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The role of movements in the development of sutural and diarthrodial joints tested by long-term paralysis of chick embryos

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INTRODUCTION

The factors regulating the development of sutural fibrous joints are a subject of controversy. Movements of muscular origin (Pritchard, Scott & Girgis, 1956), an osteogenesis-inhibiting factor (Markens, 1975) and physiological cell death (Ten Cate, Freeman & Dickinson, 1977) have been suggested. More recently, Smith & Töndury (1978) concluded that stretch-growth tensile forces in the dura mater are responsible for the development of the calvaria and its sutures, the primary determinant in their development being the form and growth of the early brain. A similar biomechanical explanation for the morphogenesis of sutures in areas of the skull where no dura mater exists was also given by Persson & Roy (1979). Based on studies of suture development and bony fusion in the rabbit palate, they concluded that the spatial separation of bones during growth is the factor regulating suture formation. Lack of such movements of developing bones results in bone fusion when the bones meet. Further, when cranial bones at suture sites are immobilised, fusion of the bones across the suture is produced (Persson *et al.* 1979).

In the normal development of diarthrodial joints, skeletal muscle contractions are essential (Murray & Selby, 1930; Lelkes, 1958; Drachman & Coulombre, 1962; Murray & Drachman, 1969; Hall, 1975), and lack of muscular movements results in stiff, fused joints.

The purpose of this study was to test whether sutural joints differ from diarthrodial joints with regard to the role of muscular movements in their development.

MATERIAL AND METHODS

The role of muscular contraction movements was tested by inducing long-term paralysis in chick embryos.

Fertilised White Leghorn hens' eggs from a local hatchery were cultured at 37 ± 1 °C and at 60 ± 2 % relative humidity with daily rotation. The start of culture was designated day 1. On day 8, long-term skeletal muscle paralysis was induced in 53 embryos according to a method described by Hall (1975). An injection of 0·1 mg of decamethonium iodide (Koch–Light Lab. Ltd, Colnbrook, Bucks, U.K.) dissolved in 0·5 ml saline was made into the air sac of each egg. Thirty nine embryos were used as controls. After injection, the pinhole in the egg was sealed with paraffin and the embryos cultured without further rotation.

At days 18 and 20, and at day 23 (experimental embryos only; Table 1) the

Table 1. *Number of embryos in different groups*

Test/control group	Cultured	Alive at examination	Sectioned at day		
			18	20	23
Test	53	27 (49 %)	10	3	2
Control	39	31 (77 %)	10	3	0

embryos were removed from the shells and tested for spontaneous movements of limbs and beaks, and also for movements following stretching of their wings. The viability of paralysed embryos was tested by opening the chest for inspection of heart beats. Only those paralysed embryos that were alive and showed consistent paralysis at the termination of the experimental period were used. The weights of all embryos were recorded after the remnants of the amniotic sac and the yolk sac had been cut off at the umbilicus.

The embryos were killed by decapitation and specimens of sutural and synovial joints processed as follows. In most embryos the head and the tibiotarsal/tarsometatarsal joint (the ankle) were partly skinned and put into cyanuric chloride for fixation (Yoshiki, 1973). After fixation for 2 days, the specimens were decalcified in EDTA, pH 7·3. With the specimens still in the decalcifying agent, further dissection was carried out. After paraffin embedding, transverse serial sections (6 μm thick) were taken through the head to include the following joints (Fig. 1): the quadrate-mandibular and the quadrate-squamosal synovial joints, and the fronto-squamosal, the fronto-parietal and the frontal (sagittal) sutural articulations. Similar transverse sections were also taken from the ankle joint. The sections were conventionally stained in haematoxylin and eosin.

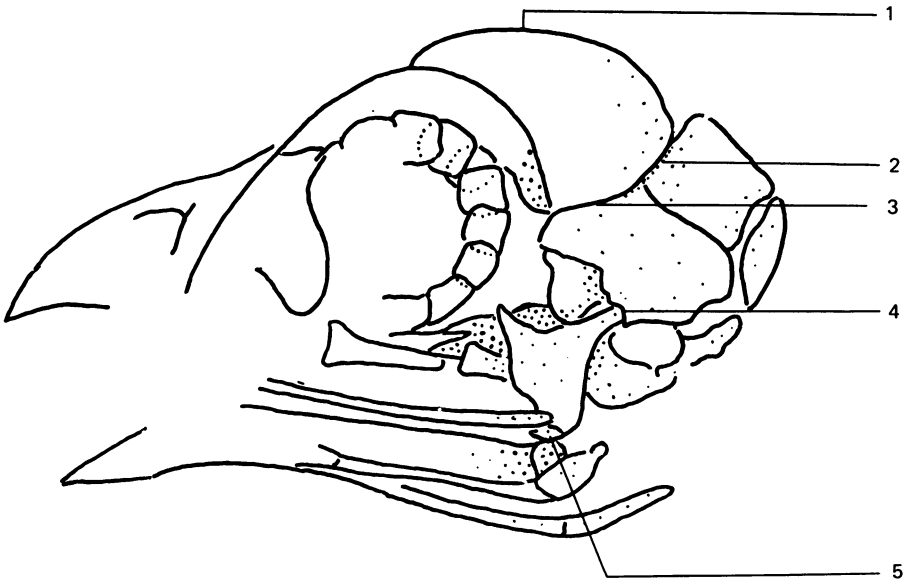
In some specimens the fronto-squamosal suture area and the quadrate bone area were dissected out and fixed in glutaraldehyde for 2 days at 4 °C. After decalcification in EDTA, pH 7·3, the specimens were embedded in Epon. These specimens were sectioned at a thickness of 2 μm and the sections stained with toluidine blue. These sections served as partial controls of the possible effect of shrinkage in the paraffin embedding procedure.

For general comparison of bone development in experimental and control embryos, an additional eight experimental and six control embryos, cultured as above, were used. Two of each group were killed at 16, 18, 20 and 23 days (at 23 days, experimental animals only). Their skeletons were stained with alizarin red S and cleared in glycerol after KOH treatment (Bancroft & Stevens, 1977).

RESULTS

Paralysed embryos differed from control embryos by a distorted position of the body, indicating retention of a more embryonic position. In some paralysed embryos, large amounts of clear fluid were found subcutaneously in the abdominal area. Apart from these observations, no obvious malformation of the trunk or limbs was seen externally in paralysed embryos. In the skull, the tip of the lower beak protruded in front of the upper beak, which is the reverse of the normal relationship (Fig. 2).

The paralysed embryos were smaller. The total body weights of paralysed embryos were significantly lower than those of controls ($P < 0{\cdot}01$), $13{\cdot}6 \pm 2{\cdot}1$ g ($n = 18$)



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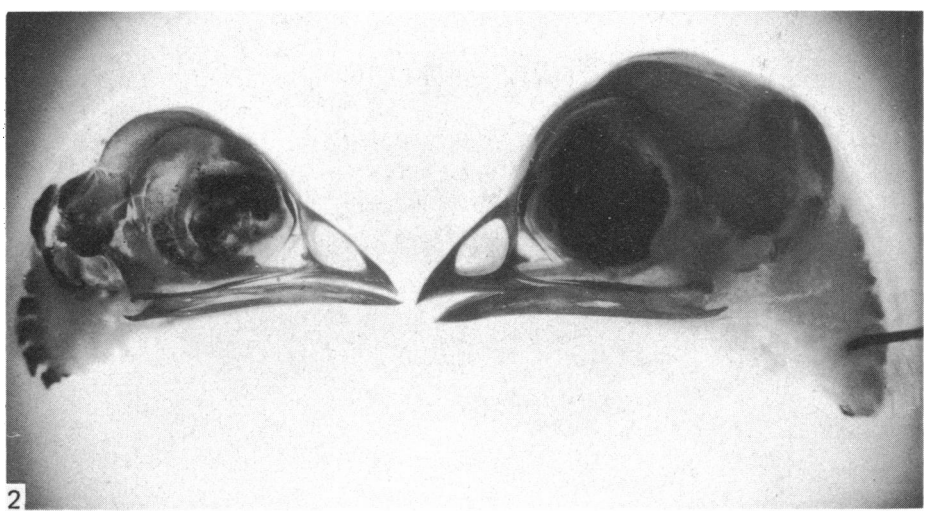

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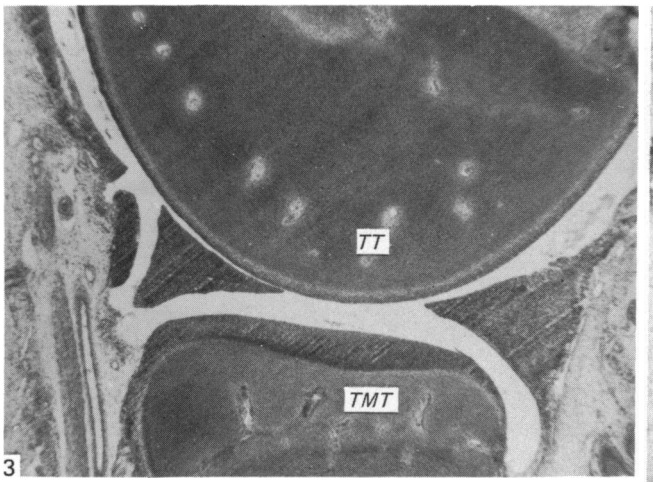
Fig. 1. Drawing of the posterior part of the skull of a normal 18 day chick embryo. The positions of the sutures and synovial joints studied are indicated as follows: 1, frontal (sagittal) suture; 2, fronto-parietal suture; 3, fronto-squamosal suture; 4, quadrate-squamosal synovial joint; 5, quadrate-mandibular (Meckel's cartilage) synovial joint.

Fig. 2. Alizarin-stained and cleared skulls of 20 day normal (right) and paralysed (left) embryos. Note the significantly smaller skull of the paralysed embryo and its protruding lower beak but the otherwise normal configuration of the head.



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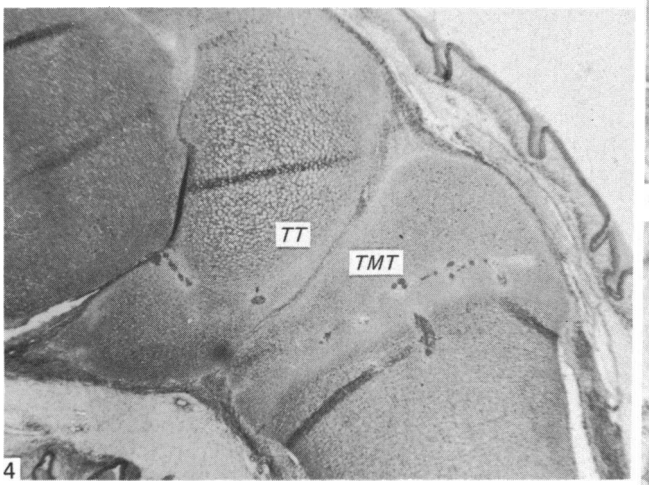

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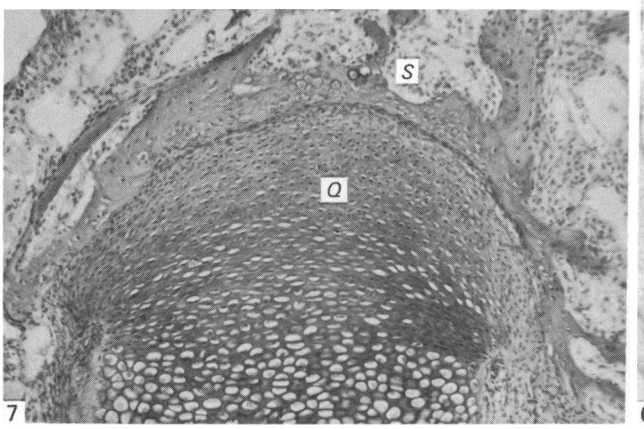

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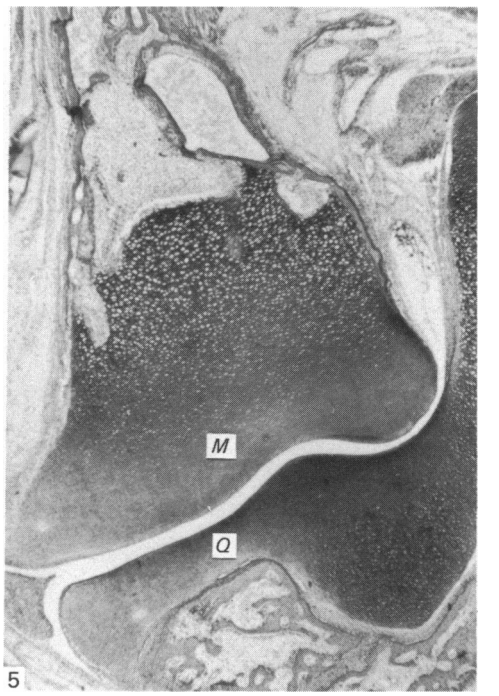

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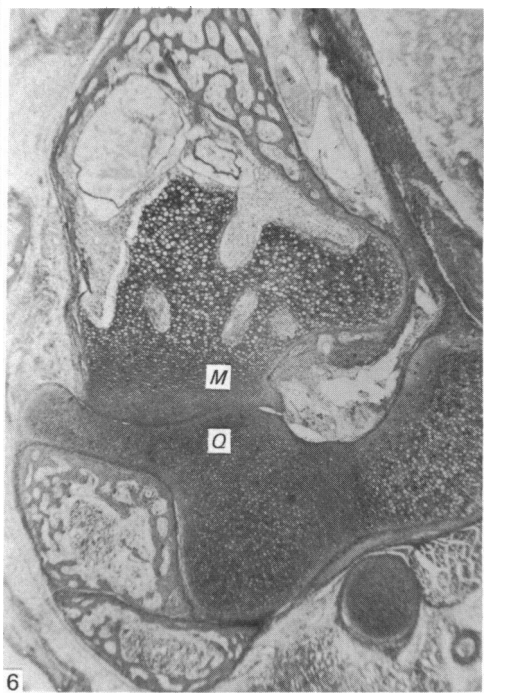

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compared to $18{\cdot}9 \pm 2{\cdot}4$ g ($n = 14$) in the 18 days old embryos. Comparison of developmental stages, based on external characteristics according to Hamburger & Hamilton (1951), indicated a slight delay in general development of the paralysed embryos. This delay in development was verified by comparisons of cleared alizarin-stained specimens, which indicated a delay in skeletal development of one to two days in paralysed embryos cultivated for 20 days. Consequently, a delay in the development of sutural articulations was also seen in these specimens. For this reason, detailed observations on the frontal suture were based on 20 days old specimens only.

No distorting effects of histological procedures on joint structures were obvious when sections from Epon- and paraffin-embedded specimens were compared.

Diarthrodial joints

The ankle joint (the tibiotarsal/tarsometatarsal joint), which served as a supplementary indicator of successful paralysis, consistently showed complete absence of an articular cavity in the paralysed embryos. Cartilages of the two skeletal elements extended across the articulation area, establishing a stiff joint consisting of cartilaginous tissue (Figs. 3, 4). The adjacent parts of the articular and epiphyseal cartilages were often distorted, as was frequently the arrangement of the cells. The malformation also involved para-articular structures, such as the capsule, ligaments and meniscus. The picture of the ankle joint in paralysed embryos was consistent with earlier descriptions of limb joint development following long-term paralysis of chick embryos.

The quadrate-mandibular articulation is formed by the quadrate bone, which is a membrane bone, and the portion of the mandible formed from Meckel's cartilage. In all control embryos, well formed cavities separated the articular process of the quadrate bone from the Meckel's cartilage, and a meniscus was always present (Figs. 5, 6). None of the paralysed embryos had patent cavities, and the specialised articular structures found in the control embryos were lacking. Cartilaginous fusion, similar to that seen in the ankle joint, mostly occurred at the normal site of articular contact. Sometimes the junction consisted partly of mesenchymal tissue.

A similar more or less complete fusion of the two skeletal elements was also seen in the quadrate-squamosal joint (Fig. 7). The articular surfaces had coalesced, the junction forming a type of synchondrosis. The site of fusion was revealed by the arrangement of the cartilage cells involved, or by remnants of the intra-articular mesenchyme.

Figs. 3–11. Paraffin-embedded 6 μm sections stained with haematoxylin and eosin.

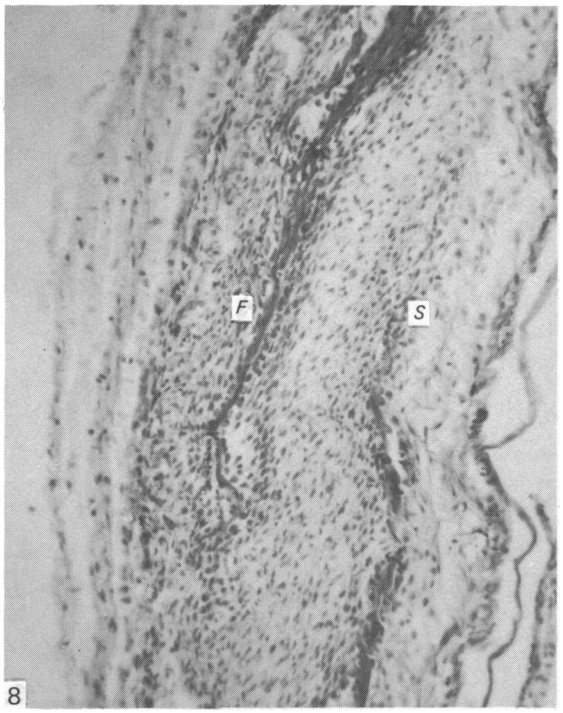
Fig. 3. The tibiotarsal (*TT*)-tarsometatarsal (*TMT*) joint (the ankle joint) of an 18 day control embryo. Normal cavity formation has taken place. × 19.

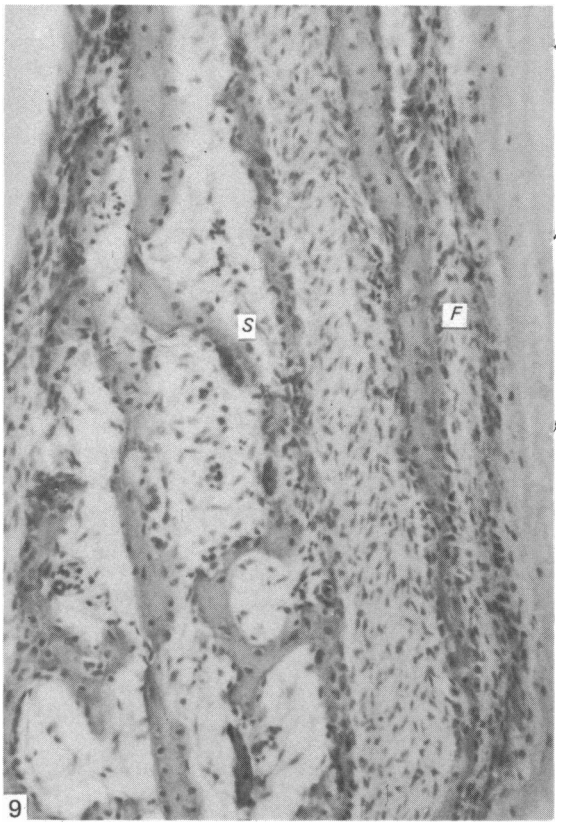
Fig. 4. The same joint as in Fig. 3 but from a paralysed 18 day embryo. Fusion of the two cartilaginous elements has occurred. × 19.

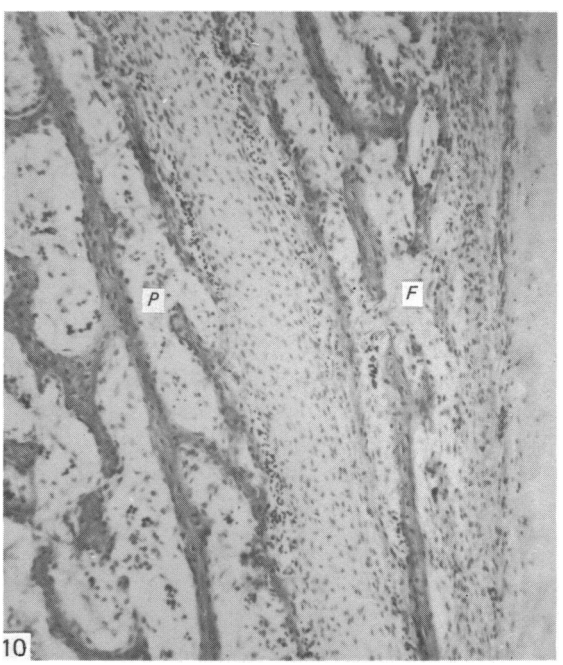
Fig. 5. The quadrate (*Q*)-mandibular (*M*) joint. Normal cavity formation between the quadrate and Meckel's cartilages in the 18 day control embryo of Fig. 3. × 19.

Fig. 6. The same synovial joint as shown in Fig. 5 but from the paralysed embryo shown in Fig. 4. Cartilaginous fusion has taken place as seen in the ankle joint in Fig. 4. × 19.

Fig. 7. The quadrate (*Q*)-squamosal (*S*) joint of the paralysed embryo in Fig. 6. Synovial cavity formation is lacking. × 82.







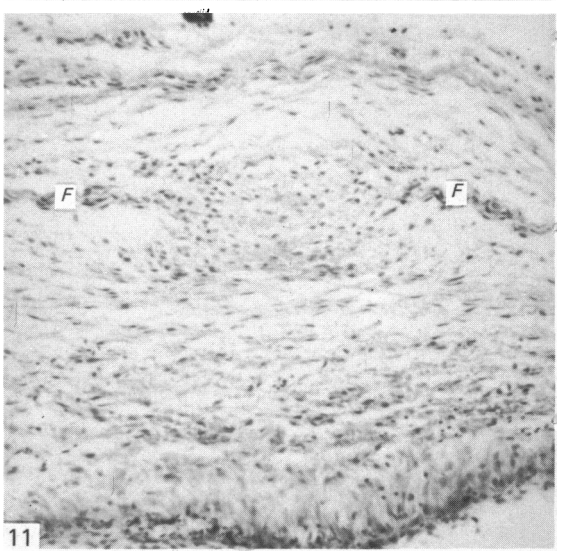


Fig. 8. The fronto (*F*)-squamosal (*S*) suture of the control embryo in Figs. 3 and 5. The suture is wide and its borders show the picture of continuing bone formation. × 88.

Fig. 9. The fronto (*F*)-squamosal (*S*) suture of the paralysed embryo in Figs. 4 and 6, and a little more medially than in the control embryo of Fig. 8. Although somewhat reduced in width, the suture shows an otherwise normal morphology, without any indications of approaching fusion. × 113.

Fig. 10. The fronto (*F*)-parietal (*P*) suture of the paralysed embryo of Figs. 4, 6 and 8. Normal suture morphology without any signs of fusion or restricted growth. × 88.

Fig. 11. The frontal (sagittal) suture area of a 20 day paralysed embryo. A normal suture has recently been organised as bundles of collagenous fibres running across the suture gap and between the edges of the two frontal bones (*F*). × 107.

Similar cartilaginous or mesenchymal fusion was also seen in other diarthrodial articulation areas found in the histological sections.

Sutural joints

In the fronto-squamosal suture, the squamous bone in control embryos slightly overlapped the frontal bone to form a squamous-type suture (Fig. 8). The sutural margins of the bones were separated from each other by the sutural tissue, which was rich in fibre bundles and arranged mainly parallel to the bone margins. The same overlapping arrangement of the bony margins was seen in paralysed embryos (Fig. 9). The fibrous tissue gap often appeared narrower than in controls, but the sutural margins never made contact with each other. Similar cellular and fibrous elements were seen in the sutural tissue of both control and paralysed embryos, and no difference in tissue arrangement was found. The osteoblast layers of opposing margins were distinctly separated along the whole course of the suture.

At the fronto-parietal suture (Fig. 10) the frontal bone met the parietal bone laterally in an overlapping manner, but more medially the bones gradually separated from each other to form the frontal (anterior) fontanelle. This was the case in both control and paralysed embryos. The sutural margins of control embryos were thicker than in paralysed embryos, due to a more advanced skeletal development, but no difference in width of the suture gap was evident in areas of established suture. Well defined osteoblasts were seen along the sutural margins of both control and paralysed embryos. The intervening sutural tissue was similarly arranged in both groups of embryos, separating the opposing osteoblast layers.

In the area of the frontal (sagittal) suture (Fig. 11), which, of the sutures studied here, is apparently the last to form, the frontal bones were close enough to form a suture line only in their most anterior part. Control and paralysed embryos differed with regard to the advancement of overall suture formation and bone development, experimental embryos being slightly delayed. Similar edge-to-edge meeting of the bones was seen in suture areas of both groups, however, separated by sutural tissue proper. The edges showed evidence of continuing bone formation in both groups, but the distance between the bone margins was maintained anteroposteriorly along the established suture. Cells and fibres of the suture showed a somewhat disorderly arrangement anteriorly but there was no obvious difference between control and paralysed embryos. More posteriorly, the suture tissue showed a more orderly arrangement between the edges of the bones. While these edges were gradually separating from each other to form the anterior fontanelle, the presumptive suture area in both groups of embryos consisted of a fibrous sheet formed by the pericranium and the outer layer of the dura mater.

DISCUSSION

The observations reported here show that absence of muscular movements in chick embryos prevents the development of synovial joints but does not cause any obvious deviation of normal suture development. If it is intended to evaluate the role of movements in joint development, then experimental paralysis of the muscular contraction of the embryo must produce no other conditions adverse to growth. In studies using neuromuscular blocking agents to produce paralysis of chick embryos *in ovo* (Drachman & Sokoloff, 1966; Murray & Drachman, 1969; Hall, 1975) general growth retardation has also been found. Apart from a shortened upper beak,

subcutaneous oedema and distortions of the skeleton caused by the paralysis, as reported here, no gross teratological action of decamethonium iodide has been found (Drachman & Sokoloff, 1966; Hall, 1975). Growth retardation *per se* has not been found to be responsible for diarthrodial joint abnormalities (Drachman & Sokoloff, 1966) and the abnormalities seen have, therefore, been assumed to be produced by the muscular paralysis.

The difference between synovial and sutural joints observed here might still be explained by a difference in the length of time for which the joint is exposed to the drug. Cavity formation of the ankle joint starts at about day 9 (Drachman & Sokoloff, 1966), and joint formation in the jaws at about day 11 to day 13 (Jollie, 1957; Murray, 1963). Data reported by Jollie (1957) and observations on the cleared alizarin-stained specimens in this study, indicate that suture formation in the vault starts at about day 14–15, thus permitting longer exposure of the diarthrodial than the sutural joints to the drug. In studies of joint cavity formation, as well as of other accessory articular structures, significant changes have been evident after 48–72 hours (Drachman & Sokoloff, 1966; Oppenheim, Pittman, Gray & Marderdrut, 1978; Ruano-Gil, Nardi-Vilardaga & Tejedo-Mateu, 1978). As no obvious effect of paralysis on suture development could be found in suture areas established for at least 3 days, it seems most likely that the difference is explained by a different role of muscular movements in the development of diarthrodial as compared with syndesmodial joint structures.

Occasional bony fusion of articulations between two bones not normally covered by articular cartilage has been reported in the experiment by Murray & Drachman (1969). This fusion, however, had occurred due to distortions of certain skeletal elements, and at extra-articular sites which do not normally make contact. Cartilage fusion of a fibrous joint has also been observed, but this articulation occurs between two 'permanent' cartilaginous elements secondarily affected by fusion of synovial structures. Such observations, therefore, do not seem to invalidate the conclusions drawn from the present results.

In the initial development of sutures, various structures have been compared with those of diarthrodial joints (Pritchard *et al.* 1956). While an outer fibrous layer has been considered to be homologous with the fibrous capsule of diarthroses, a central zone of the suture has been regarded as analogous to a synovial joint cavity. Earlier studies of sutural morphology and growth argue against this concept (Persson, 1973), and the present findings concerning the role of muscular contractions further stress the heterogeneity of developing diarthrodial and sutural structures.

Recent studies of suture formation in the fetal rabbit palate show suture formation to be consistent with a continuous displacement of expanding ossification centres, and that lack of such a displacement ultimately leads to fusion of the bones in contact (Persson & Roy, 1979). The study reported here shows that movements of neuromuscular origin play no essential role in the development of sutures. Although both diarthrodial and syndesmodial types of joints are adapted for motion, synovial joint development depends on movements of muscular origin, while suture development appears to be a response to movement dependent on growth at suture sites. The observations in this study support the conclusion reached by Smith & Töndury (1978), and the hypothesis proposed by Moss (1975), that volumetric expansion of the neural mass is the primary determinant of the development of the skull sutures.

SUMMARY

Chick embryos were paralysed *in ovo* with a neuromuscular blocking agent between 8 and 20 days of incubation. To evaluate the rôle of muscular activity in the development of sutural articulations, sutures of the cranial vault of control and paralysed embryos were studied histologically and the findings compared with the effect of the agent on the development of the ankle joint and some synovial joints of the jaws.

Paralysed embryos showed a consistent lack of development of joint cavities in synovial joints. In most embryos, fusion of opposing cartilaginous elements had occurred. In contrast to synovial joints, sutural articulation showed a micromorphology comparable to that of controls.

The findings indicate that different embryonic factors regulate the development of sutural and synovial articulations. Movements of neuromuscular origin play no essential role in the morphogenetic development of sutures, but are a prerequisite for the formation of joint cavities and other specialised structures of synovial joints.

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