Role of Chondrocytes and Hydrocortisone in Resorption of Proximal Fragment of Meckel's Cartilage: An *In Vitro* and *In Vivo* Study [1]

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ABSTRACT The possibility that chondrocytes of the proximal fragment of Meckel's cartilage may participate in resorption of the extracellular substance of the cartilage and outlive its removal, has been investigated *in vitro* and *in vivo*. Mandibles from 18 day *in utero* mouse foetuses were cultured for 14 days on an antibiotic-free chemically defined medium. When tested histochemically for acid phosphatase heavy deposits of reaction product were evident in cells in areas where resorption appeared to be occurring. When maintained on medium containing 1.0 μg/ml hydrocortisone, groups of chondrocytes hypertrophied and were reactive for acid phosphatase, and this was accompanied by loss of intervening extracellular substance. These changes were intensified by increasing the oxygen tension of the environment and by further supplementing the medium with 1.0 μg/ml triiodothyronine. Chondrocytes in the vicinity of sites of resorption could incorporate ^{3}H-proline and ^{3}H-thymidine. *In vivo*, chondrocytes in the vicinity of resorbing areas in two to three day *post-partum* animals were highly reactive for acid phosphatase, and could incorporate ^{3}H-thymidine, ^{3}H-proline, and ^{3}H-uridine. These observations have been interpreted to suggest that resorption of the proximal fragment of Meckel's cartilage is not necessarily accompanied by death of the chondrocytes, and that the chondrocytes may participate in removal of the extracellular substance. Furthermore, the response of the chondrocytes of Meckel's cartilage to hydrocortisone *in vitro* appears to differ from the response that has been reported to occur in a number of other cells *in vitro*.

The differentiation and fate of Meckel's cartilage during development of the mammalian mandible has been examined by a number of investigators (Bhaskar, '53; Bhaskar, Weinmann and Schour, '53; Richany, Bast and Anson, '56; Friant, '58, '59, '66, '68a,b; Charlier and Petrovic, '67). Differentiation of Meckel's cartilage precedes development of the mandible, but after the form of the mandible has been established, the cartilage becomes calcified in the vicinity of the mental foramen. Erosion of this area of calcified cartilage results in Meckel's cartilage being separated into two fragments, a distal fragment and a proximal fragment. The distal fragment is replaced by a process akin to endochondral ossification. The portion of the proximal fragment that lies in the developing mandible is resorbed, but there appears to be a paucity of reliable data on how this is achieved. Bhaskar, Weinmann, and Schour ('53) have stated that "The chondrocytes show pyknotic nuclei; the diameter of Meckel's cartilage becomes smaller, and in the surrounding mass of cells macrophages can be distinguished." This could imply that the chondrocytes die and that the extracellular substance is removed by invading macrophages. On the other hand, without attempting to explain how the extracellular substance is removed, Richany, Bast and Anson ('56) in an excellent study, have described the cartilage as "undergoing deorganization" and the cells as "having reverted to fibroblastic character." Unfortunately, this conclusion appears to have been arrived at on the basis of morphological observation alone.

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The investigations described above suggest the possibility that the part of the proximal fragment of Meckel's cartilage that lies in the developing mandible could be removed by one of two mechanisms, or possibly by a combination of both. Firstly, the chondrocytes could die, following which the extracellular substance of the cartilage could be removed by macrophages stemming from outside of the cartilage, and the area then be colonized by connective tissue cells also arising from outside of the cartilage or perhaps from the perichondrium. Death of cells as a normal process in development of organisms is well recognized (see, for example, Glucksmann, '51; and Saunders, '66). Alternatively, many of the chondrocytes could survive, participate in resorption of the extracellular substance that surrounds them and then differentiate into some other type of connective tissue cell. Chondrocytes are known to have the capacity to depolymerize extracellular substance (see, for example, Reynolds, '69), and there is evidence to support the belief that chondrocytes can differentiate into osteoblasts (see Hall, '70).

The present investigation was undertaken in an endeavour to determine whether firstly, some of the chondrocytes can survive resorption of the proximal fragment of Meckel's cartilage in the mandible and, secondly, whether these chondrocytes have the capacity to resorb extracellular substance. Indirect evidence obtained from *in vitro* and *in vivo* experiments suggest that the answer to both questions is in the affirmative.

MATERIALS AND METHODS

In vivo *material*

Twelve Connaught-strain mice, varying in age from 18 days *in utero* to 11 days *post-partum*, were examined by light microscopy to assess the morphological changes that occur in this strain of mouse, during resorption of the proximal fragment of Meckel's cartilage.

Thirty two to three day *post-partum* mice of the Connaught strain were used for radiobiological and histochemical studies (see below).

Explants

Pregnant Connaught-strain mice were killed by cervical dislocation when their foetuses were 18 days old. The uteri were removed aseptically and placed in sterile Waymouth's ('59) MB752/1 medium (Microbiological Associates, Bethesda, Md., U.S.A.), where the foetuses were delivered surgically. Each foetus was then decapitated, and the two mandibles dissected in fresh medium, the mandibular condyle being separated from the cranial part of the temporomandibular joint. All of the skin except that covering the chin was removed, but the muscle adherent to the mandible was not disturbed. The explants were placed on rafts of Millipore filter (pore size 1.2 μ), and the two together on expanded steel grids located in plastic Trowell-type culture dishes (3010, Falcon Plastics, Los Angeles, California). Each dish contained a single grid (Falcon 3014) spanning a well having a capacity of 1 ml, and the interior of the dish was kept humid by placing 3 ml of sterile, triple-distilled water in a moat surrounding the well. A total of 120 mandibles were cultured and examined.

In a preliminary investigation, mandibles and humeri from five foetuses were dissected. The skin covering the long bones was removed, but not the muscle. One mandible and one humerus were maintained together in each dish.

Medium and gases

The culture medum comprised antibiotic-free Waymouth's MB752/1 medium supplemented with 0.45 μg/ml ferrous sulphate and 300 μg/ml ascorbic acid (WFeA). Ferrous sulphate and ascorbic acid have been shown to be necessary for collagen synthesis (see, for example, Hutton et al., '67), and ascorbic acid has, in addition, been found to prevent waterlogging of cartilage explants maintained on a chemically defined medium (Reynolds, '66a). In some experiments WFeA was further supplemented by 1.0 μg/ml hydrocortisone-21-sodium succinate (Sigma Chemical Co. St. Louis, Mo.), (WFeAHc). In other experiments 1.0 μg/ml Triiodothyronine (Donated by Glaxo-Allenburys, Canada Ltd., Weston, Ontario, Canada) was added either to WFeA (WFeATt) or

to WFeAHc (WFeAHcTt). The media were constituted under normal laboratory conditions and were then sterilized by passage through Millipore filter (pore size 0.22 μ). Two explants were placed in each dish, and medium was pipetted into the well until it just reached the undersurface of the grid.

The dishes were placed in gas-tight Plexiglas boxes having a capacity of 12.5 L, the interiors of which were kept humid by containers of triple-distilled water. The boxes were filled with an appropriate gas mixture, sealed, and incubated in a water-jacketed incubator at 38°C. The gas-mixtures used were 95% O_2 + 5% CO_2, 40% O_2 + 5% CO_2 + 55% N_2, and Air + 5% CO_2 (Union Carbide Canada Ltd.). The oxygen tension in each Plexiglas box was checked using a Servomex Oxygen Analyser (Servomex Controls Ltd., Crowborough, Sussex, England). In some experiments, the oxygen tension in the box at the end of the culture period was again checked and was found not to have shown a measurable decrease.

In each experiment comparison was made between two different sets of culture conditions. The two explants from each foetus were maintained either on two different media but in the same gaseous environment, or on the same medium but in two different gaseous environments. The cultures were generally maintained for 14 days, but a few were harvested after seven days. Except where otherwise stated, the results described refer to observations on 14 day cultures. The medium and gas were changed three times a week. The paired explants of mandible and humerus were maintained for 14 days on WFeAHc and in 95% O_2.

Histology

At the end of the culture period most of the explants, together with mandibles from intact mice varying in age from 18 days *in utero* to 11 days *post-partum*, were fixed in Bouin's fluid and with the exception of 11 day *post-partum* animals, processed without further demineralization for the preparation of paraffin sections. Mandibles from 11 day *post-partum* animals were demineralized in equal volumes of 20% sodium citrate and 45% formic acid. Cultures and *in vivo* mandibles from which sections were to be stained by the Von Kossa method were fixed in buffered formalin, pH 7.4. All explants were sectioned serially in the longitudinal plane. Most of the sections were stained with haematoxylin and eosin, but appropriate sections were stained by the Von Kossa method for mineral salts, or with alcian blue at pH 0.5 (Lev and Spicer, '64). Twenty explants (10 maintained on WFeA and 10 maintained on WFeAHc and in 95% O_2) and ten *in vivo* mandibles aged two to three days *post-partum* were not fixed, but were frozen. These were then sectioned in a cryostat, and the sections mounted on microscope slides. The sections were fixed for 30 seconds in citrate-buffered acetone, pH 4.5, and processed by the method of Burstone (Pearse, '68) for acid phosphatase, omitting $MnCl_2$, and using red-violet LB diazonium salt. Some sections were incubated without the Naphthol-AS/BI-phosphate substrate to provide controls for the reaction.

Radioautography

1.0 μCi/ml ^{3}H-thymidine or 5.0 μCi/ml ^{3}H-proline were added on the ninth through twelfth day to the medium of some explants cultured on WFeA or WFeAHc for 14 days in 40% O_2 or 95% O_2. ^{3}H-thymidine was similarly added to WFeAHcTt. In one week cultures, isotopes were added to the medium on the third through fifth day. In a number of the ^{3}H-thymidine experiments, 0.2 mg/ml Colchicine (Sigma Chemical Co., St. Louis, Mo.) was added to the medium for the last 18 hours of culture. Two to three day *post-partum* mice were injected intraperitoneally with either 5.0 μCi ^{3}H-thymidine or ^{3}H-uridine two to three hours before death, or three successive doses of 5.0 μCi ^{3}H-uridine at six, four and two hours before death, or with 5.0 μCi ^{3}H-proline 18 hours before death. All isotopes were purchased from Amersham Searle, Toronto, Canada. In all instances sections were dipped in NTB2 nuclear track emulsion (Kodak, Rochester, N.Y.), exposed in light-tight boxes for 7–14 days for cultured material, and up to six weeks for *in vivo* material, and developed in Dektol: distilled water (1:1) (Kodak, Rochester, N.Y.) for two minutes at 13°C.

The sections were stained through the emulsion with haematoxylin and eosin.

RESULTS

A. *Morphological observations* in vivo

(a) *Eighteen day* in utero *mouse.* Examination of 18 day *in utero* mice, that is at the time when the mandibles were explanted, showed that Meckel's cartilage in the body of the mandible comprised a continuous rod. However, an area of the extracellular substance of the cartilage in the vicinity of the mental foramen was calcified. This was borne out by the appearance of sections processed by the von Kossa method. Parts of this calcified cartilage were eroded, and numerous multinucleated giant cells, which appeared to be associated with the process, could be seen (fig. 1). Further erosion of this calcified part of the cartilage resulted in separation of Meckel's cartilage into two fragments, a distal or anterior fragment, and a proximal or posterior fragment.

(b) *Two to three day post-partum mouse.* In the two to three day *post-partum* mouse much of the anterior part of the proximal fragment had been resorbed, and this fragment was now widely separated from the distal fragment which was being replaced by a process akin to endochondral ossification. At the distal extremity of the proximal fragment, and also peripherally, where resorption was thought to be occurring, the extracellular substance appeared to be disintegrating, and exhibited diminished staining by alcian blue. The extracellular substance in these sites was not stained by the von Kossa method. These areas of the cartilage also showed some increase in cellularity, and the nuclei of the cells appeared to be more prominent than chondrocytes in the rest of the cartilage. It was not possible to say whether these cells were liberated chondrocytes or whether they were cells that had originated outside of the cartilage and were now invading it. In these areas too, there appeared to be continuity between Meckel's cartilage and the adjacent soft connective tissue, whereas elsewhere there was usually a clear line of cleavage between the perichondrium of the cartilage and the adjacent connective tissue. In the latter situation, a layer of flattened cells marked the site of the perichondrium, whereas in the former these cells were absent and the perichondrium could not easily be identified (fig. 2). There was no morphological evidence of widespread death of chondrocytes in the vicinity of areas of resorption.

(c) *Six and 11 day post-partum mouse.* By six days *post-partum* most, if not all, of the proximal fragment of Meckel's cartilage in the mandible had been resorbed. No evidence of this fragment of the cartilage was ever found in the mandible of 11 day *post-partum* mice.

B. In vitro *experiments*

(a) *Culture on WFeA.* The entire proximal fragment of Meckel's cartilage was never found to have been resorbed after 14 days culture on WFeA, irrespective of the composition of the gaseous environment. The amount of cartilage that had been lost varied, but the pattern of removal and the appearance of areas where resorption was thought to have been taking place, resembled those seen *in vivo* (fig. 3). The cytoplasm of the chondrocytes appeared to be most abundant when mandibles were maintained in an atmosphere comprising 95% oxygen.

Radioautographs showed that when mandibles were maintained on medium that had contained ^{3}H-thymidine between the ninth and twelfth day of culture, a few of the chondrocytes in the vicinity of an area of resorption, and many of the cells of the perichondrium and particularly of the surrounding tissues, were labelled (fig. 4). In seven day cultures more of the chondrocytes appeared to have incorporated the ^{3}H-thymidine. Mitotic figures could be seen in some of these cells, particularly when colchicine was added to the medium. When maintained on medium that had contained ^{3}H-proline between the ninth and twelfth day, on the other hand, most of the chondrocytes and some of the surrounding extracellular substance in the vicinity of resorbing areas, were found to be labelled by the isotope (fig. 5). The density of label appeared to increase as the resorbing face was approached, and was most intense in areas from which the cartilage presumably had been resorbed and which were being replaced by cellular soft connective tissue. Histochemical tests for

acid phosphatase showed reaction product in many of the chondrocytes, but this was markedly increased in cells in areas where resorption was apparently taking place and the extracellular substance was poorly stained with alcian blue.

(b) *Culture of WFeAHc.* Addition of 1.0 μg/ml hydrocortisone to the medium produced a characteristic hypertrophy of groups of chondrocytes. However, not all of the chondrocytes were affected. The response was enhanced by increasing the oxygen tension to 95% and was most marked when explants maintained in this gaseous environment were cultured on the WFeAHc medium further supplemented with 1.0 μg/ml Triiodothyronine (figs. 6, 7, 8). When most highly developed, the hypertrophic chondrocytes exhibited large vesicular nuclei with prominent nucleoli, and abundant cytoplasm which contained haematoxyphilic thread-like material and large vesicles (fig. 8). Much of the intervening extracellular substance had disappeared, and this observation was supported by the appearance of material stained by alcian blue. The affected areas of the cartilage were very cellular. Appreciable deposits of reaction product in these cells revealed that they contained active acid phosphatase (fig. 9).

Addition of 1.0 μg/ml Triiodothyronine to the WFeA medium in the absence of hydrocortisone did not lead to hypertrophy of the cytoplasm of the chondrocytes, nor to a noticeable increase in the amount of cartilage resorbed during the culture period, even in the presence of 95% O_2.

When ^{3}H-thymidine was added to the medium between the ninth and twelfth day, radioautographs revealed that some of the nuclei of the hypertrophic cells had incorporated the isotope (fig. 10). This appeared to occur particularly where little extracellular substance remained between the cells. Mitotic figures could also be identified in some of the cells (fig. 11), and these were most easily seen when colchicine was added to the medium during the last 18 hours of culture.

C. In vivo *experiments*

Radioautographs of the resorbing proximal fragment of two to three day *post-partum* mice that had received 5.0 μCi/ml ^{3}H-proline 18 hours before death showed labelling of many of the chondrocytes and surrounding extracellular substance in the vicinity of resorbing sites. The intensity of labelling appeared to increase as the resorbing face was approached, and was most intense in areas from which the cartilage presumably had been resorbed. This pattern of labeling was similar to that seen in mandibles cultured on WFeA, but was much less intense. The veracity of the labelling was confirmed by comparing it with the degree of background labelling of the extracellular substance of adjacent trabeculae of old bone. Similarly, radioautography of sections from the same aged animals that had received ^{3}H-uridine just prior to death showed that many of the chondrocytes adjacent to resorbing faces had incorporated the isotope (figs. 12a,b). ^{3}H-thymidine administered to two to three day *post-partum* animals two to three hours before death was found to have been incorporated by a number of cells in areas from which cartilage was presumed to have been resorbed, by cells in the vicinity of the perichondrium, and by occasional chondrocytes adjacent to the face of the resorbing cartilage (figs. 13a,b).

Histochemical tests for acid phosphatase showed some reaction product to be present in almost all of the chondrocytes of the proximal fragment of Meckel's cartilage. These deposits appeared to be increased in the neighbourhood of sites of resorption and, in areas where on morphological grounds resorption was believed to be occurring, it was heavy. The heavy deposit of reaction product occurred in areas which seemed to embrace cartilage and extracartilaginous tissue, and it was not possible to differentiate between cartilage on the one hand and perichondrium and the adjacent tissues on the other. However, reaction product in these sites could be identified in chondrocytes that clearly were surrounded by extracellular substance (fig. 14).

D. *Combined culture of humerus and mandible on WFeAHc + 95% O_2*

The arrangement of the cells in the epiphyseal cartilage was fairly well maintained. The chondrocytes were not seen to have hypertrophied in the same manner

as the cells of Meckel's cartilage in the companion cultured mandible (figs. 7, 15).

DISCUSSION

1. *Fate of chondrocytes in resorbing Meckel's cartilage*

Two conclusions may be drawn from the observations made in this *in vitro* and *in vivo* investigation: firstly, that the chondrocytes of the proximal fragment of Meckel's cartilage in the mandible of the mouse have the capacity to resorb the extracellular substance that surrounds them; and secondly, that chondrocytes can survive removal of the extracellular substance. There was no evidence to support the concept that most of the chondrocytes in an area being resorbed die, and that removal of the extracellular substance is consequently left entirely to non-cartilaginous cells. On the other hand, the possibility that perichondral cells, and cells other than those of cartilage, may also participate in resorption of the extracellular substance remains.

The belief that the chondrocytes have the capacity to resorb extracellular substance is based in part on the observation that many chondrocytes were stimulated to hypertrophy *in vitro* by hydrocortisone, particularly when supplemented by triiodothyronine and in the presence of 95% O_2, and that this reaction of the cells was accompanied by depolymerization of surrounding extracellular substance. This latter conclusion was predicated not only on the morphological appearance of the tissue, but also on its loss of staining by alcian blue. No direct evidence has been obtained that the chondrocytes were responsible for removing the extracellular substance, but it is difficult to see how else this could have been achieved.

Chondrocytes have been found to secrete acid hydrolases and to be involved in resorption of extracellular substance under a number of circumstances *in vitro*. This activity has been seen in cartilage of rudiments of chick long bone exposed to excess Vitamin A, sucrose, or complement — sufficient antiserum (Dingle, '69), or to high tensions of oxygen (Sledge and Dingle, '65; Sledge, '68). It is evident that cells engaged in phagocytosis, or digestion of extracellular substance, are rich in hydrolytic enzymes (Ballard and Holt, '68; Reynolds, '69; Vaes, '69); acid phosphatase serves as a marker for these enzymes. In areas where cartilage of developing long bones is being resorbed during development, associated chondrocytes have been shown *in vitro* to be particularly reactive for acid phosphatase (Sledge, '68); and, *in vivo*, this enzyme has also been demonstrated in hypertrophic cells of the cartilage of the developing condyle of the mandible (Greenspan and Blackwood, '66). Although most of the chondrocytes of Meckel's cartilage, both *in vivo* and *in vitro* were found to be reactive for acid phosphatase, the hypertrophic cells *in vitro* and the chondrocytes in areas of resorption *in vivo* and *in vitro* were consistently found to contain appreciably heavier deposits of reaction product. This observation is thought to provide further support for the belief that the chondrocytes of the cartilage can participate in resorption of its extracellular substance. Germane to this discussion is the apparent capacity of osteocytes to resorb bone matrix that surrounds them (Bélanger, '69).

The fact that the chondrocytes apparently participate in resorption of the extracellular substance suggests that they do not necessarily die prior to, or during its removal. A number of the findings in the present investigation provide support for this assumption. Most of the chondrocytes in the vicinity of resorption sites incorporated ^{3}H-proline *in vitro*. Intensity of labelling of chondrocytes in radioautographs of these areas appeared to increase as the resorbing face was approached, and this suggests that many of these chondrocytes were metabolically active and not moribund. A similar picture was seen *in vivo*, although the intensity of labelling was lower, and fewer chondrocytes were labelled. This disparity in labelling was possibly due to a higher concentration of isotope being available to the cells *in vitro*. Many of the chondrocytes in areas of resorption *in vivo* were also found to incorporate ^{3}H-uridine and therefore to be synthesizing RNA, an activity normally associated with viable cells. Finally, occasional chondrocytes in these areas both *in vitro* and *in vivo*, were found to have synthesized DNA, and to exhibit mitotic figures,

particularly after administration of colchicine. Many of the hypertropic chondrocytes were also found to have incorporated ^{3}H-thymidine, and this was particularly noticeable in sites where little extracellular substance could be seen. This observation is consistent with that of Fell ('69), who has reported that cells that have been released from chick cartilage disintegrating in response to Vitamin A *in vitro*, can divide. Thus, although some of the chondrocytes may die during resorption of the cartilage *in vivo*, there is a body of indirect evidence to support the belief that many of them participate in digestion of the extracellular substance and outlive its loss.

This investigation has not provided any information about the role that the chondrocytes and cells of the perichondrium may play subsequent to resorption of the extracellular substance. It has been shown in epiphyseal cartilage that osteoblasts can be derived from chondrocytes (Crelin, '67; Crelin and Koch, '65, '67; Holtrop, '66, '67). Furthermore, chondrocytes of symphyseal cartilage may modulate reversibly to fibroblasts, and the alterations in functional state are influenced by hormones (Crelin, '69). Consequently, the possibility that chondrocytes of the proximal fragment of Meckel's cartilage, or their progeny, may participate in fibrogenesis or osteogenesis subsequent to resorption of the cartilage would not be unique, and should be considered. These observations and deductions allow a working hypothesis describing the life-cycle of the chondrocytes of the proximal fragment of Meckel's cartilage to be constructed. Chondroblasts differentiate from mesenchymal cells, secrete extracellular substance and lay down Meckel's cartilage. The chondrocytes so formed may divide and participate in interstitial growth of the cartilage. They subsequently take part in resorption of the extracellular substance of the proximal fragment. After release from the cartilage they divide and then differentiate into fibroblasts or osteoblasts.

2. *Effect* in vitro *of hydrocortisone upon the chondrocytes*

The appearance of the nuclei and cytoplasm of the hypertrophic chondrocytes in explants maintained on hydrocortisone — containing medium suggested that these cells were metabolically active and possibly engaged in phagocytosis. Although chondrocytes exhibiting such extensive hypertrophy were never seen in this situation *in vivo*, the finding suggests the possibility that hydrocortisone could stimulate phagocytic activity by the cells *in vivo*. It was clear from the *in vitro* study that, of the substances tested, marked hypertrophy was induced only by hydrocortisone, but the reaction was intensified by raising the oxygen tension of the environment and particularly by supplementing the WFeAHc medium with triiodothyronine (see, also, Melcher, '71a). A number of developmental processes are known to be controlled by hormones (see Saunders, '66). Because thyroid hormones stimulate the morphological changes that occur in metamorphosis of tadpole to frog, and particularly because they promote resorption of the tadpole tail, it was considered possible that triiodothyronine may stimulate resorption of the proximal fragment of Meckel's cartilage. The hormone did not appear to increase resorption of Meckel's cartilage to any marked degree *in vitro*, nor did it produce hypertrophy of the chondrocytes, but it did enhance the effect of hydrocortisone upon these cells.

When viewed in the light of previous *in vitro* work on the response of chondrocytes to hydrocortisone, the observations made here were surprising. Sledge and Dingle ('65) and Sledge ('68) have shown that chondrocytes of chick limb-bud rudiments increase production and release of acid phosphatase when exposed *in vitro* to high tensions of oxygen, and that this process can be reversed by addition of cortisol to the medium. Furthermore, Reynolds ('66b) has found that the hypertrophy of chondrocytes that occurs when developing long bones of chick are cultured on a chemically defined medium can be controlled by hydrocortisone, and that this is accompanied by considerable increase in metachromasia of the extracellular substance. This effect of hydrocortisone on the chondrocytes has been explained on the basis that the hormone stabilizes lysosomal membranes and inhibits release of acid hydrolases (see Weissmann, '69). What has occurred in the present investigation is

the reverse of these observations. Indeed, marked hypertrophy of the cells appeared to depend on the presence of hydrocortisone in the medium, and the hypertrophy was accompanied by loss of alcian blue staining of the extracellular substance. Not all of the chondrocytes responded in this way. The involved cells appeared to occur in groups, and it is conceivable that these cells were in some way "primed" to react to the hydrocortisone. The information obtained from the present experiment does not explain the difference between the results obtained here and those previously reported. Use of different experimental animals may partly be responsible for the variance. A more compelling possibility may reside in the likelihood that chondrocytes of different cartilages respond differently to the same stimulus. This explanation receives support firstly, from the finding that chondrocytes in the condylar cartilage of the same mandibles did not hypertrophy in response to hydrocortisone in the same way as did the chondrocytes of the proximal fragment of Meckel's cartilage (Melcher, '71b). Secondly, the observations made on the mandibles and humeri cultured together on WFeAHc in 95% O_2 for 14 days suggest that the chondrocytes of the epiphysis do not respond to the hydrocortisone in the same manner as do the chondrocytes of Meckel's cartilage. Finally, Levenson ('70) has shown that chondrocytes disassociated from different cartilages behave differently from one another when maintained *in vitro* under comparable conditions. The observation made here therefore suggests that hydrocortisone does not depress secretion of acid hydrolases by chondrocytes in the proximal fragment of Meckel's cartilage, in the same manner as the hormone has been shown to do in a number of other cells (see Weissmann, '69).

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PLATE 1

EXPLANATION OF FIGURES

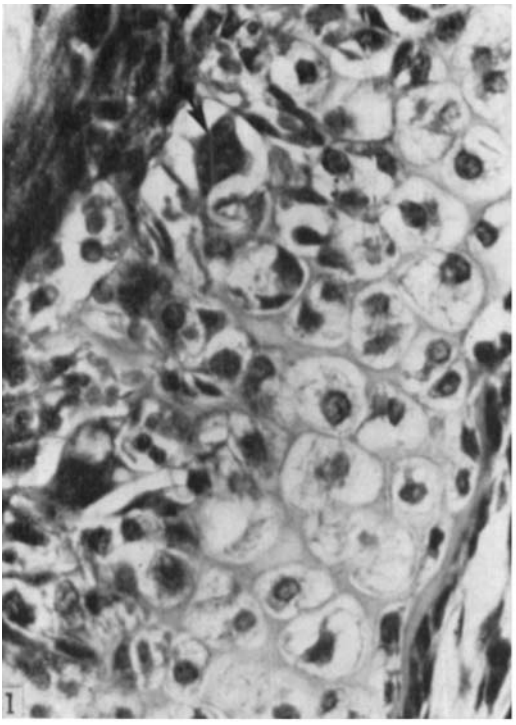
1 Erosion of Meckel's cartilage in the vicinity of the mental foramen in an 18 day *in utero* mouse. Multinucleated giant cells (arrow) are associated with the process, and most of the chondrocytes are hypertrophic. Haematoxylin and eosin. × 670.

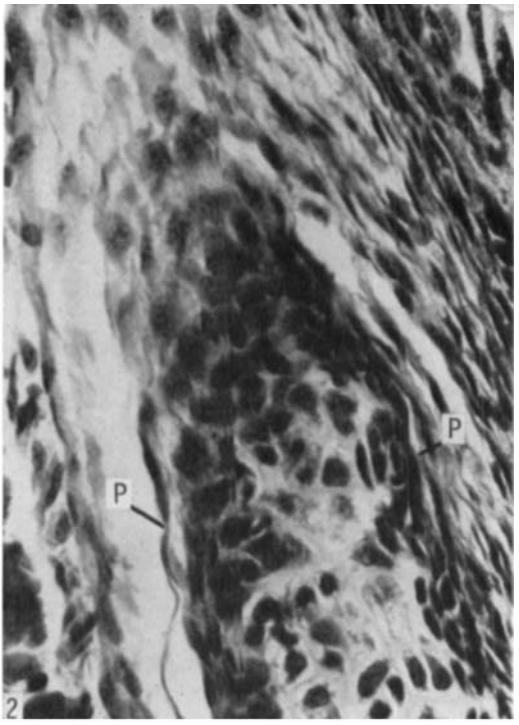
2 Resorbing anterior region of proximal fragment of Meckel's cartilage in two to three day *post-partum* mouse. There is no clear separation between the chondrocytes and the cells of the soft connective tissue. Elsewhere the two groups of cells are separated by perichondrium (P). Haematoxylin and eosin. × 670.

3 Resorbing anterior region of proximal fragment of Meckel's cartilage in explanted mandible maintained for 14 days on WFeA and in 95% O_2. Morphology of the tissue resembles that seen *in vivo* and illustrated in figure 2. Perichondrium — P. Haematoxylin and eosin. × 670.

4 Radioautograph of the anterior region of proximal fragment of Meckel's cartilage in mandible maintained for 14 days on WFeA to which 1.0 μCi/ml ^{3}H-thymidine was added on the ninth through twelfth day, and in 95% O_2. This area is similar to that illustrated in figure 3. The periphery of the resorbing cartilage is outlined by arrows. Some perichondrial cells (P) and cells of surrounding tissue (F), and occasional chondrocytes (C) are labelled by silver grains. × 670.

5 Radioautograph of the anterior region of proximal fragment of Meckel's cartilage in mandible maintained for 14 days on WFeA to which 5.0 μCi/ml ^{3}H-proline was added on the ninth through twelfth day, and in 95% O_2. This area is similar to that illustrated in figure 3. The periphery of the resorbing cartilage is outlined by arrows. Most of the chondrocytes (C) and some of the extracellular substance is labelled by silver grains. × 670.

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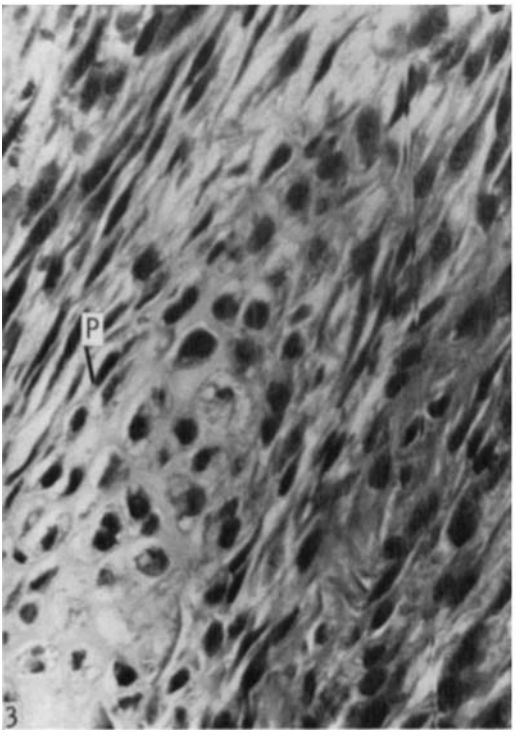
P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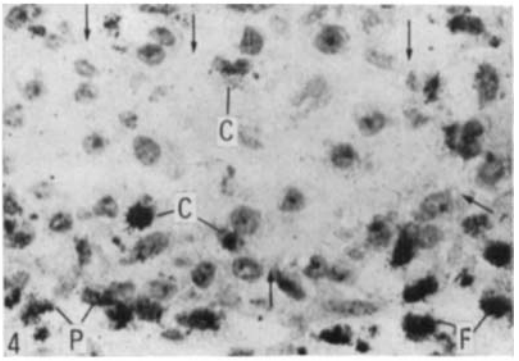
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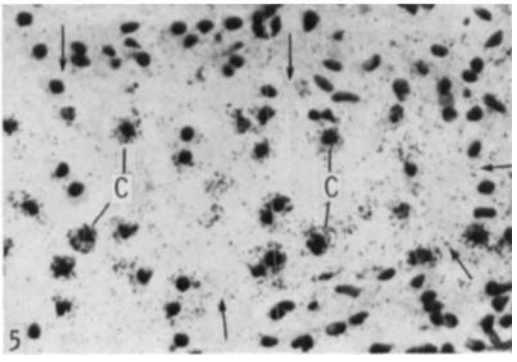
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PLATE 2

EXPLANATION OF FIGURES

Figs. 6, 7 and 8 These three photomicrographs illustrate the differing cytology of the chondrocytes of Meckel's cartilage in explanted mandibles maintained for 14 days in 95% O_2 but on different media. They are all of the same magnification × 670.

6 WFeA.

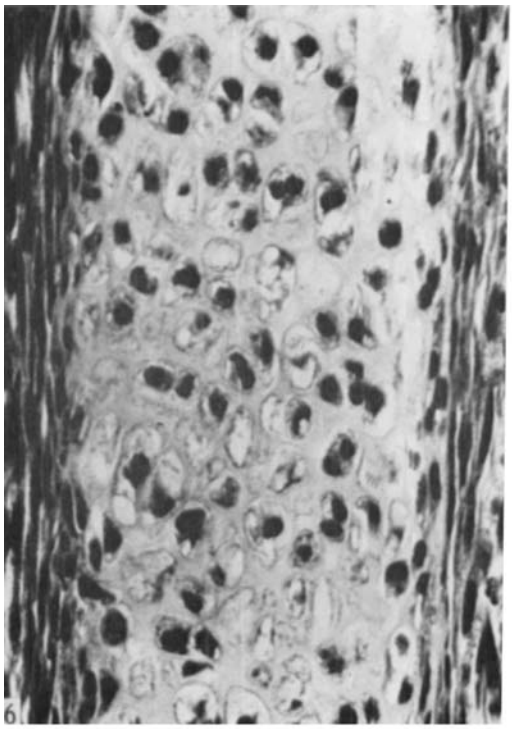
7 WFeAHc. Note the hypertrophy of the chondrocytes when compared with those illustrated in figure 6. The morphology of these cells resembles that of hypertrophic chondrocytes *in vivo* illustrated in figure 1.

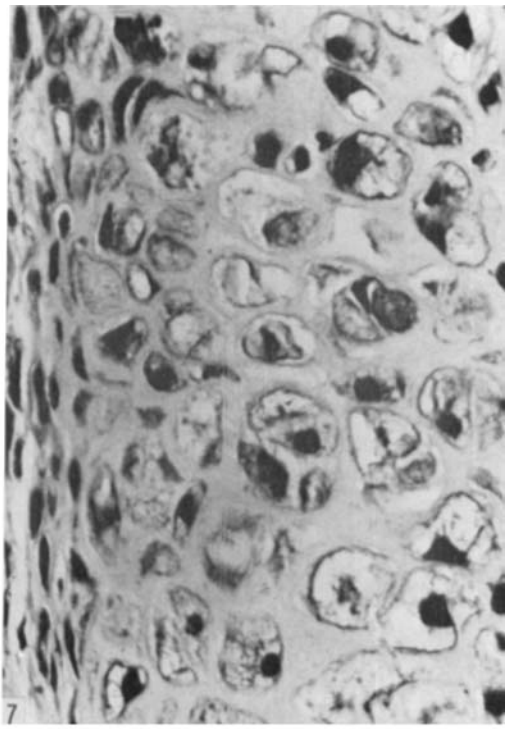
8 WFeAHcTt. Note the complexity of the cytoplasm of the hypertrophic chondrocytes when compared with those illustrated in figures 6 and 7.

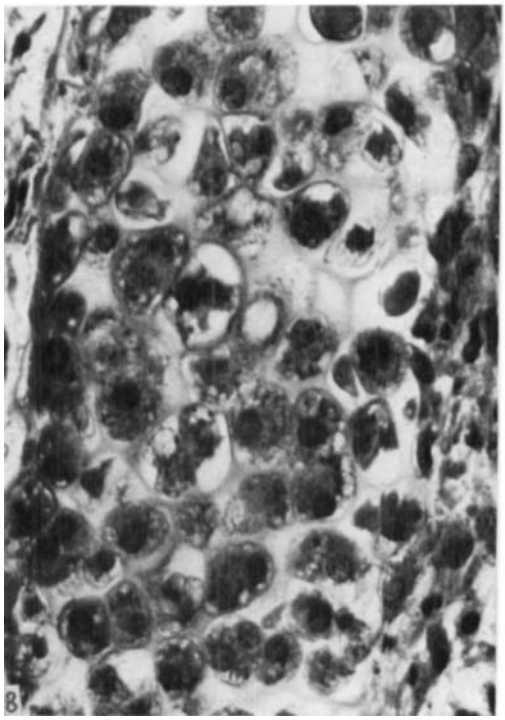
9 Hypertrophic chondrocytes in Meckel's cartilage filled with reaction product after histochemical test for acid phosphatase. Explanted mandibles were maintained for 14 days on WFeAHc and in 95% O_2 for 14 days. Cold microtome sections. Burstone's method for acid phosphatase. × 670.

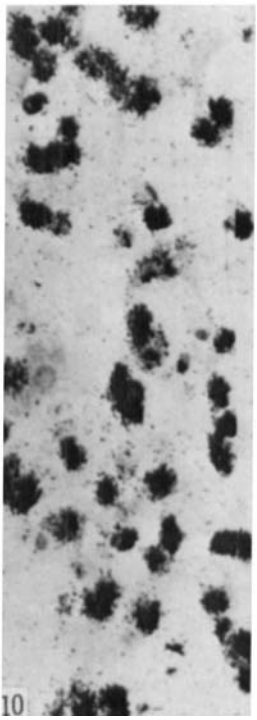
10 Radioautograph of a section adjacent to that illustrated in figure 8. ^{3}H-thymidine that had been added to the WFeAHcTt medium was incorporated into the hypertrophic chondrocytes. × 670.

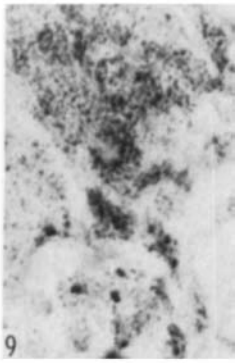
11 Mitotic figure (arrowed) among hypertrophic chondrocytes in Meckel's cartilage of an explanted mandible maintained for 14 days on WFeAHcTt and in 95% O_2. Haematoxylin and eosin. × 1,072.











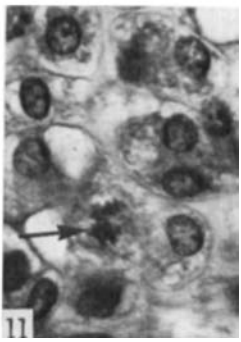


PLATE 3

EXPLANATION OF FIGURES

Figs. 12a and b Resorbing anterior region of proximal fragment of Meckel's cartilage in two to three day *post partum* mouse.

12a Haematoxylin and eosin. × 670.

12b Radioautograph of a similar area in a nearby section to show incorporation of ^{3}H-uridine, injected two to three hours before death, into most of the chondrocytes in the vicinity of the resorbing face. × 1,072.

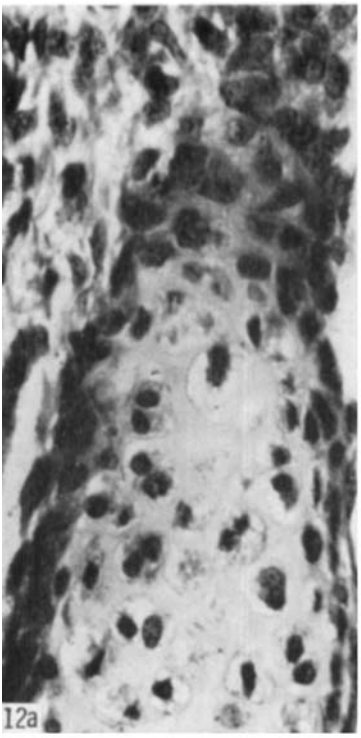
Figs. 13a and b Radioautograph of resorbing area of proximal fragment of Meckel's cartilage in two to three day *post-partum* mouse to show incorporation of ^{3}H-thymidine injected two to three hours before death into cells in the vicinity of the perichondrium (P) and chondrocytes (C).

13a × 670.

13b × 1,072.

14 Area of resorption in the proximal fragment of Meckel's cartilage of a two to three day *post-partum* mouse. Acid phosphatase reactivity has been demonstrated in chondrocytes (C), as well as in other connective tissue cells in the vicinity. Cold microtome section. Burstone's method for acid phosphatase. × 1,072.

15 Chondrocytes of epiphyseal cartilage of a humerus cultured together with a mandible, the Meckel's cartilage of which is illustrated in figure 7. The explants were maintained for 14 days on WFeAHc in 95% O_2. Comparison of this illustration with figure 7, which was photographed at the same magnification, shows that the chondrocytes have not hypertrophied in a comparable manner. Haematoxylin and eosin. × 670.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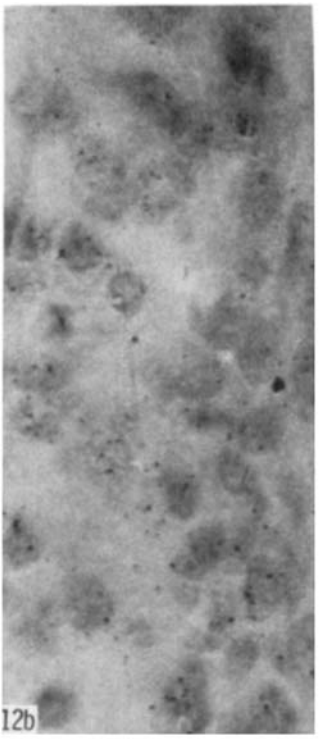
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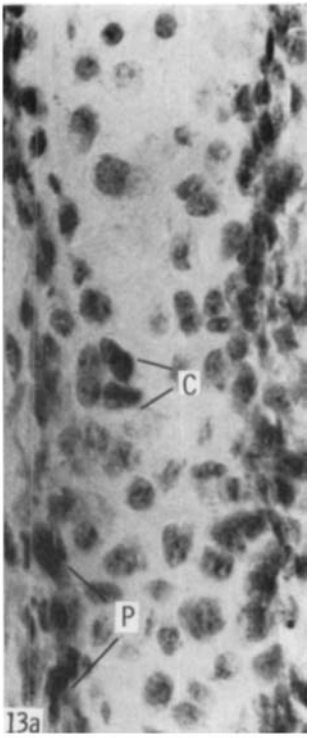

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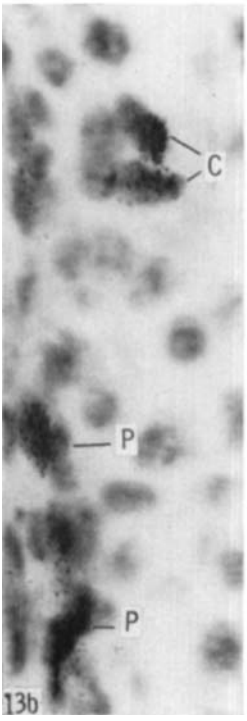

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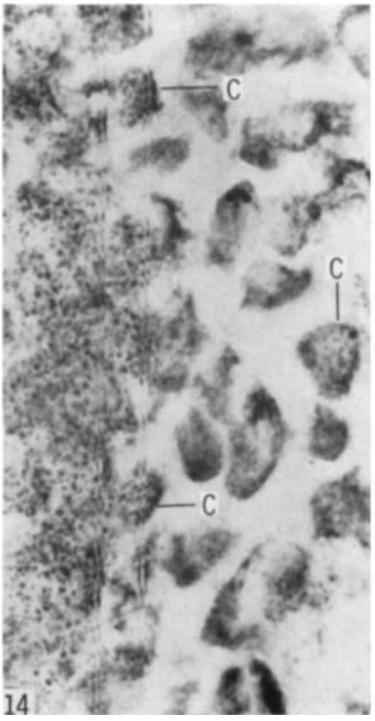

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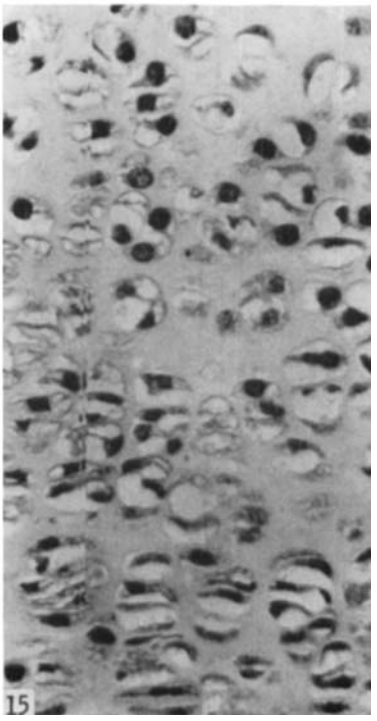
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