is at maximum 2 cm in length. Considerable microsurgical experience is necessary before undertaking this form of surgery and, above all, a familiarity with the regional anatomy is required. This can be gained only by meticulous cadaver microdissection until the operator is thoroughly acquainted with the extent and relationships of the structures he is likely to encounter.

An illustrated description of those anatomical features of special importance to surgical dissection in this area is incorporated within the account of operative technique for amygdalo-hippocampectomy. The description is based not only upon the operative experience of the senior author (MGY) but also upon cadaver dissections carried out by the authors using the operating microscope.

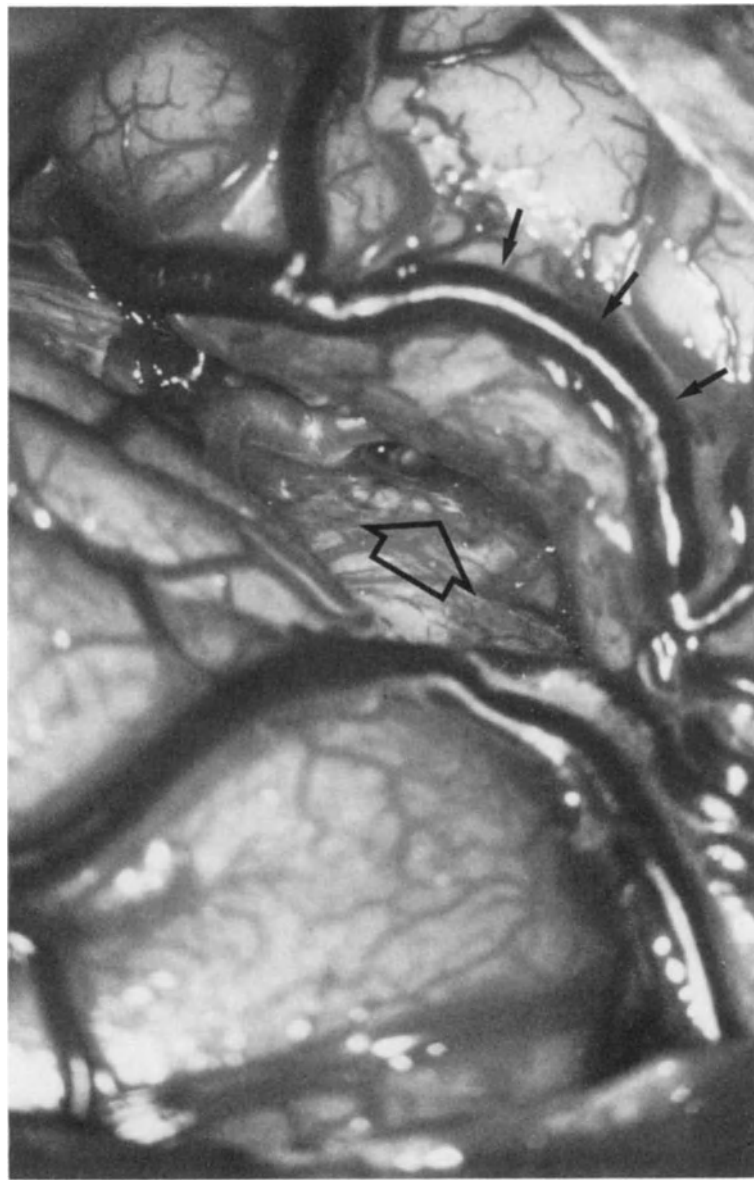

Fig. 4 A. Exploration of the right Sylvian fissure medial to the Sylvian vein (small arrows). The incision (arrow) is in the inferior portion of the insular sulcus, just medial to the superior temporal gyrus and lateral to the inferior trunk of the middle cerebral artery

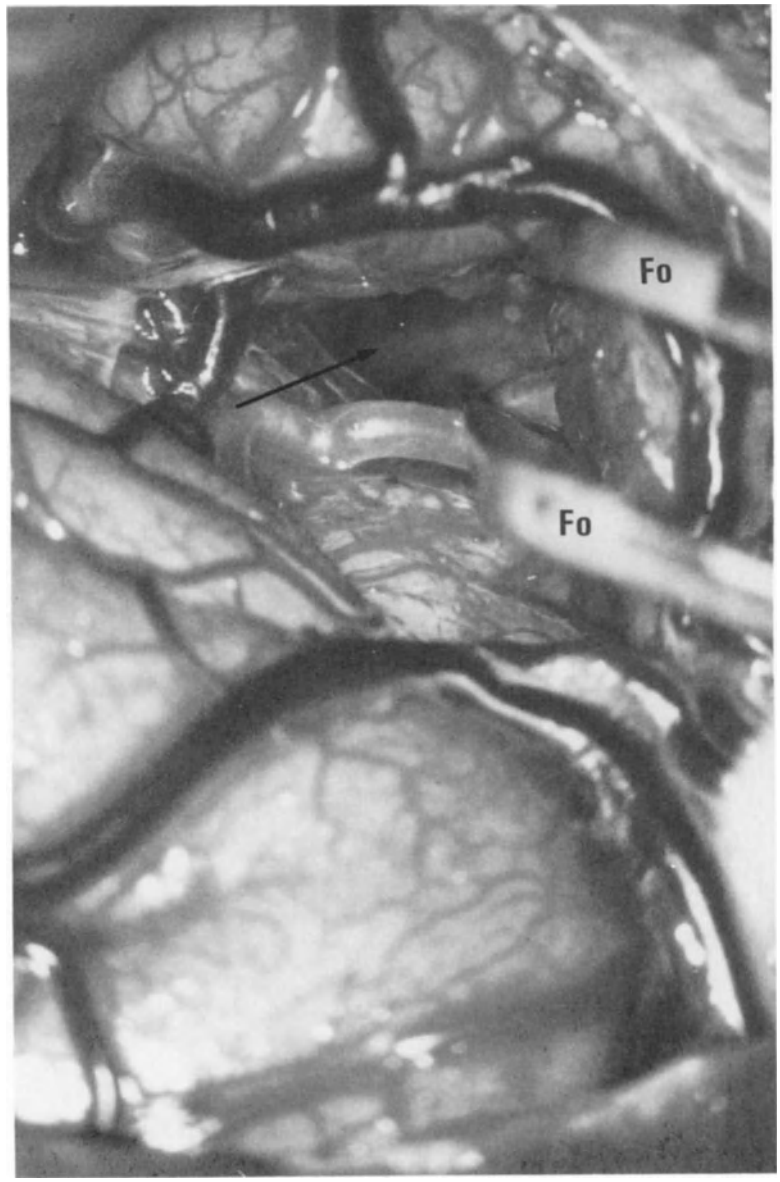

Fig. 4 B. Between the tips of the forceps (*Fo*) the small incision (arrow) is demonstrated. The body of amygdala is removed

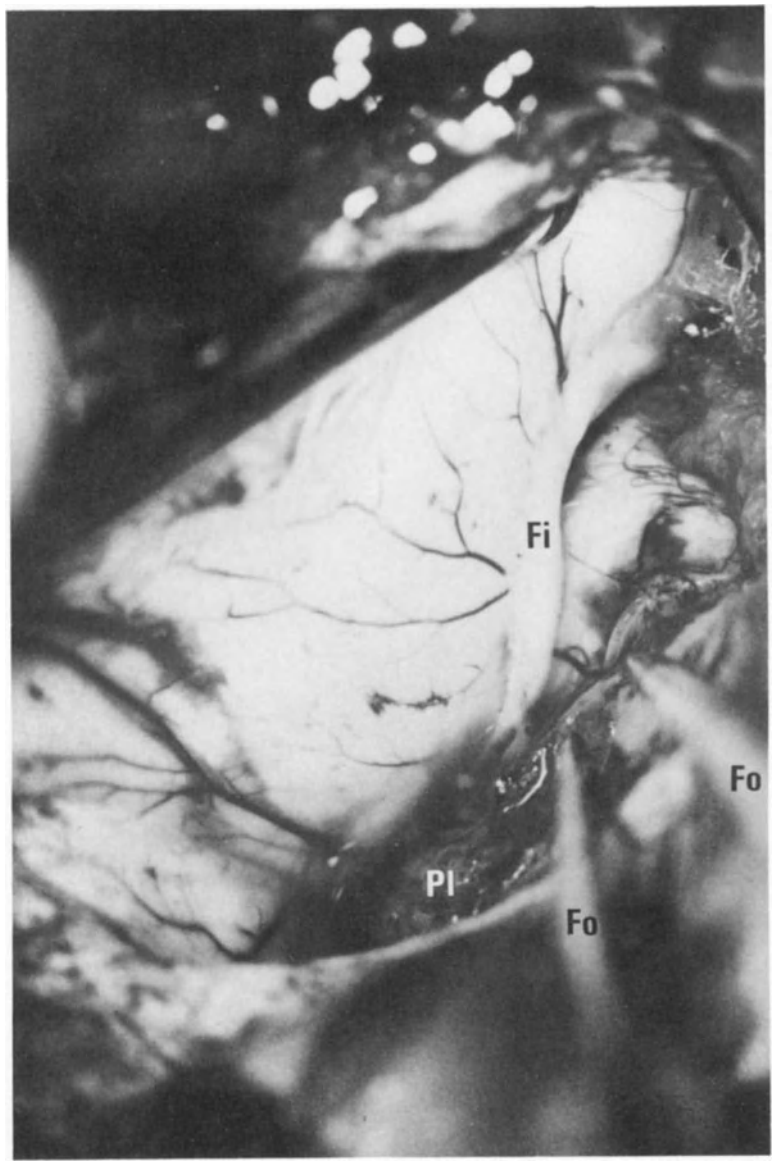

Fig. 4C. View into the temporal horn demonstrating the amygdalo-hippocampal surface from the medial aspect. Demonstration of the hippocampal fimbriae (*Fi*) and choroid plexus (*Pl*), forceps tips = *Fo*

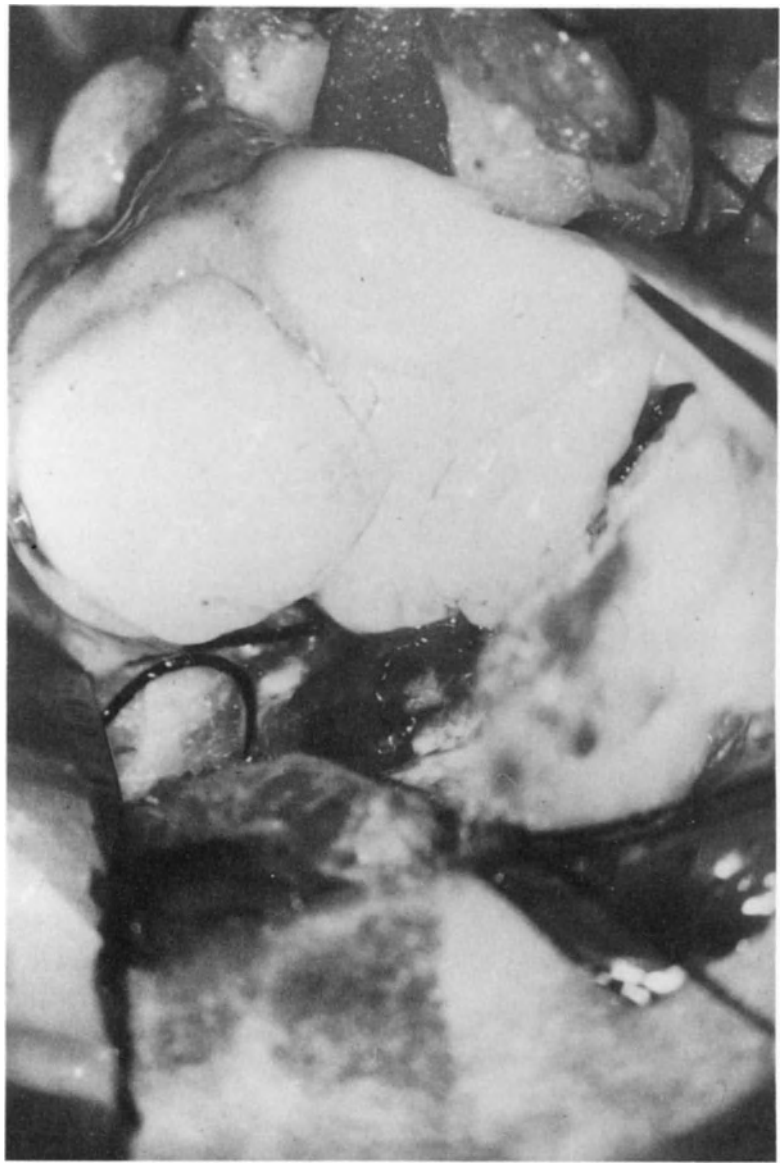

Fig. 4 D. Upon completion of amygdalo-hippocampectomy visualization of those structures in cross-section

Fig. 4 E. View into the ambient and crural cisterns following selective amygdalo-hippocampectomy. Vascular anatomy demonstrated is P-2 segment (*P2*), anterior choroidal artery with branches (arrows). Also seen is the III nerve superiorly (*III*) and the optic tract inferomedically (*Op*). The basilar vein is partially hidden beneath the anterior choroidal artery

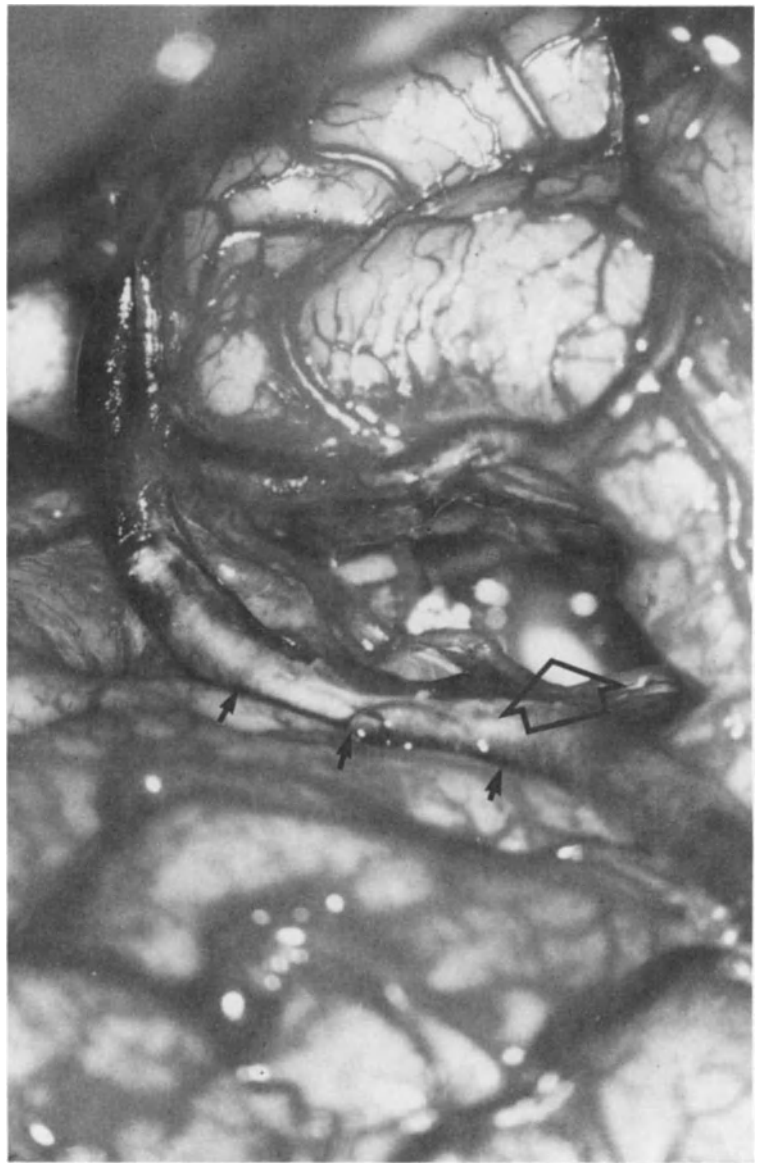

Fig. 5 A. In another case the exploration (arrow) is performed lateral to the Sylvian vein (small arrows)

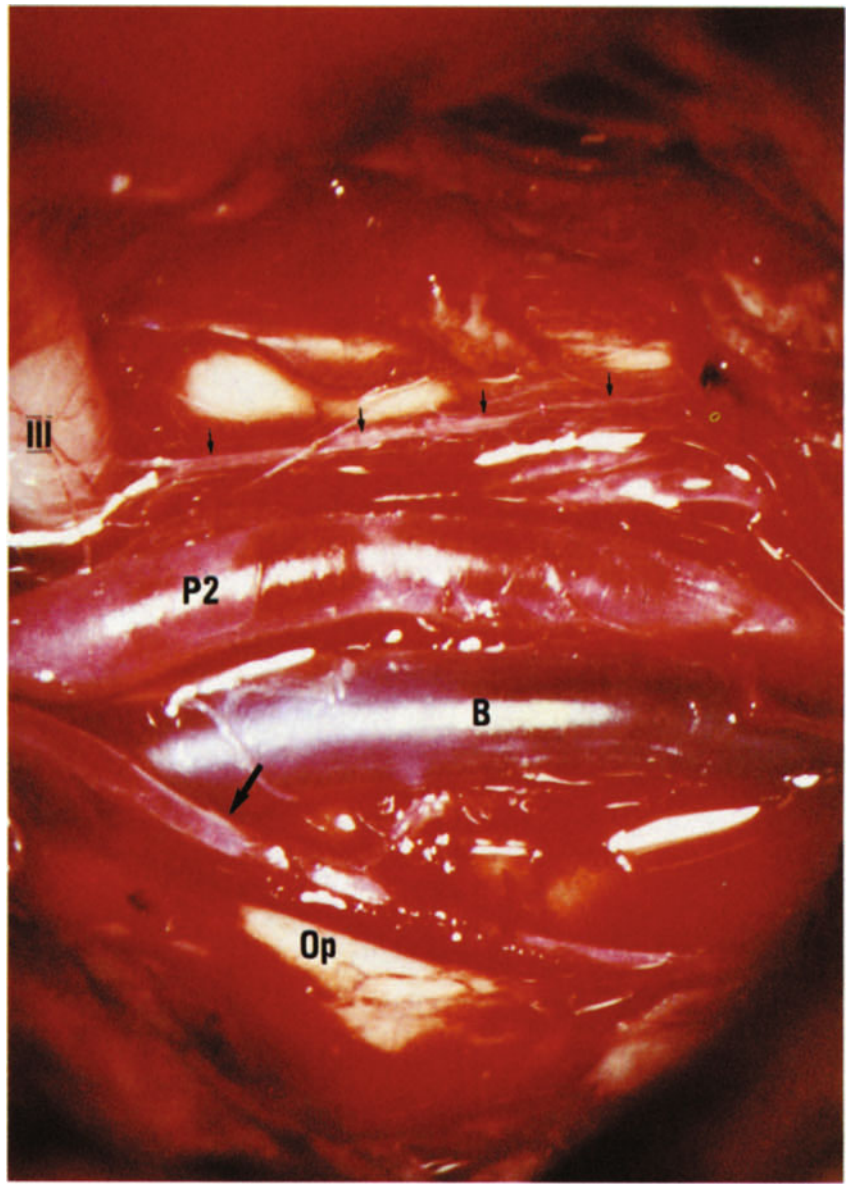

Fig. 5 B. The basilar vein (*B*) is demonstrated between P-2 segment (*P2*) and anterior choroidal artery (arrow). Also the III nerve (*III*) and the optic tract (*Op*) are seen. Tiny nerve fibers originating from the lateral peduncle (small arrows), crossing the ambient cistern, and joining the III nerve

2. Operative Technique

Craniotomy

The approach used in the operation is a variation of the now standard interfascial pterional craniotomy described previously[14]. The patient is positioned supine with the head directed about 20° vertex down, slightly elevated, and rotated about 30° to the unoperated side thus bringing the malar eminence to the superior point of the operating field. A Mayfield-Kees three point fixation devise is employed with the single prong behind the ear and placed such that none of the prongs enters the temporalis muscle which could lead both to instability and haemorrhage.

The skin incision is placed 2 cm more posteriorly than is customarily described. The craniotomy correspondingly enlarged over the temporal lobe, exposing the anterior one third of the superior temporal gyrus.

A lumbar intrathecal catheter, for perioperative drainage of CSF, is inserted in those patients in whom there is no evidence of intracranial tumour. Small dural vents are cut along the frontal and temporal portions of the proposed dural incision. This releases CSF and, together with the lumbar drainage helps to minimize subsequent brain retraction. Using a high speed electric drill under the operating microscope the posterolateral orbital roof and posterior ridge of the greater wing of the sphenoid are completely flattened down to the anterior clinoid process. Bleeding from branches of the orbitomeningeal arteries can readily be controlled with bipolar coagulation, bone wax and small pieces of muscle inserted between the bone and dura.

Inadvertant opening of the frontal sinus in this region is dealt with by mucosal stripping and sealing the bony defect with muscle, gelfoam, acrylic adhesive and finally bone wax.

Dural Opening

The dura is opened in a semicircular fashion above the Sylvian fissure and arched toward the sphenoid ridge and orbit. The dural vessels are coagulated using bipolar forceps. The temporal end of the dural incision is protected with a through and through suture to prevent tearing toward the floor of the middle fossa. The dural flap is reflected over the sphenoid ridge. The remaining dural edges are stitched through drill holes in the bony margins to prevent extradural bleeding during the procedure.

Opening the Arachnoid

Very gentle retraction is applied to the frontolateral orbital aspect of the frontal lobe to demonstrate the carotid cistern. The latter is usually opened between the optic nerve and internal carotid artery using a round-bladed

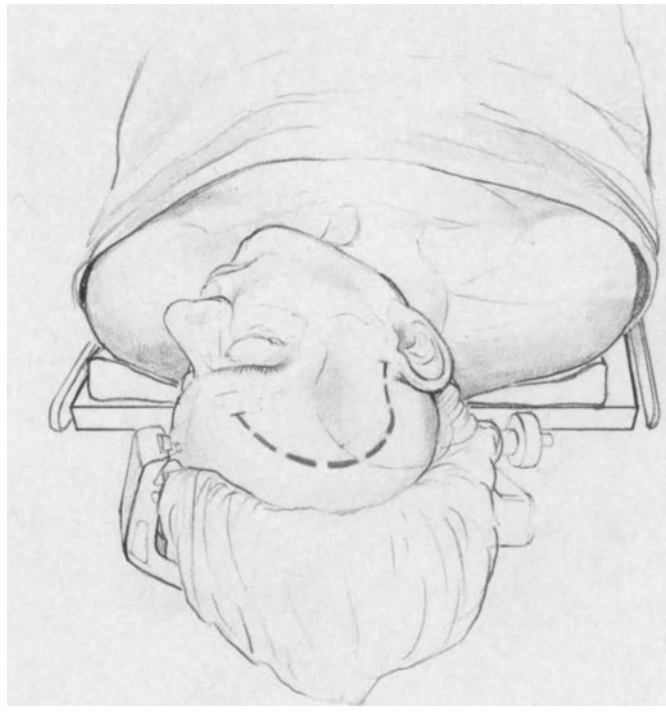

Fig. 6 A. Position of the head and location of skin incision

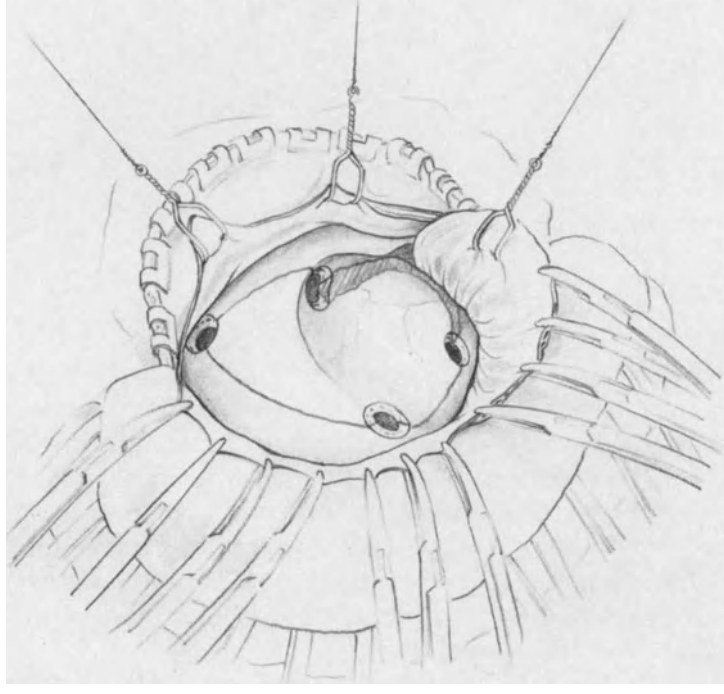

Fig. 6 B. Enlargement of standard pterional craniotomy exposing the superior temporal surface

tenotome. This manœuvre normally results in the release of generous amounts of CSF thus facilitating further dissection with minimal retraction.

The arachnoid over and lateral to the internal carotid artery and A-1 segment are opened next, releasing more CSF and enabling the surgeon to check the size, position and variations of the PcoA, AchoA, uncal artery and oculomotor nerve.

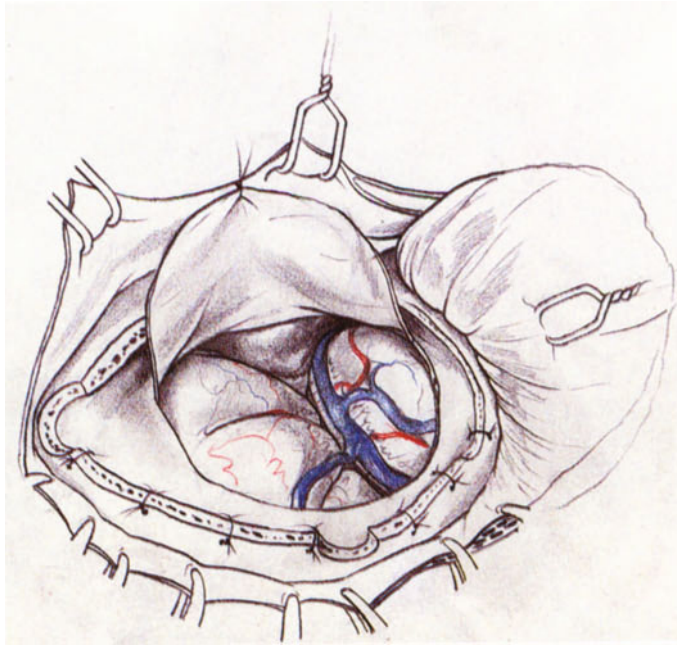

Fig. 7. Dural opening and exposure of the proximal Sylvian structures

Opening the Sylvian Fissure

The Sylvian fissure is then gently opened using sharp dissection. If the arachnoid membranes are sufficiently fine, simply a spreading action with fine bipolar coagulation forceps, is adequate. Gentle retraction with a fine sucker on a moist cottonoid sponge and, where necessary, division of thickened arachnoid bands with a tenotome or microscissors completes the dissection. The exposure thus is from the carotid bifurcation (medial to the Sylvian veins) to the middle cerebral artery bifurcation and some 1.5 to 2.0 cm beyond; exposing approximately the anterior one third of the insula and 1–2 cm of the M-2 segments.

With the arachnoid between the temporal and frontoorbital areas now well opened one may retract the frontal lobe a little more medially and

inspect the lateral aspect of the ICA more fully. The position of the uncus and parahippocampus should be noted with particular reference as to whether there is deep, medial herniation. Such was a common finding in our series of non-tumour cases.

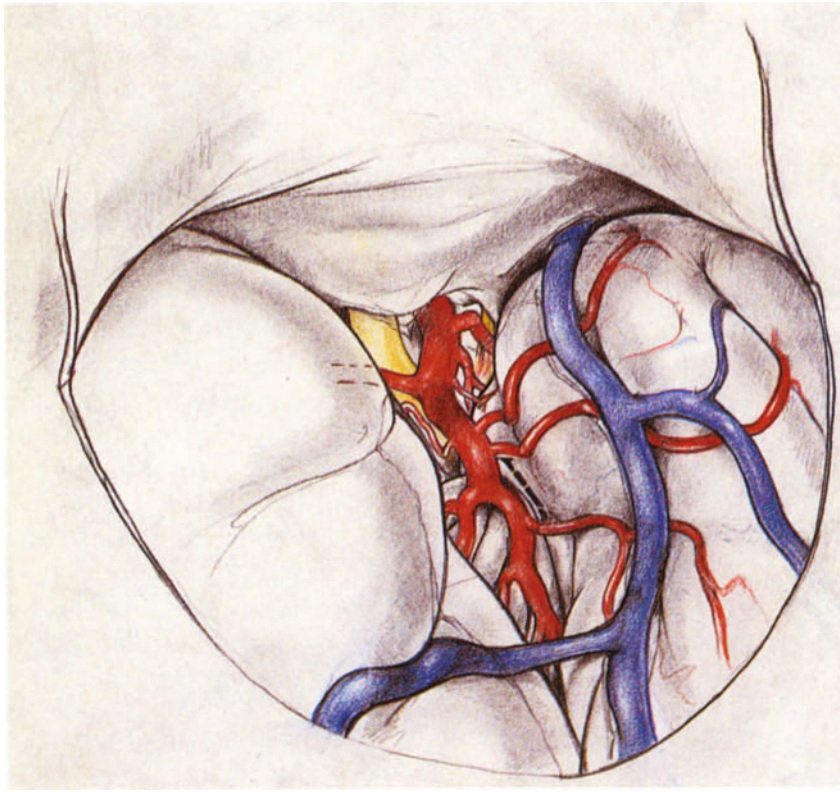

Fig. 8. Visualization of the ICA, PcoA, anterior choroidal artery, uncal artery, M-1 and M-2 segments with temporal branches. Dotted line indicates the place of incision

Inspection and Mobilization of the Middle Cerebral Artery and Its Branches

The lateral branches of the M-1 segment are now studied carefully; the temporopolar, anterior temporal and middle temporal arteries are identified (sometimes all three, but particularly the middle temporal artery, may arise from the M-2 segment).

At the peak of the limen insulae the curve of the inferior trunk of the M-2 segment is followed as it runs in the sulcus "inferior insulae". (This sulcus, which belongs to the sulcus circularis of the insula, might be more correctly

termed sulcus insulae circularis pars inferior.) The M-2 segment is gently mobilized from this sulcus. It is necessary to coagulate and divide 2–5 small perforating branches entering at this point and running to the insula.

Cortical Incision, Opening the Temporal Horn, Removal of Amygdala

An incision 1–2 cm in length is made, lateral to M-1 and anteromedial to M-2, into the "inferior insulae" sulcus opening the anterior portion of the uncinate fasciculus. The incision is placed where the perforating branches to the insula have been coagulated and lies between the temporopolar and anterior temporal arteries. The amygdala is found some few mm in depth from the cortical surface.

At this point it is advisable, using a gentle spreading action with the tips of fine forceps, to advance first in the direction of the inferior horn and to open into it. This will provide a clearer orientation as to the size, direction and extent of the amygdaloid nucleus. If uncertain of the location of the inferior horn, the amygdala may first be removed piecemeal both by microrongeur (to provide histological specimens) and gentle suction. The amygdala is removed taking great care not to extend too far mesio-basally in the direction of the optic tract. In an anterobasal direction, after removal of the most anterior aspect of the amygdala, the anterior part of the parahippocampal gyrus is identified and removed subpially.

Removal of the amygdala in the medial/mesiobasal direction proceeds with great caution until the optic tract lying medially, has been identified. The parts of the amygdala lying medially and projecting to the claustrum, putamen and pallidum must remain unresected.

Opening the Pia

Following the subpial resection of the most anterior part of the parahippocampal gyrus, the transparent curtain of pial and arachnoid membranes adjacent to the lateral part of the carotid cistern and the anterior part of the ambient cistern may readily be identified anteroinferiorly/inferoanteriorly. Upon opening the pia one identifies the uncal and anterior choroidal arteries entering the sulcus choroideus from the crural cistern. Medial to this lies the optic tract and laterally the vein of Rosenthal. The cerebral peduncle, P-2 segments, and III nerve may also be seen within the peduncular portion of the ambient cistern. Furthermore, through the delicate arachnoid membranes and fibres between the III nerve and posterior communicating artery, the P-2 and P-1 segments, with their branches, may now be identified within the interpeduncular cistern.

One can now begin to see how the combined subfrontal and insula/temporal approach described enables the surgeon to see not only the course of the anterior choroidal artery but also the optic tract. This is particularly important in those cases demonstrating deep mesial herniation.

Removal of the Hippocampus

Once the amygdala has been removed, the next stage is somewhat simpler. The earlier part of the dissection has been directed anteroinferiorly and the surgeon must now turn the microscope posteroinferiorly. The

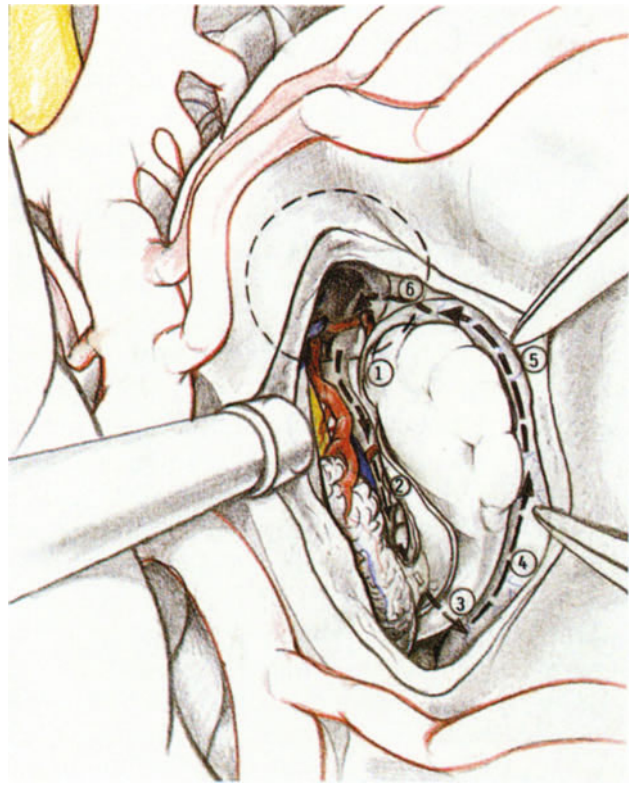

Fig. 9. The amygdala is removed (dotted lines), exposure of the entire temporal horn with demonstration of the anterior choroidal artery, basilar vein of Rosenthal and optic tract. The direction of the arrows indicate the dissection steps around the hippocampus

temporal horn is now open from its tip in an occipital direction for a distance of about 2 cm. This gives access from the tip of the horn to the trigone and an excellent view of the choroid plexus and of the pes hippocampus.

The choroid plexus may be displaced from medially to laterally to demonstrate the tela choroidea over the sulcus choroideus. Through this transparent membrane can be seen the anterior choroidal artery and ventricular tributaries of the basiliar vein.

The choroid plexus is reflected medially and the tela opened with fine forceps or a fine dissector until the taenia fimbrae of the fimbrae hippocampus and the lateral peduncle are entirely visible from anterior to posterior rim. Great attention must naturally be paid to the anterior choroidal artery with its branches running laterally to the anterior one third of the parahippocampal gyrus and uncus, then medially to the optic tract, peduncle, internal capsule and thalamus (see Fig. 13). The lateral branches of the anterior choroidal artery (so-called uncus artery) must now be coagulated and divided, whereas the main stem of the artery and its medial branches to the peduncle, optic tract, pallidus, internal capsule, thalamus, lateral geniculate body, and choroid plexus must at all costs be preserved. To avoid spasm of these vessels, papaverine is applied locally to the main trunk of the anterior choroidal artery. We should also pay attention to a further anatomical variation in which the branches to the uncus and amygdala arise separately and very proximally from the anterior choroidal artery or even originate separately from the lateral wall of the internal carotid artery. In no instance have we observed the anterior choroidal artery arising from the proximal M-1 segment.

After opening the sulcus choroideus of the hippocampus, the parahippocampus may be rotated a little laterally to inspect also around the peduncle and to visualize the entire P-2 segment and its branches. Ultimately the posteromedial choroidal artery and collicular artery, which may arise from the P-1 or P-2 segments runs very close alongside the P-2 segment.

The so-called "Ammon's horn arteries" which enter the sulcus hippocampus originate partially from the anterior choroidal artery, mainly from the P-2 segment, just proximal to the origin of the posterolateral choroid artery (see Fig. 13). The artery (arteries!) of Ammon's horn must be coagulated and divided. Transverse section of the hippocampus is then carried out at the level of the posterior rim of the peduncle as the P-2 bifurcates to form the inferolateral and superomedial trunks. From the inferior lateral trunk arise the entire inferotemporal branches (anteroinferior, inferomedial, inferoposterior, and inferotemporooccipital). The branches for the parahippocampal gyrus arise within the collateral sulcus from the inferolateral trunk or in some cases from the inferoanterior and inferomedial temporal arteries (see Fig. 13). The second trunk (superomedial) can make deep or superficial loops within the ambient cistern (Lecaque *et al.* 1978). This is the parent vessel of the calcarine and parietooccipital arteries.

Transsecting the hippocampus at this level one will encounter small hippocampal veins which are easily coagulated and divided. There will also be from one to three larger cortical veins coursing to veins in the collateral sulcus and thence to the medial cortical vein. These need to be eliminated

together with a few parahippocampal veins in the sulcus itself. The hippocampal veins drain to the inferior ventricular vein thence to the basilar vein; the inferior ventricular vein must also be coagulated and divided in a proper distance to the basilar vein (see Fig. 14).

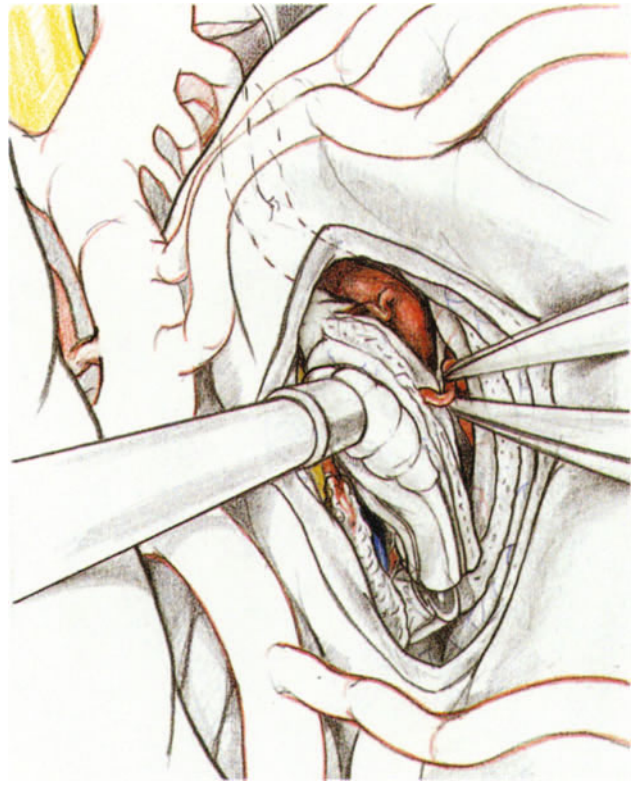

Fig. 10. The incision along the lateral hippocampus leads to the sulcus rhinale and sulcus collateralis. P-2 segments and branches are visible. The artery to the parahippocampus is coagulated and thereafter divided

At the highest point of the posterior rim of the peduncle where the P-2 segment bifurcates to form the P-3 segments one will find the terminal part of the optic tract and the beginning of the lateral geniculate body where the fimbria ascends to the splenium to form the crus of the fornix.

The posterior lateral choroidal artery may arise in the middle or terminal portion of the P-2 segment just before its bifurcation or it may arise from either the inferolateral or superomedial trunk. This occurs at the level of the posterior rim of the peduncle. The vessel continues then to enter the choroid plexus to anastomose with the choroid plexus branch of the

anterior choroidal artery. Also at this level is the origin of a separate vessel which supplies the lateral geniculate body.

Attention is now directed toward an unnamed sulcus immediately lateral to the hippocampus and lying within the inferior horn. This is an extension

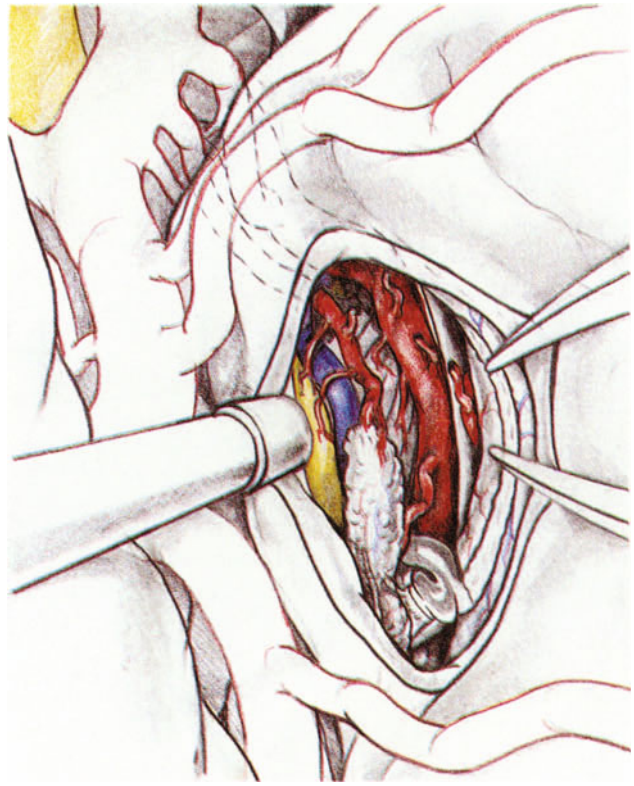

Fig. 11. Following amygdalo-hippocampectomy the important structures surrounding the lateral peduncle are visible; *i.e.*, optic tract, basilar vein, anterior and posterior choroidal arteries, P-2 segment and plexus choroideus

of the so-called eminentia collateralis, a semicircular sulcus around the pes hippocampus. Gentle spreading with fine forceps or a dissector allows this sulcus to be opened from the anterior floor of the temporal horn anteroposteriorly or posterioanteriorly in the direction of the peduncle where the rhinal sulcus and its posterior extension, the collateral sulcus, are found. This unnamed semicircular sulcus around the pes hippocampus and the bottom of the temporal horn is separated only by some 1–3 mm from the rhinal and collateral sulci. It is very easily opened in a similar fashion to the opening of the sulcus choroideus medial to the pes hippocampus.

In this lateral opening are found the several temporal vessels arising from the inferolateral trunk (P-3), not simply crossing the rhinal and collateral sulci but entering, bifurcating and leaving once again. One may observe which branches supply the parahippocampus, those which supply the gyrus fusiformis, inferior temporal gyrus and the lateral temporooccipital gyrus. Only the vessels supplying the parahippocampal complex are coagulated and divided. The parahippocampus can then be readily reflected, elevated and removed en bloc via the subpial plane. The resected specimen measures approximately 4 cm in length, 1.5 cm in breadth and 2 cm deep. In the resected area, there remain in the bed of the parahippocampus, small bleeding points from exiting pial veins. These may be coagulated without difficulty using fine bipolar forceps.

Opening the extension of the sulcus collateralis in those cases in which there is no deep mesial herniation, one will ultimately reach the tentorium some 2–5 mm from its free edge and in its anterior half. In those cases in which there is herniation of the mesial structures one must be more concerned about the line of dissection and potential damage to underlying structures. In these instances, provided one stays in the subpial plane, the parahippocampus may be removed en bloc, as the P-2 segment with its branches, the superior cerebellar artery, III nerve, and IV nerve (lying below the tentorial edge) will be protected by the pia and a double layer of arachnoid.

At no time during the procedure should retractors be inserted in the small cortical incision. This must be entered only by the sucker and forceps although the tip of the sucker placed over a moist cottonoid sponge may be used as a gentle temporary retractor. After the amygdala and parahippocampus are removed en bloc the dorsal and polar aspects of the temporal lobe should appear untouched.

Following careful haemostasis along the lines of dissection of M-1 and around the internal carotid artery the dura is closed with a running suture and the bone flap replaced in the normal fashion.

Problems

The following points should be made regarding specific problems frequently encountered during this procedure.

1. When opening the Sylvian fissure, as in all procedures using the pterional approach, dissection is carried out medial to the Sylvian vein. Occasionally, however, cases present in which the frontoorbital vein is very large and too many major branches of this vessel would be sacrified by a medial dissection. On such occasions dissection must proceed lateral to the Sylvian vein along the medial surface of the superior temporal gyrus, in an epipial plane, until the inferior portion or an area between two veins of the insular sulcus is reached.

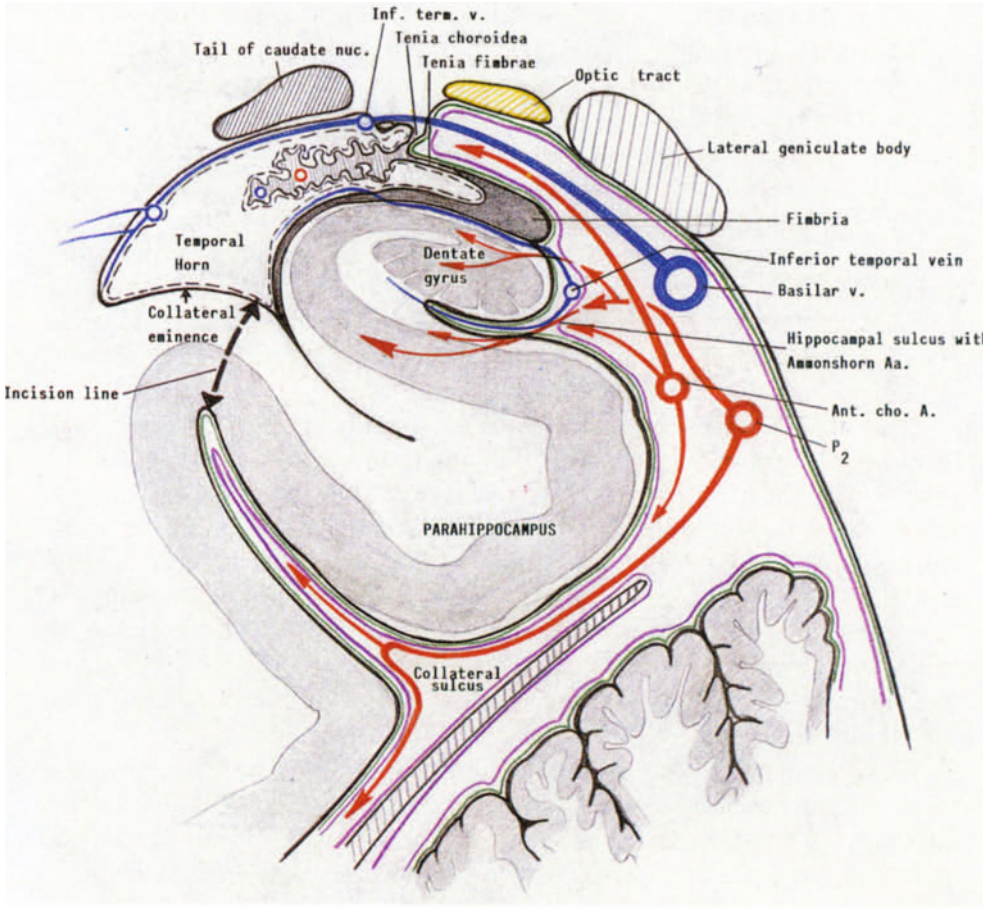

Fig. 12. Anterior coronal plane of the right hippocampal-hippocampus complex with relationship to neighbouring structures. Demonstration of important arterial and venous structures. Green line: pia, lilac line: arachnoidea. Drawing modified after Salamon and Huang, Fig. 171, page 225

2. Variations are frequently encountered in the form and distribution of the lateral branches of the M-1 segment. The surgeon must find, or by mobilization create, sufficient space to make a 2 cm incision between the two temporal arteries.

3. Vascularisation of the amygdala, gyrus parahippocampus, uncus and hippocampus is partly from the anterior choroidal artery and partly from branches of the P-2 segment of the posterior cerebral artery (see Fig. 12). The major vascular supply shows considerable regular variability [1–5, 8, 10–12, 15]. The anterior choroidal artery gives not only medial branches to the optic tract, peduncle, internal capsule, thalamus and a large branch to the choroid

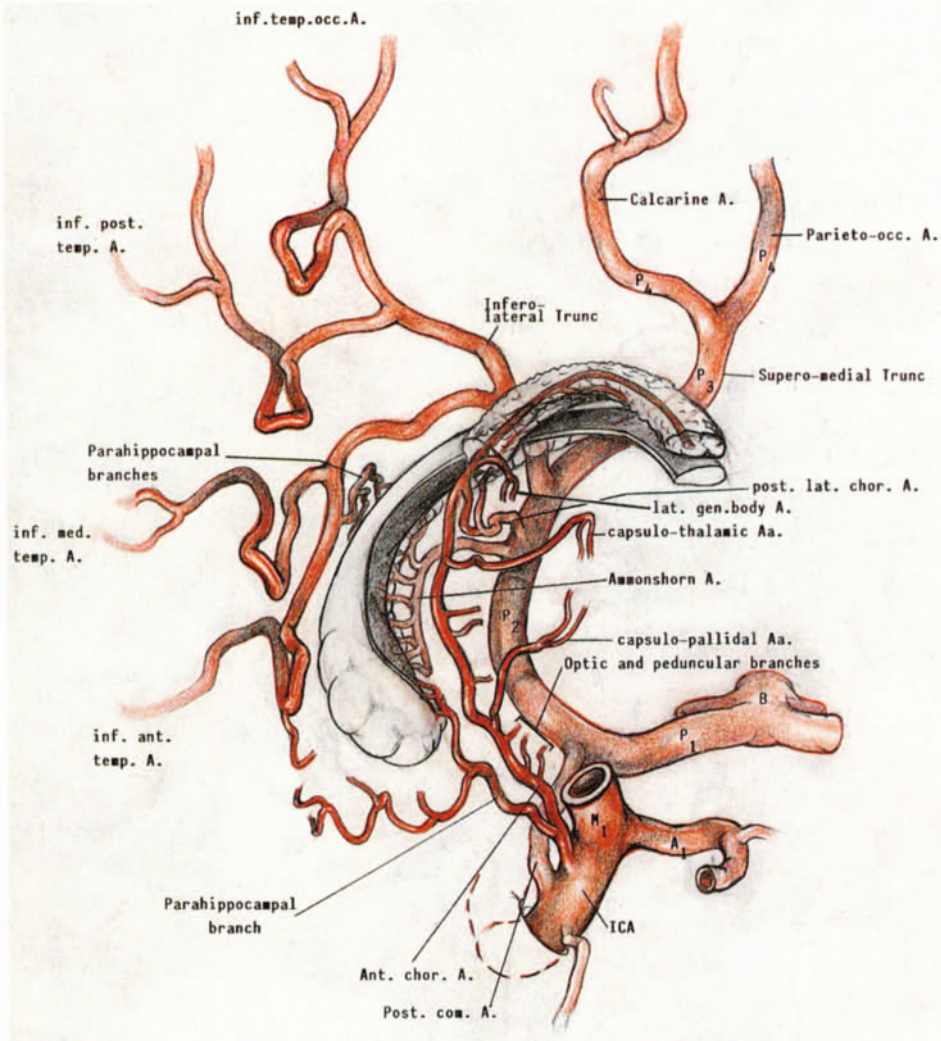

Fig. 13. Artistic demonstration of the vascular anatomy to the amygdala, hippocampus and parahippocampus

plexus but also gives a regular lateral branch to the cortical areas of the anterior one third of the parahippocampus. A small branch enters the anterior part of the sulcus hippocampus to supply Ammon's horn in its anterior third. There is also a lateral branch which may run to the uncus. This should be coagulated and divided early in the operation. The posterior two thirds of the parahippocampus receive supply from the temporal branches of the P-3 segment. There are also direct branches from the P-2

segment which enter the sulcus hippocampus to supply the posterior two thirds of the hippocampus. In only one anatomical book did we find the name of Ammon's artery together with a precise drawing of this vessel, designated Uchimura's artery. (Fig. 8.38, p. 410, P. Duus, Neurologisch-topische Diagnostik, Thieme 1983. No further information was given.) Our experience gained by surgical exploration and anatomical dissection are shown in a preliminary form in Fig. 13.

4. Removing the amygdala by the use of rongeur and sucker is not generally a bloody procedure but on approaching the ventricular wall (particularly medially) one must bear in mind the presence of veins running from the amygdala within the subependymal layer. These in turn, run subependymally to the sulcus choroideus and thence to the basilar vein. Any injury to these veins and their branches may result in torrential retrograde venous haemorrhage. Venous bleeding will obviously be more readily controlled if the patient is properly positioned with the head elevated at the beginning of the operation. Attempts at local control of bleeding from the more medially positioned veins may result in damage to the optic tract. The venous drainage of the medial temporal structures, peduncle, optic tract and thalamus to the basal cerebral vein of Rosenthal, have been precisely and elegantly demonstrated by Huang (1976), see Fig. 14.

5. The size and form of the parahippocampus can vary considerably. In some cases it is seen to bend only gently round the peduncle whilst in others it takes the form of a coiled worm. Preoperative assessment of the configuration of the parahippocampus by CT scan and air ventriculography is largely unhelpful. Perhaps the shape of the structure is determined in part by the size and configuration of the skull itself with particular reference to the pterional wing. These variations require further study by anatomists.

6. The term "selective" amygdalo-parahippocampectomy is rather misleading for several reasons:

a) The amygdala is not removed completely, particularly in its most medial part where it abuts the striatum, anterior commissure and tail of the caudate nucleus.

b) The posterior transsection of the parahippocampus is generally carried out at the level of the bifurcation of the P-2 segment to form the P-3 segments. It is possible to resect more posteriorly, but, if this is carried too far, there may be damage to the geniculate body or Meyer's loop.

7. Although the operation is safe in terms of inflicting no damage to most parts of the temporal lobe (apart from the amygdala and parahippocampus), there is inevitably some injury to the superior temporal, inferior temporal and lateral temporooccipital gyri (fusiform gyrus). Such damage cannot be well demonstrated with present imaging techniques.

8. Serious complications have not been common in the senior author's

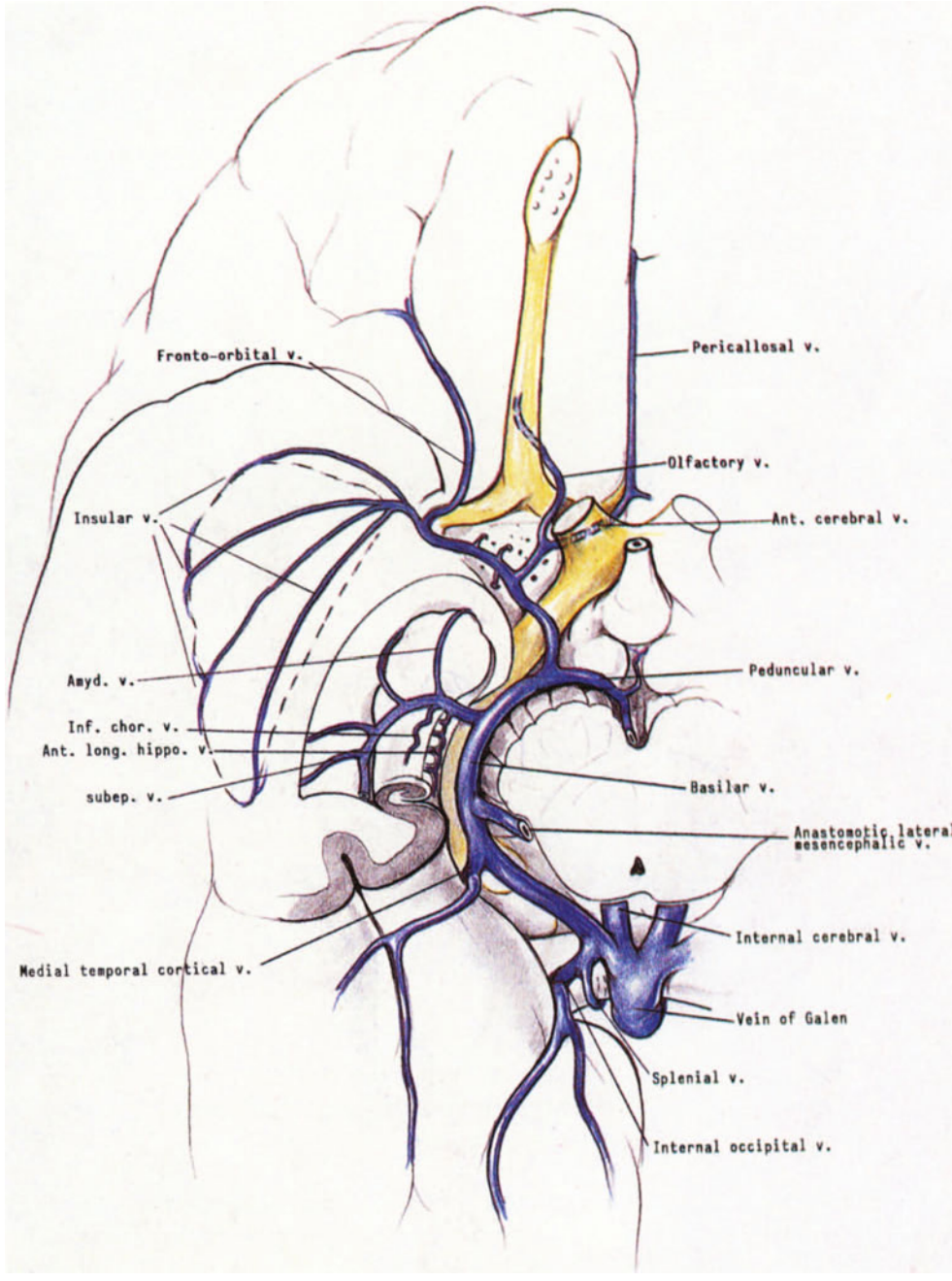

Fig. 14. Basilar vein with tributaries. Drawing modified after Salamon and Huang. (Read Amygd. v. instead of Amyd. v.)

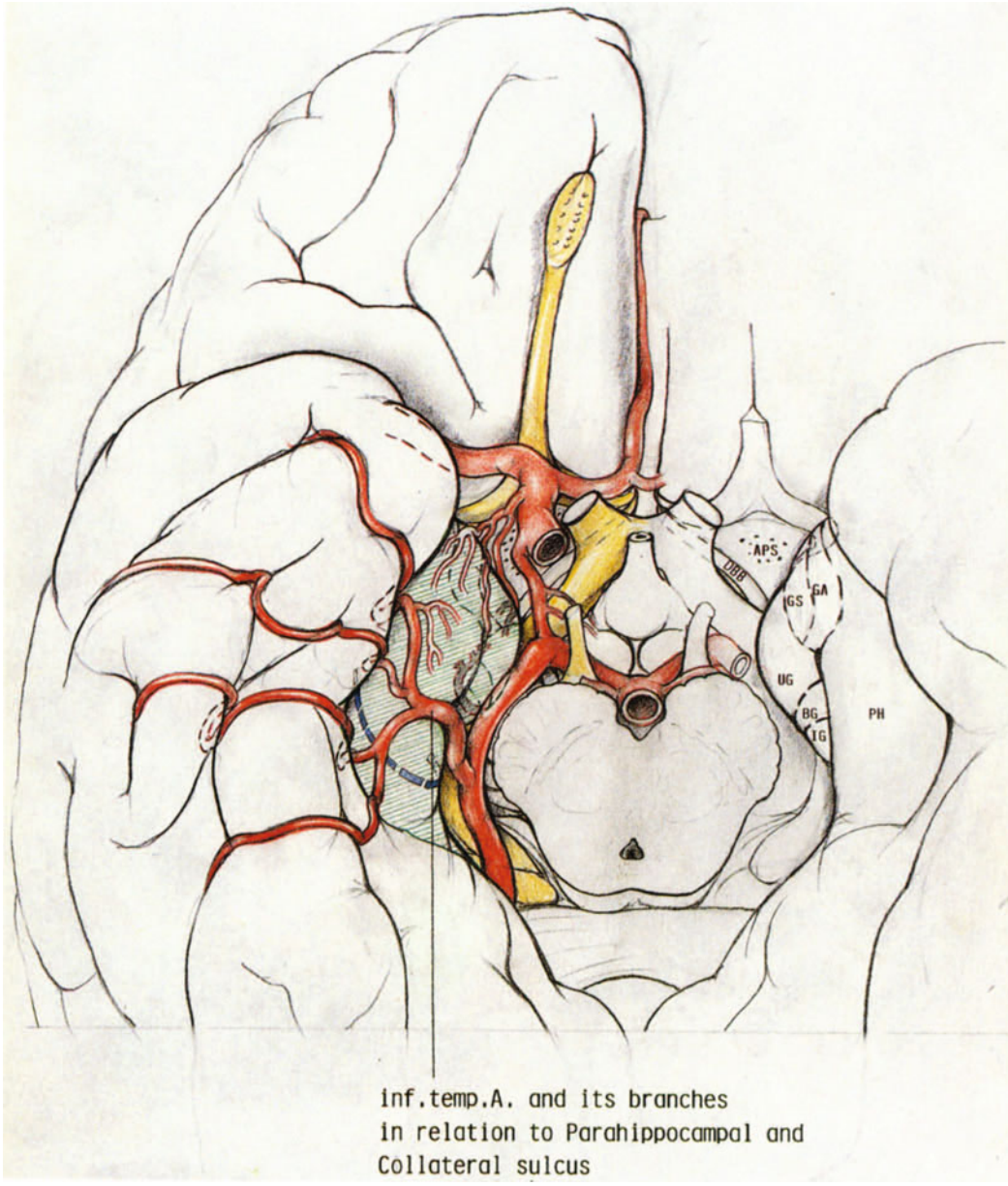

Fig. 15. Schematic representation of the basilar view of the parahippocampal gyrus and its vasculature. The inferior temporal artery and its branches surround the parahippocampus and subdivide into several branches within the collateral sulcus. The doted lines indicate the posterior limits of the approximate incision for hippocampectomy. *APS* anterior perforated substance, *BG* the band of Giacomini, *DBB* diagonal band of Broca, *GA* the ambient gyrus, *GS* the semilunar gyrus, *IG* the intralimbic gyrus, *PH* the parahippocampal gyrus. Drawing modified after P. L. Williams, R. Warwick in Gray's Anatomy, p. 934, Fig. 1.119

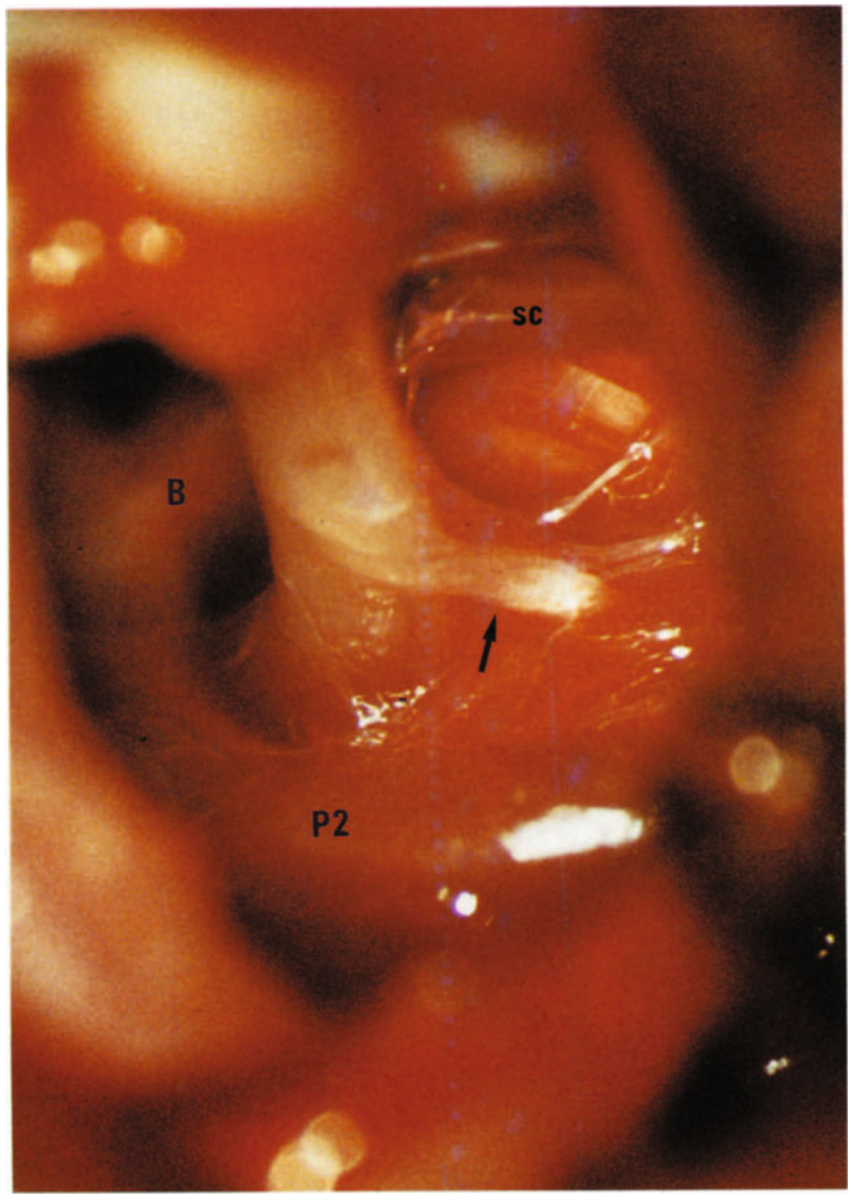

Fig. 16. Lateral roots of the right III nerve (arrow) originating from the lateral portion of the peduncle. The basilar artery (*B*), P-2 segment (*P2*) and superior cerebellar artery (*sc*) are seen

(MGY) series of over 115 selective operations. The results are described in the second part of this paper. Those which might be particularly anticipated are hemiparesis and homonymous field defects. These may be kept to a minimum if care is taken to avoid damage to the branches of the anterior choroidal artery and branches of the P-2 segment. Visual field defects are more likely to result from damage to or spasm of vessels that supply the optic tract than to direct injury to Meyer's loop.

Pertuiset *et al.* (1962) described how the ophthalmological symptoms caused by occlusion of the anterior choroidal artery depend upon the site of occlusion with respect to the lateral geniculate body. In pregeniculate lesions there is a non-congruent hemianopia and macular sparing, ipsilateral pupillary dilatation and no reaction to light in the blind half of the retina. If the geniculate branches of the anterior choroidal arteries are damaged an upper quadrantanopia is produced. Damage to the branches supplying the optic radiation, passing through retrolenticular and sublenticular segments of the internal capsule, will produce a congruent hemianopia with macula sparing.

9. As a curiosity, we observed an anatomical finding regarding the fibres of the III nerve at its origin. In 17 cases we noted a fibre branch of the nerve which came from the lateral peduncle, was 5–10 mm long and 0.2–1.0 mm thick forming a bridge over the posteromedial choroidal artery or its branches. We are uncertain as to the neurophysiological importance of this fibre bundle.

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Transoral Approach for Epidural Craniocervical Pathological Processes

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With 28 Figures

Contents

Introduction

Although there appears to be more and more evidence that transoral surgery is superior to any other approach to ventral craniocervical lesions this method does not seem to have reached its well deserved place in the neurosurgical armamentarium.

Disappointing results can be seen in the literature using other kinds of treatment and there are obviously many more not reported. This makes one wonder why this method so ingeniously suggested by our masters a few decades ago has been neglected.

The feasibility of transoral approach to the upper cervical spine was demonstrated in dogs by German in 1930 (Greenberg *et al.* 1968). For the ENT surgeon it was natural to drain a retropharyngeal abscess through the mouth (Thomson and Nagus 1947) or use the transoral route with palatal incision in reaching a nasopharyngeal process (Wilson 1951). The credit for the idea of transoral neurosurgery, however, has to go to Scoville. In his original paper on platybasia (Scoville and Sherman 1951) he writes: "It is the authors' concept that the angulation of the medulla over the abnormally high odontoid process is the chief offender in causation of the neurologic signs and disability. Posterior decompression simply prevents adhesive arachnoiditis and hydrocephalus. Future surgical advance lies in the development of a successful removal of the odontoid itself, possibly through the mouth." In footnotes he adds: "This has been found feasible on a cadaver."

Scoville not only proposed a theoretical possibility but has proven it in clinical practive and reported two successful cases of atlanto-axial dislocation operated on via his originally advised route with Greenberg and Davey some 17 years later.

I should like to dedicate this paper to his memory. I hope the reader will be convinced of the usefulness of this approach.

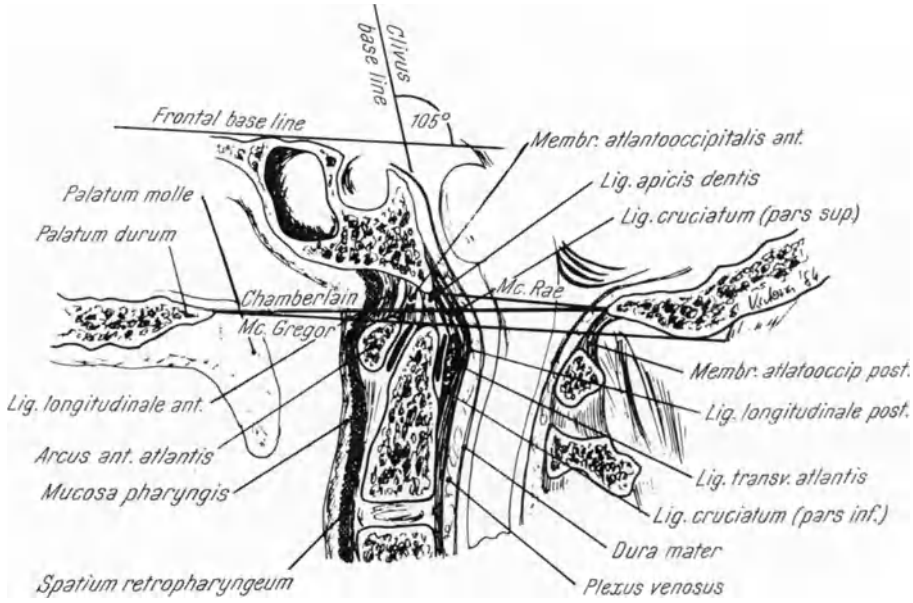

Fig. 1. Sketch of the anatomical structures of the craniocervical region in the sagittal plane

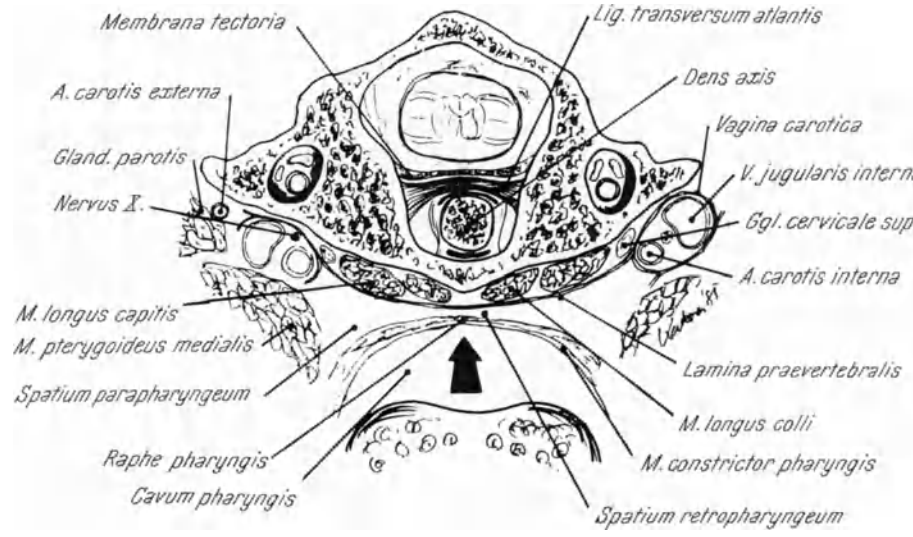

Fig. 2. Sketch of the anatomical structures in the horizontal plane at the level of C_1

Anatomy

The anatomical relationships of the ventral craniocervical and upper cervical region are considered here. Exact knowledge of structures described below is essential for precise execution of the operation and to avoid post-operative complications (Figs. 1 and 2).

Deviating from common anatomical practice the structures will be listed in sequence as they appear one after another during transoral surgery.

1. Soft Palate

This acts as a valve separating epipharynx from mesopharynx in the process of deglutition or formation of particular consonants or vowels. It is a laminar structure about 1 cm thick in continuation with the hard palate.

Between the two mucosal layers covering the soft palate (*i.e.*, nasal and oral mucosa) there are mixed salivary glands, a few taste buds, muscle fibres, fatty tissue and a complex of connective tissue fibres originating from the aponeurosis of tensor veli palatini muscle.

Damage to the function of the soft palate is minimal if the incision is made in the midline (avoiding the uvula) incising mainly connective tissue saving the functioning muscles.

2. Pharynx

This is a sack-shaped cavity open anteriorly located behind the nasal (pars nasalis, epipharynx), oral (pars oralis, mesopharynx) and laryngeal (pars laryngea, hypopharynx) cavities.

The posterior wall of the pharynx takes its upper origin from the pharyngeal tubercle of the occipital bone and is continued forward to the medial pterygoid plate of the sphenoid. Below it suddenly narrows at the level of C 5–6 as it becomes the upper part of the oesophagus. The transoral approach therefore is suitable to gain access to the clivus as well as to the area of the uppermost cervical bodies.

Layers of pharyngeal wall: 1. tunica mucosa; 2. tela submucosa; 3. layer of mainly elastic fibres; 4. muscles: longitudinal elevators of the pharynx (levators) and transversal and oblique fibres of superior, middle and inferior constrictors telescoped into each other. The muscles insert in the midline in the pharyngeal raphe.

Incision and blunt retraction of the deeper layers of the pharyngeal wall in the exact midline does not bring about muscle injury.

The pharynx is in contrast posteriorly with the praevertebral fascia. Between the two there is a layer containing loose connective tissue (retropharyngeal space) which continues caudally into the posterior mediastinum. This is an important point as in cases of inflammation of the

spine infection can extend into the mediastinum, and this must be prevented postoperatively.

Next layer in the sequence of the operation is, depending on the angle of the approach, the anterior aspect of the clivus, the anterior atlanto-occipital membrane originating from the inferior part of the clivus, or the anterior longitudinal ligament extending down from the arch of the atlas of covering cervical certebral bodies and intervertebral joints.

When the atlanto-occipital membrane is cut transversally the tip of the odontoid appears just above the upper rim of the atlas together with the ligaments inserting here: the alar ligament—running from the odontoid tip bilaterally upwards and sidewards to the occipital condyles and the apical ligament—spreading in the midline from the odontoid tip to the interior rim of the clivus.

It is generally preferable to keep the width of the approach no more than 10 mm from the midline in order not to interfere with the atlanto-occipital joints while resecting the atlas itself (see below).

In cases of tumorous destruction of these bony structures the surgeon must be aware of the vertebral arteries which are approximately 25 mm from the midline (expect if pathological displacement of these vessels is seen on angiograms).

3. Atlas

The anterior arch (relevant to this exposure) is 9 mm high and 4 mm thick on average. The posterior surface of the anterior arach articulates with the anterior surface of the odontoid by a small round synovial joint. This is opened during resection.

4. Odontoid Process

This is 13 mm high and 10 mm thick on average. Following the removal of the odontoid the joint between the posterior aspect of the odontoid and the transverse ligament is opened and the transverse ligament proper, fixing the odontoid to the arch of the atlas, comes fully into view. Behind that are the cruciate ligament and the tectorial membrane originating from the inferior rim of the clivus and within the anterior rim of the foramen magnum. This continues caudally as the posterior longitudinal ligament and covers the anterior aspect of the cervical vertebral bodies and intervertebral joints on the anterior aspects of the spinal canal. The ventral epidural space and the dura are immediately behind this complex ligamentuous structure.

5. Muscular Structures

Muscles located at the anterior surface of the cervical spine are:

1. Rectus capitis anterior—a small muscle running from the anterior

arch of the atlas to the base of the skull in front of the area of the atlantooccipital joint.

2. Longus colli which originates from the side of the upper thoracic vertebral bodies and inserts into the side of the upper cervical vertebral bodies. It contributes to flexion of the head.

3. Longus capitis which originates from the transverse processes of the lower cervical bodies and inserts into the basilar part of the occipital bone. It is also a flexor of the head.

The last two muscles do not reach the midline. This is why only the medial margin of the long cervical muscles has to be tetracted in transoral approach.

From this description it is clear that fewer anatomical structures are disturbed to reach the dura at the level of the clivus or second to fourth cervical vertebrae than at atlanto-axial level.

The upper cervical vertebral bodies are 15–16 mm high (C_2 is 5–6 mm higher), 23–25 mm wide and 17–19 mm deep on average. The average distance from the midline to the intervertebral foramen where the roots leave the spinal canal is 12–13 mm. The foramen transversaria, creating a canal for the vertebral artery, are found 25 mm from the midline at C_1 20 mm at C_2, and 15 mm at C_3 respectively.

The length of the clivus measured from the tip of the dorsum sellae to the ventral rim of the foramen magnum is 45 mm in average.

Surgical Pathology and Indications for Transoral Surgery

Thus review deals only with those ventrally placed lesions of the craniocervical junction and pathological processes of the upper cervical vertebrae which we consider suitable for transoral surgery.

1. Developmental Anomalies

The clinical symptoms of bony abnormalities of the craniocervical junction appear usually after the second or third decades (Wollin 1963, Davis and Gutierrez 1977, Holmes and Hall 1978, White and Panjabi 1978). However, they may appear also at a younger age and Menezes *et al.* (1980 a) call our attention to the possibilities of active treatment of these conditions even in childhood.

a) Primary basilar impression is a developmental anomaly of the chondrocranium. There is a wide variety of forms and severity. Usually the rim of the foramen magnum, the condyles and the odontoid process are invaginated upwards into the posterior fossa. From our point of view the most important variant is that which presents a severe bony deformity with medullary compression. The malformation may be combined with other

bony, nervous tissue and meningeal anomalies: Klippel-Feil syndrome, non-fusion of the atlas, Arnold-Chiari malformation, blockage of the cisterna magna, syringomyelia, dural fibrous ring (Spillane *et al.* 1957, Greenberg 1968, Michie and Clark 1968, Menezes *et al.* 1980 b).

b) The most important causes of secondary basilar impression are: osteomalacia, Paget's disease, osteogenesis imperfecta, hyperparathyroidism (Bonney 1970).

c) Disgenesis of the odontoid process. The unfused odontoid process (os odontoideum) or the ossiculum terminale may lead to clinical symptoms by upward displacement (Menezes *et al.* 1980 b, di Lorenzo *et al.* 1982). The separated os odontoideum may be fused to the anterior arach of atlas (Miller and Parent 1984). The odontoid hypoplasia may cause compression myelopathy because of atlantoaxial displacement (Greenberg *et al.* 1968, Kopits *et al.* 1972, Hakuba 1985).

d) In spondylo-epiphyseal dysplasia the occipital condyles are hypoplastic and the skull may glide forwards (White and Panjabi 1978).

e) In some cases of Marfan's syndrome (hypermobility of the joints) the congenital disorder of the mesenchyme may be associated with atlanto-axial dislocation (Levander *et al.* 1981).

f) In cases of neurofibromatosis, as a maldevelopment of neuroectoderm and mesoderm, atlantoaxial dislocation has also been observed among several other deformities of the vertebral column (Isu *et al.* 1983).

In the majority of patients with craniocervical malformation, skeletal traction and/or posterior craniocervical bony and dural decompression with or without stabilisation is sufficient to produce significant improvement. However, in cases of ventral compression the posterior decompression carries a high operative risk (Scoville and Sherman 1951, Alexander *et al.* 1958, Dastur *et al.* 1965). Anterior decompression by the transoral approach has proved to be effective and in recent studies far safer (Bonney 1970, Derome *et al.* 1977, Lamas *et al.* 1977, Greenberg *et al.* 1968, Pásztor *et al.* 1980, 1984, Menezes *et al.* 1980 a, b, di Lorenzo 1982, Miller and Parent 1984).

2. Traumatic Conditions

The pathological anatomy of traumatic deformities of the upper cervical vertebrae and articulations is based on the nature and mechanism of injury. Their treatment on the other hand, depends quite substantially on the presence and features of neurological deficits and pathological dislocations which can be hazardous to neural structures. The common traditional management of fractures and dislocations of the upper cervical spine are external immobilization or operative posterior fusion, or both.

a) Odontoid fracture. This is one of the most important of all traumatic conditions. More than one tenth of patients with cervical injury display abnormalities of the odontoid.

Forces which can cause odontoid fracture are combinations of flexion, extension and rotation of the craniocervical junction. The actual shape and position of the fracture and the final dislocation of the odontoid will be determined by the resultant of the different vectors of forces on the actual position of the neck and head when the impact took place.

The odontoid can be sheared from the C_2 vertebra by its ligaments or may be distracted from it by its apical ligament. The three main types of odontoid fractures are illustrated by Anderson and d'Alonzo (1974).

The optimal treatment remains controversial. Most of the patients can effectively be treated by immobilizing them in a halo device and only few, especially the long-standing ones, require posterior surgical fusion (Prols *et al.* 1973, Apuzzo *et al.* 1978, Ekong *et al.* 1981, Tator *et al.* 1982).

Others suggest, that in so called type two fractures (fracture at the junction of the odontoid with the body of the axis) early posterior cervical fusion (wiring and onlay bone graft) is more effective (Maiman and Larson 1982). Schiess *et al.* (1982) believe that surgical fusion should be considered as the initial treatment of all types of odontoid fractures.

b) Antero-posterior dislocation of the atlantoaxial segment without fracture of the odontoid process is more frequent than was previously recognized (Maiman and Cusick 1982). Patients usually require posterior cervical wiring and fusion (Alexander *et al.* 1958).

c) Atlanto-occipital fracture-subluxations are the consequence of overstretching of the occipital-atlantoaxial ligament-complex. According to Cooper *et al.* (1979) and Cooper and Chalif (1983), once reduction has been achieved a posterior occipital-cervical fusion should be done followed by immobilization in a halo vest.

d) Burst fractures of C_1 (Jefferson fractures) appear when the impact hits the vertex combined with hyperextension. They are usually managed with external immobilization (Seljeskog 1978).

e) Traumatic spondylolisthesis of the axis (Hangman's fracture) consists of a fracture of the arch of the axis, which subluxation between C_2 and C_3 and an intervertebral disc displacement resulted mostly from hyperextension of the upper cervical spine. The treatment is usually non-operative: skeletal traction and a halo ring.

In some cases of odontoid fractures however, antero-posterior dislocations, anterior compression of the cord by displacement of parts of the fractured C_2 body, transoral surgery may be indicated (Fang and Ong 1962, O'Laoire and Thomas 1982, Lee and Fairholm 1985). In most of the reported cases the traumatic changes were not acute. Anterior compression of the medulla constituted the basis of clinical symptoms, the alignment of the spine remained disturbed, the clinical symptoms either developed or worsened after posterior surgical fixation.

3. Inflammatory Processes

a) Rheumatoid Arthritis

86% of patients with long standing disease have pathological changes in the cervical spine (Bland 1974).

The progressive nature of the disease makes early surgical intervention of questionable value. Therefore, surgery should be restricted to patients with neurological deficits or severe pain resistant to conservative methods. Menezes (1984) however, has suggested that surgical immobilization (posterior occipito-cervical fusion) is best performed before severe neurological or vascular damage occurs.

The inflammatory granulomatous invasion results in laxity of ligaments or subluxation and displacement of the odontoid process. Vertical subluxation takes it's origin from erosive changes with accelerated bone resorption at the occipital condyles and the lateral masses of the atlas. Pathological changes at the atlanto-odontoid joint after inflammatory invasion can be identified by forward, posterior, upward and lateral dislocations (la Montagna *et al.* 1981). Therapeutic results with posterior fixation are not quite satisfactory (Conaty and Mongan 1981), yet some authors still propose this kind of intervention (Christophidis and Huskisson 1982, Menezes 1984, Lesoin *et al.* 1985).

Transoral surgery has been reserved for cases with significant ventral compression, because only this approach allows removal of the displaced odontoid if it is a bar to reduction of an atlanto-axial dislocation (Sukoff *et al.* 1972, Davidson 1977, Wood *et al.* 1980, Smith *et al.* 1980). Transoral surgery may be combined with different forms of anterior fixation (Thompson 1970, O'Laoire *et al.* 1982, Fang *et al.* 1983, Hakuba 1985), or may be followed by an occipito-cervical fusion (Crockard *et al.* 1985a, Menezes 1984). In cases with ascending luxation of C_1 and C_2 and subluxation of the upper cervical vertebrae, removal of the dislocated vertebral bodies was performed by transclival transcervical approach. Acrylic was moulded around the metal rod running between clivus and C_3 or C_5 (Lesoin *et al.* 1984, 1985).

b) Tuberculosis

Tuberculosis in the upper cervical spine is rare. In a recent study of 587 cases of tuberculous spondylitis there were 42 cases with cervical involvement and in 18% the affected vertebrae were C_1 and C_2 (Fang *et al.* 1983).

Osteolytic erosion of the bones, destruction of the joints and ligaments, granulation tissue, swelling of soft tissue and abscess formation constitute the basis of pathological changes.

As the pathological process is rarely localized in the posterior arches of the vertebrae, it is evident that debridement (excision of the affected bone,

synovium and granulation tissue and drainage of abscess) must be carried out by the anterior, transoral approach (Wang 1981, Fang *et al.* 1983).

4. Tumours

Most of the tumours here dealt with are extradural in the ventral craniocervical or upper cervical regions, at least in origin.

In its early stages, the tumour usually erodes the bone causing only local or radiating pain. Later on compression causes symptoms of cranial nerve, spinal root, medullary or spinal cord lesion.

The primary and secondary tumours of the spine are summarized in the papers of Paillas *et al.* (1979) and Torma *et al.* (1979).

a) Osteoma

Osteoma is a benign circumscribed tumour which is not always easy to resect. The upper cervical location is infrequent, but in some cases may be accessible via transoral route (Southwick and Robinson 1957).

b) Chondroma and Osteochondroma

Chondromas are rare, they represent only 5% of all bone tumours and 4% of them are located in the spine (Slowik 1968). The tumour is usually sharply delimited from surrounding tissue. Osteochondromas are also rare. The most frequent spinal location is cervical. These tumours are benign and rarely recure even after subtotal removal (McGee 1979, Fortuna *et al.* 1983).

c) Giant-Cell Tumour (Osteoclastoma)

The histogenesis of this tumour is not clear: it probably originates from mesenchymal cells of the bone marrow. Both of the above names are correct, as histologically it contains giant cells and has a osteolytic character. The tumour is rarely located in the spine (Lesoin *et al.* 1982). The character of this tumour is semimalignant and surgical resection gives good results in most cases (Pásztor *et al.* 1984). However, about 10–15% of these tumours have a malignant course.

d) Osteoblastoma

About 40% of these rare tumours are located in the spine. They are solitary with osteoblastic proliferation and capillary vascularization and have a reactive compact margin. The tumour is usually benign. Two such cases in the upper cervical spine have been operated upon by the transoral route in our hands (Pásztor *et al.* 1984).

e) Chordoma

These tumours arise from remnants of the embryonic notochord and half of them occur in the craniocervical region. Survival is generally disappointingly short, but long term remission and useful life can be achieved by surgical resection combined with radiation therapy.

Most clivus chordomas have been operated upon via a temporal craniotomy in the past, but recurrent tumours, and more recently also unoperated cases have been subjected to attempted resection by a transoral approach (Guthkelch and Williams 1967, Wood 1980, Delgado *et al.* 1981, Pásztor *et al.* 1984). Stevenson *et al.* (1966) used a transcervical, transclival approach in a case of chordoma. In chordomas of the upper cervical vertebrae, transoral surgery is the appropriate method (Bathia and Yadav 1965, Mullan *et al.* 1966, Pásztor *et al.* 1984).

f) Eosinophilic Granuloma

This benign bone tumour is characterized by infiltrates of mononuclear cells and eosinophils; is usually avascular and most commonly located in the skull. In the upper cervical area the transoral approach is again very helpful (O'Laoire *et al.* 1982).

g) Extramedullary Tumours of the Foramen Magnum

These are mostly benign tumours, meningiomas or neurinomas, rarely with sarcomatous change (Dodge *et al.* 1956, Cohen and MacRae 1962, Guidetti and Spallone 1980). Only two transorally treated cases have been reportedly successful (Mullan 1966, Crockard and Bradford 1985). In a presentation of Isu *et al.* (1983) neurofibromatous tissue was found around the odontoid process causing an atlanto-axial dislocation in a case of neurofibromatosis.

h) Metastases

Metastases are rarely located in the upper cervical vertebrae. Laurence (1969) presented one case with metastases of breast carcinoma into the axis, operated on successfully by the transoral approach. The transoral route provides also the possibility to obtain histological samples from the body of the upper three cervical vertebrae in dubious cases (Grison 1967).

5. Vascular Processes

Operative approach to aneurysms located in the lower half of the basilar artery or the vertebrobasilar junction is difficult (Drake 1978 a, b). Sano (1966) and Yaşargil (1969) used the transoral approach. The route has been thought appropriate and is claimed to give easy access to the aneurysmal

neck in these cases, carrying however, the disadvantage of causing high incidence of postoperative infectious complications (CSF fistulas).

Several authors have tried to improve the technique of closure of the transoral operative field (Yamaura *et al.* 1979, Hayakawa *et al.* 1981, Litvak *et al.* 1981, Hitchcock and Covie 1981), while others have turned to the use of a transcervical transclival approach (Wissinger *et al.* 1967, Fox 1967) to lower basilar aneurysms.

Clinical Features

Most of the pathological conditions mentioned above can cause a wide variety of clinical symptoms and an even wider range of degree in their severity from the symptom-free state through disablement to life-threatening states such as respiratory failure as in some traumatic cases.

Symptoms may arise from compression of the medulla, the spinal cord, nerve roots, afferent vasculative and/or venous drainage. In most cases a combination of these is seen. Common to all of these factors is that they are located anterior to the cord even when symptoms they elicit suggest lateral or dorsal pathology.

Following the sequence of clinical neurological examination there can be disturbances of lower cranial nerves; difficulty in swallowing, nasal speech, atrophy and weakness of muscles innervated by the accessory nerve. Upper cervical nerves can be involved in a series syndromes of neck pain. This however is only occasionally encountered in transoral surgery.

Motor deficit due to anterior compression of the craniocervical medulla is usually attributed to a uni- or bilateral pyramidal tract lesion. Peripheral motor signs can also contribute to upper cervical motor syndromes.

Long-standing compression of motor structures may cause chronic quadriparesis of varying severity with spastic myelopathy. In cases of lateral compression or as more often happens, when mainly the spinal arteries are involved, unilateral pyramidal symptoms may dominate the clinical picture. Motor signs are accompained by sometimes quite remarkable muscle wasting that can occur distally or entirely in the upper limb(s) depending on the extension and time course of the underlying pathology.

The sensory system is highly sensitive and therefore may dominate the picture. Sensory loss usually involves touch, pain and temperature modalities. These can be limited to cervical areas uni- or bilaterally.

Symptoms can be caused by the disturbed function of the spinocerebellar co-ordinating complex. Ataxia and dysdiadochokinesis with dysmetria are common findings and can be the first dysfunctions the patient reports. They are often associated with nystagmus and different manifestations of spinal and truncal ataxia.

In the later stage of diseases affecting the ventral craniocervical region

vegetative disturbances may appear. These are sphincter disturbances sometimes associated with vasomotor disturbances, sympathetic or parasympathetic in limbs and viscera.

Symptoms attributable to intracranial hypertension such as headache, mental deterioration, depressed consciousness are indisputably part of the clinical picture that a progressive ventral craniocervical lesion can bring about. The narrow CSF spaces at this point can easily be subject to compression. Disturbance in CSF flow, especially complete block, may cause significant elevation of intracranial pressure.

Neuroradiological Investigations

Basic pathology is well elucidated by radiological investigation as a rule. In some cases simple plain X-rays show the pathology and severe disease can be discovered when still causing only mild radicular pain.

1. Radiography and X-Ray Tomography

Plain X-ray films of the craniocervical region including the "open mouth projection" are essential. There are many anatomical and projectional variations of the cervical spine in this region. It is essential to be familiar with these in order to distinguish normal from pathological (Wackenheim 1974). Nicolet *et al.* (1984) for example described an apparent hyperostosis of the C_2 body which was found to be a projectional variant and not an osteoid osteoma.

X-ray tomography must be multidirectional (Russin and Guinto 1976).

The distance from the posterior aspect of the anterior arch of the atlas to the anterior aspect of the odontoid process is less than 3 mm in adults. A larger distance bespeaks atlantoaxial dislocation. The antero-posterior diameter of the spinal canal in the atlanto-axial region is 20–26 mm in men and 19–25 mm in women. According to Apuzzo *et al.* (1978), patients develop clinical symptoms when the anteroposterior diameter is less than 14 mm at C_1–C_2 level.

In neutral position the odontoid is usually about 5 mm directly beneath the anterior lip of the foramen magnum. Craniocervical abnormalities can be identified by reference to the clivus baseline, Chamberlain's line (Taveras and Wood 1976), or McGregor (1948) and McRae's line (McRae 1955). The angle between clivus and cervical canal (clivus-line + line through the axis) is normally greater than 130 degrees (Dolan 1977). Details of these lines are given in standard radiological texts.

Traumatic lesions, fractures, osteolytic erosion of tumorous and inflammatory origin are readily detectable on tomograms. In inflammatory processes there may be an increased width of the retropharyngeal soft-tissue space on lateral view.

2. Gas or Water Soluble Contrast Myelography

These studies can reveal additional soft-tissue component to the bony compression and identify a syrinx or Chiari malformation.

3. Computerized Tomography and Myelography

In cases of basilar impression, CT of the cranium and upper cervical spine can image the invaginated odontoid process and other bony anomalies. A typical CT finding is a complete dense ring of bone on the lower cut of the posterior fossa at which level in normal cases there is still brain tissue (Boles *et al.* 1977). Moreover, such accompanying anomalies as syrinx, Chiari malformation, hydrocephalus are also detectable (di Chiro and Schellinger 1976, Nakagawa *et al.* 1977, Lee *et al.* 1978, Oberson and Azam 1978, Carella *et al.* 1981, Isu *et al.* 1983).

CT myelography can reveal the presence of increased pathological soft tissue compressing the medulla or spinal cord (Laasonen *et al.* 1985).

High resolution scanners allow detection of pathological changes in the spine more precisely even without injection of any contrast media (Ethier *et al.* 1979, Coin 1980, Capesius *et al.* 1981, Burguet *et al.* 1985).

4. Magnetic Resonance (MR) Scan

Because of the ability to image directly in three orthogonal planes (horizontal, sagittal and coronal) MR imaging simplifies the assessment of some clinical problems involving the region of the craniovertebral junction (Hawkes *et al.* 1983).

Sagittal MR scan in patients with basilar invagination shows associated brain stem deformity. Chiari malformation and other abnormalities of the craniovertebral junction are also demonstrable (Bradley 1984).

In rheumatoid arthritis the narrowing of the foramen magnum by subluxation of C_1 and C_2 can be visualized. Moreover, a mass with soft tissue signal intensity can be seen posterior to the dens in the region of joint synovium and transverse ligament (Modic *et al.* 1984).

Because cortical bone gives a zero signal bone defects are difficult to delineate. However, cancellous bone, with a marrow cavity within the vertebral bodies, exhibits high signal intensity. In tumours of the vertebral bodies destruction and marrow replacement by tumour significantly alters the signal intensity, collapse of the body may be seen and the associated paraspinal mass visualized (Han *et al.* 1983).

There are however, certain limitations of this new investigation at present. Access to MR scanners is fairly limited in many countries. Considering also the fact that bony details are not readily shown on MR, conventional X-ray methods and "bone window" images an CT are unlikely to became absolate.

5. Vertebral Angiography

Vascularized tumours of the spine can be verified by angiography.

If symptoms of interruption of the vertebro-basilar circulation are present (*e.g.*, in cases of Jefferson fracture) vertebral angiography is mandatory. In severe subluxation of the atlanto-occipital joint damage to the vertebral artery is rather common.

In basilar impression marked angulation of the vertebral artery can to be seen. An abnormal position of the PICA may identify the Chiari malformation.

6. Arthrography

The atlanto-axial joints have been visualized radiologically recently by percutaneous puncture of the articular space (Dirkeimer 1977, Mellström *et al.* 1980).

Surgical Procedure

Pre-operatively the patient is examined for oral infection. Nasal, oral and pharyngeal swabs are taken for bacterial culture and sensitivity. The antibiotic combination with the greatest in vitro effect is administered for some days prior to surgery, intravenously during surgery and also in the immediate post-operative period.

Under general anaesthesia tracheostomy is performed and ventilation continued via a cuffed tracheal tube. The patient is placed in the recumbent position, the skin of the mouth and nose and mucosa of the oropharynx are cleaned with iodine solution. A Whitehead retractor is placed into the mouth for a possible maximal retraction and the tongue depressed by a special attachment. The naso- and hypopharynx are packed off to prevent blood entering the paranasal sinuses or the oesophagus during operation.

The tuberculum of the atlas is identified by palpation and an X-ray image intensifier used to identify anatomical landmarks for the appropriate direction of approach. At this stage a decision must be made whether incision of the soft palate is necessary or not. If so (*e.g.*, direction up toward the clivus), the soft palate is incised in the midline avoiding the uvula. It is dissected a short distance on each side from the hard palate and the flaps retracted laterally with soft rubber bands. In cases where the incision is not necessary (direction down to upper cervical vertebrae) two rubber catheters are passed through the nose into the nasopharynx and the soft palate is elevated on both sides by tightening and knotting the catheters. The tip of the uvula is pulled laterally by a silk suture (Figs. 3 and 4).

The next steps of the procedure depend on the nature of the pathological process itself.

The length and site of the incision on the pharyngeal wall depends also on the pathology. In any case, we recommend a longitudinal incision and

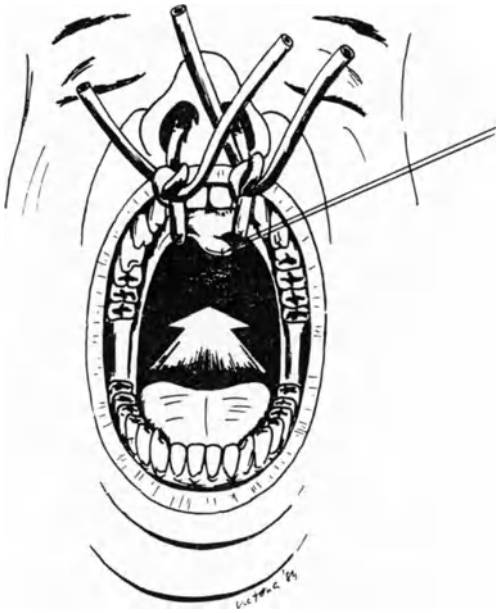

Fig. 3

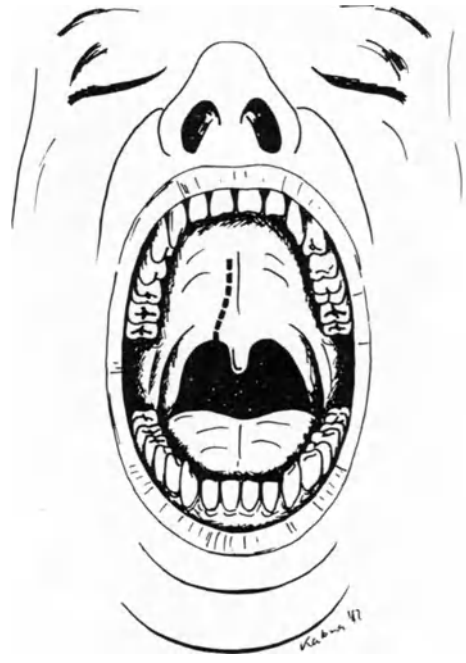

Fig. 4

Fig. 3. The soft palate elevated on both sides by rubber catheters

Fig. 4. The soft palate incised avoiding the uvula

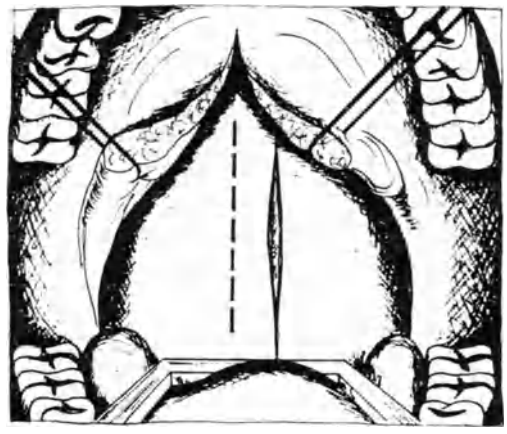

Fig. 5. Double incision of the pharyngeal wall and deeper structures to facilitate safer closure and better healing

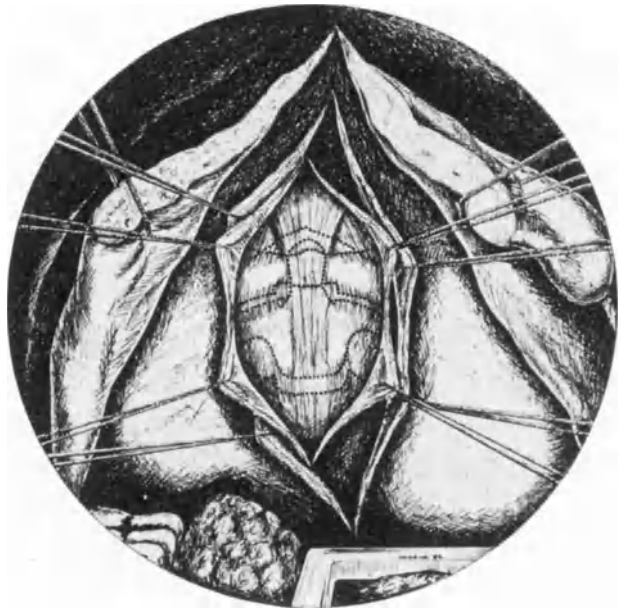

Fig. 6. The two flaps of the pharyngeal wall and the deeper structures are retracted laterally. The spinal structures are partly recognizable

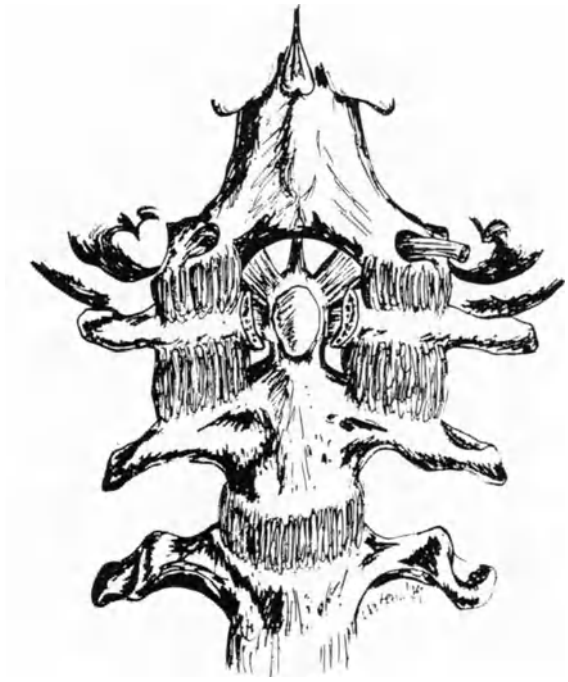

Fig. 7. Resection of the anterior arch of the atlas in a curved line to avoid injury to the atlanto-occipital or atlanto-axial joints

separation of two flaps. The first layer consists of mucosa and submucosa and the incision is here near the midline. The second flap consists of the tendinous muscular layer, the anterior longitudinal ligament and the periosteum together, which are incised a few millimetres away from the former incision in the midline (Fig. 5). Preparing two flaps and making the longitudinal incisions at a distance facilitate safer closure and better healing. Both flaps are retracted laterally by sutures (Fig. 6).

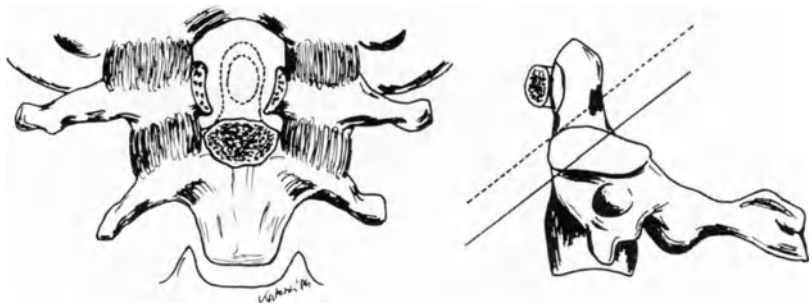

Fig. 8. The resection plane of the odontoid proposed by us. In the case of a high upward and backward projecting odontoid process we advise starting the resection of the odontoid 4–5 mm deeper than its neck, in the central part of the body of the axis

The anterior arch of the atlas is removed piecemeal using a high speed drill under the surgical microscope with X-ray image intensifier control.

During these manipulations it is of vital importance to know the exact dimensions of the structures seen under the microscope. The average size of some important bony structures in millimetres is given. We recommend the use of a small ruler or an instrument with precisely calibrated dimensions which when placed in the operative field aids precise measurement (*e.g.*, the width of a bony resection or the depth of a cavity). Resection of the anterior arch of the atlas should not be carried further than 8–10 mm from the midline because of the proximity of the atlanto-occipital joints.

The resection surface should be curved with its lower part being more medially (Fig. 7), for although the medial border of the atlanto-occipital joint is about 10 mm from the midline, the atlanto-axial joint can already be inadvertantly entered at 8 mm from the midline.

The odontoid process is also removed with the high speed drill and diamond burr. The latter prevents soft tissue (ligamentous) damage and is used in the deeper parts of the bony structures.

In our experience resection of the high upward and backward projecting

odontoid process is the most complicated part of this surgery. Whilst avoiding injury to the dura mater total resection of the odontoid must be carried out. If the resection cannot be achieved it is often due to an inappropriate angle of approach. Therefore, we suggest that resection of the odontoid is begun 4–5 mm deeper than its neck, in the central part of the body of the axis (Fig. 8).

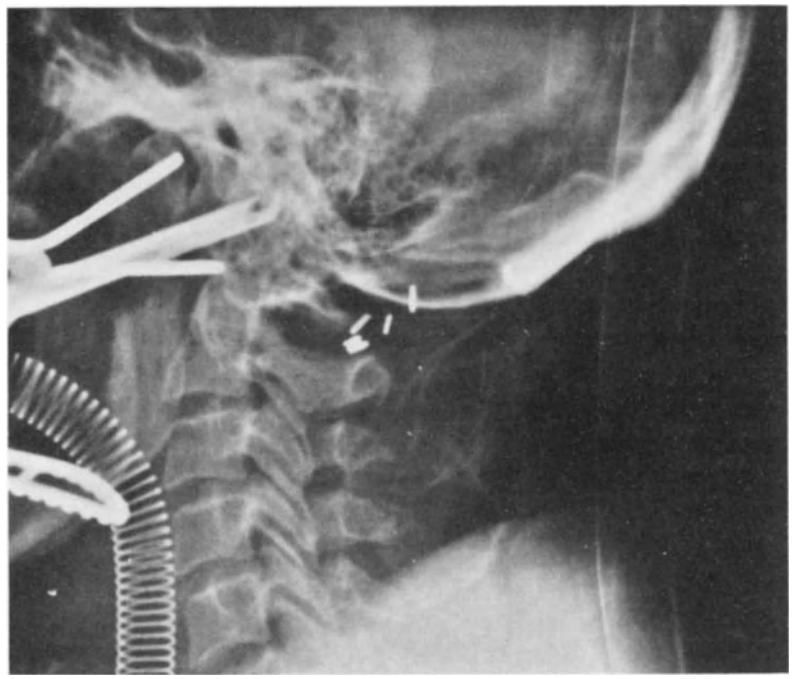

Fig. 9. Removal of the lower part of the clivus with upcutting bone forceps. Picture was taken during operation of case no. 9

For the resection of the lower part of the clivus (the anterior margin of the foramen magnum) the diamond burr and upcutting bone forceps are used (Fig. 9).

In tumour cases after the pharyngeal incision it is usually easy to enter the tumour itself, which is then removed with tumour forceps, microcurettes (Fig. 10) and suction. In one of our C_2 tumour cases (osteoclastoma) an interbody fusion was performed using iliac autograft and smaller bone chips placed into the cavity evacuated in the vertebral body.

In intradural processes the dura may be opened in a cruciate fashion or by a "U" shaped flap. Venous bleeding from the dura is easily controlled with diathermy. Closing of the dura is a great technical problem and will be

considered in detail later. The author has no personal experience in intradural cases.

In extradural processes the closure is simpler. A thin gelfoam layer is placed on the deepest part of the exposure and the posterior wall of the

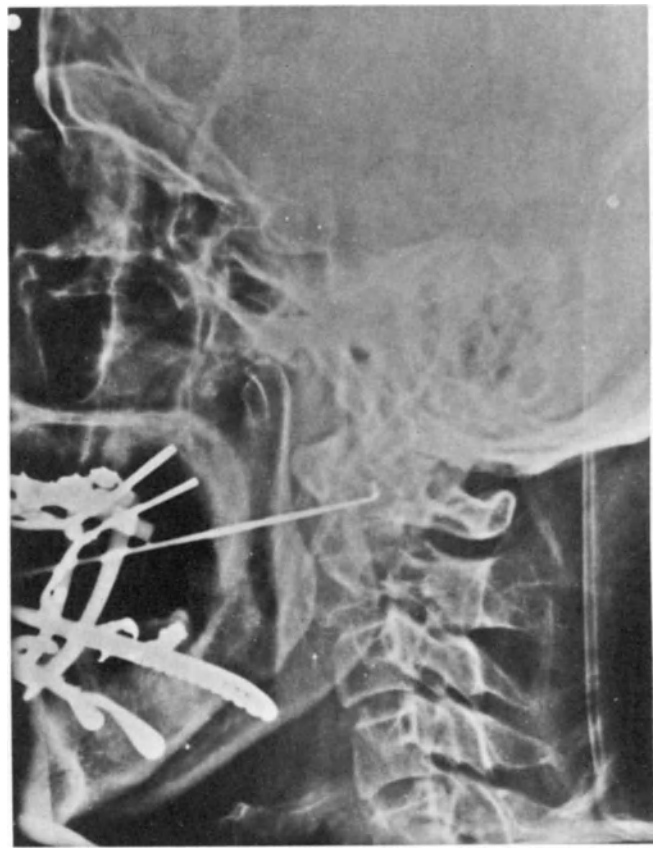

Fig. 10. Tumour (osteoclastoma) was removed with microcurette from the body and dens of the axis. It was possible to excavate the odontoid without resection of the anterior arch of the atlas. Picture was taken during operation of case no. 6

pharynx closed meticulously in two layers. The soft palate, if incised, is replaced and sutured.

At the end of the operation a nasogastric tube is passed and is usually required for four days. The oral cavity is packed for a few days to prevent post-operative haematoma. The tracheostomy tube remains for 4–5 days, depending on the oedema of the palate and tongue. The combined antibiotic therapy commenced preoperatively, is continued in the post-operative period.

There is a general consensus of the necessity of microsurgical technique in transoral surgery. However, controversy remains among authors in the last decade concerning some details of the operation.

Most surgeons give prophylactic antibiotics as we do, before surgery. Apuzzo *et al.* (1978), however, argue that if normal oral and nasal flora have been obtained, no antibiotic cover is necessary.

An endotracheal tube need not constitute an obstacle at the transoral operation. Some surgeons therefore declare tracheostomy unnecessary (Derome and Guiot 1979, Spetzler *et al.* 1979, Hitchcock and Cowie 1983). We have considered tracheostomy important for adequate airway control in the early post-operative period. However, after greater experience and in suitable anatomical conditions, we have omitted the tracheostomy in our last two cases without ill effect.

Formerly the incision of the soft palate was performed in different ways, but most of surgeons in recent years agree on the longitudinal incision.

According to the opinion of Delgado *et al.* (1981) lesions located in the upper part of the clivus are better reached by the labio-mandibular approach, because this gives a shorter working distance and a larger operative field. This approach was originally described by Wood *et al.* (1980). They extended the surgery with glossotomy if the lesion extends further down to C_2 or below. We have not used this approach as we have found it possible to resect tumour even in cases of upper clivus or C_3 chordomas without such extended surgery.

Menezes *et al.* (1980) gained remarkable experience in children with abnormalities of the craniocervical junction. They emphasized that in children, at the resection of the odontoid it is important to leave the transverse portion of the cruciate ligament complex intact as well as the cruciate notch on the axis. This provides better post-operative stability and may allow for future bone formation, since the periosteum is intact. They were able to demonstrate reformation of the odontoid process after such surgery.

In transoral surgery radiographic control is vital. Spetzler *et al.* (1979) even proposed continuous monitoring of somatosensory cortical evoked potentials during transoral microsurgical odontoid resection. This method of control makes the surgery safer in the deep structures near the medulla.

In intradural processes various authors use different methods of closure. Delgado and Buchheit (1982) make no attempt to suture the dura. They proposed replacement of the bone removed from the clivus with an abdominal fat graft or muscle pledgets. Hayakawa *et al.* (1981), Crockard and Bradford (1985) stressed the importance of complete post-operative closure of the nasopharyngeal mucosa, long term nasopharyngeal packing and continuous spinal drainage. Hitchcock and Cowie (1983) were able to suture the "U" shaped dural flap using 9/0 nylon.

In cases of atlanto-axial dislocation operated by the transoral route stabilisation of the spine is a crucial point. There are methods to achieve this fixation anteriorly. De Rougemount *et al.* (1966) placed a bone graft anteriorly from the ventral rim of the occipital bone to the level of the body of the axis. Thompson (1970) proposed the same fixation with bone chips after denuding the anterior surface of the bony structures. Estridge and

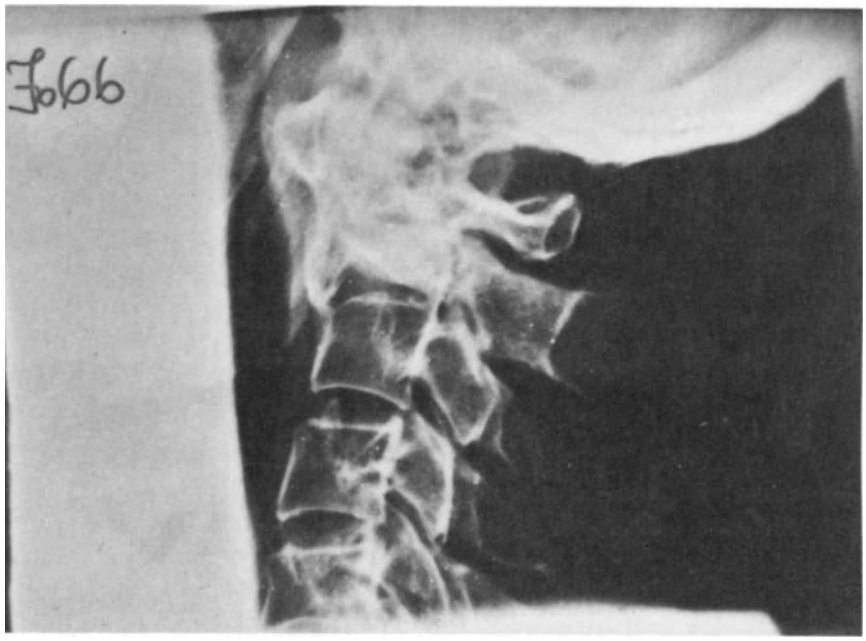

Fig. 11. After resection of the tumour (osteoclastoma) from the C_2 body and odontoid bone chips were placed into the cavity. One year later control X-ray showed excellent fixation without any further need of surgical procedure (case no. 6)

Smith (1967) after the resection of the dens prepared a bed for the iliac graft which was inserted into the cavity and guaranteed good fixation. O'Laoire and Thomas (1982), Fang *et al.* (1983) achieved good results with anterior fixation using bone chips placed between the articular facets of the lateral joints.

Having resected the tumour from the body of the C_2 or C_3 vertebrae (chordoma in Mullan's cases, osteoclastoma in one of our cases) a bone graft or bone chips can be placed in the cavity, which gives excellent fixation (Fig. 11).

Potential complication of transoral surgery occur predominantly in

cases of intradural pathology (Derome and Guiot 1979) cerebrospinal fluid leak and meningitis being the commonest. In entirely extradural lesions wound infection is rare. Tongue and pharyngeal swelling usually resolves without special treatment. Anterior bone grafts became displaced in two of the five tuberculous patients of Fang *et al.* (1983). Crockard *et al.* (1985) reported nine patients with rheumatoid arthritis: two of those required resuture of the soft palate. Of the fifteen transoral operations performed on our thirteen patients only one post-operative complication occurred. The soft palate of a patient with basilar impression had to be resutured, but healed thereafter.

Some of the space-occupying lesions of that area include malignant or semi-malignant tumours, surgeons must therefore be prepared to reexplore those recurrences suitable for reoperation. Although no references can be found in the literature on transoral secondary surgery our material seems to prove that in cases of recurrent chordomas the transoral route is again sufficient to remove much of the recurrent tumour tissue. Principles of transoral reoperation are similar to those of the primary ones. Scars and adhesions are not significant obstacles in finding the necessary layers and anatomical structures and even the dura can be found with safety.

Summary of Cases

This series includes thirteen patients operated on by the transoral approach. In two patients transoral surgery was repeated 2 years later because of tumour recurrence. The clinical data of these patients are summarized in Table 1.

There was no operative mortality in cases of primary surgery. One of our patients (case no. 5) died in two weeks after an urgent transoral reoperation necessitated by sudden medullary respiratory disturbance. Although neurological symtoms improved remarkably in the early post-operative period we lost the cachectic patient from pulmonary and meningeal infections.

Case Reports

Case No. 11

G. M. a 44-year-old lady had an accident in 1980 when she hit the nape of her neck. Since then she had felt numbness in her fingers and tongue and also noticed progressive unsteadiness of gait. She was investigated in a County Hospital where basilar impression was discovered. She was admitted to our Institute in February, 1983. Examination revealed mild diabetes mellitus and hypertension. Neurological examination revealed a limited ability to move the head associated with the neck held stiffly toward the left. The gag reflex was absent. Bilateral nystagmus and lingual atrophy

Table 1. *Summary of Clinical Course in*

Case no.	Sex, age (years)	Pathology	Disease localization	History and time before admission	Symptoms
1	m, 46	basilar impression	odontoid, clivus, C_1	15 years myelopathy; 5 years radicular pain	cranial nerve dysfunction; radicular symptoms; myelopathy
2	m, 29	osteo-blastoma	C_2, C_3	6 years injury, pain; 5 years injury, tetra-paresis, dorsal decompression; bone graft	myelopathy
3	m, 27	osteo-blastoma	odontoid, C_2	3 months pain, decompression	myelopathy; radicular symptoms
4	f, 27	chordoma	clivus	5 years cranial nerve dysfunction, dorsal surgery; 3 years pain	cranial nerve dysfunction
5	f, 57	chordoma	C_{2-4}	5 years cranial nerve dysfunction; 3 years epipharynx surgery	cranial nerve dysfunction; myelopathy
	60	recurrent chordoma	C_{2-5}	3 years transoral surgery	rapid bulbar lesion
6	m, 41	osteo-clastoma	odontoid, C_2	3 months pain	none
7	m, 46	chordoma	odontoid, clivus	2 years pain; cranial nerve dysfunction; epipharynx surgery	cranial nerve dysfunction

Thirteen Patients with Transoral Surgery

X-ray findings	Angiography	Myelography	CT scan	Transoral and other treatment	Outcome and follow-up period
basilar impression	VA displaced	not done	no	clivus, odontoid, C_1 resection	excellent: 7 years
C_{2-3} destruction	VA displaced; tumour stain	CSF space no compression	no	tumour removal	excellent: 5 years, then died from other causes
odontoid, C_2 destruction	VA displaced	not done	no	tumour removal; posterior skeletal fixation	excellent: 5 years
clivus destruction	none	not done	no	partial tumour removal	4 years, died of tumour
C_{2-4} destruction	not done	CSF space compression	destruction and hypodensity	tumour removal	excellent for 3 years
C_{2-5} destruction	not done	not done	hydrocephalus	acute surgery following respiratory arrest, subtotal removal	died in 2 weeks of pneumonia and meningitis
odontoid, C_2 destruction	not done	not done	destruction and hypodensity	tumour removal, irradiation; iliac bone graft	excellent: 4 years
clivus, C_{1-2} destruction	not done	not done	destruction and hypodensity	tumour removal irradiation	excellent: 2 years

Table 1 (continued)

Case no.	Sex, age (years)	Pathology	Disease localization	History and time before admission	Symptoms
	48	recurrent chordoma	odontoid, clivus	2 years transoral surgery	cranial nerve dysfunction
8	f, 22	ectopic thyreoid adenoma	meso-pharynx	1 year subtotal thyoidectomy	disturbance of swallowing
9	f, 41	basilar impression	odontoid, clivus, C_1	8 years myelopathy; 4 years surgery, dorsal decompression	cranial nerves radicular symptoms myelopathy tetraparesis
10	m, 44	chordoma	clivus	5 months diplopia; 3 months lagophthalmus	nerve III paresis, nerve V hypaesthesia
11	f, 44	basilar impression	odontoid, clivus, C_1	4 years trauma; 4 years gait and sensory disturbance	stiff neck myelopathy paresis right leg cranial nerves, bulbar symptoms
12	m, 30	odontoid fracture	odontoid	1 month trauma, neck pain and stiffness	no neurological symptoms
13	f, 34	basilar impression	odontoid, C_1	4 years headache; 1 year gait disturbance obscuration	cranial nerves ataxia

CT = computerized tomography, VA = vertebral artery, BA = basilar artery,

X-ray findings	Angiography	Myelography	CT scan	Transoral and other treatment	Outcome and follow-up period
clivus	BA displaced	not done	clival and epipharyngeal density	tumour removal; irradiation	improved 1 year
40 × 20 mm soft-tissue shadow	vascularized tumour in mesopharynx	not done	not done	tumour removal	excellent: 3 years
basilar impression	not done	not done	basilar impression	clivus odontoid, C_1 resection	improved 2 years
clivus destruction	retro-, parasellar dislocation	not done	destruction and hypodensity	tumour removal, irradiation	excellent: 2 years
basilar impression	VA displaced	not done	basilar impression	clivus odontoid, C_1 resection	improved $1^1/_2$ years
odontoid fracture and dislocation, C_1 dislocation	not done	not done	not done	odontoid, C_1 resection, posterior skeletal fixation	excellent: 1 year
basilar impression, C_{2-3} block vertebrae	not done	not done	basilar impression	odontoid, C_1 resection	excellent: $^1/_2$ year

CSF = cerebrospinal fluid.

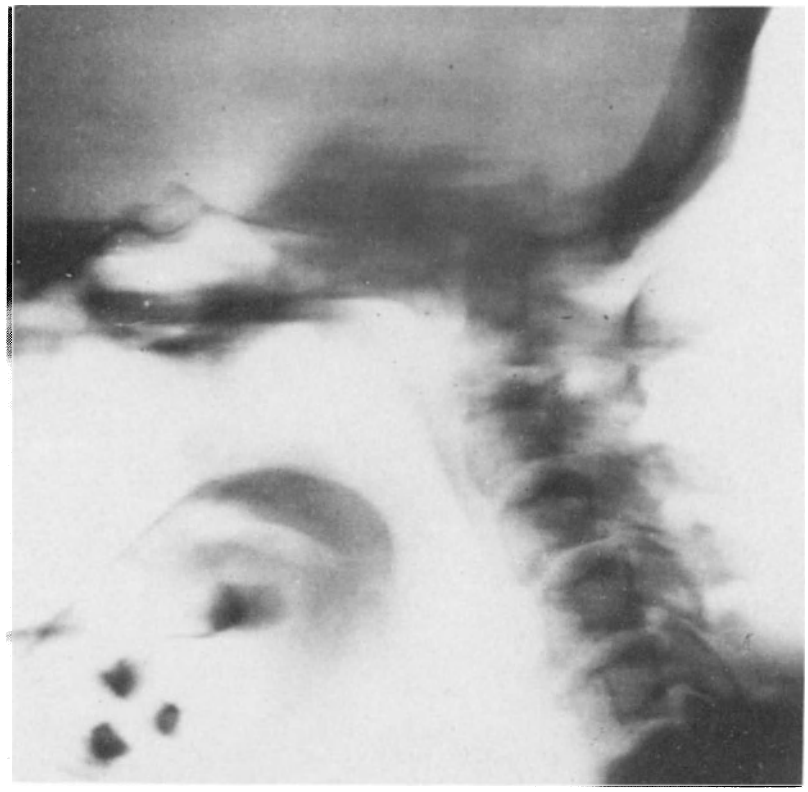

Fig. 12. Case no. 11. Pre-operative tomogram of craniocervical region shows marked narrowing of foramen magnum caused by the odontoid invaginating and tilting backwards into the medulla

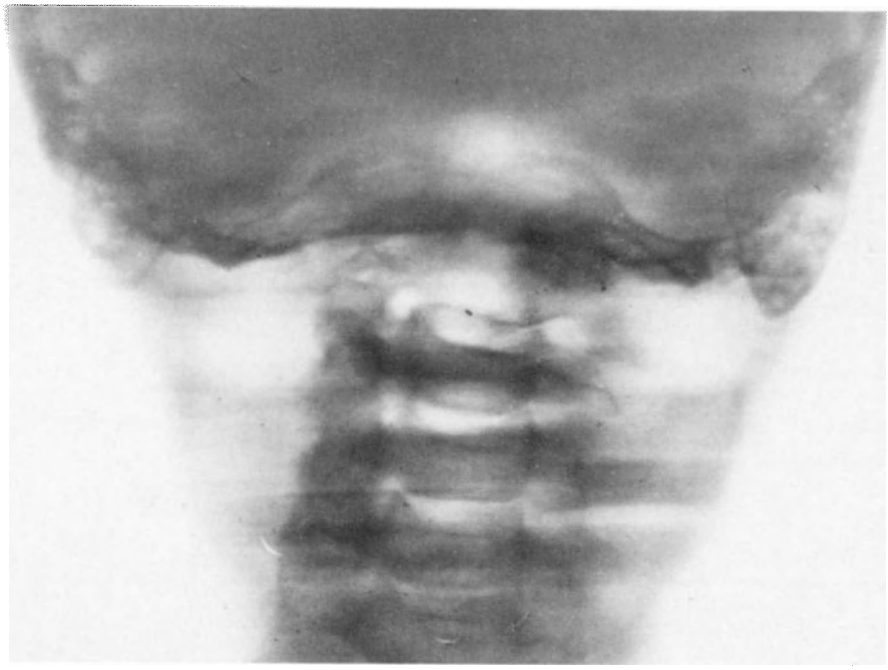

Fig. 13. Case no. 11. Pre-operative AP tomogram demonstrates the elevated foraminal rim of the occipital bone

with fasciculation was revealed. She had nasal speech, exaggerated right reflexes, mild paresis in the right leg and hypaesthesia in the left arm. Skull X-ray showed marked platybasia with an overdeveloped odontoid indenting the medulla (Figs. 12 and 13). On CT scan dislocation of the fourth ventricle to the left and backwards was demonstrated. The odontoid process

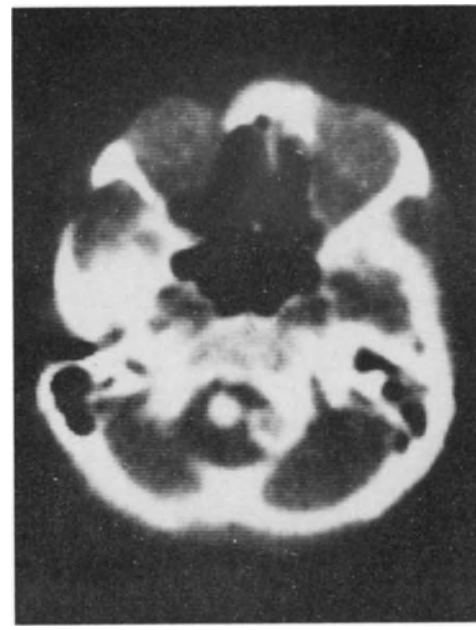

Fig. 14. Case no. 11. CT slice at the level of the basal part of the posterior fossa shows the indenting dense ring of the foramen magnum and the invaginated odontoid process

appeared at the level of the foramen magnum on the left side, intracranially (Fig. 14). Left sided retrograde brachial angiography showed a curved deformation of the left vertebral artery as it entered skull (Figs. 15 and 16). She was operated on under general anaesthesia via tracheostomy. The soft palate was divided and retracted. A paramediam incision on the left side about 5 mm from the midline in the "double layer" fashion was made from the atlas, to the C_2 body on the posterior pharyngeal wall. After removal of soft tissue the anterior arch of C_1 was explored and its central one centimetre was resected. The odontoid process was progressively decreased in size from its base with the uppermost part of the C_2 body drilled away to avoid medullary damage from early mobilization of the odontoid (Fig. 17). After

internal removal of the odontoid, the entire process was extirpated. She improved remarkably but still presented mild difficulties in swallowing when seen one and a half years post-operatively.

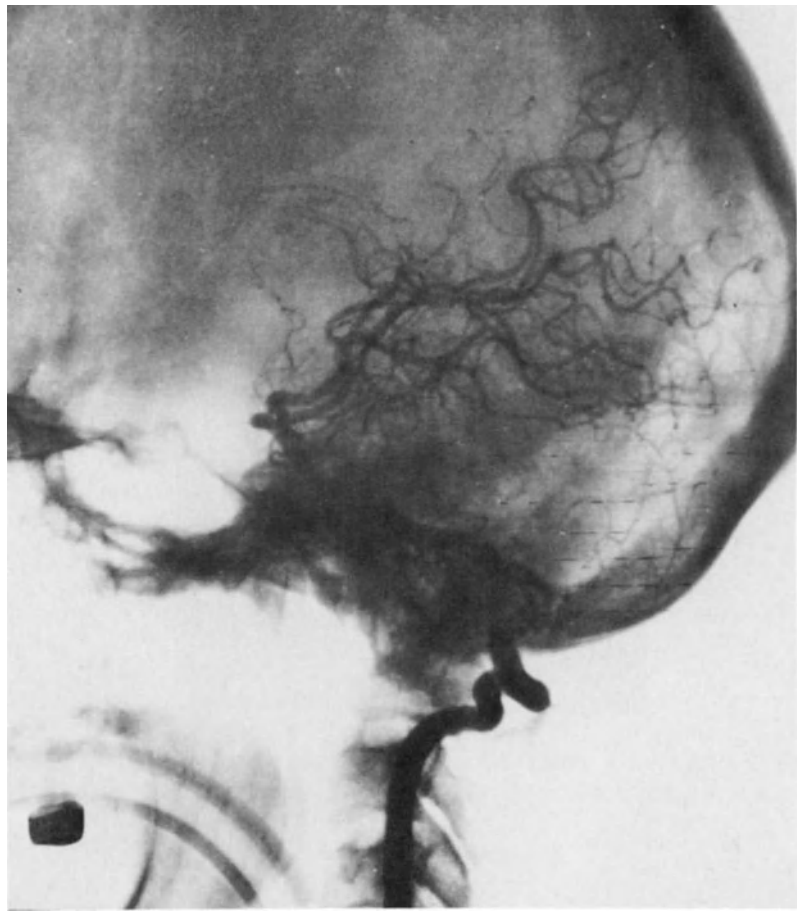

Fig. 15. Case no. 11. Lateral angiogram shows the left vertebral artery displaced over the extremely elevated rim of the foramed magnum

Case No. 12

A. D., a 30-year-old man fell from a ladder and lost consciousness for a short while in July, 1984. A few hours later he felt cervical pain. Investigating the cause of the persistent neck pain it became evident that his odontoid process had broken and tilted to the right and the axis was displaced backward. Attempts at closed reduction failed and he was placed in an external fixation device. He was admitted to our Institute in August,

1984. Examination revealed no neurological signs. Transoral resection of the fractured odontoid was decided upon (Figs. 18 and 19). General anaesthesia was induced via tracheostomy. A midline incision of 3 cm was made on the posterior pharyngeal wall. The anterior cervical ligament was

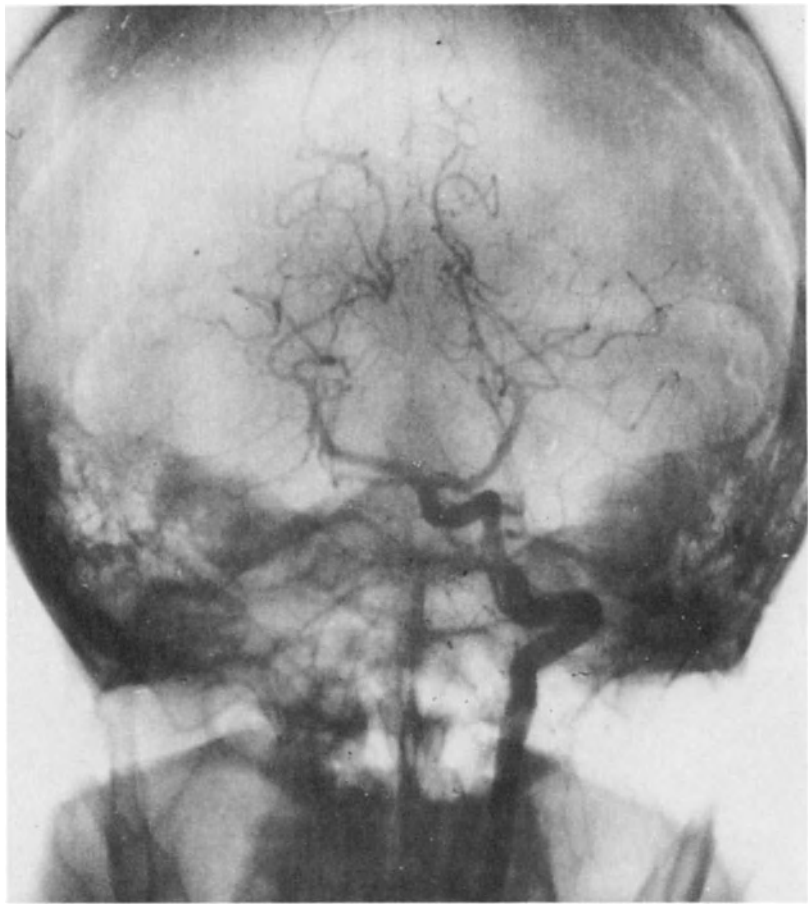

Fig. 16. AP view of Fig. 15

removed. The anterior arch of the atlas was removed under fluoroscopic control. The dislocated odontoid process was removed piecemeal with care to avoid injury to the dura. Post-operative recovery was uneventful, the external plaster fixation was reinstituted, a control X-ray films showed successful removal of the dislocated odontoid. He was transferred to a Trauma Unit, where an internal C_1–C_3 fixation was established using wires and lateral bonegrafts (Fig. 20). On a follow-up examination one year later he showed no neurological deficit, of all his cervical pain disappeared. He still excercises actively.

Fig. 17. Case no. 11. Post-operative tomogram of the craniocervical region demonstrates the total removal of the odontoid, partial resection of the anterior arch of the atlas, and lower part of the clivus. The ventral surface of the medulla has been freed

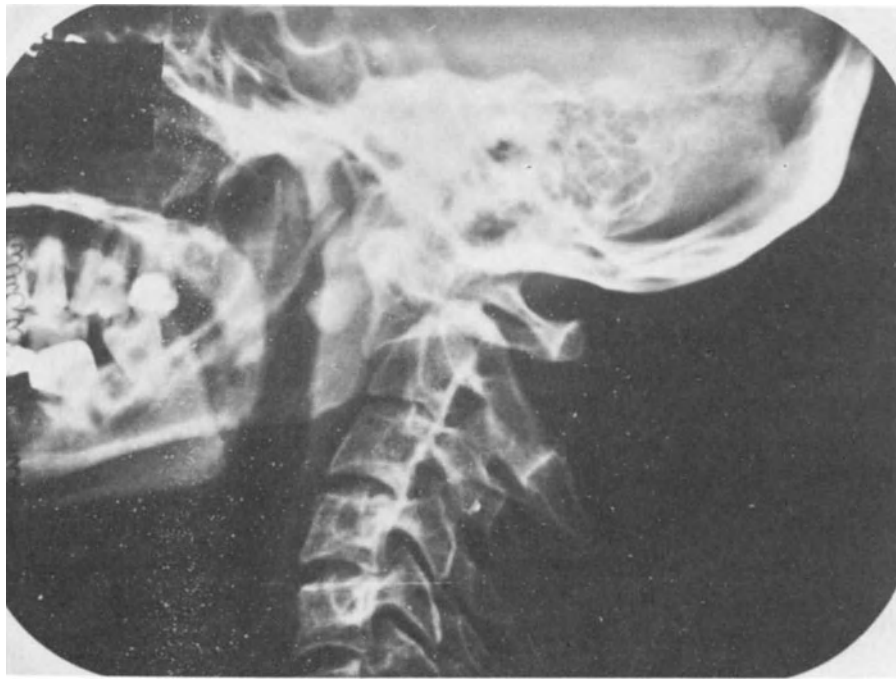

Fig. 18. Case no. 12. Pre-operative lateral X-ray of the craniocervical region illustrates the fracture of the odontoid accompanied by posterior dislocation of C_2

Case No. 10

P. D. This 44-year-old man presented with a history of diplopia of 5 months duration accompanied by gradual lagophthalmos and hypaesthesia in the ophthalmic nerve territory on the right side, developing over the

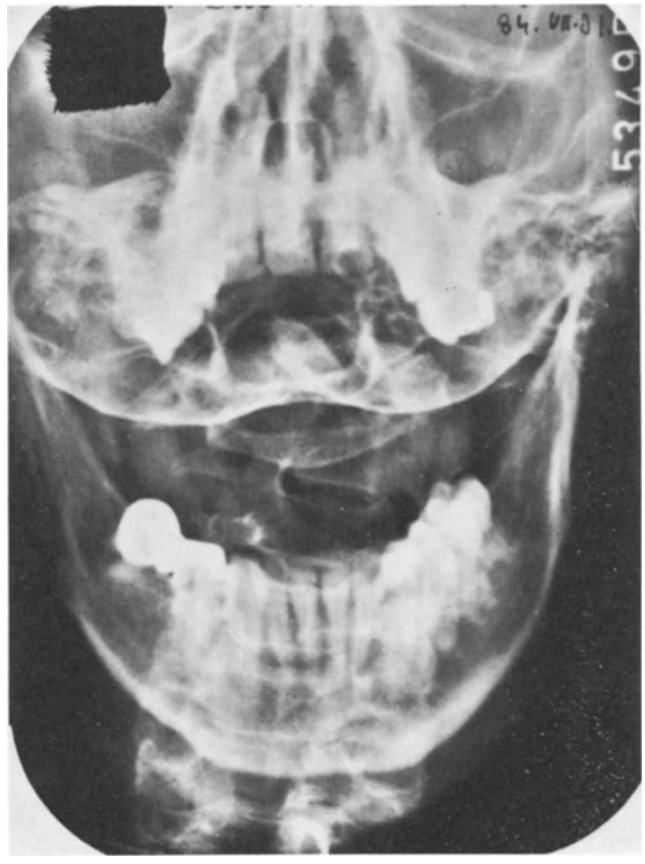

Fig. 19. AP X-ray film of the same patient shows the broken odontoid tilted to the right

following 3 months. He was admitted to our Institute in September, 1983. Examination revealed partial paresis of the third nerve and sensory loss on his forehead on the right, with mild reduction of the corneal reflex on the right. Tomograms of the skull revealed immense destruction of the clivus involving the basal part of the sella and right petrous apex (Fig. 21).

CT showed marked loss of bony structure in the upper two thirds of the clivus with predominance on the right and also of the apex of the right

petrous pyramid. The posterior part of the sphenoid sinus was filled with material of soft tissue density. The whole area of destruction enhanced strongly with contrast. Surgery: general anaesthesia was induced via

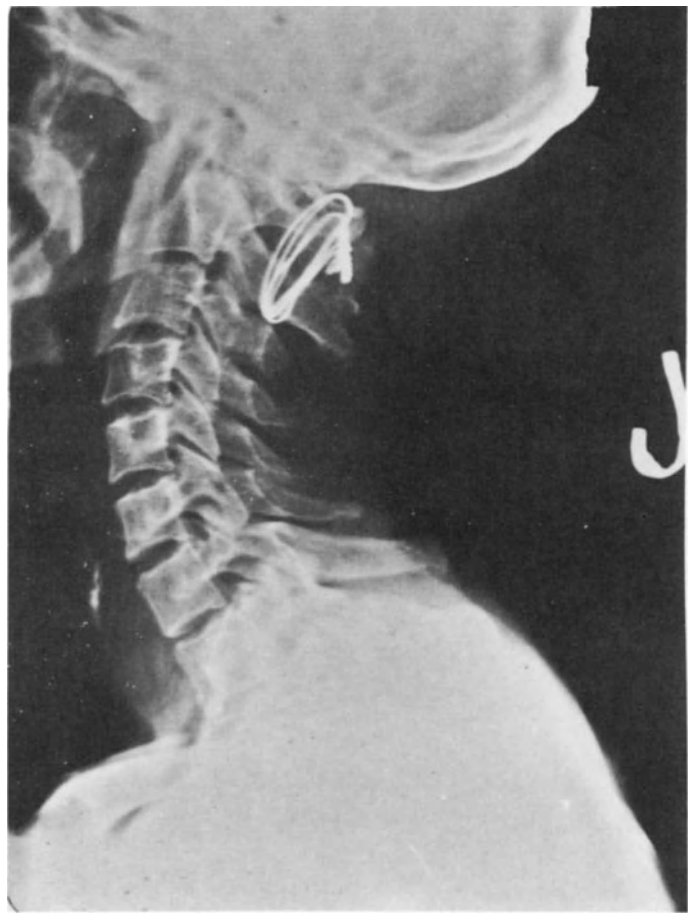

Fig. 20. Case no. 12. Post-operative lateral X-ray film shows complete removal of the odontoid and posterior internal fixation with bonegrafts and wires between C_1 and C_2

tracheostomy. The oral cavity was opened by Whitehead retractor and the base of the tongue depressed. The soft palate was divided, the hard palate was freed from all connective tissue covering. The posterior rim of the hard palate was removed piecemeal. An incision half a cm paramedian, from the caudal border of the epipharynx on the posterior pharyngeal wall through mucosa and periosteum revealed a greyish largely avascular tumour which was removed by suction and curette (Fig. 22). Meticulous closure was

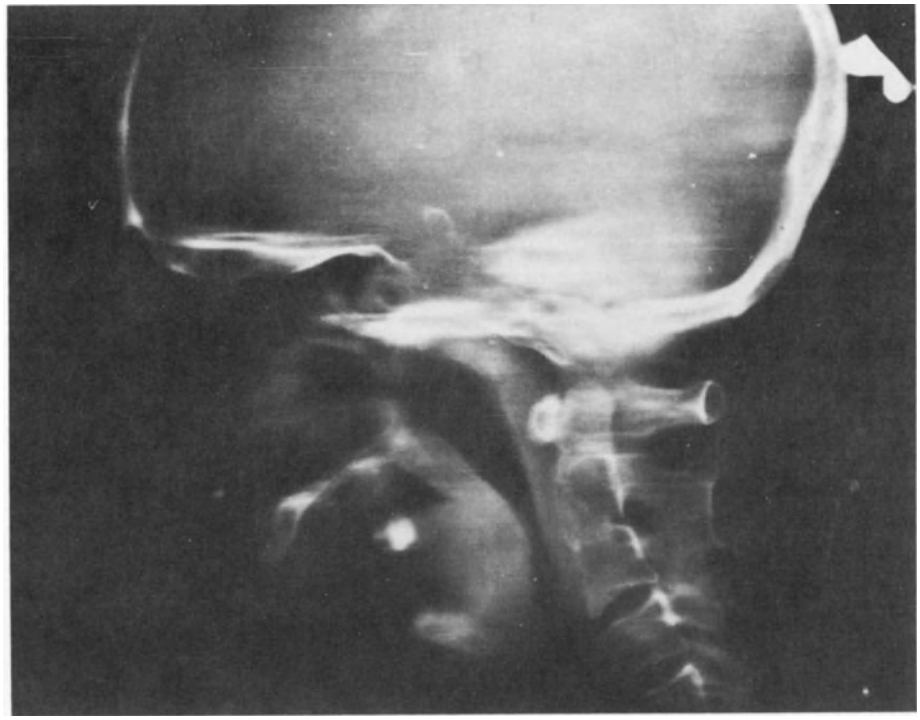

Fig. 21. Case no. 10. Pre-operative X-ray tomogram shows loss of sellar and clival bony structure

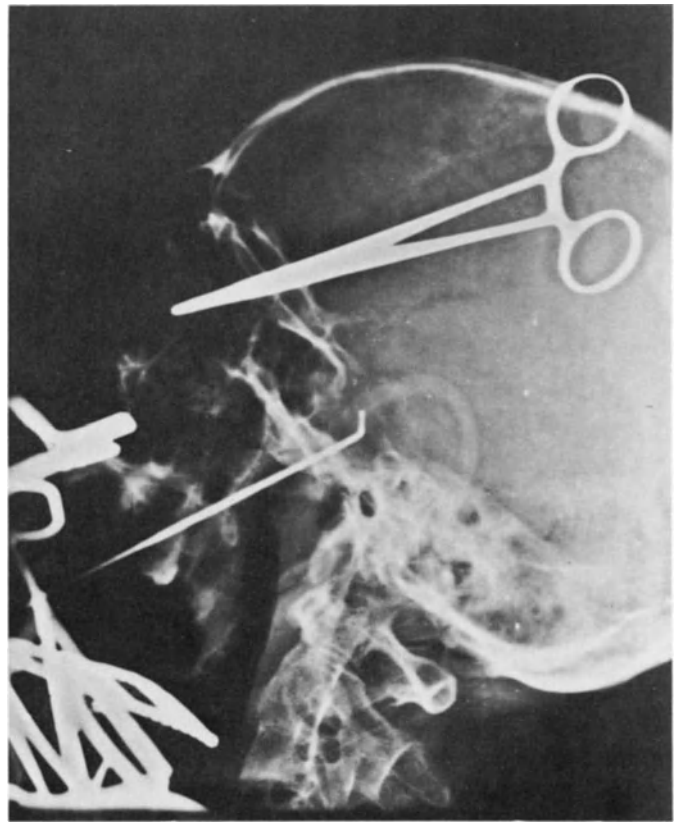

Fig. 22. Intraoperative X-ray film illustrates the feasibility of the transoral route in removing clival tumour using sharp curette

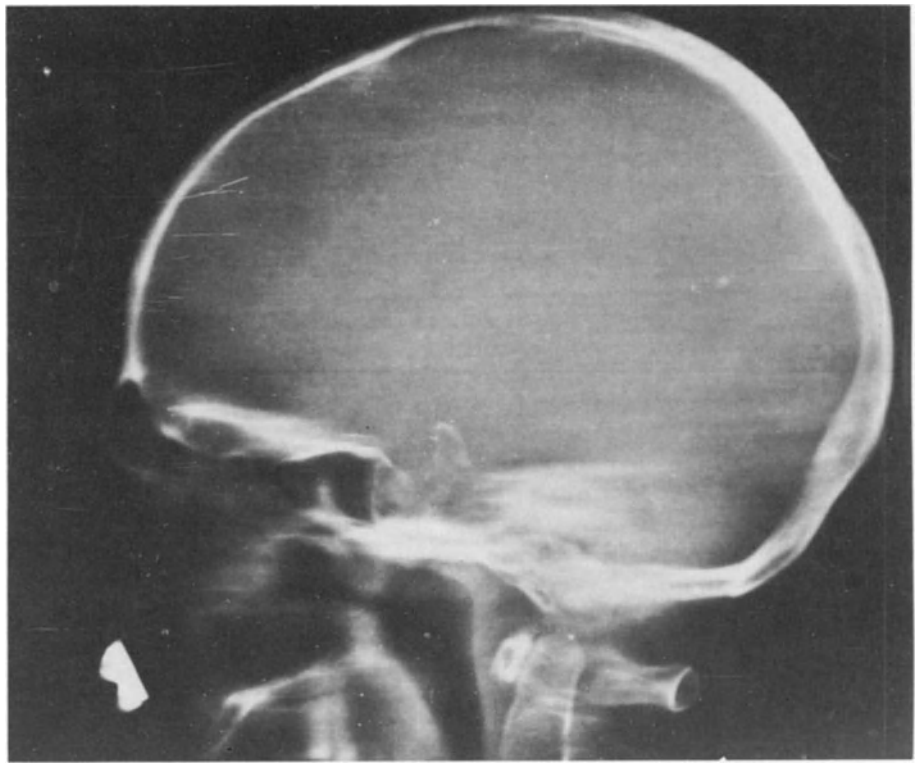

Fig. 23. Post-operative X-ray tomogram from the same patient demonstrates the process of re-ossification with higher density in the sellar and clival area

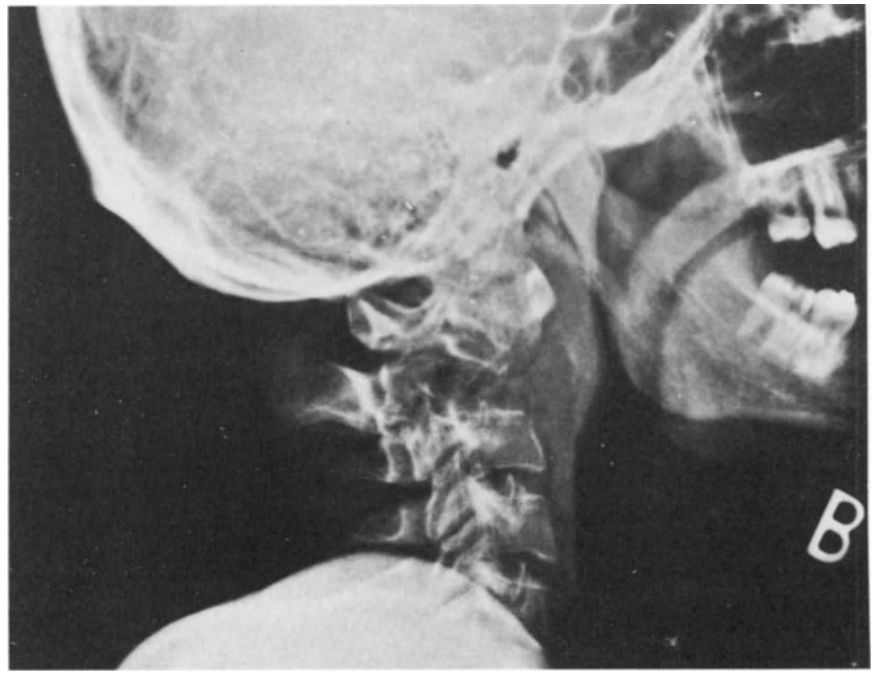

Fig. 24. Case no. 3. Pre-operative lateral X-ray film shows the entire disappearance of the C_2 body. The anterior arch of the atlas is slightly thickened

instituted in layers. Histology revealed chordoma the post-operative period was uneventful. The patient was put on antibiotics selected on the basis of bacterial culture taken from the oral cavity. No signs of infection occurred. He was discharged on the 12th post-operative day and was transferred to an Oncoradiological Unit. After irradiation (6,000 rads) third and fifth nerve function improved. Control X-ray tomograms showed signs of re-

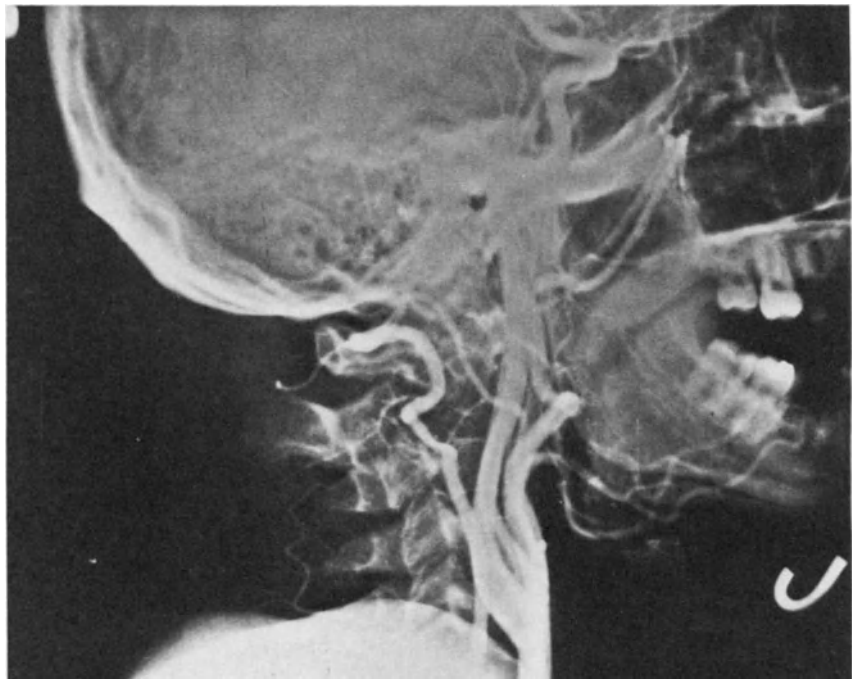

Fig. 25. Pre-operative lateral angiogram of the same patient shows the right vertebral artery displaced and stenosed at the level of the tumorous C_2

ossification in the clival and sellar area (Fig. 23). Two years post-operatively he has returned to work without neurological deficit.

Case No. 3

P. S. Z. A 27-year-old man who complained of painful stiffness of his neck for 3 months. Routine cervical X-ray showed some loss of bony structure in the C_2 body; he was placed in a cervical collar for partial fixation. His complaints increased. 2 months later repeat X-ray showed total loss of the C_2 body (Fig. 24). He was admitted to our Institute in 1980. Neurological examination revealed tactile hypaesthesia in the area of the left C_2, and hyperreflexia all over the extremities; X-ray tomograms showed severe destruction of the body of C_2 and of the odontoid process. The

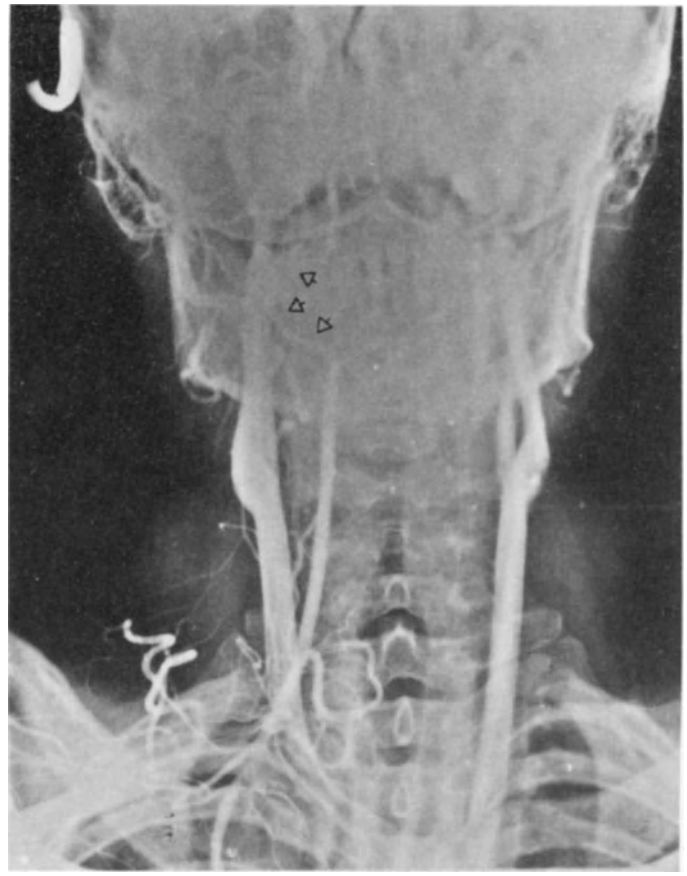

Fig. 26. AP view of the same angiography. Arrows show the curved displacement of the right vertebral artery

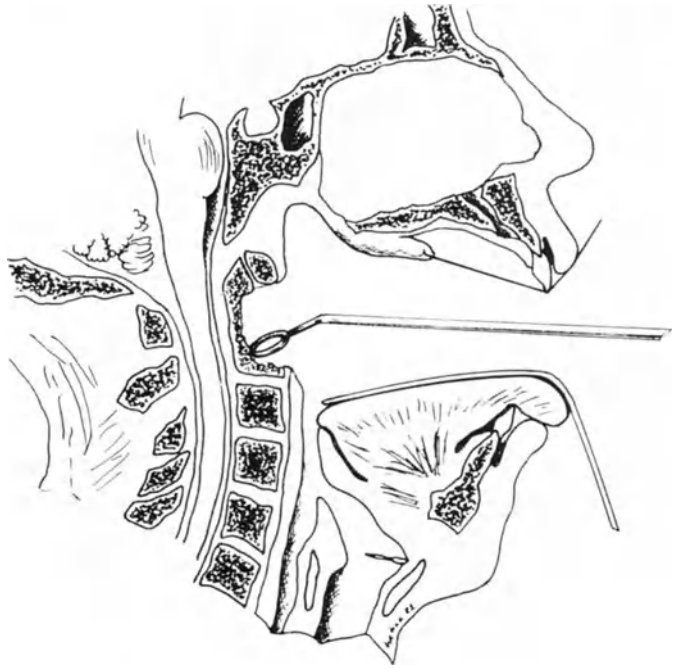

Fig. 27. Sketch of the operative situation of the same patient. Sharp curette used to remove the tumour from the C_2 body

remnants of C_2 and the anterior arch of C_1 were dislocated upwards and forwards. Right retrograde brachial angiography revealed marked narrowing and lateral displacement of the right vertebral artery from the level of C_2 cranially (Figs. 25 and 26). At operation by transoral approach the

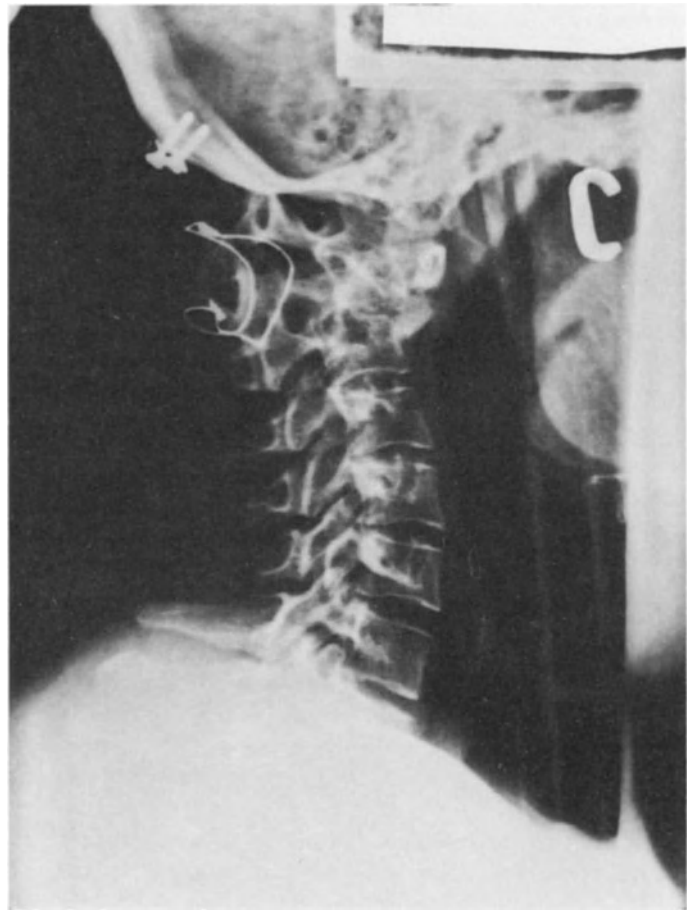

Fig. 28. X-ray control $2^1/_2$ years post-operatively shows re-ossification in the C_2 body. The posterior internal fixation using wires can be seen. The screws shows where the bilateral bone grafts have been fixed to the occipital bone

posterior pharyngeal wall was incised under fluoroscopic control in "double layer" fashion. The soft tumor in the C_2 body was removed by suction and curette (Fig. 27). Histology showed osteoblastoma. The post-operative period was uneventful. After 5,200 rad. irradiation the collar was replaced by posterior fixation using wire with bilateral bone grafts connected to the occiput (Fig. 28). His complaints resolved dramatically. He has remained well for 5 years.

Summary

Transoral surgery for ventral craniocervical pathology is an integral part of modern neurosurgery. This approach should be considered in many more cases than in current practice.

On the basis of our experiences with 15 operations in 13 patients we feel able to improve the surgical technique in some small details, as 1. the double, two flap incision of the posterior wall of the pharynx, 2. the method of "deep" resection of the odontoid in its high upward and backward position, and 3. insertion of bone grafts and chips in the cavity of tumorous vertebrae for fixation.

Admittedly, these cases are not encountered every day in neurosurgical units. An exact knowledge of the anatomical, neurological, pathological, radiological and surgical details is of vital importance and constitutes the basis of this account.

This method should not be confined to specialised regional neurosurgical centres, but is within the technical capacity of all trained neurosurgeons.

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