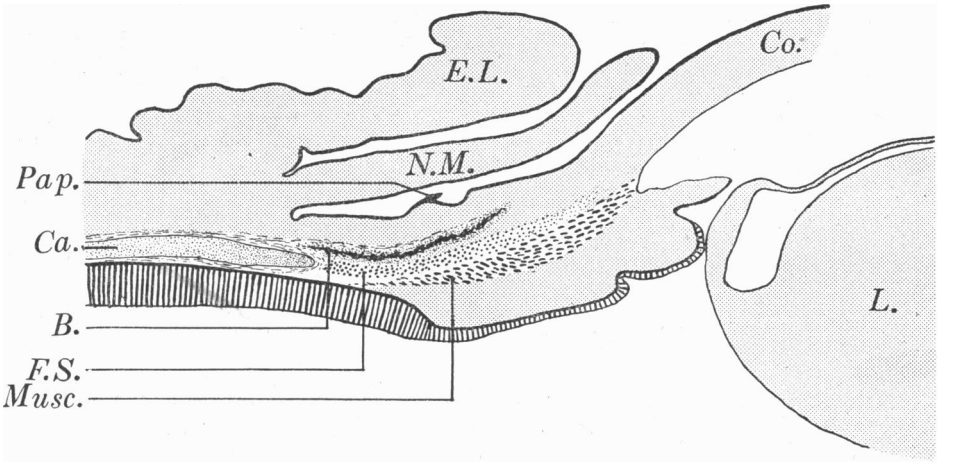
THE DEVELOPMENT OF THE CONJUNCTIVAL PAPILLAE AND OF THE SCLERAL BONES IN THE EMBRYO CHICK

By P. D. F. MURRAY, *Department of Biology, St Bartholomew's Hospital Medical College*

It is well known that a series of fourteen epidermal papillae is present on the conjunctiva of the chick embryo between the seventh and fourteenth days, arranged in a ring round the pupil. Somewhat later, from about 10 days, the fourteen scleral bones begin to appear, one developing in the mesenchyme beneath each epidermal papilla. The development and fate of the papillae have been described by Nussbaum (1901). His account, though in large part confirmed by Dabelow (1927), is in important respects erroneous, and a new description is necessary. The present paper contains a description of the development and fate of the papillae, of the early development of the scleral bones, and of a structural relation, previously described by me in *Nature* (1941), between each papilla and the bone developing beneath it.



Text-fig. 1. A diagram showing the position of the epidermal papillae and scleral bones with reference to other structures. *B.* bone; *Ca.* scleral cartilage; *Co.* cornea; *E.L.* eyelid; *F.S.* fibrous sclera; *L.* lens; *Musc.* ciliary muscle; *N.M.* nictitating membrane; *Pap.* epidermal papilla.

Nussbaum found that the papillae appear at about 6 days of incubation and that at 13 days they are only recognizable microscopically, while at hatching no trace of them remains. This accords with my own observations.

Text-fig. 1 is a diagram, based on a section of a 12-day embryo, showing the topographical relation between the papillae, the bones and the neighbouring parts of the eye. The scleral bone lies between the cartilage and the cornea, overlapping the cartilage peripherally. Above it, the papilla is shown rising from the epidermis, and below it is indicated a ring of fibrous tissue which extends from the cartilaginous part of the

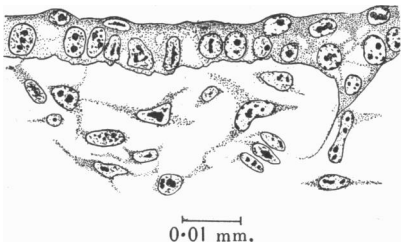
sclera into the cornea. It will be referred to as the 'fibrous sclera'. Beneath this again is the ciliary muscle.

MATERIAL

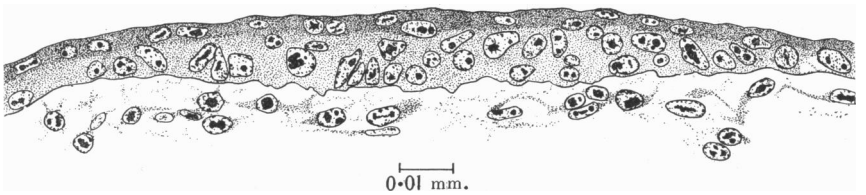
I have studied the following material:

5-day embryo: one eye;
6-day embryos: one eye from each of three embryos;
7-day embryos: one eye from each of five embryos;
8-day embryos: one eye from each of two embryos;
9-day embryos: both eyes from two embryos;
10-day embryos: one eye from each of two embryos;
12-, 17-, 19-day embryos: one eye each.

Fixation was in some cases with Zenker, in others with Susa; the stain was in all cases Azan, except for a few slides from the 19-day embryo which were stained with haematoxylin and eosin.



Text-fig. 2. Unmodified conjunctival epidermis and some of the mesenchyme beneath it. 7 days. Azan. Camera lucida.



Text-fig. 3. A papilla early in stage 1, with a little of the mesenchyme beneath it. 7 days. Azan. Camera lucida.

OBSERVATIONS

No papillae were found in the 5- and 6-day embryos; this agrees with Nussbaum and Dabelow.

The development and regression of the papillae can be arbitrarily but conveniently divided into six stages.

Stage 1 (Text-figs. 2, 3, 9A)

Early stages in the development of the papillae can be found at 7 days. At this time the unspecialized epidermis between the papillae has the structure shown in Text-fig. 2. It is a two-layered epithelium, the cells of the lower or basal layer being approximately cubical, while those of the upper layer or periderm tend to be flattened. It retains this structure without important change at least as late as 17 days of incubation.

One of the earliest papillae which I have seen is shown in Text-fig. 3. It is a flat thickening of the epidermis in which the periderm cells appear to be unchanged while the basal layer is two or three nuclei deep, the whole being scarcely twice the thickness of the unaltered epidermis. In slightly more advanced papillae, the periderm becomes two or three cells thick. The order of magnitude of these thickenings may be illustrated from one of them, which could be traced through seventy-four sections 5 μ thick and in this dimension (approximately tangential to the pupil) therefore measured 370 μ. The length of the thickening in the plane of the sections (approximately at right angles to the tangent to the pupil) was about 180 μ.

In this stage mitoses can be found rather sparsely in the upper and lower layers of the epithelium both in the thickened region and elsewhere; but in the thickened region very nearly all, if not all, are in the peripheral part of the thickening and not near its centre. The axis of the mitoses is usually horizontal or nearly so. The greatest thickness of each papilla is at its centre, to which cells appear to be migrating from its more peripheral parts (see stage 2). The degenerative phenomena which play so important a part in later stages are not seen in stage 1. This statement must be qualified by reference to a single papilla in a 7-day chick, in which the structure of the papilla as a whole is typically that of stage 1, but the whole of the periderm is in active degeneration. This does not usually happen until stage 2.

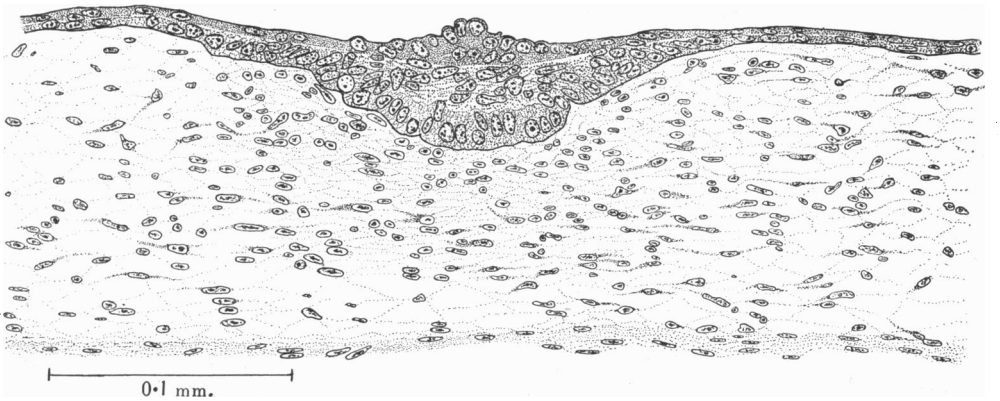
Casual examination gives the impression that the mesenchyme beneath the epidermal thickenings is slightly denser than in neighbouring regions, but an attempt to confirm this by counting nuclei in this region and in the mesenchyme in regions between epidermal thickenings failed to show any difference. An attempt to find a difference in the size of mesenchyme nuclei led to a similar negative result. It seems, therefore, that if there is any condensation in the mesenchyme at this stage it is very slight.

Stage 2 (Text-figs. 4, 9B; Pl. 1, fig. 2)

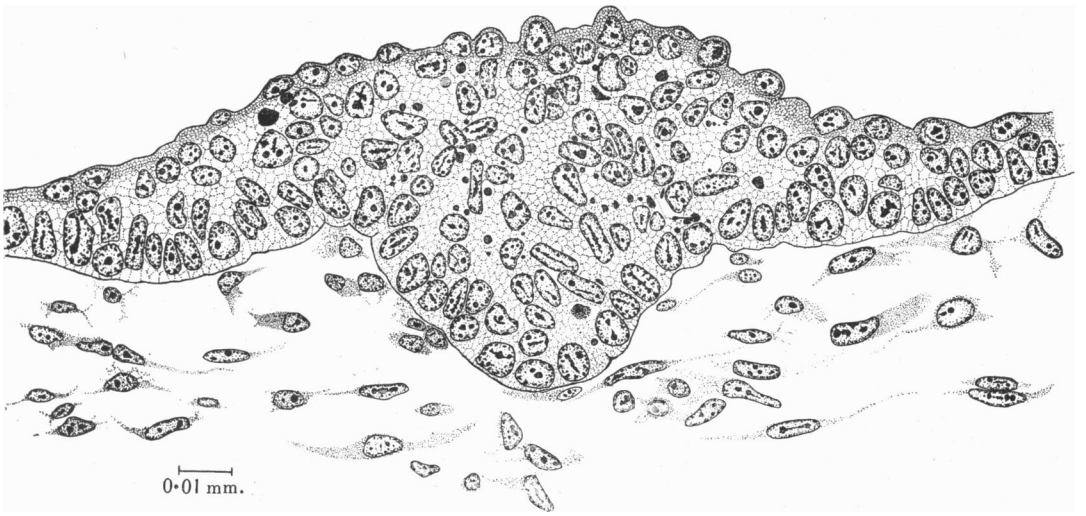
I have found papillae of stage 2 only in 7-day chicks, except for one in an 8-day chick. In this stage there appears a differentiation between the central and peripheral parts of each thickening. The central region increases in thickness, beginning to bulge downwards into the mesenchyme, and, to a less extent, upwards above the general level of the epidermis (Text-fig. 4). The cells responsible for this increase in the thickness of the central region seem, from the histological picture, to have originated as basal layer cells in parts of the thickening a little away from the central region; they appear to migrate out of the lower layer, and then centripetally between the periderm and basal layers, so that they form a 'central mass' which causes the downward bulge. At the same time the basal layer cells covering the bulge become more regularly columnar. When it is first forming, the downward bulge may be of irregular form, consisting of several rounded protuberances.

The periderm may now be two, three or even four nuclei deep and the cells rounded or cubical, not flattened as they are in unmodified parts of the epidermis and in the papillae during stage 1. During stage 2 degenerative changes begin in these cells. Details of these changes resemble those seen on a larger scale in other parts of the papillae in later stages, and some description is given of them under stage 3. The uppermost nuclei tend to project above the general surface of the epithelium as though being extruded, and this probably is the fate of some. These changes are confined to the periderm and to its more central part; in the peripheral parts of the papillae the

cells remain healthy. The occurrence of mitoses is as in stage 1. During stage 2 the mesenchyme underlying the papilla begins to show definite condensation, which is densest close to the downward projection of the central part of the papilla and gradually thins out in all directions (Pl. 2, fig. 1). The cells are more numerous than



Text-fig. 4. A papilla in stage 2, 8 days, showing an early stage in the formation of the 'tongue', the limiting cells of the lower layer becoming columnar, and the papilla beginning to project slightly above the rest of the epidermis. There is a diffuse condensation in the mesenchyme beneath the papilla. Azan. Camera lucida.



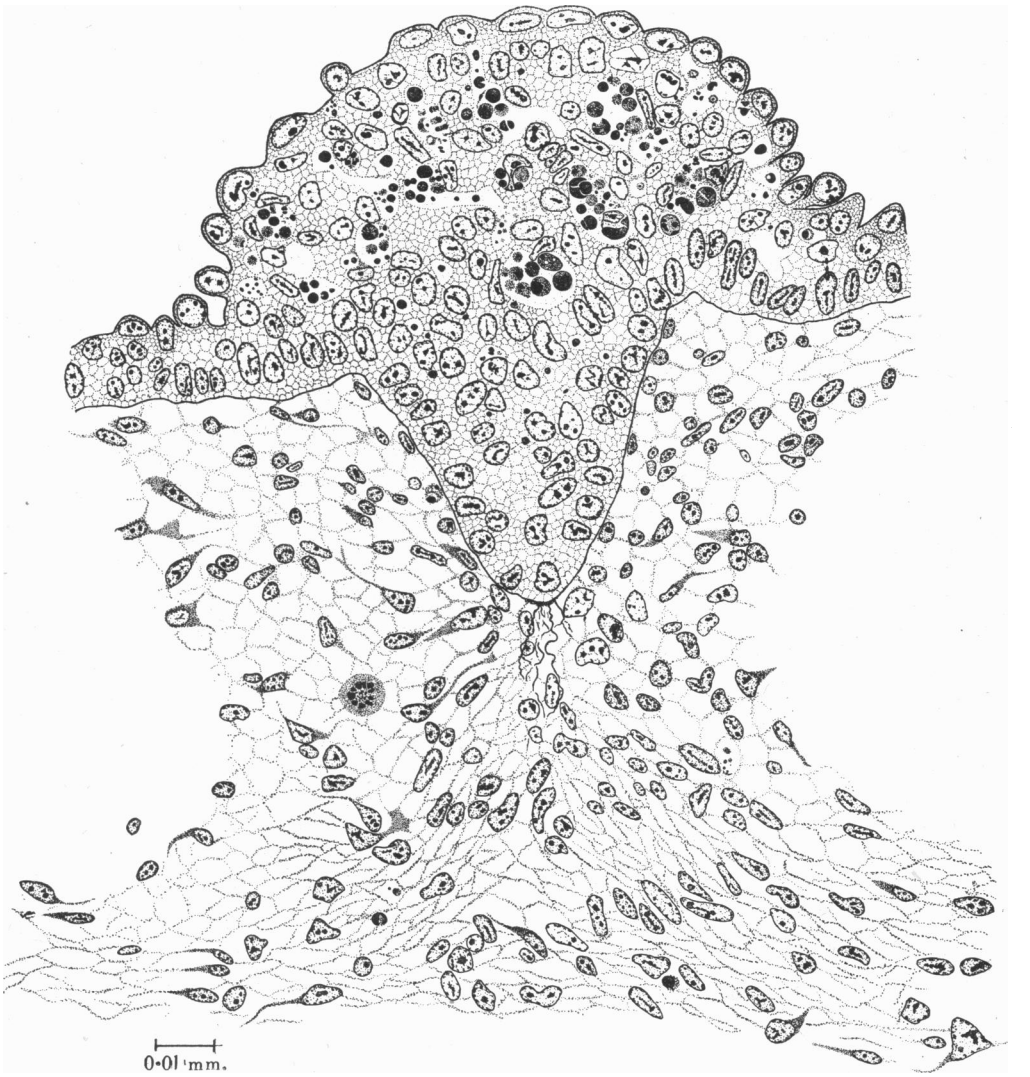
Text-fig. 5. Stage 3, 8 days. Formation of the tongue. The entire papilla except for a single superficial layer of cells is derived from the lower layer of the epidermis. Degeneration granules are appearing. Azan. Camera lucida.

in neighbouring regions, but show with Azan no differentiation of form or structure. They are mostly fusiform with their long axes roughly parallel to the plane of the epidermis. The nuclei vary from oval to elongate, and have one or two nucleoli. The cells lie in a very delicate fibrillar stroma which takes aniline blue feebly, if at all. In contrast with later stages this stroma shows no disturbance beneath the papilla

except in being pushed downwards as the downward projection of the papilla becomes longer.

Stage 3 (Text-figs. 5, 6, 9C; Pl. 1, fig. 3)

This stage, which I found in chicks of 7 and 8 days' incubation, is marked by an increase in the size of the downward projection from the papilla, which becomes a



Text-fig. 6. A more advanced specimen of stage 3, 8 days. There is much degeneration in the central mass of cells at the base of the tongue, with the formation of vacuoles. Slender collagen fibres arising from the tongue run among the cells of the mesenchyme condensation; this is the 'descending strand'. There are a few degenerate cells in the mesenchyme. Azan. Camera lucida.

large conical structure (Text-figs. 5 and 6). It is here called the 'tongue'. At the same time the papilla begins to project more above the general surface of the epidermis. These changes are expressions of the increase of the central part of the papilla, brought

about by the continuation of the centripetal migration of cells mentioned in stage 2. At the height of stage 3 (Text-fig. 6) the papilla consists of (*a*) a superficial epithelium of cells derived from the original periderm, (*b*) on the surface towards the mesenchyme a fairly regular columnar epithelium of basal layer cells, (*c*) the 'central mass' filling the tongue and lying between it and the periderm, derived from the basal layer cells which migrated here from their original positions nearer to the periphery of the papilla.

Mitoses can still be found in the papillae at this stage, but only in their more peripheral parts, and the number varies considerably from papilla to papilla. In five papillae 1, 1, 4, 5 and 8 mitoses respectively were found.

In stage 2 degeneration occurs among the cells of the periderm, but not elsewhere. In stage 3 the periderm cells tend to project above the general surface, the nuclei being in their free ends, but they show less degeneration than in the preceding stage, and less in the more advanced papillae of stage 3 than in the less advanced. Basophil granules may be found in their cytoplasm, but the wave of cellular destruction which is apparent in stage 2 has apparently receded. The degenerating cells have either been cast off, which is strongly suggested by certain papillae of stage 2, or have recovered, which is almost certainly not the case. There is, however, as stage 3 progresses, increasing degeneration in the cells of the central mass. It begins in the base of the tongue or above it, the cells forming the tongue itself being at first less affected. Its onset is marked by the appearance in the cells of basophil granules (staining with azo-carmine). These seem to originate in two ways: in the cytoplasm, which contains granules varying in size from tiny specks hardly visible with an oil-immersion objective to particles larger than nuclei, and from the nuclei themselves. In nuclear degeneration, the nucleus seems first to stain more deeply with azo-carmine and also more diffusely, and then to lose all visible structure as the basophil substance increases. Finally, the nuclei probably become indistinguishable from particles of cytoplasmic origin. At first the basophil granules are found rather sparsely in scattered cells (Text-fig. 5), but the number of affected cells increases, and in the more advanced specimens of stage 3 there are large vacuoles which seem to have bounding membranes and which are filled with basophil detritus (Text-figs. 6, 7). The columnar layer bounding the tongue against the mesenchyme remains unaffected. Its cells are now more regularly columnar than in stage 2.

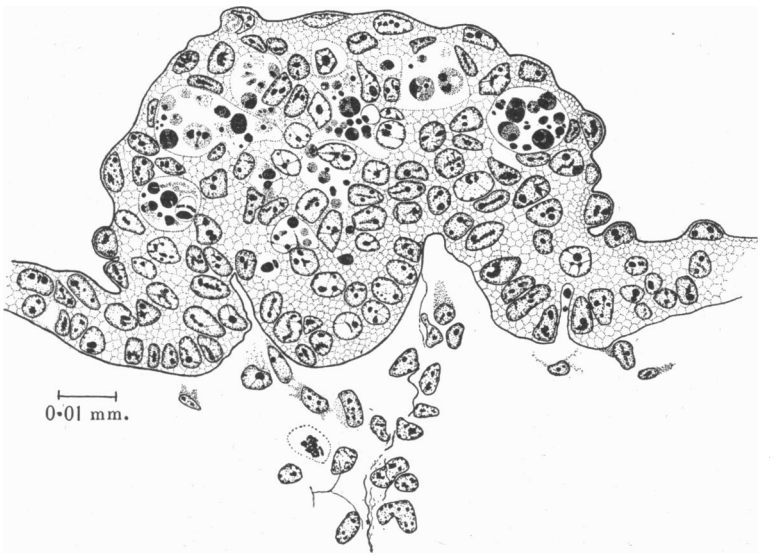
The condensation of the mesenchyme below the papilla is more obvious than in stage 2 (compare Pl. 1, figs. 2, 3). During stage 3 it changes in form and in the arrangement of its cells and fibrous stroma. In stage 2 there is a diffuse condensation whose cells lie with their long axes parallel to the general plane of the conjunctiva (Text-fig. 4; Pl. 1, fig. 2), and the form of the whole condensation is roughly that of a thick disc, the condensation occupying nearly the whole space between its tongue and the fibrous continuation of the sclera. Later, and especially in stage 4, the condensation ceases to be disc-like and arranges itself as a thick column, with its longest dimension at right angles to the plane of the conjunctiva (Text-fig. 6; Pl. 2, fig. 5, a papilla of stage 4), while the cells lie, with their long axes more vertically than horizontally, in a fibrillar stroma which is similarly orientated (Text-fig. 6). A few mitotic figures can be found in the condensed mesenchyme and also occasional degenerating cells (Text-fig. 6).

An important observation at this stage is the presence of fine collagen fibres arising from the basement membrane of the tongue and spreading out among the cells of the

condensation (Text-fig. 6). These fibres are distinct from those forming the stroma of the mesenchyme, for they stain with aniline blue while the stroma scarcely stains at all; they do not form a harmonious part of the stroma, but run among the cells of the mesenchyme without reference to its arrangement. In stage 3 they stain only weakly, though quite decisively, with aniline blue, and are very fine, frequently by no means easy to see; they become a more prominent feature in later stages.

Stage 4 (Text-figs. 7, 9D; Pl. 2, fig. 5)

The principal change in this stage is the regression of the tongue, and this is accompanied by a further increase in the elevation of the papilla above the general surface of the conjunctiva. I have found papillae of stage 4 in 8- and 9-day embryos, and one in a 7-day embryo.



Text-fig. 7. Stage 4, 8 days. The papilla, a few cells of the mesenchyme condensation; and the upper part of the descending collagen strand. The tongue is sinking into the mass of liquefying cells at its base. Azan. Camera lucida.

The disappearance of the tongue is a consequence of the continued degeneration of its cells and of cells forming its foundation in the central part of the papilla. This degeneration, which begins in the central mass at the base of the tongue, spreads into the substance of the tongue itself, so that soon the whole central part of the papilla, except its upper and lower limiting layers, is in active degeneration, and it becomes a spongy mass riddled with large vacuoles (Text-fig. 7). Into this the tongue, reduced by the progressive degeneration and liquefaction of its cells, subsides. The degeneration of the cells which form most of its bulk causes the aspect of the papilla facing the mesenchyme to become more and more concave, except for the still projecting tongue. Finally, the tongue disappears altogether, and the papilla thus becomes a hollow structure whose cavity opens on to the mesenchyme (Pl. 1, fig. 1). This is the beginning of stage 5.

An attempt to count mitoses in six papillae of this stage showed them to be rare.

Three were found in one papilla, two in another, and in the remainder none, a total of five in six papillae as against nineteen in five at stage 3. As in earlier stages, the few found were situated in the peripheral parts of the papillae.

The mesenchyme condensation is still arranged as described in advanced specimens of stage 3 (Text-fig. 6; Pl. 2, fig. 5), as a sort of column extending from the papilla down to the fibrous continuation of the sclera. In the axial part of this column the stroma is particularly dense and its orientation is vertical to the plane of the conjunctiva; from this region it sprays out on either side to become continuous with the general stroma of the connective tissue, which is orientated parallel to the conjunctiva. The descending strands of collagen fibres, which were first detected in stage 3 running from the tongue into the mesenchyme condensation, are now somewhat more prominent and more numerous, and can be traced further into the mesenchyme. Some of them are shown in Text-fig. 7. They run predominantly in the axial zone of the condensation, but spray outwards from it, losing themselves in the more peripheral parts.

In the most advanced specimens of this stage the papilla stands high above the epidermis, but the tongue is so reduced that it no longer projects beyond the concavity into which it has sunk. The mesenchyme condensation is dense, and its form is beginning to change again, for as it is followed downwards below the papilla it begins to spread or flatten outwards. The collagen strands from the tongue run through it spraying out among its cells, but I suspect that, in addition to this, tiny sprigs of collagen have here and there been formed in the mesenchyme as the very first indication of the approaching formation of bone.

Stage 5 (Text-figs. 8, 9E; Pl. 1, fig. 1, Pl. 2, fig. 6)

Papillae in stage 5 were found in 8- and 9-day embryos. Pl. 1, fig. 1 and Pl. 2, fig. 6* show a papilla rather early in this stage. The tongue has disappeared completely, the surviving part of the papilla is elevated higher above the general level of the epidermis than in earlier stages and it is concave below, having a cavity which is widely open to the mesenchyme and is occupied by mesenchyme cells. The cells which originally formed the central mass and the substance of the tongue are greatly reduced in quantity, but some still exist, forming the greater part of the wall of the papilla. Basophil granules and vacuoles show that their degeneration is still going on. From the cavity of the papilla collagen fibres run down and spray out among the cells of the mesenchyme condensation. Later in stage 5 (Text-fig. 8) the form of the papilla changes, becoming more elongate, so as to be still further elevated above the general surface of the conjunctiva, while at the same time it narrows, especially at the base. Its cavity is in its lower half and opens by a small aperture on to the mesenchyme below. The cavity still contains mesenchyme cells, and from it the bundle of collagen fibres runs down into the mesenchyme. The papilla still consists of the surface epithelium of periderm cells, an irregularly cubo-columnar epithelium of basal layer cells lining the cavity, with some degenerate remains of the 'central mass' between the two.

A search for mitoses in twelve papillae showed three in one papilla, one in another, and in the remainder none. The two in which mitoses were found were both young specimens.

* Both these figures show the same section, Pl. 1, fig. 1 being a retouched photograph which is a mirror image of the correctly orientated Pl. 2, fig. 6, an untouched photograph.

In the earlier specimens of this stage the mesenchyme condensation has the vertical orientation and columnar form described in stage 4, but later this changes. The condensation begins to spread outwards, flattening itself above the fibrous sclera. In other words, the columnar condensation of stage 4 becomes disc-like again. This

Text-fig. 8. Stage 5, 9 days. Filiform papilla, still with a cavity. The descending collagen strand is very prominent. Notice the degeneration in the mesenchyme near the descending strand, between the papilla and the young bone, which is not shown. Azan. Camera lucida.

flattening process is not completed in stage 5, and even in the more advanced specimens condensed mesenchyme intervenes between the base of the papilla and the mesenchyme disc; this is not the case in stage 6.

Numbers of collagen fibres, in more advanced specimens staining brilliantly with aniline blue, run from the cavity of the papilla down through the core of mesenchyme, spraying out among its cells (Text-fig. 8; Pl. 1, fig. 1). Mitoses occur in the condensed

mesenchyme. The degenerate cells seen in the mesenchyme condensation in earlier stages are now much more numerous, and are found associated with the descending strands of collagen fibres (Text-fig. 8), but are rare or absent in the flattened part of the condensation.

In the flattened part of the condensation of the more advanced specimens very delicate blue-staining collagen fibrils are now appearing between the cells. These are the beginnings of the collagen fibrils of the scleral bones. Their orientation is in harmony with the pre-existing stroma, which does not stain with aniline blue or only stains extremely feebly, and they are probably formed in part by a thickening of its fibrils. They are not found in any part of the condensation except its basal region, precisely in the position to be occupied by the scleral bone. The much thicker and more striking descending collagen strands coming down from the papilla can be traced right through the condensation to this 'bone' (Pl. 1, fig. 1).

Stage 6 (Pl. 2, fig. 4)

In stage 6, which was found in 9- and 10-day embryos, the final regression of the papillae commences and the developing bone becomes firmly established.

The first change in the papillae is the reduction of the cavity to a narrow canal and then its final disappearance, so that each papilla becomes a solid structure arising from the slightly thickened conjunctiva. The layer of cubo-columnar cells which lined the cavity may for a time persist as a core of nuclei in the axis of the papilla, but their arrangement becomes irregular and soon they can no longer be recognized. The collagen fibres which lay in the cavity can still be found after it has disappeared, penetrating into the base of the papilla (Pl. 2, fig. 4), and running between its cells for varying distances, sometimes through about half the length of the papilla. It frequently ends in a little knot of collagen, in about the position which must earlier have been occupied by the end of the cavity. In younger specimens in which it is still possible to recognize the remains of the cubo-columnar lining of the cavity, the papilla retains its earlier structure, viz. a superficial epithelium covering a mass of degenerating cells between it and the remains of the cubo-columnar layer. Later, the degenerating material seems to disappear, and in more advanced specimens (Pl. 2, fig. 4) the papilla is reduced to a rather flattened filiform structure, two or three cells thick near the base and only one cell thick near the apex, with few or no obvious signs of degeneration such as vacuoles or basophil granules. It seems to consist wholly of periderm cells, all the cells derived from the basal layer having degenerated and disappeared. In length the papillae vary. Some are tiny structures consisting of only a few cells, like that shown in Pl. 2, fig. 4, but in another the length was 135 μ, its width near the base being 76 μ and near the apex 55 μ, so that it has a rather ribbon-like form. The papilla often ends distally in a little vesicle. Degenerative changes can still be found in the area of thickened epidermis from which the papilla proper arises; indeed, it has not hitherto been noticeable in the neighbouring thickened epidermis. Mitoses do not occur in the papilla at this stage, but they do occur in the unmodified epidermis of the conjunctiva.

In the younger specimens of stage 6, although most of the mesenchyme condensation is now flattened out in the position of the future bone and is beginning to ossify, a small cone of condensation still extends upwards to the base of the papilla. In this region the stroma is vertically orientated and the descending strands of collagen fibres

pass downwards in harmony with it; but when the condensation is fully flattened out and becoming ossified, the stroma of the connective tissue between the epidermis and the developing bone is orientated in planes parallel to the epidermis and the descending collagen strands cross it disharmoniously, without reference to its orientation, as shown in Pl. 2, fig. 4.

The descending collagen strand itself emerges from the base of the papilla usually as a single thick fibre which runs downwards, fraying out as it goes into smaller fibres and fibrils, which continue to diverge and branch as they approach the developing bone, with whose fibres they can be seen to be continuous. In the mesenchyme around the descending strand there are usually found, especially in the younger specimens, a number of degenerating mesenchyme cells, but these are less numerous in such advanced specimens as that shown in Pl. 2, fig. 4.

The cells of the bone rudiment itself are at first mesenchymatous in character, with very delicate collagen fibrils between them, but as the fibrils multiply and thicken the cells take on the character of osteoblasts, increasing the quantity of their cytoplasm and becoming irregularly rounded or polyhedral. In the more advanced specimens (Pl. 2, fig. 4) the bone fibres have so increased in number and staining power that a coherent sheet of bone is present, in whose denser parts it becomes difficult to distinguish individual fibres. On both surfaces it is thickly covered with osteoblasts, between which run the delicate new collagen fibres which are being added to the matrix. A few bone cells are already imprisoned in the developing bone, becoming osteocytes. At its end remote from the pupil the bone anlage just overlaps the edge of the scleral cartilage (Text-fig. 1), and here fades out into a layer of connective tissue which extends peripherally just external to the perichondrium of the cartilage. Later this layer of the connective tissue is added to the perichondrium. At its central end (towards the pupil) the bone fades into a slight condensation of mesenchyme into which fine collagen fibres extend from it; this is evidently merely the central end of the original mesenchyme condensation, not yet ossified. The bone has not yet any clearly defined periosteum; on its surface towards the conjunctiva a zone of the mesenchyme is flattened against it, and this is evidently the beginning of the fibrous component of the periosteum. On its opposite surface the same is true, but the bone lies so close against the fibrous sclera that it is not easy to say whether the periosteum should be distinguished from it or not. Later, no such distinction is possible.

Later stages

In an eye of a 12-day embryo only four papillae were found, the remainder had disappeared. A descending collagen strand could be found with certainty in only one of these cases, and in this it could not be traced to the bone. In two of the other cases scraps of collagen were present embedded in the epidermis at the base of the papilla, and a few collagen threads in the mesenchyme were perhaps remains of the descending collagen strands.

The bone is as described in stage 6, but a little further advanced. Some osteoblasts have been included as osteocytes. Since the bone stains with aniline blue and not at all with azo-carmine it is apparently still uncalcified.

At 17 and 19 days no papillae were seen. The bones in these later stages were very much thicker, being about 25 μ at the thickest part by comparison with less than 10 μ at 12 days. Each bone, which is calcified, appears to be a simple plate without cavities

or eminences. With both Azan and haematoxylin and eosin the bone substance appears homogeneous in texture and no attempt was made to study its fibrillar structure; it is not lamellar. Osteoblasts are present on both outer and inner surfaces, and there is an accumulation covering the central edge (near the cornea), and to a less extent the radial edges; on the other hand, at their peripheral ends (i.e. remote from the pupil), the bone ends in connective tissue without osteoblasts. It is evident from this that growth is in thickness and in surface area, and the growth in area is centripetal but not centrifugal. The growth in thickness seems to be more rapid on the lower surface (that towards the retina) than on the upper, for on the upper surface the osteoblasts are more flattened and the surface of the bone smoother, while the lower surface is made irregular by the bays in which lie osteoblasts fixed in the process of becoming osteocytes.

The bones lie between two layers of fibrous periosteum which are common to them all; there is no fibrous periosteum between two bones, and where they overlap one another there is only a loose and delicate connective tissue between the two sets of osteoblasts. On the upper surface the fibrous periosteum continues into the outer layers of the perichondrium, and that on the lower surface is identical with the fibrous sclera.

DISCUSSION

(1) *The work of Nussbaum and Dabelow*

The earlier and more complete of the two antecedent descriptions of the development of the papillae is Nussbaum's. It is difficult to understand how Nussbaum arrived at the views he expresses. With his description of the early condition, when the papillae are flat thickenings (my stage 1), I am in accord, but thereafter, according to him, the papillae develop in two different ways according to whether or not the underlying mesenchyme takes part. Generally, he says, it plays no part, and in these cases the solid epidermal papilla projects both above and below the epidermis. This accords well with my stages 3 and 4. Nussbaum described in detail the degenerative changes in the papilla, but he thought that the degeneration led to the disappearance of all the cells except those lining the tongue against the mesenchyme, and that the result was the formation of a deep crater full of degenerating cells. These are cast off, leaving a flat pit or pocket, lined by epithelium, sinking from the epidermis down into the mesenchyme, and having a lumen which opens on to the surface. I have never seen anything resembling this. In Nussbaum's figures of this stage (his Figs. 12 and 13) the mesenchyme is omitted and reference has to be made to the description of figures to find out which side of the epithelium is supposed to be towards the mesenchyme and which away from it. It is perhaps significant that if the correct orientation of the figures were the reverse of that stated by Nussbaum, they would satisfactorily represent papillae in my stage 5. The second mode of development described by Nussbaum, in which the connective tissue does play a part, he believes to be less common. Short cylinders are formed which extend above the epidermis like feather germs, and they have a cavity containing connective tissue cells. He figures only one papilla in this stage (his Fig. 10); it and the description agree well with my stage 5.

In my material I find no evidence that there are alternative modes of development or that Nussbaum's hollow pockets opening on to the surface exist at all. I have made drawings of over fifty papillae and have studied more, and all that I have

examined fit satisfactorily into the single developmental story outlined in the descriptive section. All Nussbaum's and Dabelow's figures fit too, except Nussbaum's Figs. 12 and 13, which would be more convincing if the mesenchyme were shown.

(2) *Epidermal thickenings and mesenchyme condensations*

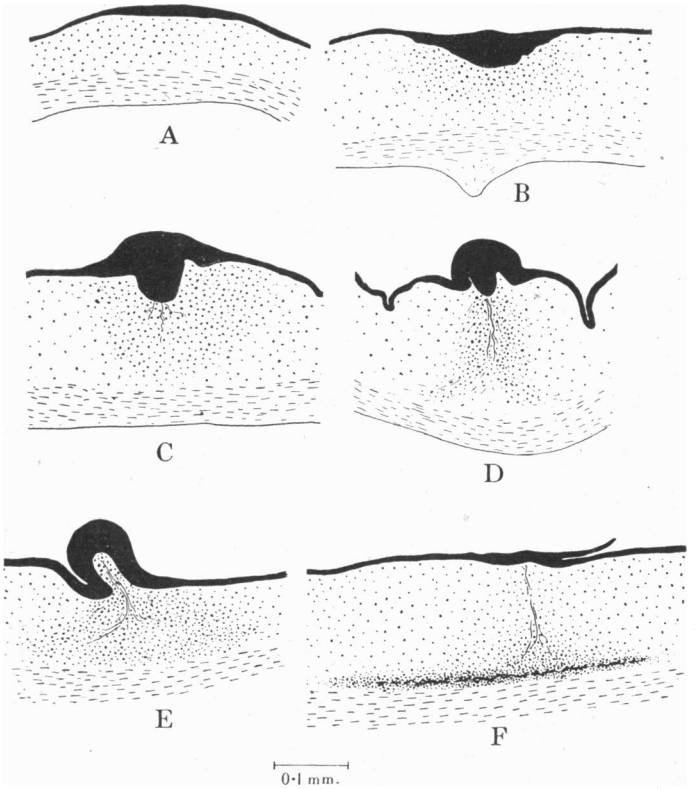
The more interesting observations described in the present paper are: (1) the recognition of the osteogenetic cells first as a mesenchyme condensation, not at the site of their subsequent differentiation, but separated from it by a short distance and associated with a transient epidermal thickening in whose development and fate cellular degeneration plays an important part; (2) the presence of the 'descending strands' of collagen fibres, whose development increases as the cells move towards the site of their differentiation; and (3) the occurrence of degeneration among the osteogenetic cells particularly during this movement. It is interesting to notice similar events in other structures.

In the developing mandible of the chick Jacobson & Fell (1941) found that the osteogenetic cells, which will later form the dermal bones, originate in mesenchymal proliferation centres which lie below two thickenings of the epidermis. Although these thickenings are much smaller and less striking structures than the scleral papillae, the resemblance is sufficient to hint at a probable underlying similarity in the physiology of development. Another instance is probably the epidermal thickening found at the apices of limb-buds in many vertebrate embryos; in these the occurrence of cellular degeneration (Glücksmann, 1934) accentuates the resemblance to the scleral papillae. A third is the association, described by Pehrson (1922), between early stages in the development of certain dermal bones in fish and neuromast organs. Moy-Thomas (1941) found that the dermal bones develop normally even when the formation of neuromast organs is prevented, but Westoll (1941) has suggested that the experiments should be repeated on more primitive forms. The nearly normal development of isolated proximal fragments of limb-buds of 3- and 4-day chicks, which necessarily lack the thickened epidermal cap because this is at the distal end of the limb-bud, similarly seem to deny any significance to the association. Nevertheless, it is difficult to believe that a resemblance between such diverse animals and structures is wholly accidental.

(3) *Cell degeneration and collagen fibres*

Since the works of Glücksmann (1930, 1934), of Fell (1939) and of Jacobson & Fell (1941), there can be no doubt of the importance of cell degeneration as an event in morphogenetic processes. The degeneration here described in the scleral papillae resembles those described by Glücksmann in the epidermal thickening at the tips of limb-buds and by Fell in the mid-ventral epidermal ridge in birds. Degenerative changes in the mesenchyme were reported by Jacobson & Fell in the mandible and by Fell in the avian sternum, in both cases associated with cell migrations. In the present work the degenerating mesenchyme cells were found around the descending strand of collagen fibres, where the mesenchyme migrates downwards to its definitive position, but they are found rarely if at all in early stages before there has been any migration or in the final position where migration has ceased. The collagen strands first appear in stage 3 but become prominent only in stage 4, when the downward migration of the mesenchyme begins, and reach their maximum development in stage 5. Thus there seems to be a connexion between cell degeneration, the formation of strands

of collagen fibres, and the migration of cells. Very similar collagen fibres were described by Fell in her work on the sternum, again in association with migration.



Text-fig. 9. Diagrams summarizing the history of the papillae and of the mesenchyme condensations; all the diagrams are based on actual sections. A, stage 1; B, stage 2; C, stage 3; D, stage 4; E, stage 5; F, stage 6.

SUMMARY

1. The epidermal papillae on the conjunctiva of the embryonic chick are present at 7 days' incubation as flat epidermal thickenings. In 7- and 8-day embryos the central part of each thickening has increased greatly and projects downwards into the mesenchyme as a conical mass (the 'tongue'). The papilla also projects slightly above the general level of the epidermis. Degeneration soon sets in among the epithelial cells of the papilla, especially in and at the base of the tongue, and this part of the papilla liquefies. As a result, the tongue retracts and disappears, while at the same time the elevation of the papilla above the general level of the epidermis increases, and it changes from a solid mass projecting principally downwards into the mesenchyme, into a hollow, more or less filiform structure, projecting upwards from the epidermis. Its cavity is open to the mesenchyme and contains mesenchyme cells. Finally, the papillae lose their cavities and dwindle away; by 12 days most have disappeared.

2. The rudiment of the bone is first recognizable as a diffuse condensation of mesenchyme cells below and around the tongue, separated by a short distance from the site

at which it later differentiates into bone. At about the time when the tongue of the papilla is regressing, the cells of the condensation begin to move downwards, and finally flatten out in the position of the future bone. During the migration collagen fibres appear running from the basement membrane of the papilla, especially in the tongue, among the mesenchyme cells. These fibres are called 'descending collagen strands'. Associated with them are numbers of degenerating mesenchyme cells. After the disappearance of the tongue, when the mesenchymal precursor of the bone has arranged itself as a flat sheet, its cells transform themselves into osteoblasts and the development among them of matted collagen fibrils is the beginning of the bone matrix. The descending collagen strands now run from the papilla to become continuous with the developing collagen of the bone. Degeneration among the mesenchyme cells ceases.

3. In later stages the bone grows in thickness and in surface area. Growth in thickness is principally on the lower surface, growth in area at all the edges except that remote from the pupil; the bone thus grows centripetally but not centrifugally. The whole series of bones is contained between a single upper and a single lower fibrous periosteum. The upper periosteum is continuous with the upper perichondrium of the scleral cartilage, while on the lower side the fibrous periosteum is identical with the fibrous continuation of the sclera towards the pupil. At the end towards the cornea, both layers of periosteum become merged with its fibrous systems. Where the bones overlap one another the two osteoblast layers are separated only by a delicate fibrillar sheath.

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EXPLANATION OF PLATES 1 AND 2

Plate 1

Fig. 1. A papilla and underlying mesenchyme early in stage 5, 9 days. The tongue has disappeared and the papilla contains a cavity which is open to the mesenchyme below and contains mesenchyme cells. The descending strands of collagen fibres run from the cavity of the papilla down into the mesenchyme, and towards the bottom right-hand corner of the figure join the delicate fibrils of very early bone. The figure is a retouched photograph. Azan.

Fig. 2. Stage 2, 8 days. A photograph showing the diffuse mesenchyme condensation beneath the papilla, and beneath this again the fibrous sclera. Azan. × 150.

Fig. 3. Stage 3, 8 days. The mesenchyme condensation is more pronounced than in fig. 2. The tongue is more strongly developed. Azan. × 150.

Plate 2

Fig. 4. Stage 6, 10 days. The papilla has lost its cavity. The formation of bone is advanced and the descending collagen strand is traceable from the papilla into the collagen of the bone. The figure was drawn, with a camera lucida, from two adjacent sections. Azan.

Fig. 5. Stage 4, 8 days. The tongue is beginning to regress and the mesenchyme condensation is arranged like a broad column beneath the papilla. The papilla is shown in detail in Text-fig. 7. Azan. × 150.

Fig. 6. Stage 5, 9 days. The mesenchyme condensation is flattening out against the fibrous sclera. The papilla is shown in greater detail in Pl. 1, fig. 1. Azan. × 150.

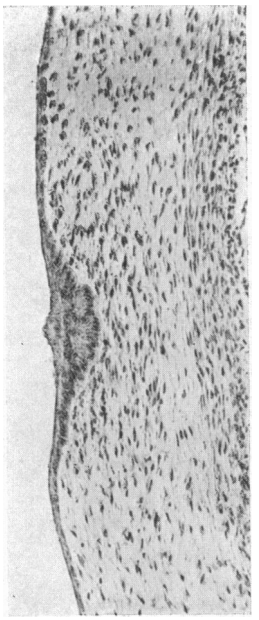

Fig. 2

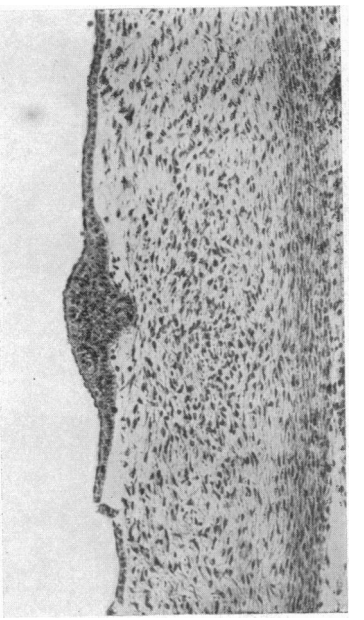

Fig. 3

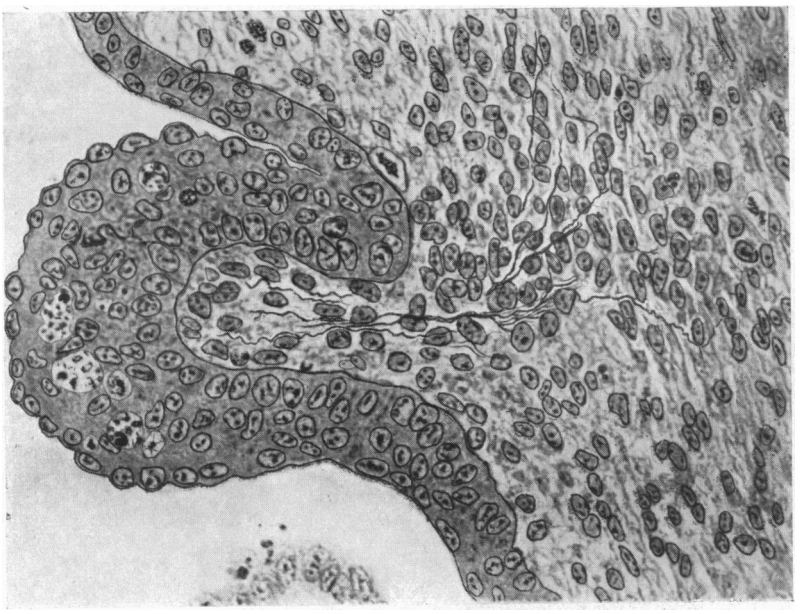

Fig. 1

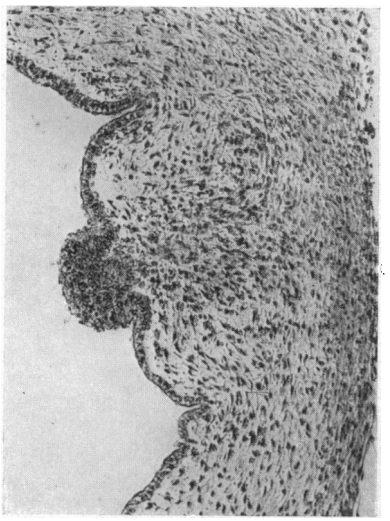

Fig. 5

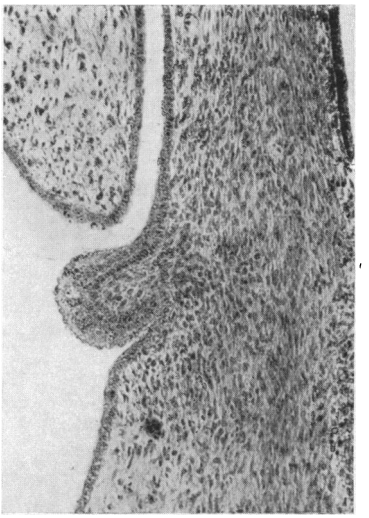

Fig. 6

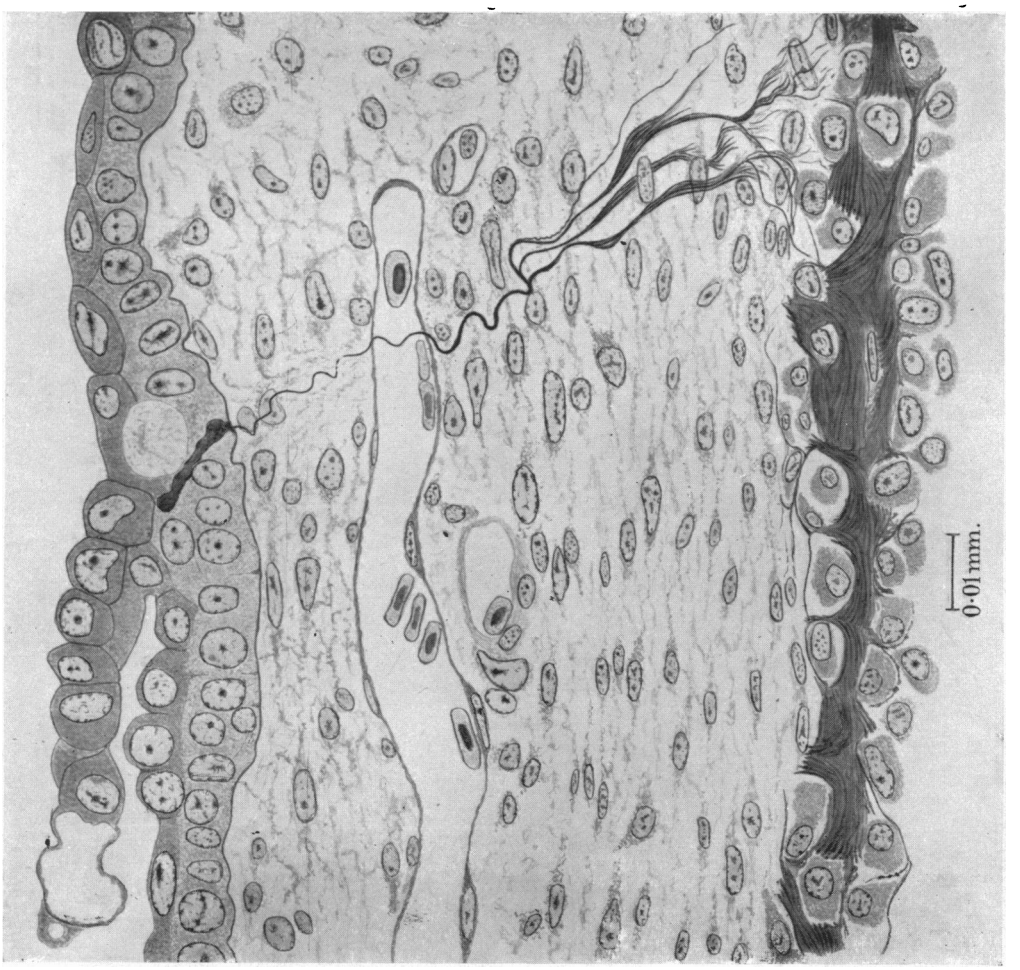

Fig. 4