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Sutural closure in rabbit and man: a morphological and histochemical study

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INTRODUCTION

After cessation of growth many bones of the skull fuse and the intervening sutures are obliterated by calcified tissue (synostosis). If a cranial suture closes prematurely, i.e. before growth of the skull has ceased, this may result in a marked malformation of the skull (craniostenosis). The nature and mechanism of sutural closure are still obscure (cf. Hoyte, 1966; Herring, 1974) and histological studies of sutural closure are strikingly few (Hauschild, 1921; Loeschke & Weinnoldt, 1922; Sitsen, 1935; Moss, 1958; Kokich, 1976).

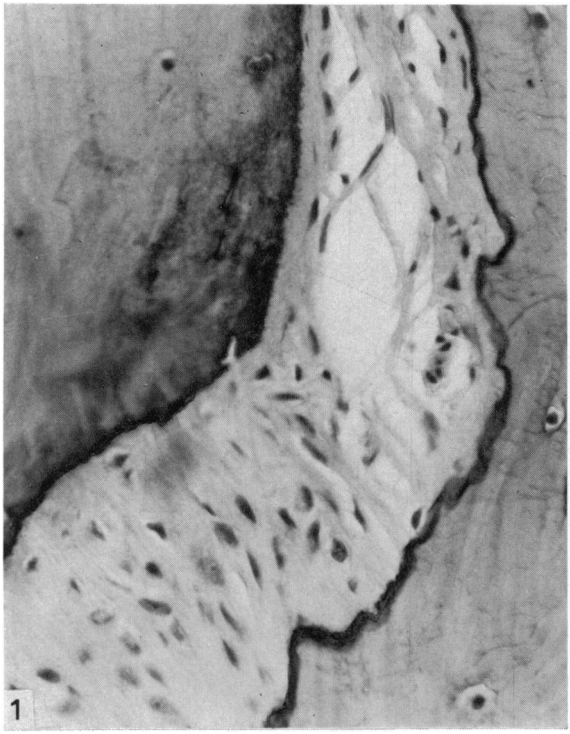
Studies of the morphological changes leading to fusion of the interfrontal suture in the rat showed closure to occur via cartilage formation and replacement as in fracture healing (Moss, 1958). Furthermore, from experimental surgical procedures in the same species, Moss (1960) concluded that this fusion of the bones was extrinsically governed.

Cartilage has been found in several suture areas of the young cranial vault and facial skeleton (Sitsen, 1933; Pritchard, Scott & Girgis, 1956; Persson, 1973; Friede, 1973). Normal closure of palatal sutures, however, appears to proceed in the absence of cartilage (Persson & Thilander, 1977). Moreover, when growth of the sutural margins comes to an end, strong collagenous fibre bundles run uninterruptedly across the suture in specific areas (Persson, 1973), indicating that changes in the structural organization of the fibrous tissue precede the obliteration process.

The object of the present study was to describe certain morphological features of early stages of sutural closure in man and in an experimental animal. In order to shed light on the role of structural components in the obliteration process the initial stage was evaluated using both histological and histochemical criteria.

MATERIAL AND METHODS

The *human material* consisted of necropsy specimens from 24 males and females, 15–35 years old, all of whom died suddenly. The specimens, taken at post-mortem examination, included the intermaxillary and transverse palatine sutures of the palatal vault (further information on the material is given by Persson & Thilander, 1977). They were fixed in a mixture of 95% ethanol and concentrated formalin (3:1), after which they were decalcified in a formic acid and sodium formate mixture (45% and 15% solutions respectively, in equal volumes). For paraffin embedding a double embedding technique was used, and sections were taken with the microtome set at 7 μm.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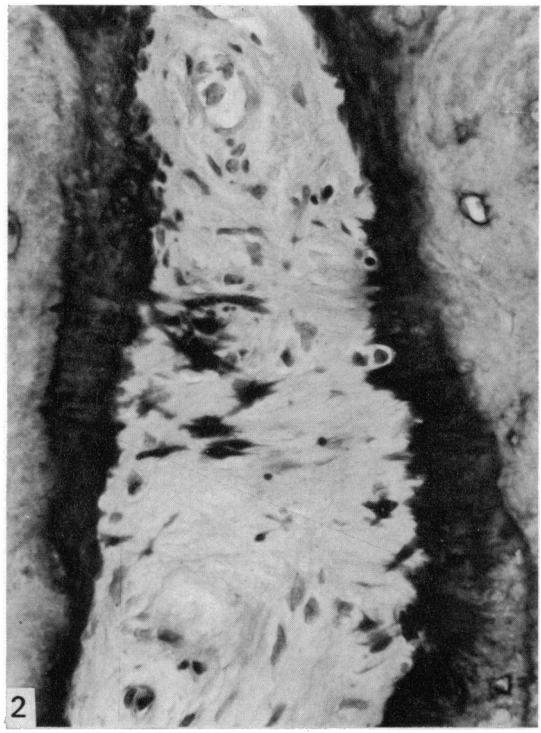
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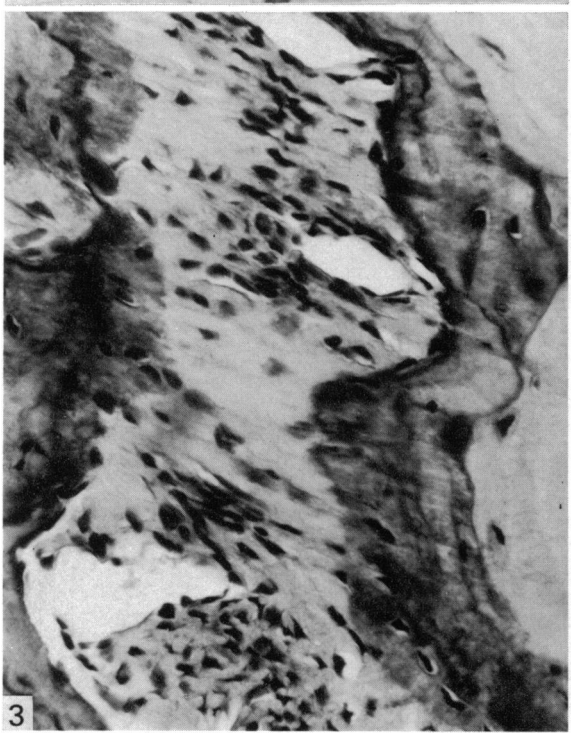
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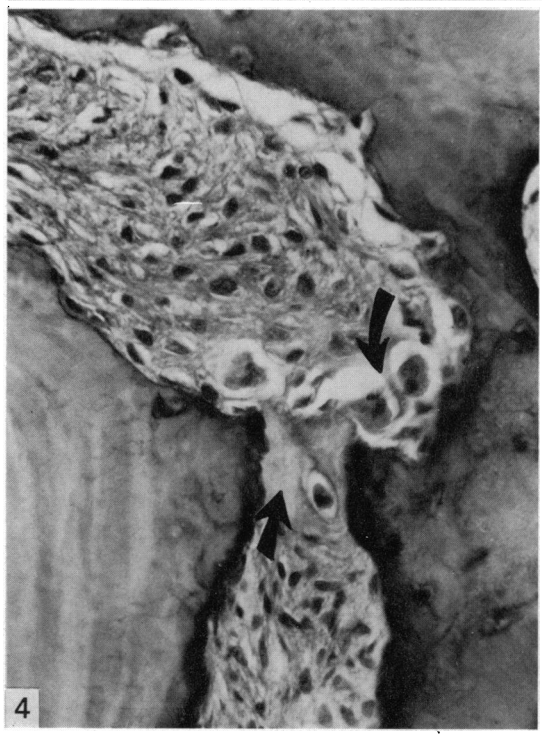
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The *animal material* consisted of the sagittal and interfrontal sutures of rabbits, 25–36 months old. Some specimens were fixed in alcoholic formalin as above and prepared for paraffin histology. Others were transferred immediately after death to a 10% NA_2EDTA (disodium-ethylenediamine tetraacetate) solution containing 7·5% PVP (polyvinylpyrrolidone, mol. wt. 10 000) and demineralized at +4 °C according to the method of Fullmer (1966). After this they were mounted on cryostat chucks and frozen in isopentane pre-chilled to about −140 °C with liquid nitrogen. Sectioning (8 μm) of the frozen material was made in a cryostat (Kryostat 64, System Dittes-Duspiva, Germany) at −20 °C.

The following staining methods were used. The paraffin sections were stained in Mayer's haemalum–eosin and in Van Gieson's connective tissue stain (Romeis, 1948). A few sections were stained with Astra blue (Bloom & Kelly, 1960). The frozen sections were incubated according to standardized methods for $NADH_2$-diaphorase, glutamate dehydrogenase, $NADPH_2$-diaphorase (Chayen, Bitensky & Butcher, 1973), glucose-6-phosphate dehydrogenase (Altman, 1968), acid phosphatase (Barka & Anderson (1965), azo dye and lead method), leucine aminopeptidase (Nachlas *et al.* 1960) and ATPase (Padykula & Herman (1955), Ca-method at pH 9·4, Wachstein & Meisel (1957), Pb-method at pH 7·2; Sundström & Mörnstad (1975), Pb-method at pH 9·4).

As controls for the enzyme histochemistry incubations were carried out in media lacking the appropriate substrates. The specificity of the ATPase technique was also tested by using Na-β-glycerophosphate as substrate instead of ATP.

RESULTS

General morphology of obliterating sutures

Both in rabbit and man two main structural patterns were observed in different parts of the sutures: one where the fibres were essentially perpendicular, the other where they were more or less parallel, to the sutural margin (Fig. 1). In areas of mainly perpendicular organization, most frequently observed in tortuous parts of a suture, thick bundles of collagenous fibres appeared to cross the suture uninterruptedly. More marked in rabbit than in man, there were dense bundles which were tendon-like with few immature fibroblasts (Figs. 2, 3). A different type of uncalcified tissue which frequently obliterated the suture had the appearance of compressed,

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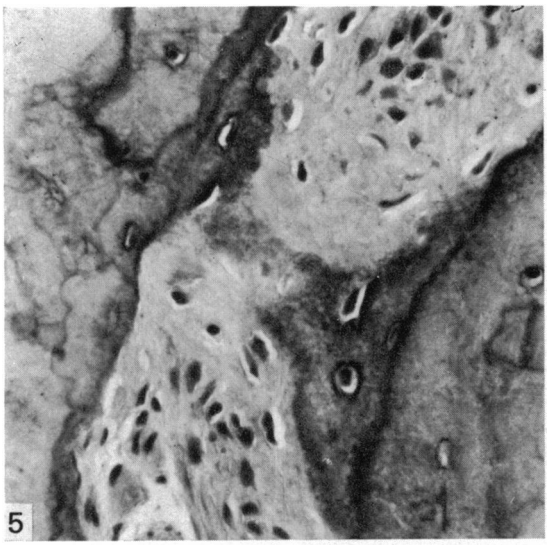
The photomicrographs were taken from the intermaxillary suture in man and from the interfrontal suture in rabbits.

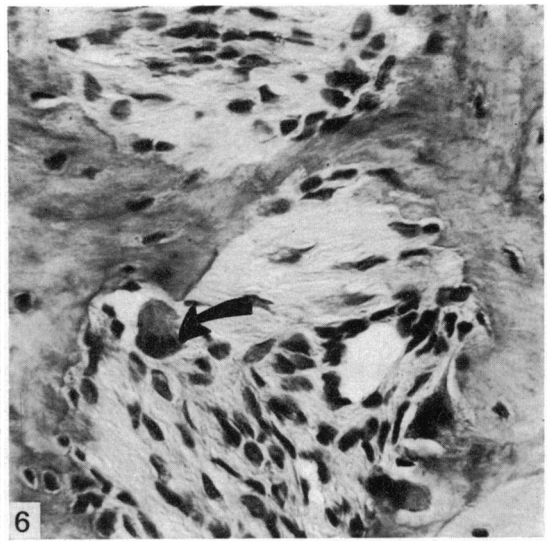
Fig. 1. Human (male, 23 years), showing the two main patterns of structural organization observed in the non-obliterated, adult suture. In the upper part of the figure cells and fibres are more or less parallel to the bony surfaces; in the lower part the suture shows a mainly perpendicular arrangment of fibres. Cells of the former pattern appear more mature. Mayer's haemalum–eosin, ×250.

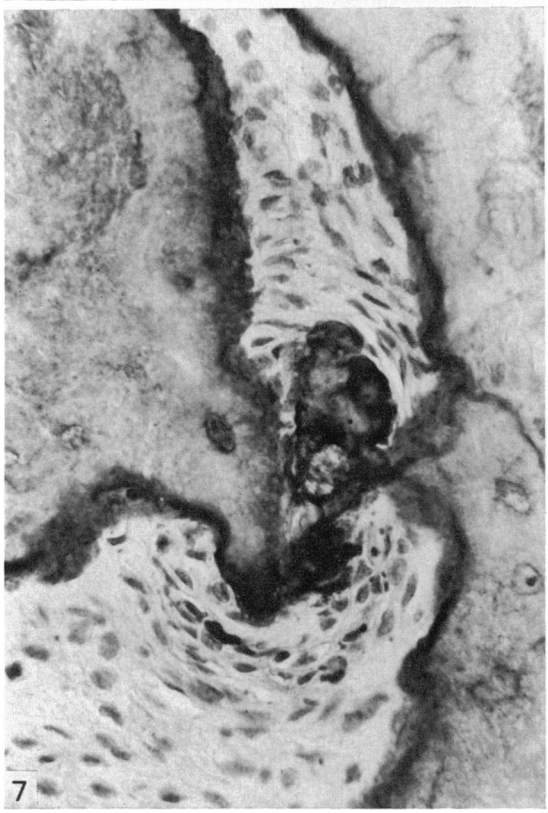
Fig. 2. Human (male, 25 years) showing coarse bundles of fibres running across the suture. Heavily basophilic spicules project from the bony surface along the fibre bundles. Mayer's haemalum–eosin, ×250.

Fig. 3. Rabbit (28 months), showing tightly packed, collagenous bundles forming a tough fibrous cord between the bony margins of the suture. Mayer's haemalum–eosin, ×250.

Fig. 4. Human (male, 26 years), showing a part of the suture filled by a more or less cell-free structureless tissue (straight arrow). The suture appears to have been 're-opened' by undermining osteoclastic resorption from above, followed by ingrowth of a more immature mesenchymal tissue (curved arrow).







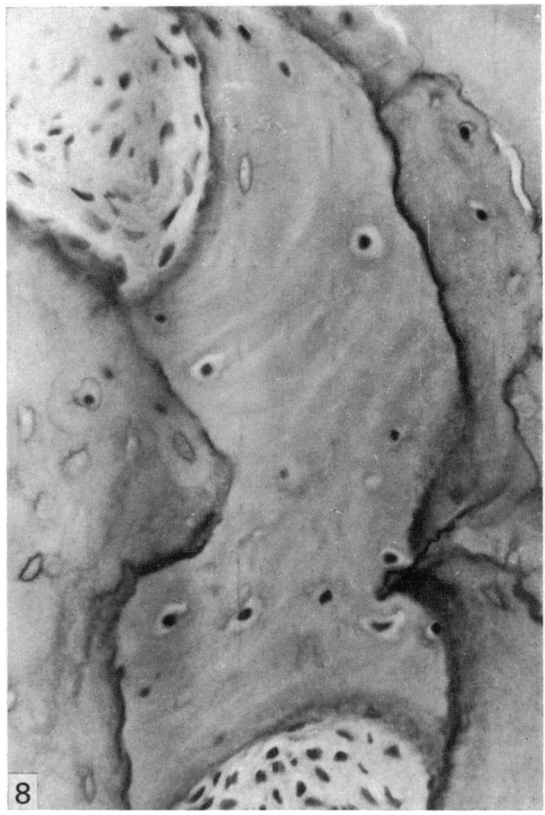


Fig. 5. Human (male, 25 years). A slender bridge of calcified tissue runs across the suture in an area of tightly packed fibre bundles. Osteocytes are included in the synostosing bone. The margin of the earlier quiescent suture is as a basophilic cement line. Mayer's haemalum–eosin, ×250.

Fig. 6. Rabbit. The sutural margins are united by a bony bridge in an area of transversely arranged tissue. Osteoclasts (curved arrow) appear to be removing the obliterated area. Mayer's haemalum–eosin, ×250.

Fig. 7. Human (male, 25 years). The suture is partly obliterated by an irregular, calcified mass at the site of a fracture of a bony trabecula which previously bridged the suture. Mayer's haemalum–eosin, ×250.

Fig. 8. Human (male, 23 years). Bony obliteration of a suture. There are basophilic cement lines at the sites of the earlier suture margins. Mayer's haemalum–eosin, ×250.

mainly cell-free, bionecrotic tissue (Fig. 4). Its tissue components were arranged mainly parallel to the margins, and were found in various stages of degeneration or else were undergoing restitution by vascular, mesenchymal tissue.

Morphology of obliterating areas

Two main patterns of initial obliteration were found in both the human and the rabbit specimens.

One type showed slender bone spicules extending from the sutural margins to partly or completely bridge the suture gap (Figs. 5, 6). These spicules, single or in groups, were localized specifically to suture areas of trans-suturally orientated, dense collagenous tissue. Few osteoblasts were observed adjacent to the spicules, but osteocytes of normal appearance were frequent. Spicules were more often found in older specimens in which the suture was rich in completely ossified areas.

The spicules, like the sutural margin, were strongly basophilic (Figs. 5, 6). The tissue into which spicules were projecting gave a strong positive staining reaction with Astra blue, unlike non-obliterating parts of the suture. Neither in man nor in the rabbit were cartilage cells noted in these areas.

The other type of initial obliteration was found almost exclusively in the human palatal suture. The suture gap was occupied by an irregular, inhomogeneous but mainly acellular mass with a clearly demarcated outline (Fig. 7), present either as a free nodule in the suture or as a mass attached to short spicules extending from the margins. The structure was that of several calcified bodies, sometimes coalesced to form a solid mass, and characterized by a staining reaction which varied from basophilic to strongly eosinophilic. The centre often gave the picture of bionecrotic tissue, and sometimes contained a cyst-like space. The bodies did not stain with Astra blue, but some specimens demonstrated a strong positive staining reaction in adjacent tissue. In other areas woven bone of normal appearance bridged the suture (Fig. 8).

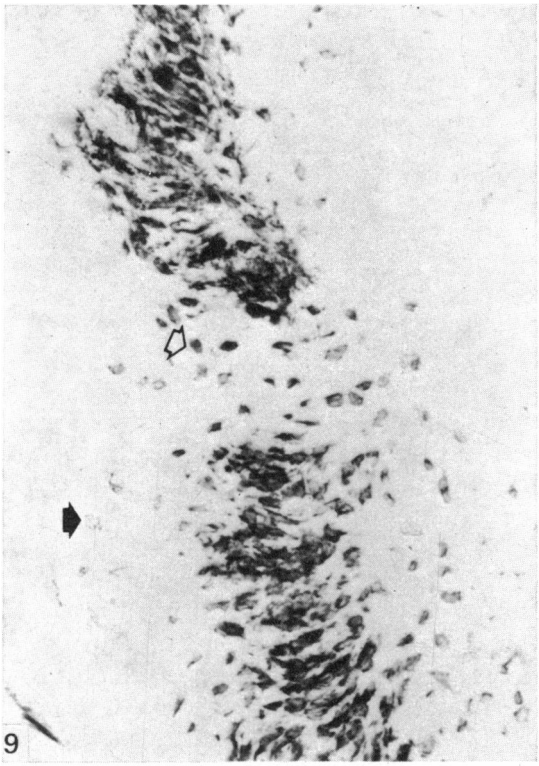
Osteoclastic resorption was frequently observed adjacent to both types of obliteration (Figs. 4, 6) and appears to contribute to a reopening of the obliterated suture by an undermining resorption of the suture margins on both sides of the calcified bridge.

Enzyme histochemistry in obliterating areas

All connective tissue cells in the suture showed high activities of $NADH_2$ diaphorase (Fig. 9), $NADPH_2$ diaphorase (Fig. 10) and glutamate dehydrogenase. No differences could be observed between the fibroblasts and the osteoblasts, but the activity of the osteocytes in the surrounding bone was considerably lower. Glucose-6-phosphate dehydrogenase activity was observed both in osteocytes and in cells of the suture.

Intense staining reactions for oxidative enzymes ($NADH_2$ diaphorase, $NADPH_2$ diaphorase and glutamate dehydrogenase) were recorded in cells of the dense collagenous areas transversing the suture (Fig. 10) and in young osteocytes in bony bridgings (Fig. 9). The osteocytes in close relation to the bony bridges showed a greater activity than osteocytes further away (Fig. 9). In some transversely arranged suture tissues, bands showing positive staining for leucine aminopeptidase could be seen bridging the sutures (Fig. 11).

Differences in staining reactions for oxidative enzymes were also observed in relation to differences in the structural organization of the tissue. In areas where



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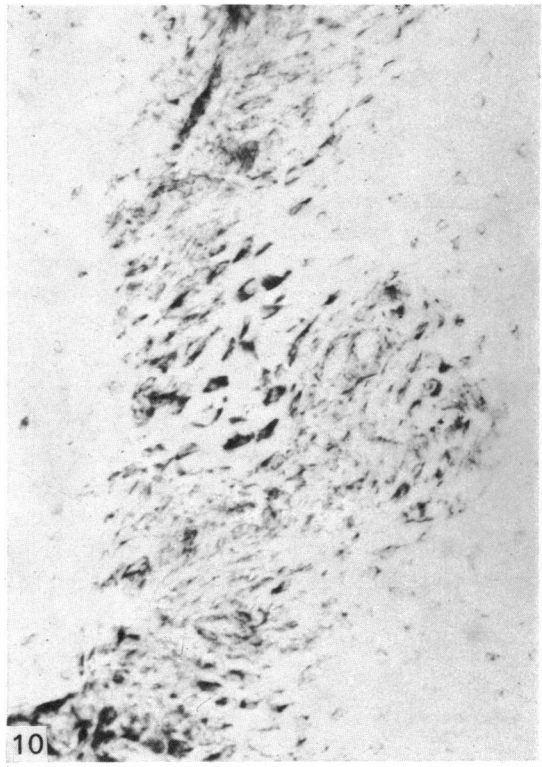

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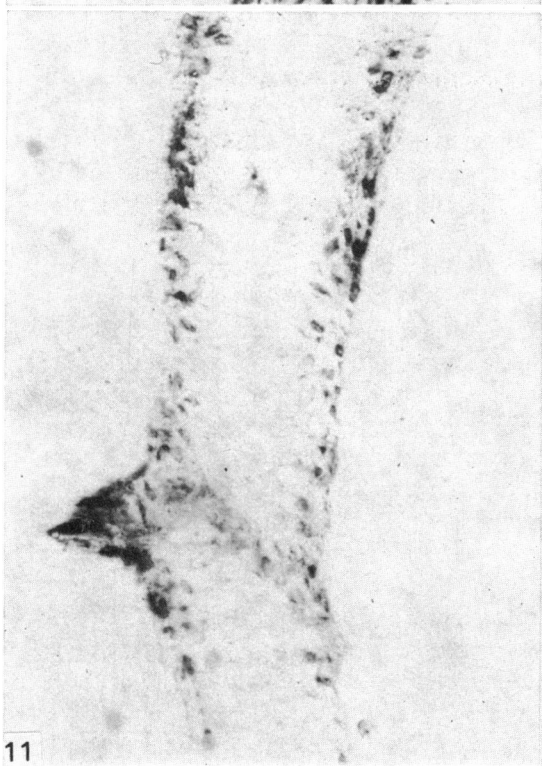

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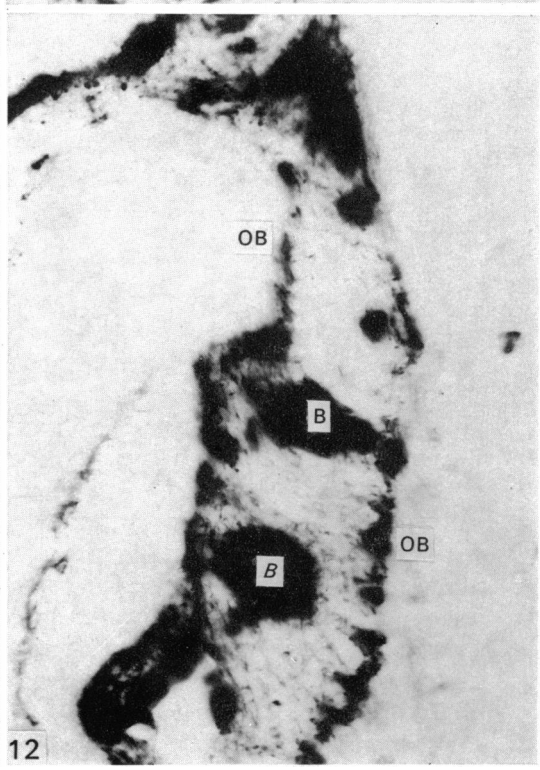

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cells and fibres were arranged more or less parallel to the sutural margins, osteoblasts and fibroblasts demonstrated weak activity, while similar cells in areas with a transversely arranged tissue structure showed moderate activity.

Acid phosphatase activity was moderate in most of the sutural cells with the exception of the osteoclasts, which showed intense activity; osteocytes on the other hand showed only slight staining. Over wide areas the cells bordering the suture revealed a high activity of leucine aminopeptidase (Fig. 11). Moderate to high activity was recorded in osteoclasts, while the staining of the osteocytes was slight. The results obtained with the different ATPase technique were similar. However, the lead techniques, especially that at pH 9·4, gave more heavily stained precipitates. The most intense staining was seen in the walls of the blood vessels and this revealed the high vascularity of the sutural tissue (Fig. 12). Sutural osteocytes showed slight, osteoblasts high, and fibroblasts moderate ATPase activity. Incubations using Na-β-glycerophosphate as substrate gave no positive staining at all.

DISCUSSION

Closure of sutures has been related to vascular, hormonal, genetic and mechanical factors, and to specific local conditions. Opinions by mainly European authors are cited by Schmitt & Tamáska (1970), while mainly American studies are summarized and discussed by Herring (1974).

High metabolic activities in sutures have been demonstrated autoradiographically in growing rats (Persson, 1973) as well as histochemically in human fetuses and neonates (Friede, 1975). In the present study, metabolic activities indicating functional pathways for glycolysis, the citric acid cycle and the pentose–phosphate shunt, as well as capacity for synthesis of amino acids were demonstrated in adult sutures. These activities are found in cells associated with mineralization (Fullmer, 1966) and can also be associated with a continuous remodelling of the sutural margins, as demonstrated by Kokich (1976).

Necrotic areas were observed locally in sutures in this study. However, the morphology of the bony spicules, and the high oxidative enzyme and ATPase activities of the sutural cells indicate active hard tissue formation (Severson & Tonna, 1970; Göthlin & Ericsson, 1973; Magnusson, Heyden & Arwill, 1974). Thus, suture closure cannot be attributed to dystrophic calcification.

The current morphological studies reveal that the closure of human palatal and rabbit vault sutures proceeds without participation of cartilaginous tissue. Sections incubated with ATP as substrate provide a clear demonstration of the high vascularity of the obliterating tissue. Since chondrogenesis is favoured by relatively avascular conditions, while osteogenesis requires greater vascularity (Bassett, 1962; Andersen, 1970), the histochemical findings are in keeping with the histological evidence.

Fig. 9. Rabbit. $NADH_2$ diaphorase activity in the soft tissue cells of the suture. Note the marked reactions in the young osteocytes (open arrow) of the bony bridge contrasted with the slight reactivity of the osteocytes (filled arrow) further away, ×250.

Fig. 10. Rabbit. Diaphorase ($NADPH_2$) activity is high in cells related to collagenous bundles running across the suture, ×250.

Fig. 11. Rabbit. Leucine aminopeptidase activity is seen in cells bordering the suture and also in a band bridging the suture, ×250.

Fig. 12. Rabbit. The blood vessels (*B*), as well as the osteoblasts (*OB*) bordering the suture, reveal high ATPase activity, ×250.

Tensional forces may stimulate the formation of bony bridges across the suture (Moss, 1958, 1960; Herring, 1974). The observation of collagen fibre bundles crossing the suture is consistent with recent studies (Persson, 1973; Kokich, 1976) and is thought to reflect tensile forces. Osteogenesis was observed to be restricted to such areas of transversely arranged collagen bundles. Moreover, the intense oxidative enzyme activities in the bony bridge, and in areas of densely arranged collagenous bundles, support the idea that the bundles are preliminary to bony bridgings. However, the mechanical properties of bone vary according to the plane tested (Ascenzi & Bell, 1972). At the same time as tensional forces are stimulating the formation of bony bridges across the suture, forces developing in a slightly different direction are in a position to disrupt the initially weak bony bridges. Such forces may explain our frequent observation of fractured spicules in human palatal sutures.

SUMMARY

Some aspects of normal obliteration of sutures in rabbit and man were studied histologically and it was found that suture closure took place by intramembranous ossification in specific areas of trans-suturally arranged, tendon-like tissue. Histochemically these areas demonstrated high activity of oxidative enzymes.

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