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The Yolk-Sac and Allantoic Placenta in Perameles

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The Yolk-Sac and Allantoic Placenta in Perameles.[1]

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With Plates 9–11 and 4 Text-figures.

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[1] The present communication was awarded the University Medal when presented to the University of Sydney as a thesis for the degree of Doctor of Science. The absolute necessity, in the present time, of reducing publication costs to the minimum, has resulted in the omission of some of the original figures, these being reduced from fifty-two to the present limit.

1. Introduction.

It is proposed in the present communication to initiate the publication of a series of papers dealing with marsupial embryology. The facts and interpretations embodied will be based on result of an investigation into an amount of marsupial material now collected in the Biological Laboratory of the University of Tasmania and fairly representative of the Tasmanian fauna.

It is evident that any attempt to solve the phylogenetic problems presented by the mammalian group without having at our disposal a large number of the facts relating to the morphology and embryology of the Metatheria will be extremely unsatisfactory.

Yet in the not very distant future, with the spread of settlement and the almost incredible devastation caused among these animals by fur-hunters and trappers—a depletion but little compensated for by restrictive legislation—it may be considered certain that the connected stages necessary for the elucidation of their intra-uterine development will be practically unobtainable.

In this connexion it may be instructive to quote the words of Hubrecht (1909), who says, in discussing placental arrangements in the Marsupialia, that 'the early ontogenetic events and the different phases in the mutual relation of the blastocyst and mucosa ought to be fully known in order to furnish us with all the data that can be brought to bear on this important question. And it is to be fervently hoped that those genera that are very rapidly diminishing in their native land, some of them even on the verge of disappearance, may yet be fully investigated before they have been exterminated, and have thereby become as mute on this important point as are their fossil predecessors.'

Of such disappearing genera two may be mentioned, Thylacinus and Sarcophilus, both certainly primitive in the morphology of their genital organs, and for that reason alone possessing important possibilities with regard to their intra-uterine nutritional arrangements. Both genera are absolutely on the verge of extinction. The intra-uterine development of Thylacinus will probably never be known, and it is extremely unlikely that detailed investigation will be possible in the case of Sarcophilus.

Under these circumstances it is a pleasure to acknowledge gratefully any help extended towards an investigation of the ontogenetic and phylogenetic relationships of this fast disappearing fauna.

First, in this way, I must express my most grateful thanks to the Trustees of the John Ralston Bequest and particularly to their chairman, Dr. L. G. Thompson, these gentlemen having placed at the disposal of the University of Tasmania a sum of money to be used chiefly in marsupial investigation. It is mainly by their financial aid and unswerving support that the important collection of embryological material in the University of Tasmania has been brought together.

Secondly, I am indebted to the Committee of the British Association for the Advancement of Science, who, at their Australasian meeting in 1914, placed at my disposal a grant with the object of procuring material for the study of the brain and embryology of marsupials.

The preliminary portion of the examination of the material of the present paper was carried on in my own laboratory, but lack of facilities for consulting almost all the literature, and other disadvantages of isolation in Tasmania, led me to consult my old friend Professor S. J. Johnstone as to the possibility of accommodation in one of the laboratories of the University of Sydney.

This was arranged and the work was carried on under these altered conditions.

Still more recently, by the courtesy of Professors Hill and Watson, I have been accommodated at University College, London. I am indebted to the Senate of the University of Sydney and to the Research Committee of that University for a grant from the McCaughey Bequest to defray the cost of the plates illustrating this paper.

Finally, I must not omit to pay a tribute to the friendly interest which has been shown in my work by my former teacher, Professor W. A. Haswell, who has been always ready to help with kindly criticism and advice. To him, also, I am indebted for the loan of literature otherwise inaccessible to me.

2. Review and Criticism of Previous Work.

The discovery by Hill of a true allantoic placenta in the two forms, Perameles obesula and nasuta, was of the greatest importance in regard to the phylogeny of the marsupial group and its relationship to the remainder of the mammalia. The actual placental material, upon which Hill was able to base his conclusions, consisted of three stages. The first of these, Stage C, concerned an embryo 7 mm. long. This was followed by a Stage D describing the placental connexion in embryos 8 to 8·75 mm. long. In a subsequent paper Hill described the similar phenomenon in a 12·5 mm. embryo which was also designated D. For the sake of clearness, when speaking of these stages, I will designate them by their length in each case.

In his earliest (7 mm.) stage the fixation of the embryo was already completed and the allantoic placenta was well on the way towards being fully established.

From an examination of these three stages Professor Hill showed that the epithelium of the uterus becomes converted into a vascular syncytium, the nuclei of which arrange themselves in groups in the lower portion of this layer. 'At the same time maternal capillaries pass up between the syncytial lobules, penetrate the syncytial protoplasm, and form a network on and just beneath the surface' (1897, p. 387).

The fixation of the embryo is brought about in the usual way by means of the chorion. The ectodermal portion of this membrane, by which actual approximation of the foetal membranes to the uterine wall is first achieved, consists of a single layer of large cells by means of which the attachment is brought about.

With the somatic mesothelial layer of the chorion, the splanchnic mesoderm of the allantois fuses, and the vessels ramifying on the surface of the allantois thus come to lie immediately below the layer of true chorion. Up to this point the development of the placenta may be regarded as being fairly typical, but, from now on, the development of this organ, according to Hill's account, results in the appearance of such structural peculiarities and modifications as to give rise to the general impression that the placentation of Perameles, at any rate as concerns its more intimate development, is without parallel in the whole mammalian group.

The allantoic placenta is completed 'by the gradual degeneration and resorption of the enlarged chorionic ectoderm cells over the placental area proper. These cells thus take no further share in placental formation.' The result of this is the close apposition of maternal and foetal blood-vessels, the two bloodstreams being 'now only separated by their thin endothelial walls and perhaps a thin layer of syncytial protoplasm' (p. 388).

A yolk-placenta is present, formed by the close apposition of the vascular area of the yolk-sac to the highly vascular uterine syncytium outside the allantoic placental area.

It is not necessary at this stage to enter into any discussion as to the significance of the presence of an allantoic placenta in Perameles other than to indicate that it has been definitely accepted by most embryologists, that there is now no reason to doubt the common origin of Metatheria and Eutheria from a primitive placental stock. Hill says it is 'exceedingly improbable that an allantoic placenta should have been twice independently acquired and in such a fundamentally similar manner within the limits of the mammalian class' (p. 433).

Now, outside the intrinsic interest of the presence of an allantoic placenta in a member of a group formerly regarded as aplacental, it would be expected, on a priori grounds, that the occurrence would be of importance in elucidating the phylogeny of the mammalian placenta or, at least, in giving us some means of arriving at a definite idea of the method of placental formation in the original protoplacental group.

It need hardly be said that in these respects the placentation of this animal, as interpreted by Hill, is extremely disappointing.

The combination of a persistent uterine syncytium with a degenerating chorionic ectoderm is without parallel in the Eutheria, and any attempt at a comparison of the placentation of the two groups results in a deadlock.

Although Hill's results have been accepted by many, nevertheless Hubrecht (1909) ventures to question whether, morphologically, the placenta of Perameles will not prove on further investigation to be more comparable with some one or other of the various placental styles found in the Eutheria. And that there is some foundation for Hubrecht's opinion is undoubtedly apparent from an examination of Hill's figures alone.

Before passing to a consideration of this question it will perhaps be necessary to have before us a short account of some special details of the placentation of this marsupial as described by Professor Hill.

His most important and peculiar point concerns the history of the foetal ectoderm. The portion of this which is concerned in the fixation of the embryo is said to disappear almost completely, being represented in the 12·5 mm. stage by a few scattered cells in the original ectodermal position. The disappearance is stated to be of the nature of a degeneration, this, apparently, not being inaugurated until the allantoic attachment has taken place and the allanto-chorionic fusion completed. In the earliest placental stage (Stage C, 7 mm.) the allantoic placenta is already on the way towards full establishment, the allantoic vesicle being already attached to the chorion by its placental face. Excellent figures are given of this stage (1897, Pls. xxix and xxx, figs. 5 to 12).

At a later stage (D, embryos 8–8·75 mm.) the ectoderm has, apparently, almost completely disappeared. In the description of Stage C, it is stated that the cells towards the central portion of the foetal ectoderm are 'of a very varying size and shape, and, in places, through the disappearance of the outlines between adjacent cells, *large multinucleate cells* have been formed. . . . In many of the ectoderm cells shown in fig. 9 the nuclei are also seen to be in various states of disintegration. Many of them stain only slightly; the nuclear membrane is becoming indistinct, while the chromatin is found broken up and diffused in the form of small granules throughout the delicate nuclear reticulum. Eventually the position of the nucleus is only marked by a few straggling irregularly thickened remnants, which finally become diffused through the protoplasm and lost to view' (p. 404). [Italics mine.]

In this stage (embryos 8–8·75 mm.) the ectoderm is represented centrally, 'only by more or less isolated degenerating cells' (p. 413).

In the 12·5 mm. embryo Hill states that 'over the placental area usually single, much degenerated, and deeply-staining chorionic ectoderm cells are still to be found' (1899, p. 9).

From the above it appears that one of the causes of the disappearance of the ectoderm is the loss of the chromatic constituents of the nucleus, these being absorbed into the surrounding cytoplasm.

As to the other possible reasons for the presumed degeneration and disappearance of this ectodermal layer, Hill states further that, in some cases, 'the inner ends of the cells are greatly vacuolated, a fact which suggests that a process of vacuolation may also play a rôle in the retrogression of the chorionic ectoderm' (1897, p. 404).

Further, referring again to this layer, he is 'inclined to believe that the allantoic capillaries, so closely related to its inner surface, are by no means the least active agents in effecting its removal. *Of direct fusion of the degenerate ectoderm with the underlying syncytium there can be no question. All the facts negative such a view*' (1897, p. 404). [Italics mine.]

From the above it appears that Hill is of the opinion that the layer of chorionic ectoderm disappears, and that the following processes mutually assist in causing this removal:

(*a*) Degeneration in situ with or without vacuolation.

(*b*) Removal by allantoic capillaries with or without previous degeneration.

Thus it is definite enough, according to Hill's views, that the chorionic ectoderm takes no share in the formation of the placenta proper.

The completion of the placenta is brought about by the allantoic capillaries coming into intimate relationship with the maternal capillaries ramifying on the surface of the maternal syncytium.

The above is, I am inclined to think, an accurate précis of Hill's results. His interpretation of the facts and his strongly-expressed opinion that there is nothing of the nature of a fusion between the foetal ectoderm and maternal tissue has given rise to the idea—almost generally accepted—that the placentation of Perameles is of 'a peculiar type not met with anywhere else' (Jenkinson, 1913, p. 216).

The correctness of Hill's conclusions has been questioned by A. A. W. Hubrecht in his famous essay on 'The Early Ontogenetic Phenomena in Mammals' (Hubrecht, 1909).

Hubrecht's criticism:—

The opinion of this gifted investigator was arrived at evidently on somewhat theoretical grounds, but he was confirmed in his ideas by an examination of material placed at his disposal by Professor Hill. It is important to understand Hubrecht's standpoint thoroughly, because, in my opinion, it is to some extent justified. He believes that the foetal ectoderm, so far from disappearing, penetrates into the maternal syncytium to form a 'mixed syncytium', corresponding to what Schoenfeld has described (1903) for the dog. 'The Perameles placenta may be said to be a somewhat simpler—because thinner—form of placenta than that of the Carnivora, but at the same time to approach more closely to that type; whereas amongst the Insectivora, Sorex provides us with an example of a yet more extensive proliferation of the material uterine epithelium before the allantoic attachment of the blastocyst comes about than even Perameles. At all events, the placentation of Perameles, characterized by so intimate a fusion between foetal and maternal elements, should never be classified amongst those forms of placenta which are either primarily primitive (as yet unknown to us) or secondarily simplified (Ungulates, Lemurs, Cetacea, &c.)' (1909, p. 117).

With similar material before each of them hardly could two authors come to more different conclusions. Whereas Hill is of the opinion that there is no interfusion of maternal and foetal tissues other than that caused by the intergrowth of allantoic tissue—which is comparatively insignificant—Hubrecht is just as emphatic that an invasion of the maternal syncytium by the foetal chorion does occur and that therefore the type of placentation in Perameles corresponds closely with that of some of the Eutheria, notably the Carnivora.

Interpretation of Professor Hill's Figures:—

Without as yet adducing any evidence from my own material, I may be permitted to say that from the evidence of Hill's figures alone there appears to be a considerable defence for Hubrecht's standpoint in this matter.

According to Hill's account (1897, 1899) the histology of the placental area is extremely simple. With the exception of the syncytial nuclei, endothelial nuclei, and leucocytes, the only elements of this region are the cells of the foetal chorion, which in no case enter into the constitution of the placental thickening but degenerate and disappear.

With this interpretation before us it will be possible to proceed to examine his figures, particularly those of Pls. xxix and xxx, figs. 7, 8, and 9 (1897), representing portions of the placental area with the chorionic ectoderm attached. These figures are all representative of Stage C (7 mm.).

As Hill has shown, there is a marked difference in the condition of the chorionic layer dependent more or less on its distance from the centre of the fixed area. In general terms it may be stated that the farther from the centre of the attached area the less alteration is evident.

Examining Pl. xxix, fig. 7, representing the edges of the placental area of Stage C, it will be seen that the ectoderm is almost intact throughout. At certain localized points, however, the ectodermal cells are deeper than the average. Some cells have become multinucleate and the nuclei are in many cases situated basally, such cells being deepened considerably compared with their original condition. Below the ectoderm the syncytial nuclei are arranged in their lobules. These nuclei are rounded, and although membranate the chromatic contents are spare. Each nucleus contains usually a well-defined nucleolus. Examination of the syncytial nuclei of the extra-placental area shows that the suppression of the chromatin contents and the presence of a single rounded nucleolus is a general characteristic. Even with iron-haematoxylin staining only a very faint network can be made out. It is quite otherwise with the nuclei of the cells of the ectoderm layer. A reference to Hill's Pl. xxx, fig. 9, will serve to show that the nuclei of these cells contain quite a well-developed chromatin network with a number of karyosomatic aggregations, even as many as half a dozen in some cases. Further, these nuclei are seldom spherical, but irregularly elliptical, ovalish, or lenticular, and generally of large size.

The difference in shape and histological characteristics between these and the syncytial nuclei is most marked, and in my preparations they can immediately be distinguished from one another.

Bearing in mind, then, the difference between the rounded, bead-like, maternal syncytial nuclei—'typical resting nuclei'—now congregated in groups in the syncytial lobules whether within or without the placental area—and the more robust layer of irregularly-shaped nuclei of the foetal ectoderm, each with its network of easily-stained chromatin, it will be possible to follow out their migration and rearrangement in a general way.

In Hill's Pl. xxix, fig. 7, which represents a marginal portion of the placental area with the chorion attached (embryo 7 mm.), the foetal ectoderm (*ch.ect.*) is apparently complete and unbroken, while on the lower side of the figure are the syncytial lobules containing groups of syncytial nuclei. Between these two sets of elements are to be seen a number of

nuclei of the origin of which nothing is said by Hill. Apparently he leaves it to be inferred that they are of the nature of syncytial nuclei which have not yet reached the lobules.

In my opinion it is quite definitely indicated that these nuclei originate from the foetal ectoderm through its active proliferation, and such centres of proliferation are to be seen in this figure. It needs, I think, no other evidence than that of Hill's fig. 7 to show conclusively that such a process of proliferation is in progress.

Any possible doubt, however, must be dispelled by an examination of figs. 8 and 9 of Pl. xxx of the central portion of the placental area. These are drawn at a greater magnification than fig. 7, and show the features I have indicated above with more certainty. I cannot see that any other conception than the one I have suggested can be possible. Particularly is this evident in the case of fig. 9. Here a most active proliferation and migration of the chromatically rich trophoblastic nuclei is quite apparent. They have so far advanced as to invade the syncytial lobules. In the latter position the original maternal epithelial nuclei are being overwhelmed by the advancing ranks of foetal nuclei. In some cases but one or two trophoblastic nuclei have entered the syncytial nests; in extreme cases maternal syncytial nuclei appear to be entirely absent, their place being taken by the newly-arrived, evidently phagocytic, foetal nuclei. One result of this is that the original chorionic ectoderm is now no longer a perfectly discrete layer. Another is the inclusion of the maternal capillaries by the cytoplasm of the advancing trophoblast and their consequent approach towards the stratum of allantoic capillaries.

The above statements being granted, it will be easy enough to apply the new interpretation to the remainder of Hill's stages and figures. To the further consideration of this I will return in the descriptive portion of the present paper.

Gland Alteration.—There is still, however, one point to which I would like to draw attention at this stage. It concerns a drawing of a gland in Pl. xxxi, fig. 18, one of Hill's many figures in which a gland is depicted. In the whole of the paper, no mention is made of any gland alteration, nor would any such be likely to occur under Hill's conception of a degenerating chorion with a passive syncytium—yet distinct traces of such alteration is evident in this figure. It will be seen that up to the point where the gland enters the presumable syncytium its epithelium consists of the somewhat low cubical cells so characteristic of many of the glands of this stage. From this point to the opening of the gland, however, there is abundant evidence of degeneration. Apparently this consists of a syncytialization similar to—though not as marked as—that occurring in the Carnivora. It was the evidence of this phenomenon in my own material which first drew my attention to the possibility of the occurrence of a more complex process in Perameles than was described by Hill, and in itself lends sufficient colour to the view I have expressed above that there is something more to be reckoned with in the placentation of this animal than a simple degeneration of the foetal ectoderm.

Accepting the fact that the trophoblast of the placental area in Perameles proliferates and that the uterine epithelium after a preliminary preplacental extension remains afterwards passive, then we can come to the conclusion that placental phenomena in Perameles can now be brought more or less into line with similar phenomena in the Eutherian mammals. Here, further, I may be allowed to state that I will be able to show by the aid of my own material that the chorionic ectoderm after attachment proceeds to form by proliferation two structures :

(*a*) a plasmodiblast, plasmoditrophoblast, or plasmodium,

(*b*) a cytoblast or cytrophoblast,

and that Hill failed to recognize the presence of the plasmodiblast nuclei, the structure which he calls the chorionic ectoderm being really only the cytoblastic portion of that layer.

3. Material.

At my disposal for the examination of the foetal membranes of Perameles, I have two intra-uterine stages both of which are important. The younger one, a specimen of Perameles obesula, shows the first fixation of the chorion ; the older one, which belongs to the Tasmanian form, P. gunni, is a stage in which the first attachment of the allantois is in progress.

Both specimens were preserved in Hill's fluid (picro-nitro-aceto-osmic). Sections were stained, sometimes with Ehrlich's haematoxylin, sometimes by the iron-haematoxylin method, counter-stained in each case by means of eosin.

In addition, I have been able, by the courtesy of Messrs. L. Harrison and E. A. Briggs of the Zoological Department of the University of Sydney, to refer to several excellently-preserved sections in the collection of that department and representing some of the material mounted by Dr. Hill of the stages described in his paper. This very important collection is as follows :

Perameles obesula, 7 mm. stage, one microscope slide containing one representative section of the uterine wall, showing the placental and extraplacental areas and the attachment of the allantois.

Perameles obesula, 12·5 mm. stage, one slide with five sections similar to the above.

Perameles nasuta, post-partum stage, one slide with one section.

All the above are stained with haematoxylin and eosin.

Hill's Stage D, representing embryos 8–8·75 mm., is, unfortunately, not represented in the Sydney University collection.

4. Terminology.

The expressions 'omphalopleure', 'vascular omphalopleure', and 'bilaminar omphalopleure' first used by Hill are so convenient and expressive as to need no apology for their continued employment.

The term 'chorion' or 'true chorion' will be used by me in the same sense as by Minot and Hill to indicate that part of the extra-embryonic somatopleure which remains after separation of the amnion.

I will follow the example of most embryologists in using

Hubrecht's term 'trophoblast' for the outer ectodermal layer of the mammalian blastocyst without, however, associating it with the more recent theoretical and hypothetical meaning with which Hubrecht has invested it.

I shall also use Minot's expression 'trophoderm' for that portion of the trophoblast which proliferates and enters into relationship with the maternal epithelium. As will be seen later, this, in Perameles, undergoes changes comparable to those occurring in the Eutheria. Similarly we find a complementary structure, the maternal 'trophospongia', used to indicate (Hubrecht, 1909) 'maternal cell proliferation, specially intended for the fixation of the blastocyst'.

5. Descriptive Account of Material.

Stage 1. Perameles obesula, 6·1 mm.

This is perhaps the most important stage which has yet been examined in the placentation of Perameles. Its investigation definitely shows the fundamental connexion between the placentation of this animal and that of the Eutheria.

The specimen of Perameles obesula, on the examination of which the following account is based, was trapped by myself some miles from a small town in the Tasmanian midlands. It had apparently been dead in the trap for about an hour, but no sign of post-mortem change was to be detected. It was dissected on the spot and both uteri were found to be swollen. Conditions at the time made any dissection of the uteri inadvisable. They were opened slightly and placed in fixing solution (Hill's fluid). Later detailed examination showed that the uterus of the right side was pregnant, but, unfortunately, the delicate foetal membranes had been somewhat damaged. The general condition was that the chorion was already attached to the uterine syncytium over a small area, but that the allantois had not yet come into relation with the conjoint layer so formed.

Pregnant Uterus.—This was found to contain an

embryo of 6·1 mm. direct length, attached to the uterine epithelium by a portion of the true chorion.

In the uterus the allantoic placental area at this stage is distinguishable by the fact that its surface is marked by folds noticeable at once by their depth and distinctness.

To one of these folds the foetal trophoblast is attached.

Sections show that the uterine epithelium has become converted by loss of cell outlines and by proliferation and migration of the nuclei into a syncytium as described by Hill.

I will proceed first to give a description of the maternal structures afterwards passing to those more concerned with the embryo.

Morphology of the Syncytium.—This varies greatly in character according to the locality in the uterus. Over the main wall of the uterus the syncytium is thin, 0·035 mm., while in the region of attachment of the trophoblast it measures as near as can be judged about 0·07 mm.

In the allantoic placental region it is that the complexity of the syncytium has reached its maximum. Here, as Hill has already shown and as happens over the remainder of the uterus to a less degree, the nuclei of the original epithelium have proliferated and migrated to the deeper portion of the layer, which has now markedly thickened. The result is the formation of a syncytium in which the deeply-situated nuclei assume a particular form and arrangement. These nuclei become aggregated mainly in rounded masses or nests situated in lobular projections of the syncytial protoplasm. The lower surface of the syncytium has a wavy appearance due to the presence of these lobules.

The syncytial nuclei at this stage are rounded with a well-defined membrane, a distinct nucleolus, and indefinite chromatin network which, however, is slightly more evident than it is in later stages. Their lack of staining qualities makes them easily distinguishable from the newly-formed trophoblastic nuclei. Careful investigation of the arrangement of the epithelial nuclei in each lobule shows that, when finally at rest, they are more or less definitely arranged round a central cavity. This arrangement is, in many cases, somewhat irregular, as is shown in figs. 1 and 2 (*cav.*), but a glance at the further fig. 3, which depicts quite a common arrangement, will show that in many cases the syncytial nuclei come to form a more or less definite layer round the central space.

The latter becomes filled by infiltrated lymphatic material (fig. 3, *inf.*), which in fact is copiously distributed throughout the syncytium in and between the lobules. At this stage also the syncytium is well vascularized, each capillary being enclosed in its delicate endothelial layer. Endothelial cells and leucocytes are a well-marked feature of the syncytium. They will be referred to in more detail later on.

The syncytium outside the allantoic placental area gradually decreases in thickness. In the region opposite the bilaminar omphalopleure the arrangement of the nuclei is essentially in groups similar to those of the placenta area, yet these aggregations are not so distinctive nor so individual as in that area.

This portion of the uterine epithelium is also extremely well supplied with capillaries, many of which reach the surface. Lymph also finds its way into the uterine lumen through the thin portions of the uterine epithelium between the ill-defined syncytial nests.

Leucocytes are also present in this region but not nearly so plentifully as in the allantoic placental area.

Remainder of the Mucosa.—The main portion of this consists of the much-branched connective-tissue cells, the branches of which are extremely delicate and contain in their meshes abundant lymph material. In the stroma are contained glands and blood-vessels, and around these the connective tissue is condensed to form a thin investing layer.

One feature of this stage is that the glands in the allantoic placental region are narrower and more closely packed than in the remainder of the mucosa. No doubt proliferation of the glands has occurred, their length has increased, and their courses become more tortuous without there being as yet a sufficiently accommodating increase in the thickness of the mucosa. They measure in this region, on the average, ·05 mm. in diameter while outside this region the average width is 0·065 mm. A result of this is that the mucosa of the former region has a more compact appearance than in the latter.

Another feature to which attention should be drawn is the presence of at least one branched gland, a photograph of which is shown in fig. 10 and an outline, obtained by superimposing a number of sections, in Text-fig. 1. This figure by no means represents all the branches of the gland. This, I believe, is the first record of any but simple glands in the marsupial uterus.

TEXT-FIG. 1.

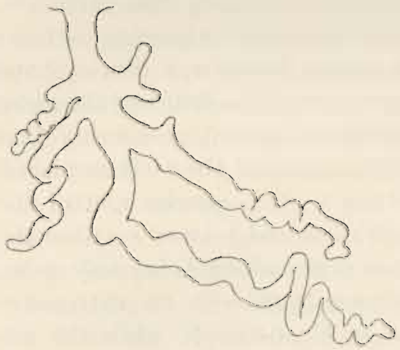


Diagram of a branched gland from the uterine mucosa of Perameles.

In all cases the glands in their lower portions are narrower and more coiled than in the upper, where in general they widen out and retain their epithelium unchanged up to the point of opening into the uterus. This latter characteristic, however, is not shown by glands opening into that portion of the syncytium to which the chorionic ectoderm is attached.

The gland epithelium is of the usual character, consisting of a single layer of cells with peripherally situated dark-staining nuclei. The secretory activity of the glands is most marked, particularly in the allantoic placental area. Migrating through the glandular epithelium, to be added to the secretion, are numbers of leucocytes.

Abundant in the connective tissue of the placental regions are cells containing pigment in the form of black streaky and granular deposits. These cells occur throughout the whole wall of the uterus. They are very abundant in the serosa and are found distributed through the muscularis, the connective tissue, and the glandular epithelium. Such pigmented cells have often been noted in the virginal and pregnant uteri of Eutherian mammals.

Foetal Structures.—In general the arrangement and histology of the foetal membranes are in agreement with the description given by Hill, and I find I can add nothing of importance to his description of these structures.

Allantois.—The vesicular portion of this is a somewhat flattened body, taking, however, a curved shape corresponding to the dorsal curvature of the trunk of the embryo. In surface view it is somewhat elliptical, measuring 5 mm. by 3·1 mm. The point of attachment of the stalk is placed a little nearer the posterior than to the anterior end of the vesicle. The difference in thickness and texture between the placental and coelomic surfaces of the allantois is easily seen with the naked eye. The coelomic surface is an extremely tenuous sheet bearing the larger blood-vessels, while the outer or allantoic surface is more opaque and abundantly supplied with a network of capillaries derived from or supplying vessels which pass round the margin in the manner described by Hill. For a full description of these allantoic vessels I would refer the reader to Hill's account. The allantoic stalk has the usual relations and structure.

Fixation of the Embryo.

The importance of this stage rests on the fact that, over a very small area, the trophoblast is now attached to the thickened maternal syncytium (trophospongia). This portion of the foetal ectoderm is, of course, the outer layer of the chorion, which consists, in addition, of somatic mesoderm. The latter is a thin mesothelial layer consisting of flattened cells with oval, somewhat deeply-staining, nuclei.

The chorionic ectoderm typically consists also of a single cell-layer as it undoubtedly does in the marginal free portions. Over the area of fixation to the uterine syncytium, however, an important and highly significant alteration has been impressed upon the ectoderm by which it becomes converted into a typical trophoderm (Minot) or ectoplacenta (Duval) so characteristic of this layer in the Eutheria.

At certain points in this portion of the trophoblast, cell proliferation takes place. The cells of the original layer divide to give rise to nucleated groups in which the cell outlines have disappeared. These cytoplasmic aggregations possess an irregular contour due to the presence of pseudopodial processes, so there is distinctly present here a layer definitely homologous with the plasmodial structure (plasmodium, plasmodiblast, or plasmoditrophoblast) so characteristic of the placentation of the Eutheria. The appearance of the plasmodiblast at this stage is shown in figs. 4, 5, and 6. At various points the plasmodial nuclei invade the uterine syncytium. The soldering of the foetal trophoderm to the maternal syncytium is brought about by the above-mentioned pseudopodial processes, in the meshes of which numerous spaces are enclosed. The remaining basal cells of the trophoblast layer form the cytoblast or cytotrophoblast. This is by no means at first a definite cell-layer. It is apparently not till a little later that the basally-situated nuclei divide in a regular way to form the more definite cellular layer known as the cytoblast.

A point of the greatest significance, and one to which I shall later refer, is the fact that the localities of proliferation are determined by the presence of the syncytial nests, and it is into these that the plasmodial masses pass. Fig. 4 shows this phenomenon, while it is also indicated in fig. 8, in which, however, the nests are not quite cut centrally. The effect of the growth of the attached trophoblastic cells on the maternal structures is shown in figs. 4 and 7. In fig. 4 the chorionic ectoderm cells show but little departure from their original linear arrangement, but have already begun to give off pseudopodial processes which immediately phagocytically attack the syncytial nuclei of a neighbouring syncytial lobule, only part of which latter is shown in the drawing. Only the nuclei of the nest outside the range of attack are seen to preserve their original shape and structure, the others being in the state of degeneration. Apparently this consists in the loss of contour through the breaking down of the nuclear membrane followed by loss of chromatin and virtual disappearance. Figs. 5 and 6 show stages in the proliferation of the chorionic ectoderm cells. In all cases they give the impression of thrusting forward wedge-shaped plugs which penetrate into the aggregations of maternal nuclei.

There happens to be but one gland present in the small area to which the trophoblast is attached at this stage. This is shown in fig. 7, which depicts a section through the actual opening of the gland. Its uterine mouth is seen to be blocked by the overlying chorion, the ectodermal portion of which may now be regarded as forming two distinct portions, a basal portion, the cytoblast (*cyt.*), and a plasmodial portion, the plasmodiblast. The growth of the plasmodium has extended a considerable distance, particularly on one side, where the protoplasmic processes have caused degeneration in the gland epithelium cells similar to that which occurs in the maternal nuclei. Here, as before, the nuclei have lost their contour, their position in the cell-line, and their chromatin. They decrease in size and disappear, being evidently ingested. Further degeneration of the gland-cells is foreshadowed by the presence of protoplasmic processes involving them. Also in this figure will be seen remnants of syncytial nuclei and other remains, haematids and leucocytes.

That the cells of the plasmodiblast are phagocytic cannot be doubted. Their effect on the syncytial and gland nuclei is some evidence of this, but the presence of numerous rounded granules such as those shown to the left of fig. 7 (*ing.*) and other cellular débris makes the matter certain.

Pigmented cells are present in the trophoblast. This pigment is black and is arranged either as minute granules or as an aggregation of streaky lines, usually in the neighbourhood of the nucleus.

From the above account it will be evident that the method of fixation of the embryo to the uterine wall in this marsupial is fundamentally similar to the general type occurring in the Eutheria. If, for example, the figures of this stage which I have given (figs. 4, 5, 6, 7, 29, and Text-fig. 4) be compared with the essentially similar drawings of the comparable phenomena recorded by Schoenfeld for the dog (1903), (Pl. xxii, figs. 14 to 17) (see Text-fig. 3), it will be seen that the difference in the two forms rests mainly, in the early stages, on the behaviour of the uterine epithelium. This, in the dog, remains as a distinct layer until it undergoes degeneration as a result of the inroads of the plasmodiblast.

The Non-pregnant Uterus.—This was examined in sections and found to have undergone changes corresponding to those which had occurred in the right. The ovary of this side, however, had a well-developed corpus luteum. I will, therefore, not enter into a detailed description of the structure of this uterus further than to mention the following:

The epithelium has developed into a syncytium abundantly supplied with capillaries, many ramifying on the surface, some of which discharge their blood by extravasation into the lumen. A point of interest is the fact that, on the dorso-mesial side of the uterus, the syncytium is somewhat thickened and the mucosa is here developed into deep folds. At its edges this thickening passes off gradually into the rest of the syncytium. This thickened portion possibly represents the maternal trophospongia. Throughout the whole uterus the glands show no degeneration. They are in an active state of secretion and the inner ends of the gland-cells are torn and frayed out through abundant breaking off of cellular secretion. Cilia, therefore, are not to be found.

(*a*) Significance of the Uterine Syncytium in Perameles.

Proliferation and syncytialization of the uterine epithelium is a well-marked feature of maternal preparation for allantoic placentation in many Eutheria. In many cases the preparatory proliferation is soon interfered with by the destructive action of the blastocyst on the uterine epithelium.

Hill (1897, p. 393) institutes a comparison between Perameles and Sorex in the matter of the proliferation of the uterine epithelium. As a consequence, however, of his belief in the degeneration of the chorionic ectoderm and the persistence of the uterine epithelium in Perameles he could not carry the comparison far enough.

In Sorex, Hubrecht states that over the future allantoidean and omphaloidean placental areas the epithelium undergoes a tremendous proliferation and development into a cell aggregate of relatively great thickness.

The history of this maternal formation in the allantoic placental area of Sorex is, I think, worthy of particular consideration and of comparison with what happens in Perameles. Hubrecht shows (1894, p. 492 seq.) that, in the shrew, the nuclei of the epithelial proliferation become arranged in fan-shaped groups at comparatively regular distances, the centre of each group being without nuclei (1894, fig. 69).

'In the following stage', says Hubrecht, 'this arrangement becomes converted into a functionally more important one. The centre of the fan-shaped structure becomes an open crypt, the protoplasm breaking up and the peripheral nuclei forming the epithelial lining of the crypt. The uterine epithelium breaks away from under the crypt and the inner lining of the crypt solders with the surrounding epithelial surface at the lower border' (p. 493).

It is quite easy to see the resemblance between the shrew and Perameles in respect of the phenomenon here described. In both there is intense epithelial proliferation, particularly in the placental area. The resulting nuclei or cells are in both arranged in nest-like groups. While in the bandicoot these groups remain practically unaltered, in Sorex they are transformed into epithelial crypts. Nevertheless, as I have shown above, the proliferated nuclei in Perameles take on a more or less definite arrangement as a layer bounding a central space. This is indicated in figs. 1, 2, and 3, whilst in other figures, 4, 7, and 9, I have drawn attention to the fact that the trophoblastic proliferations bear a definite relation to the groups. Particularly in the next stage, it will be seen that the foetal nuclei invade and fill the nest with consequent more or less complete disappearance of the maternal nuclei. Under these circumstances it does not require any extraordinary stretch of imagination to recognize in this highly characteristic and important phenomenon the remains of a much more elaborate system of placental formation. The conclusion is certainly obvious to me that here in Perameles, in the formation of the peculiar syncytial groups, there is to be recognized an abortive attempt at the formation of crypts such as occur in the placental area of Sorex, and further, while in the latter crypt-building is confined to the placental area, in Perameles the comparable phenomenon occurs at all points of the uterine epithelium, although in a lesser degree opposite to the omphalopleure than in the placental area.

(b) General Remarks on the Fixation of the Embryo.

Here it will be convenient to interpolate a few remarks on the method of fixation of the blastocyst and on the general terms used to express the nature of the structures taking part in it.

Fixation is brought about in Perameles as in others by the junction of a circumscribed portion of the trophoblast, the chorion, with a corresponding area of preplacentally proliferated maternal tissue, the trophospongia.

There is a fundamental difference in the character of the two uniting layers—the foetal being an active, the maternal a quite passive layer. To a mobile, virile formation of the former type—of foetal origin—the general term plasmodium is applied, while the corresponding multinucleate structure, of maternal origin, usually acting as a pabulum for the foetal plasmodium, is known as a syncytium (see Schoenfeld, 1903).

It is evident enough that in all cases of placentation where the uterine epithelium is not immediately destroyed, the area of fixation consists for a longer or shorter time of a conjoint layer of foetal and maternal epithelia, each having the characteristics outlined above. Development of the former and degeneration of the latter proceed in Perameles side by side, and it would be convenient if a single expression could be coined to denote the composite layer consisting of the two. No convenient term seems to exist, and I propose to use the name 'diploplasma' to indicate the conjoint layer consisting of foetal chorionic ectoderm and maternal trophospongia.

The diploplasma consists in Perameles of three zones. Along the line of junction of foetal and maternal tissues, the syncytium is undergoing degeneration and resorption by the plasmodium. Such a degenerating syncytium is called a symplasma (Schoenfeld, 1903), a term which can be correctly applied only to maternal structures of a degenerate nature contained in the plasmodium. Recently, however, Willey has suggested its use to indicate the junctional portion where there is an intimate mixture of active foetal elements and degenerating maternal material.

Accepting this suggestion (Willey, 1914), the three zones of the diploplasma in Perameles consist of the following: a middle junctional layer composed of mixed foeto-maternal tissue (symplasma) with, on one side, a pure layer of foetal, and on the other side, pure maternal material.

The foetal portion differentiates early into a basal layer, the cytoblast, and a plasmodial layer, the plasmodiblast. Contrary to what has been stated by Willey for other mammals, the cytoblastic layer in Perameles is well in evidence before the time of attachment of the allantois.

The plasmodiblast has a twofold duty concerned with (*a*) attachment, (*b*) nutrition. Both functions are performed with the aid of root-like pseudopodial processes which attack the maternal elements converting them into symplasmatic débris which is ingested. Such nutritional material is passed on to the cytoblast, the cells of which exercise a secretory and selective rôle.

The cytoblast appears to have as one of its duties the amplification of the area of attachment. Cell outlines are always distinct between the cytoblastic cells due to radial divisions. These result in the formation of new cells and consequent increase of the area of attachment. As a result, new marginal zones of plasmodial formation are brought into being, and these attack fresh sources of nutrient material in the maternal trophospongia. This areal increase is subject, of course, to certain limitations, and in Perameles, as will be seen later, the growth in thickness of the plasmodial formation is determined also by the thickness of the proliferated maternal epithelium. In many mammals, however, as is well known, the plasmodiblast extends much further, even in some cases as far as the muscularis, causing the degeneration and disappearance of practically all the structures in its track.

A second function of the cytoblast cells is, as indicated above, the elaboration of a secretion which is passed into the extra-embryonal coelome. They therefore exercise a certain selective capacity on the material passed to them by the plasmodium.

Abundant evidence of this secretory activity is to be seen in sections, the actual secretion being easily observed. From the above it will be seen that structurally and physiologically the proliferating trophoblast of Perameles over the allantoic placental area is quite comparable with the corresponding layer in the Eutheria.

Stage 2. Perameles gunni, 6·6 mm.

The specimen on which the following description is based was brought to me by a trapper on September 17, 1919, about two hours after being trapped. Both uteri were swollen and found to be pregnant. The left uterus after being opened was fixed in Hill's fluid, and the whole uterus with embryo in situ was later sectioned.

The right uterus was examined in salt solution. It was carefully slit open along the ventral side without any injury to the foetal membranes. It was noted that although there was a close apposition of the yolk-sac wall to that of the uterus, the folds of the former fitting into the hollows of the latter in a very intimate way, yet there was absolutely no sign of fusion or organic connexion. It will be remembered that Hill, at first of the opinion that there was a protoplasmic connexion between the yolk-sac wall and the uterine syncytium, found later that this was not the case.

In the case of P. gunni it was possible by means of careful manipulation to make out the details of the yolk-sac circulation.

This, at this stage, is in a state of considerable activity, a condition no doubt to be correlated with the fact that the allantoic placental connexion and circulation is now just on the way towards completion and definite establishment. By removing the lower portion of the bilaminar omphalopleure and reflecting the remainder over the embryo a full view of the vascular area was obtained, and the whole course of the vessels could be made out in detail. After these had been sketched and photographed the lower portion of the yolk-sac was removed, whereupon it was found that the connexion between allantoic vesicle and uterus was so slight that the whole embryo with its allantois could be removed intact. The point of attachment was visible in the case of this embryo as a somewhat central raised area on the placental surface of the allantoic vesicle. The elevation of this area was due to the adherence of a slight amount of maternal tissue brought from the uterine wall.

This being the only pregnant uterus of P. gunni so far known, I may be excused from entering with some detail into the description of the relation of the embryonic and maternal structures.

General Relations of Embryo and Uterus.

Foetal Membranes.—These agree essentially in their arrangement with what Hill has described for other species of Perameles. For that reason I will concern myself in the

following account only with those features which seem to be peculiar to the Tasmanian form.

The Allantois.—This agrees in all respects with that of other species.

In the vesicle the distinction between the outer or placental wall of the allantoic vesicle and the inner or coelomic wall is obvious. A feature worthy of note is the somewhat dense look of the allantoic vesicle in surface view. This is due to the thickness of its walls, the mesodermal layer of which is much denser and thicker than in the species examined by Hill.

The Vascular Omphalopleure.—The vascular omphalopleure (fig. 23, *v. omph.*) is essentially similar in structure to that of other species. It is, however, as well to point out that the tenuity of the ectodermal layer of this area, on which Hill laid so much stress, is not so pronounced a feature in P. gunni as in the forms examined by him. It is, however, still much thinner than in Didelphys (Selenka, 1886–7). In P. gunni the ectoderm cells are somewhat columnar with ovalish nuclei, basally situated. The free end of the cell-body is frayed out into pseudopodia-like processes. In the vascular omphalopleure is contained a large proportion of the extra-embryonal vascular system which in the stage under discussion forms a vascular absorptive organ of some complexity and size. No marsupial so far described appears to have a yolk-sac circulation of such an elaborate type as is characteristic of this stage of P. gunni.

Bilaminar Omphalopleure (fig. 23, *bil. omph.*).—This of course consists of two layers, trophoblastic ectoderm and yolk-sac entoderm. The histological details of a portion of this wall are shown in fig. 22. It will be seen that the ectodermal layer consists of cells which are large and are remarkable for the immense amount of vacuolation which occurs in their cytoplasm.

These vacuoles are of all sizes occupying in the aggregate almost the whole of the interior of each cell, separated from one another by thin bridges of protoplasm in a special condensation of which the nucleus is contained. The outer ends of the cells are often rounded, but in other cases possess an irregular profile suggesting the presence of pseudopodial processes.

No doubt the vacuolation of the ectodermal cells of this region of the blastocyst is to be associated with an active absorption on the part of these cells of carbohydrate (? glycogen) in process of being transferred to the embryo per medium of the yolk-sac. The cells closely resemble the glycogenic cells figured by authors for certain other mammals (e. g. Jenkinson, 1902, I. vi).

The entoderm of this region consists of a layer of cells somewhat darkly staining with haematoxylin. These are sometimes rounded, sometimes flattened. I have found the entoderm cells occasionally slightly vacuolated in the manner described by Hill for his species.

It is a well-known fact that the vacuolation of cells concerned in embryotrophic processes varies greatly at different stages. Hill found that the entoderm cells of the yolk-sac, but slightly vacuolated in early stages, became greatly so in his 12·5 mm. specimen. This he regarded as a forerunner of degeneration, but I rather think it is to be associated with the active absorption and internal transmission by the yolk-sac wall of substances (probably carbohydrate) secreted by the mother and destined for the nutrition of the foetus.

The Yolk-sac Placenta.—As stated above, this is brought about by the intimate apposition of the vascular omphalopleure to the extra-placental portion of the uterine wall. Fig. 23 shows the details of this circulation and fig. 24 is a somewhat diagrammatic representation of the area with regard to the blastocyst wall and the embryo.

In fig. 23 most of the bilaminar omphalopleure has been removed and the remainder of the blastocyst wall has been reflected over the embryo. The vascular area is therefore seen from its inferior aspect. The anterior end of the embryo is contained in the proamnion (*proa.*), here large and persistent.

The vitelline artery (*vit.a.*) is a very large and thick trunk which, after leaving the yolk-stalk, keeps at first a little to the left side of the body and passes over the surface of the tail to reach the right side. Here it passes into the outer leaf, i. e. from the yolk-sac splanchnopleure to the vascular omphalopleure. In the course of its passage along the body it gives rise to the numerous fine and extremely characteristic vessels which extend in their peculiarly parallel manner into the yolk-sac splanchnopleure which they supply. These branches are extremely long and pass for some little distance over into the vascular omphalopleure where they alternate with corresponding venous factors to the vitelline vein.

Immediately on entering the vascular omphalopleure the vitelline artery divides to form the sinus terminalis (*s.t.*). Each portion of the sinus passes forward and at first has the usual course. Instead, however, of passing directly forward in the usual way, each branch, at about the level of the fore-limb, takes a sudden turn ventrally so that the anterior portion of each lateral branch forms another curve with a ventral convexity. The artery on each side unites with its fellow in front of the head by anastomosis.

The sinus gives off to the area vasculosa a wonderfully rich plexus of branches quite as complex or even better developed than in any marsupial so far described.

The peculiar arrangement of the sinus terminalis results in the division of the vascular area on each side into two regions, anterior and posterior, the former being somewhat smaller in area than the latter. Each of these areas is drained by a separate factor of the vitelline vein on each side. The posterior factor from the posterior area is the larger, and after receiving numerous fine capillaries and branches travels along the dorsolateral aspect of the vascular omphalopleure, parallel to and some little distance from its dorsal edge till the vein reaches the height of the neck flexure of the embryo, where it passes over into the yolk splanchnopleure. Here it receives the anterior factor which drains the anterior area. The branches and capillaries which go to make up the latter are extremely rich. Each lateral vitelline vein (*vit.v.*) formed of the factors just mentioned passes down and unites with its fellow just before entering the body. Between them in this region is the permanently non-vascular area spoken of by Semon and by Hill.

It should be mentioned that the posterior factor of each lateral vitelline vein receives on its inner side branches from the yolk-sac splanchnopleure corresponding to the fine branches from the vitelline artery supplying that membrane. These pass upwards in the splanchnopleure, and then continue outwards into the omphalopleure to join the posterior factor as stated.

The measurements of the vascular area are as follows:

Across anterior portion	7·5 mm.
Across posterior portion	9·9 mm.
Greatest length, anterior portion . .	4 mm.
Greatest length, posterior portion . .	5 mm.

Formation of the Allantoic Placenta in Perameles gunni.

Various stages of this are shown in figs. 13–16, 19.

Maternal Structures.—The wall of the uterus is divisible, as stated above, into two general portions, placental and extra-placental regions.

Placental Region.—The mucosa varies greatly in thickness, from 0·60 to 1·1 mm., due to folds in the uterine wall. The glands are numerous, long, and tortuous, measuring in diameter from 0·037 to 0·051 mm. They are of the usual type, being unbranched and lined by a single layer of columnar cells, with deeply-staining nuclei, peripherally situated. I am not able to detect any trace of the cilia which Hill and O'Donoghue have observed in Perameles. The glands are in a highly active state of secretion, their basal portions being filled with cellular and other material.

The inter-glandular tissue is extremely thin and tenuous, but condensed where it immediately surrounds glands and blood-vessels. Distributed through the connective tissue is an abundance of lymph material. This is particularly evident just below the uterine epithelium, in which position there is a space up to 2 mm. wide filled with lymph which bathes the lower surface of the uterine syncytium. Here and there this space is crossed by glands and blood-vessels. I have called this space the sub-epithelial lymph-space. Owing to the presence of this coagulum and the sparsity of cellular elements the whole stroma presents a very homogeneous appearance. Scattered through it, however, are numbers of leucocytes, and here and there are erythrocytes which have escaped from the capillaries by extravasation.

Attached to and intimately united with the epithelium of this area is the embryonic chorion.

Epithelium.—The condition of the epithelial elements of the allantoic placental mucosa shows, unquestionably, that in P. gunni the same process of syncytialization has occurred as in other species, and that here is a similar aggregation of the syncytial nuclei into nests arranged in lobular masses of the syncytial protoplasm. The distribution, as I have suggested above, I believe to represent an abortive attempt at the formation of simple uterine crypts. Between these nests the capillaries and leucocytes of the remainder of the mucosa gain access to the syncytium.

The Extra-Placental Region.

Mucosa.—In all essential respects this resembles the mucosa of the placental area, being highly vascular and containing a large quantity of lymph. Here are to be found leucocytes and red corpuscles, but neither are so numerous as in the placental portion. The glands are in an active secreting phase and their contents—cellular detritus and other nutritive substances—are poured into the uterine cavity to be received into the trophoblastic layer of either region of the omphalopleure.

The sub-epithelial lymph-space, as such, becomes less evident the farther we proceed from the placental area and the glands are somewhat smaller in diameter.

Syncytium.—This has been formed by a similar process to that which has resulted in the modification of the epithelium of the placental area. The difference is mainly one of degree. Thus the extra-placental syncytium is thinner, being about

0·043 mm. opposite the vascular omphalopleure and 0·023 mm. opposite the bilaminar. Similar proliferation and migration of the nuclei have taken place but the lobular nests are much smaller and less individual, and, further, more and more nuclei remain outside the nests until in some places lobules and nests as such are scarcely distinguishable. The nuclei are quite similar to the syncytial nuclei of the placental area being rounded, bead-like, and vesicular with little chromatin.

Chorion.—This shows, both in the amount of area of attachment and of proliferation, a considerable advancement on the condition found in the last stage. The fusion with maternal tissue is so complete that the resulting layer is one in which occasionally there is some difficulty in distinguishing maternal and foetal cytoplasm. Here, as before however, we have the infallible test of the difference in structure, shape, and fate of the maternal and foetal nuclei.

The Diploplasma.—The thickness of this layer measures now from 0·135 to 0·190 mm.

This increase is due mostly to the great growth of the chorionic ectoderm, which consists of a well-marked basal cellular layer, the cytoblast, and a much thicker portion in which cell outlines are not visible, the plasmodiblast. The maternal portion of the diploplasma consists of the remains of both the maternal syncytial protoplasm and of the syncytial nuclei. When unaltered the latter have the characteristics which have been before noted, viz. small rounded vesicles in which the chromatin is suppressed but containing a more or less prominent nucleolus (see figs. 13, 14, and 19, *syn.n.*). They have an exactly similar appearance to the nuclei of the extra-placental area.

At this stage, however, nests are seldom found intact. In almost every case they have been invaded by plasmodiblast nuclei (*pb.n.*), so that by this time a fair proportion of maternal nuclei have degenerated and disappeared.

The Trophoderm.—This is divisible as stated above into cytoblast and plasmodiblast.

The Cytoblast (*cyt.*) is a remarkably definite layer of cells with distinct cell outlines. The cytoplasm is granular and the nuclei large and plump. Their chromatin contents are in the form of an extremely rich network with a number of karyosomes. A nucleolus may be present, and if so is of large size. At the edge of the placental area the cytoblast (*cyt.*) passes over into the ectoderm of the marginal chorion, whose cells gradually decrease in size till they attain, at the junction with the vascular omphalopleure, the normal dimensions and appearance of trophoblastic ectoderm cells. Some idea of the difference in size of the cells of this layer may be obtained from the fact that, at the margin of attachment, the cells are twice, while in the centre of the area of attachment they are eight times the height of the ordinary trophoblastic ectoderm cell.

The cytoblast cells, therefore, have already the appearance, particularly in the centre of the placental area, of typical placental megalokaryocytes.

Intense proliferation is to be seen in the cells of the cytoblast of the whole of this area. I have not been able to observe mitotic figures, but it must be remembered that in the case of my material as well as that of Hill, fixation was not possible until a couple of hours after death.

Proliferation of the cytoblast (*cyt.*) and its results are shown in figs. 13–16, 19. The plasmodiblast nuclei (*pb.n.*) resulting from this division are usually more darkly stained than the original cytoblastic nuclei and for this reason are easily distinguishable, particularly from the original syncytial nuclei (*syn.n.*). The plasmodiblast nuclei are occasionally isolated, but more often are arranged in clumps to form multinucleated masses (*g.c.*) of sometimes large size. These resemble the giant or monster cells which have been found in similar positions in connexion with the placentation phenomena of higher Eutheria. The main result is that the plasmodiblast makes its way into the nests of syncytial nuclei, in which one or two or, in most cases, a large number of these nuclei can be distinguished. In such cases the syncytial nuclei have to a great extent disappeared, the tendency being to replace them in their nests by the newly-arrived foetal nuclei.

The pseudopodial processes characteristic of the plasmodium of the previous stage are greatly in evidence in this stage also. The result is that spaces occur in the diploplasma giving it a very uneven appearance. The arrangement of these plasmodial spaces suggests that new nuclei are formed in a somewhat spasmodic manner in waves one after the other.

One result of the advance of the plasmodium is the inclusion of the maternal capillaries which pass up between the remains of the syncytial lobules and ramify below the layer of cytoblast. Each capillary is contained in its delicate endothelial layer. Further than this in the symplasmatic zone of the diploplasma are to be found remains of maternal nuclei, maternal blood corpuscles and various granules. These are all obtained phagocytically. An evidence of this action in the plasmodiblast is the strong affinity which some of its nuclei have for pigment. In this stage much of the pigment found in other cells of the previous stage has disappeared. It is now almost entirely confined to the cytoblast and plasmodiblast. Here it appears usually as black granules deposited round the nucleus, but in other cases as narrow irregular lines in the cytoplasm (figs. 25–27). A similar pigment (related to haematoporphyrin?) is found in Ungulates, and is regarded by Jenkinson and Duval as being the remains of ingested haematids whose iron has been passed on to the embryo. The result is that the pigment remains in the trophoblast increasing in amount up to the time of parturition.

Leucocytes.—These are extremely characteristic of the uterine mucosa, particularly in the placental regions. They are found in the connective tissue, from which they migrate through the epithelium of the glands and mingle with their secretion. They also pass into the diploplasma, from which they reach the extra-embryonal coelome. They can be seen abundantly present in all sections of this region at this stage. In the connective tissue they are small, taking the form of small mononuclear leucocytes. In the diploplasma, however, and particularly when they reach the cytoblast, they have increased greatly in size, forming large mononuclear leucocytes or macrocytes. In this position they occasionally displace cytoblast cells. All the leucocytes carry well-defined pigment. Isolated patches of pigment occur occasionally in the plasmodium.

The coagulable material (proteid, lymph) passing through the epithelium of the uterus is particularly abundant in the placental area. The secretion of the cytoblast cells is visible on their coelomic faces as small rounded swellings which break off to form spherical bodies floating in the coelomic fluid.

I can find no evidence of fat secretion either in the cytoblast cells or in the gland cells, but it must be remembered that no special means have been employed for the detection of fat, glycogen, or iron.

The chorionic mesoderm, where it can be made out, is a thin mesothelial layer consisting of flattened cells with ovalish darkly-staining nuclei. I must confess that I find it impossible to discern this layer over most of the placental area.

The free margin of chorion forms the connecting link between the fixed chorion on the one hand and the vascular omphalopleure on the other. The mesoderm here is similar to that of the central portion of the chorion, but the trophoblastic layer consists of somewhat flattened cells near the vascular omphalopleure, these increasing greatly in height as they approach the fixed portion.

Gland Alteration.—A word about the condition of the glands in this stage. The body of the gland is lined by an epithelium which is somewhat lower than in the preceding stage. In its upper portion the gland swells out and becomes somewhat barrel-shaped. The whole of this portion enclosed in the diploplasma suffers a degeneration of its epithelium. The result is that here the gland appears to be a mere space lined only by plasmodiblast. The gland-mouth is closed by a layer consisting of the cytoblast plus a certain amount of plasmodiblast. The latter by no means takes the form of a plug as in the last stage, but passes down on either side apparently causing the disorganization of the gland epithelium on its way. There appears to be a struggle here between the downward force of the growing plasmodiblast and the upward pressure of the gland secretion, evidenced by the swelling of the gland and the formation of lateral fissures in the plasmodium. Such appearances are characteristic of all the glands in this stage.

When such glands are sectioned obliquely or transversely they appear in sections of the diploplasma as irregular spaces without any definite epithelial bounding layer.

Attachment of Allantois.—At this stage this has occurred over an approximate area of 0·5 by 0·21 mm. The attachment is due to an intimate fusion of the splanchnic mesoderm of the allantois with the somatic mesoderm of the chorion. Apparently the allantois spreads its area of attachment very rapidly, and one of its almost immediate results is a quickening of the proliferating activity of the cytoblast cells.

In the centre of the area of attachment the distinction between the plasmodial layer and the cytoblast to a great extent breaks down, the cell outlines of the latter disappearing and its nuclei being converted into plasmodiblast nuclei. It is in these positions that apparently the union of the allantois with the chorion is best effected. This dissolution of the cytoblast allows maternal capillaries to come closer to the surface of the diploplasma, and this can be seen in some cases even before the allanto-chorionic fusion is in being (fig. 14).

Stage 3, *Perameles obesula*, 7 mm.

This is Hill's Stage C, being his earliest placental stage. It is represented in the Department of Zoology of the University of Sydney by a single slide containing one section. The specimen, however, is well stained and mounted, and shows all the details of the relations of the foetal membranes to the uterine wall.

The excellent general account given by Hill of the foetal membranes of this stage renders any further description unnecessary. I will therefore confine myself to a consideration of the maternal and foetal structures associated with the allantoic placentation.

The attachment of the allantois may now be said to be complete, the allanto-chorion being united with the maternal epithelium over an area whose diameter, following the well-marked uterine folds, is some 13–14 mm.

The area of attachment is not co-extensive with the whole of the chorion since there is a marginal zone of the latter quite free, as in the 6·6 mm. stage.

Structural Details of the Allantoic Placenta.

As would have been expected from the description of the last stage there is by this time a most intimate fusion of the maternal and foetal tissues in the diploplasma, the proliferation of the trophoblastic ectoderm and its invasion of the maternal syncytium having advanced considerably beyond the condition found in the 6·6 mm. embryo of *P. gunni*.

Here again, therefore, in the trophoblastic proliferation there can be recognized two portions, a basal cellular layer corresponding to the cytoblast or cytotrophoblast of higher mammals, and externally to this a proliferating plasmodial portion, the plasmodiblast or plasmoditrophoblast.

The cytoblast is throughout its extent fairly distinct. Over a fair area towards the centre, however, it has already begun to lose its integrity—having disappeared in many places as a cellular layer and become converted here into plasmodiblast (fig. 18). It was upon this condition of the cytoblast in its central portion that Hill based his suggestion of the degeneration of the chorionic ectoderm.

In the marginal portion (fig. 21) the cytoblast still consists of high columnar cells separated from one another by distinct cell walls. Externally, however, the boundaries of these on their plasmodial aspect are indistinct. Where intact, they have somewhat elongated nuclei arranged at right angles to the surface of the uterus. These nuclei stain well and are rich in chromatin. They do not, however, stain so darkly as the more granular plasmodial nuclei to which they give origin.

At the edge of the placental area the development of plasmodiblast from cytoblast is in its minimum condition of activity, as is shown in the figure (fig. 21).

Here it can be seen that the arrangement of the syncytial nuclei of the extra-placental area is similar to that of the preceding stages. The presence of an internal space, free of nuclei, in the syncytial nest should be noted.

Passing from this region into the placental area the gradually increasing activity of the proliferating chorionic ectoderm becomes evident. More and more of the foetal nuclei occupy the nests. The plasmodial nuclei in a large number of cases form multinucleate groups. In many cases there are but two nuclei in each group, these being comparable to the binucleate cells 'diplokaryocytes' described for some Eutheria (for example in Ungulates, Assheton, 1906). More often three or more nuclei are contained in one plasmodial mass.

An examination of a more central section shows that in this portion of the placental area many nests are now quite filled with the plasmodiblast nuclei (fig. 18). So closely are these packed in the nests that instead of being oval or irregularly shaped they take a polygonal form due to mutual pressure. The condition of the original epithelial nuclear nests can be gauged from the fact that of twenty-seven observed in one central portion of the allantoic area four were untouched; eleven were partly, twelve completely filled by invading nuclei, the nests having the appearance of solid multinucleated masses.

In such positions also it is that the original cytoblast layer has practically disappeared. It is evident, therefore, that a large proportion of the maternal epithelial tissue has been replaced by intruding foetal material. One effect of this is that the maternal capillaries of the allantoic area become enclosed by the advancing plasmodium, and in many cases are now to be found at the surface of the diploplasma, where they directly underlie the allantois and even come into contact with the allantoic vessels.

Pigment is not so common as it is in the previous stage but it is still to be found, particularly in leucocytes and in the cytoblast cells. Here and there in the plasmodium are to be seen isolated patches of pigment pointing to an active ingestion of maternal cellular material. Haematids are often to be found contained in the plasmodium in process of being absorbed.

Glands.—Most of the glands of the allantoic placental area have undergone certain characteristic changes. Instead of being narrow and tortuous, they have now become generally straighter and wider.

The necks of the glands are particularly spacious, and often the gland on its entrance into the diploplasma shows a barrel-like enlargement. The aperture of the gland in the placental area is closed by the cytoblast, while the plasmodiblast has disappeared from its mouth but bounds this portion of the gland on either side where it has been responsible for the disorganization of the gland epithelial cells (fig. 28). The cells of the body and neck of the glands have now been transformed into a low cubical epithelium, and their nuclei instead of being oval are rounded. The gland-cells throughout are ciliated. Occasionally some of the glands swell to a relatively enormous size. A further feature of note is that a number of glands have penetrated into the muscularis.

The Allantois.—For the general description of this I would refer the reader to Hill's account. At this stage the splanchnic mesoderm of the allantois is found fused with that of the chorion over the full extent of the placental area. There is but a small amount of penetration of allantoic tissue into the plasmodiblast, and this only becomes possible where gaps have occurred in the disorganized cytoblast. The complex 'interlocking system'—by which apt phrase Hill describes the mutual apposition of foetal and maternal capillaries—is brought about by short finger-like downgrowths of the allantoic mesoderm combined with the opposite (inward) tendency of the maternal capillaries. This mutual process becomes more and more easy the more the basal cytoblastic layer loses its integrity and becomes converted into plasmodiblast. Where the cytoblast remains practically intact as at the margins (fig. 23), the approximation of foetal and maternal vessels does not occur. The maximum penetration of the allantois appears to be a little more than the thickness of the cytoblast. One result of this penetration is the tendency to form occasional cytoblastic islands, containing usually one or two nuclei, isolated by the growth round and behind them of the allantoic capillaries (fig. 24).

Stage 4. Perameles obesula, 8–8·75 mm.

Of this stage I have no material available, so will content myself by stating that Hill's figures (1897, figs. 15 to 21) show that the various processes of allantoic placental formation which have been initiated in the case of the younger embryos can be recognized as being continued in this.

The growth of the plasmodiblast has gone on apace, with a corresponding diminution of the amount of maternal tissue contained in the diploplasma. This results in a very homogeneous appearance of the tissue of the placental area. In Hill's figures very few syncytial nuclei are recognizable with certainty. On the other hand, it can be seen unmistakably that, by this time, most of the cytoblast has been converted into plasmodiblast, and that in this formation giant multinucleate cells are a very prominent and characteristic feature (see especially his fig. 17).

Here again the greatest activity is being shown towards the centre of the placental area. Of the still remaining basal cytoblast cells Hill says (p. 414), ' in some cases they are multinucleated . . . or the single nucleus is also hypertrophied and vesicular,' a statement which well accords with the facts to which I have already drawn attention in the preceding pages.

Many of these remaining cytoblast cells have the appearance of diplokaryocytes. Some of them plainly show a tendency towards plasmodial formation, as can be seen in Hill's fig. 21, where the cell marked *ch.ect.* has very much the appearance of a giant cell with plasmodial processes.

Stage 5. Perameles obesula, 12·5 mm.

Of this stage I possess five consecutive sections stained in haematoxylin and eosin.

Here, as before, I will confine myself to a description of the structural modifications associated with the formation of the allantoic placenta.

A section through the very folded allantoic placental area reveals the presence of an extremely homogeneous granular layer with lobed lower margins, indicating the positions of the original syncytial nests.

Unfortunately, the gap between this and the preceding stage is too wide to make it possible to closely follow and be certain of the histolytic changes which have taken place in the placental region.

Within the lobulated portions of the diploplasma are very irregular groups of nuclei corresponding in position to the groups of the original syncytium.

An examination of such an aggregation at this stage shows that it consists mainly of plasmodiblast nuclei which are in a marked stage of degeneration. One simple group is shown in fig. 26 of this paper, while many others are depicted by Hill (1899, Pl. xliii, figs. 6 and 7).

One particular type of degeneration change in the case of a plasmodial nucleus is shown in fig. 17. This corresponds closely with what has been recorded by Jenkinson (1902, figs. 24–5) for the megalokaryocytes of the mouse. The original robust nucleus loses its contour through shrivelling of the nuclear membrane and escape of the nuclear sap. The chromatin becomes irregularly arranged. Thereupon the whole nucleus flattens and becomes of the nature of an extremely thin rod, which by further absorption is seen as a few darkly staining particles in the general ground-mass, finally disappearing.

There are to be found also degenerating vesicular elements which no doubt are the remnants of the formerly numerous maternal epithelial nuclei. On this important point, in view of the paucity of the material available, I regret I am not able to make any certain statement.

If, however, this interpretation be correct, then Hubrecht's suggestion (1909) that the placenta of Perameles consists in its final stage of a ' mixed syncytium ' is not very far from the truth.

It is difficult to suggest a cause for the degeneration and resorption which has taken place in the case of these nuclei, but it is a significant fact that associated with the degenerating groups are to be found abundant leucocytes of the small and large mononucleate types.

Situated just internally to the allantois are to be found occasional large trophoblastic cells, being remains of the cytoblast. These also are undergoing degeneration.

In places the homogeneous nature of the ground-tissue is less evident, and here multinucleated masses of protoplasm are still in evidence. Apparently the foetal chorionic cells have performed their function, viz. the fixation and a portion of the preliminary nutrition of the embryo, and are now in a process of degeneration and disappearance.

The maternal vessels are extremely numerous, and their finer branches now ramify at the surface of the diploplasma, where they come into intimate apposition with the vessels of the allantois in the way Hill describes.

The allantoic mesoderm has penetrated but little into the trophodermic layer. No spaces are formed in which maternal blood flows, all maternal vessels being contained in definite endothelial walls. The two blood-systems, however, are elaborately interlocked, and very often foetal and maternal blood-streams are separated merely by the thickness of two endothelial walls.

As regards the glands of the placental area, they are now characterized by the possession of a low cubical epithelium. Their cavities are wide, particularly near the opening of the gland. The cells are ciliated. The mouth of each gland is sometimes closed by the allantois with its numerous vessels, at other times by degenerating remains of the trophoblast. The condition of the portion of the gland contained within the diploplasma is similar to that characteristic of the preceding stage.

Outside the placental area the maternal nuclei of the epithelium still take the form of rounded vesicles in which it is difficult to distinguish even a nucleolus. The glands of this portion of the uterine wall possess the usual columnar epithelium and are also ciliated. They are narrower than the glands opening into the placental area.

Seeing that the newly-born young of Perameles measures but 14 mm. in length, the condition of the allantoic placenta in the 12·5 mm. stage may be accepted as being practically that of the full-term placenta.

Stage 6. Perameles nasuta, post-partum.

With regard to this stage the only way in which I can supplement Hill's description is by pointing out that the amount of foetal tissue left behind in the uterus is considerably more than would be the case if Hill's conception of the placentation of this animal were correct. Instead of consisting merely of the allantois—with the addition of a few remaining foetal cells left behind after the degeneration of the chorionic ectoderm—there is really comprised in the contra-deciduate portion, in addition to the allantois, the whole thickness of the diploplasma, of which undoubtedly the greater part is foetal.

6. SUMMARY OF CONCLUSIONS.

The conclusions arrived at in the preceding pages may be summarized as follows:

1. Allantoic placenta.

(*a*) The fixation of the embryo is brought about by means of the chorionic ectoderm at a time when the embryo measures about 6 mm. direct length.

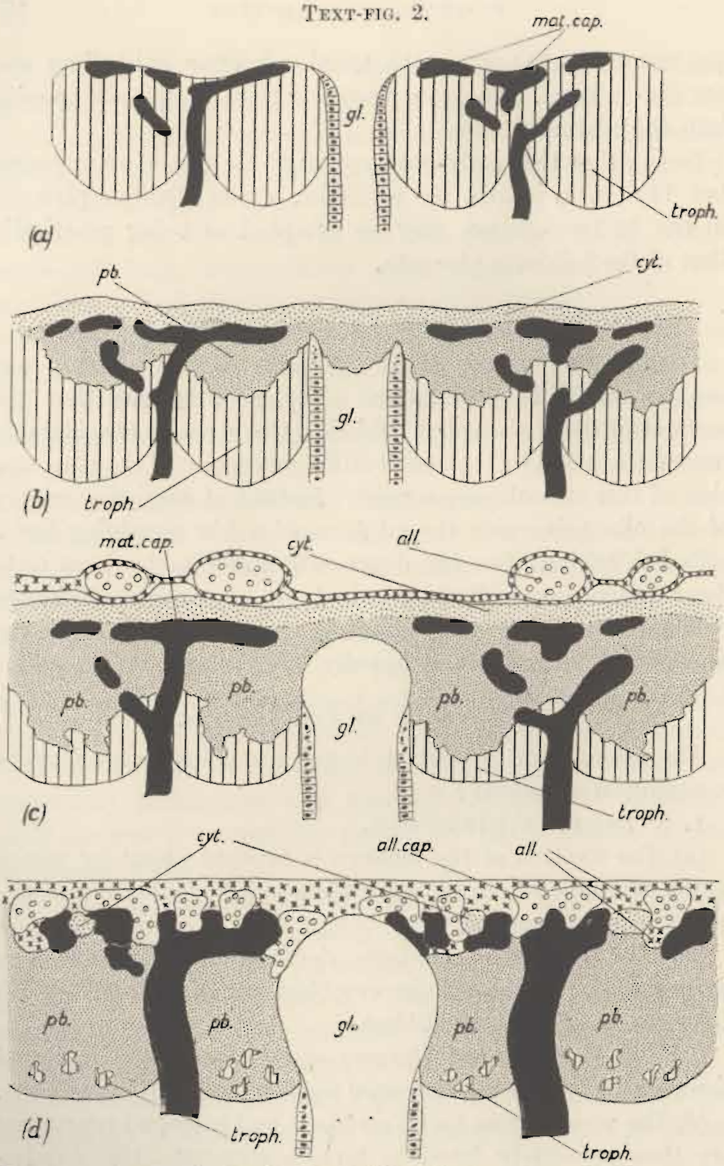
(*b*) The chorionic ectoderm develops directly into two portions, (1) a basal cellular cytoblast which by proliferation gives rise to (2) a plasmodiblast.

(*c*) The plasmodiblast phagocytically attacks the maternal tissues, particularly the maternal nuclear aggregations or nests.

(*d*) The proliferating foetal ectoderm and maternal syncytium are thus intimately fused to form a structure, the diploplasma.

(*e*) Sooner or later most of the cytoblast layer disappears, being converted into plasmodiblast.

TEXT-FIG. 2.



Diagrams showing the development of the allantoic placenta in Perameles.

(*a*) Shows the character of the preplacental maternal trophospongia with its lobules, between which vessels and glands penetrate. The capillaries ramify at the surface.

(*b*) Chorionic attachment has now taken place and the chorion has proliferated to form a basal cytoblast and an external plasmodiblast. The proliferations of the latter are related to glands and nuclear nests. The capillaries ramify just below the cytoblast layer.

(*f*) The outgrowth of foetal plasmodium does not extend any farther than to involve the proliferated maternal epithelium.

(*g*) The attachment of the allantois is effected when the embryo has attained a length of approximately 6·5 mm.

(*h*) The outward migration of the basal cytoblast cells (when converted into plasmodiblast) gives opportunity for the maternal and foetal vessels to come into intimate apposition.

(*i*) In the final stage the foetal nuclei in the placenta are found to be in a state of degeneration.

(*j*) Remains of the maternal epithelium still probably exist in the full-term placenta.

(*k*) The uterine glands persist throughout gestation, but the portion of their epithelium within the diploplasma disappears.

(*l*) All maternal vessels have definite endothelial walls: hypertrophy of the endothelial cells does not occur and lacunae are not formed.

(*m*) An allantoic placenta is recorded for P. gunni.

2. Yolk-sac Placenta.—A virtual yolk-sac placenta is present in P. gunni as in other species of Perameles, brought about by the intimate apposition of the complex system of vessels in the vascular omphalopleure with the highly vascular portion of the uterine syncytium just beyond the placental area.

(*c*) Allantoic attachment is now in its first stages. Further proliferation of the foetal trophoblast has taken place and the plasmodiblast now occupies the greater part of each nest. Within the diploplasma the gland epithelium has disappeared and the gland is closed in above by cytoblast plus plasmodiblast. Capillaries still ramify below the cytoblast.

(*d*) The foetal plasmodium now occupies the whole of each nest with the exception of (possibly) a few remains of maternal tissue. The cytoblast layer has broken down, having mostly been transformed into plasmodiblast. A few islands of trophoblastic tissue are, however, still left in the cytoblast position. The very much branched and interlocked foetal and maternal vessels have now come into intimate apposition. The gland epithelium in the diploplasma has entirely disappeared, and the glands are closed in above by allantoic vessels alone.

all., allantois; *all. cap.*, allantoic capillary; *cyt.*, cytoblast; *gl.*, gland; *mat.cap.*, maternal capillary; *pb.*, plasmodiblast; *troph.*, trophospongia.

Further, considerable vacuolation of the cells of the bilaminar omphalopleure at certain stages mark it as being an absorptive organ of some importance in embryotrophic processes.

7. Concluding Remarks.

Now that we have some clear idea of placentation in Perameles, the question arises what relation exists between this and the placentation of other marsupials and of Eutheria generally? Further, can any light be thrown on the phylogeny of the mammalian allanto-placenta by a consideration of this question?

Before, however, entering on a discussion of these, it is, I think, necessary to get a clear idea of the morpho-physiological conception of the term 'Placenta'.

(*a*) The Placental Conception.

It is inevitable that the conception of placentation which has arisen in the minds of investigators should, until fairly recently, have been associated with the very complex and highly advanced structure developed in the intimate fusion or apposition of the foetal membranes of the commoner and best-known Eutheria with the uterine mucosa.

It might, however, be taken for granted that—in the case of an organ so prominent in mammalian developmental processes and rightly regarded ontogenetically and phylogenetically as of the highest importance—there would be no two opinions as to its definitive structure or its physiological significance. But such a supposition would be wrong, and a very superficial examination of the writings of the more recent investigators soon shows that they hold radically different views as to what is understood by the term 'placenta'.

All the important recent works treating of the comparative anatomy of the placenta to which I am able to refer (Strahl, 1905; Grosser, 1910; Jenkinson, 1913) are insistent that the fundamental idea of placental formation lies in the apposing of two blood-streams—one foetal, one maternal—to form a structure by which the physiological processes intended for the well-being of the embryo can be carried out.

Such a conception makes no allowance whatever for the work of the bilaminar omphalopleure of marsupials—itself non-vascular but physiologically of considerable importance.

Even less acceptable is the suggestion of Professor Hubrecht (1909), who insists that 'fusion of embryonic with maternal tissue is a conditio sine qua non, and so we must admit a placenta in the case of Didelphia (Perameles) and deny it to certain Monodelphia (Equus, Sus, Nyticebus, Galago, and others)'. As Assheton has pointed out, under this scheme 'the sheep is a placental, a cow a non-placental mammal'.

Reaction between mother and embryo, or rather dependence of the latter on the mother for food, oxygen, and the removal of its waste products, may be said to commence from the time of the first appearance of the ovum in the uterus. 'The mammalian ovum,' says Hill (1910, p. 113), 'already in the monotremes greatly reduced in size as compared with that of reptiles, and quite minute in the Metatheria and Eutheria, contains within itself neither the cubic capacity nor the food material necessary for the production of an embryo on the ancestral reptilian lines. We accordingly find that the primary object of the first developmental processes in the mammals has come to be the formation of a vesicle with a complete cellular wall capable of absorbing nutrient fluid from the maternal uterus.'

Our knowledge of the physiology of the early stages of mammalian intra-uterine development is admittedly as yet very incomplete. Nevertheless, it is quite certain that the amount of nutrient material present in the ovum is absolutely insufficient for even the most elementary developmental processes, and has to be supplemented, from the very beginning, from outside sources.

Even a cursory consideration of the above will serve to indicate that in the uterine development of the viviparous mammals there occur two distinct phases, differing entirely in the means by which the necessary physiological processes of the embryo are arranged for. In the first of these, extending over the time of cleavage and of blastocyst formation, absorption and exchange are performed solely by means of the trophoblast.

When blood-vessels appear and are functional, quite a new phase is inaugurated, lasting to the end of pregnancy, during which these vessels come to the aid of the trophoblastic layer in more quickly and efficiently performing the necessary embryonic services.

Grosser's (1910, p. 94) terms 'embryotrophic' and 'haemotrophic' could have been conveniently employed to indicate these periods, but, unfortunately, his use of the word 'exclusively' in the definition of the latter term has made it applicable alone to haemochorial placentae, and it is even doubtful if the definition would be strictly correct in their case.

Nor does Resink's (1902) arrangement suit the case any better. This author regarded the intra-uterine life of the hedgehog as falling into two well-defined periods as follows:

(*a*) Preplacental period, during which maternal and foetal preparation for the allantoic placentation takes place; the trophospongia and ectoplacenta are formed and the embryo fixed. Broadly, this period may be said to occupy the earlier portion of intra-uterine existence up to the time of attachment of the allantois.

(*b*) Euplacental period, in which the allantoic attachment is made and the placenta completed.

The weakness of this arrangement as applied to mammals generally is to be found, in my opinion, in the extreme importance given to the allantoic placentation and the inclusion of the yolk-sac (vascular) placentation in the first of these periods. Such a scheme becomes difficult of application, particularly to the Metatheria, in which an allantoic placenta occurs, so far as is known, in but one genus, various methods of trophoblastic attachment in others, in many no attachment whatever, a yolk-sac placenta in all.

It is therefore apparent that allantoic placentation is only of the greatest importance in one group of mammals, the Eutheria, and it is due to the fact that the most detailed investigation has been expended on this group and to the prominence of the allantoic placenta in it that other features of embryonal intra-uterine life have been for so long overlooked.

Viewed in the light of what we already know of the morphology and physiology of the foetal membranes in the two groups of viviparous mammals, it is evident that placentation, as generally understood, is but part of a much larger conception which has to do with the whole physiological intimacy, during intra-uterine life, between foetus and mother.

Thus I am fully in accord with Assheton's suggestion (1909) that the term 'placenta' should be applied to all organs consisting of an intimate apposition or fusion of the foetal membranes with the uterine wall for the purpose of carrying out physiological processes destined for the well-being of the embryo.

Such a conception would include the following types of placenta:

(*a*) That in which the trophoblast is vascularized from the allantois—allantoplacenta.

(*b*) That in which the trophoblast is vascularized from the yolk-sac—omphaloplacenta.

(*c*) That in which no foetal blood-vessels are concerned. This is the case of the bilaminar omphalopleure of marsupials whether there is a fusion of part of this with the uterine mucosa (Dasyurus, Phascolarctos), or merely, as is more usual, intimate apposition. For this type of placenta I propose the term 'metrioplacenta'. These may be illustrated by referring to Perameles, the genus which is the subject of investigation in the present paper.

(*b*) Placental Phases in Perameles.

Preliminary Phase.—During this period the blastocyst is formed and the physiological processes are carried on by means of the trophoblastic cells. There is no union in

Perameles between the trophoblast and the uterine wall, and there is no absorption by means of foetal blood-vessels.

The work of this phase is carried on in later intra-uterine life by the bilaminar omphalopleure.

Intermediate Phase.—This is the stage of the vascular yolk-sac placenta. It comes into being with the functional formation of the vascular area. There is a close apposition between the foetal and maternal blood-vessels. This phase reaches its most active condition before the attachment of the allantois, and although, maybe, less efficient, endures, with the existence of the vascular area, until the end of pregnancy.

Final Phase.—The allantoic attachment takes place and the allantoic placenta is completed.

It is evident that in marsupials, with the exception of Perameles, the preliminary and intermediate stages are the more important, in fact the only ones present, while in general, in Eutheria, the preliminary and the final phases are of the greater value.

Under these circumstances we can denote the placental periods in Perameles as metricoplacental, omphaloplacental, or allantoplacental, according to the type of placenta which is the dominant one for the period concerned.

(c) Placental Pheuomena in Marsupials generally.

In reviewing these I will commence with the most specialized groups.

Macropodidae.—The works of Owen (1834–7, Macropus major), Semon (1894, Aeprymnus rufescens), and Hill (1895, M. parma, M. ruficollis, M. robustus, and M. major) emphatically show that in these forms the allantois throughout life remains small, buried in the splanchnocoele. From my own observations I am able to state that this is also the case for Potorous tridactylus and Bettongia cuniculus. It is possible that in some Macropods the allantois reaches the chorion, although Caldwell's statement (1884) that there is such a union in the case of Halmaturus ruficollis, as well as his testimony of a fusion between the bilaminar omphalopleure and the uterine wall, have not yet been confirmed. An omphaloplacenta is well developed in Macropods.

Phalangeridae.—In Trichosurus vulpecula (Hill, 1889) and Petaurus sciureus (Semon, 1894) the allantois is similar to that of Macropods. I am also able to state that this is the case for Pseudochirus cooki. Here again the embryo depends on the work of the trophoblast both of the vascular omphalopleure and of the bilaminar omphalopleure.

Phascolarctus.—This genus is particularly interesting in possessing, according to Caldwell (1883) and Semon (1894), a respiratory allantois. There is a well-developed omphaloplacenta and also a union in the metrioplacenta between an annular zone of the bilaminar omphalopleure (just outside the sinus terminalis) and the uterine mucosa.

Didelphys.—The allantois does not meet the chorion. The omphaloplacenta is well developed. Certain portions of the ectoderm of the bilaminar omphalopleure are stated by Selenka to form absorptive proliferations similar to those found in certain Eutheria, for example Manis (Weber), and Equus (Ewart).

Dasyurus.—The allantois shows interesting stages in degeneration. At a particular stage it becomes applied to the chorion which is itself in intimate association with the uterine mucosa. Later the allantois withdraws from the chorion and degenerates considerably, its vascular system practically disappearing. An omphaloplacenta is present as well as a similar annular fusion of the bilaminar omphalopleure with the uterine wall as occurs in Phascolarctus.

Perameles.—This is a most primitive form possessing a well-developed allantoic placenta and an omphaloplacenta, and there is considerable evidence of absorption in the bilaminar omphalopleure.

From the above abstract it will be seen that we can, as yet, hardly be said to have a detailed knowledge of the structure, physiology, and ontogeny of the foetal membranes of most marsupials. Particularly in such primitive genera as Thylacinus and Sarcophilus, it may be expected that investigation will help to shed a clear light on the phylogeny of the placenta in this group.

Another point of importance (of greater value, I think, than Assheton would have had us believe) lies in the behaviour of the uterine mucosa. Of this our knowledge in the marsupials is particularly meagre. Yet it is extremely important, since there is naturally a mutual reaction of embryo and uterus. An investigation of the modifications of the uterine mucosa during pregnancy would, there is not the slightest doubt, be of great value in shedding a light on ancestral placental arrangements in marsupials.

Pseudochirus cooki is instructive in this regard. Preliminary investigations which I have already made in the case of this diprotodent marsupial have shown that the uterine epithelium in a very early stage of pregnancy consists of a single layer of very high columnar cells with correspondingly elongated deeply-staining nuclei. Below the epithelium the connective tissue is condensed to form a layer in which run the capillaries. This stage can be recognized as being very similar to one occurring in many Eutheria.

At a later stage of gestation, cell outlines have disappeared and a vascular syncytium is formed similar to that of Perameles, except that it is composed apparently not only of the epithelial cells but of those of the sub-epithelial capillary layer. These capillaries now ramify at the surface as is the case in Perameles.

Here without doubt can be recognized the remains of an ancestral trophospongial proliferation.

From the consideration of the above facts, particularly as regards the condition of the foetal membranes in Perameles, Dasyurus, and Phascolarctus, bearing in mind the complementary modifications of the uterine wall where they are known, it must be evident that these conditions in marsupials represent a degeneration from a more complex system

of placentation which undoubtedly obtained in the original protoplacental group.

Into a full treatment of this there is no need for me to enter. It has been ably discussed by Hill and the facts and conclusions embodied in the preceding pages can only be regarded as confirming and strengthening his expressed opinions.

(*d*) The Relation of the Allantoic Placentation of Perameles to that of the Eutheria.

This question I will discuss but briefly, reserving its full treatment for some future occasion when adequately fixed and preserved late gestation stages of Perameles may perhaps be available.

It is with some pleasure that I have been able to bring the method of allantoplacental formation of Perameles into line with that occurring in the simpler Eutherian forms. In fact it may be said in general that the only difference between the two is one of degree. There are the same characteristics of passivity of the uterine epithelium and activity of the trophoblast with a division of the latter into a cytoblastic and plasmodial layers. After preliminary diploplasmatic preparation the allantois becomes fixed and an apposition of the two blood-streams becomes effected. I might here briefly refer to the resemblances between the earlier stages of allantoplacentation in Perameles and the dog and rabbit. In Text-fig. 3 I have indicated the main points of Schoenfeld's fig. 14 (1903) representing an early stage of chorionic invasion in the dog. A somewhat comparable stage in Perameles is represented by Text-fig. 4. The agreement in the method of foetal invasion is evident. In the dog, however, according to Schoenfeld, the uterine epithelium does not form a syncytium.

In the rabbit, on the other hand, as in many other Eutheria, such a maternal syncytium is formed, and here the early stages show an even more significant resemblance to those occurring in Perameles. Particularly I may refer to Schoenfeld's (1903) figs. 4, 5, and 6, Pl. xxi, and those of Maximow (1900, figs. 1 and 2, Pl. xxx).

The phylogenetic importance of the presence of large multinucleate masses of foetal origin in the allantoplacenta of Perameles, the dog, the rabbit, and others cannot be overestimated.

Bearing in mind the accepted origin of the Metatheria and Eutheria from a primitive diphyodont protoplacental stock (Hill, 1897, p. 432), it is possible to state with certainty that, in that early group, the same conditions of passivity of the uterine epithelium and active phagocytic quality of the trophoblast were already in existence; with the further differentiation of the latter layer in its placental portion into two distinct layers, respectively cellular and plasmodial.

TEXT-FIG. 3.

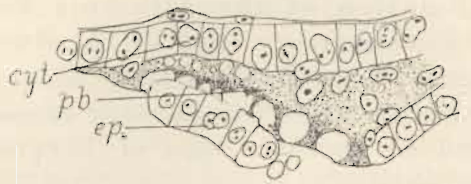


Fig. 3.—An early stage of development of the dog showing chorionic attachment (after Schoenfeld). *cyt.*, cytoblast; *pb.*, plasmodiblast; *ep.*, uterine epithelium.

TEXT-FIG. 4.

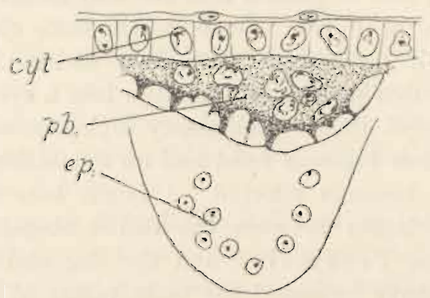


Fig. 4.—A stage in Perameles comparable with that of the dog in Text-fig. 3. Lettering as in Text-fig. 3.

The foregoing facts make this conclusion inevitable. This being so, those Eutheria, particularly the Ungulata, in which there is no union between the trophoblast and mucosa in the allantoic placental region, must have reached this condition, in the course of their phylogeny, by a process of secondary simplification.

It is evident enough then that the attempt made by Strahl (1906) to group Perameles with the Ungulates and others in the Semiplacenta breaks down. At the same time it is not easy to suggest any arrangement by which Perameles will take its proper place in placental classification.

The only possible course at this stage appears to be to examine briefly what is the relation of the allantoic placentation in Perameles to some one or other of the groupings at present in use.

Assheton's suggestion to divide the Placentalia into Placentalia cumulata and plicata seems to be the most promising, since in addition to having a structural basis these divisions are to some extent physiological. Assheton has given a table of the characteristics of the two groups as he conceived them (1909), and even a cursory glance at these will indicate that the allantoplacenta of Perameles structurally and physiologically occupies a place somewhere between the two but more primitive than either.

Thus in the 'heaping up' of the trophoblast—a very fundamental point—it agrees with the cumulate type, while in many other features, absence of lacunae and the mildness of attack on maternal tissues, it approaches the plicate type. The secretion of the placental glands appears to be at first of minor importance in Perameles, but, later, absorption by the allantoic vessels is direct, increasing the value to the embryo of glandular secretion tremendously.

It is apparent that if we regard the above grouping as a rational one, the allantoic placenta can be regarded as being of a central primitive type from which development in either direction might easily have proceeded.

And the very real relation of the allantoplacentation in Perameles to that of the Carnivora—the relation of the simple to the slightly more complex—shows that the Carnivora, as suggested by Hubrecht and upheld by Assheton, exhibit, of the Eutheria, the most undifferentiated arrangement. The trophoblastic 'heaping up' is common to the allantoplacenta of both Perameles and the Carnivora. This is fundamental, and is sufficient evidence of the fact that the cumulative type of placenta is the more primitive.

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9. DESCRIPTION OF FIGURES.

The outlines of all figures have been drawn with the aid of Zeiss's camera lucida, then enlarged by means of the pantograph and details filled in by freehand.

List of Reference Letters.

all., allantois. *all.cap.*, allantoic capillary. *all.ent.*, allantoic entoderm. *all.mes.*, allantoic mesoderm. *all.pen.*, penetration of the allantoic mesoderm. *all.st.*, allantoic stalk. *all.ves.*, allantoic vesicle. *amn.*, amnion. *ana.*, anastomosis of the two portions of the sinus terminalis. *bil.omph.*, bilaminar omphalopleure. *cav.*, cavity contained in syncytial nests. *ch.mes.*, chorionic mesoderm. *ch.ect.*, chorionic ectoderm. *coel.w.*, coelomic wall of the allantoic vesicle. *cyt.*, cytoblast (cytotrophoblast). *emb.*, embryo. *ex.coel.*, extra-embryonal coelome. *ex.syn.*, maternal syncytium of the extra placental area. *ex.syn.n.*, syncytial nuclei of the extra-placental area. *g.c.*, multinucleate giant cell. *gl.*, gland. *gl.ep.*, gland epithelium. *inf.*, infiltrated material (? lymphatic) contained in the cavity of a syncytial nest. *ing.*, material ingested by the plasmodiblast. *leuc.*, leucocytes. *mat.cap.*, maternal capillary. *m.ch.*, marginal chorion. *muc.*, uterine mucosa. *musc.*, muscularis. *pb.*, plasmodiblast. *pb.n.*, plasmodiblast nuclei. *pgm.*, aggregations of pigment in the plasmodiblast. *plac.*, allantoic placenta. *pl.syn.*, maternal syncytium of the placental area. *proa.*, proamnion. *syn.n.*, nuclei of the maternal trophospongia. *troph.*, maternal trophospongia. *vasc.omph.*, vascular omphalopleure. *v.omph.*, vascular omphalopleure. *vit.a.*, vitelline artery. *vit.v.*, vitelline vein. *yk.cav.*, yolk-sac cavity. *y.s.*, yolk-sac. *yl.spl.*, yolk-sac splanchnopleure.

EXPLANATION OF PLATES 9–11.

Plate 9.

Figs. 1–8, 10–12, Perameles obesula, 6·1 mm. Fig. 9, Perameles obesula, 12·5 mm.

Figs. 1, 2, and 3.—Sections showing the arrangement of syncytial nuclei as a more or less irregular layer round a central cavity. Note the vesicular shape and chromatic characteristics of these nuclei. Note also intruding leucocytes.

Figs. 4, 5, and 6.—Phases in the earliest growth of the plasmodiblast. In fig. 4 the syncytial nuclei are already undergoing degeneration under the effect of the plasmodial advance processes.

Fig. 7.—Plasmodial attack on a gland. Note the breaking down of the gland epithelium on one side. Here also ingested material is evident in the plasmodiblast.

Fig. 8.—Another stage in plasmodiblast formation. The cytoblast is a well-defined layer. Note presence of leucocytes.

Fig. 9.—Shows the vesicular and degenerate appearance of foetal nuclei at this stage with close apposition of maternal and foetal vessels.

Figs. 10, 11, and 12.—Photomicrographs of sections through a branched gland just outside the area of the first fixation of the chorion (see Text-fig. 1).

Plate 10.

Figs. 13–16, 19, Perameles gunni, 6·6 mm. Figs. 17, 18, Perameles obesula, 12·5 mm. Figs. 20, 21, Perameles obesula, 7 mm.

Fig. 13.—Placental area towards the centre, showing the commencement of the disorganization of the cytoblast allowing maternal capillaries to approach the surface. The very superficial position of one of these capillaries is, however, very exceptional for this stage. Note the penetration of the plasmodiblast nuclei into the syncytial nests.

Fig. 14.—Section of somewhat more peripheral portion of the same area. A distinct cytoblast is present and a plasmodiblast in which giant cells and ingested material are outstanding features. This figure should be compared with Maximov's fig. 1 of the rabbit.

Figs. 15, 16, and 17.—Show attachment of allantois and disorganization of cytoblast to allow of the apposition of foetal and maternal blood-vessels.

Figs. 17 *a*, *b*, *c*, *d*, *e*.—Stages in the degeneration of a foetal nucleus.

Fig. 18.—Section of the central portion of the placenta showing the following: breaking down of cytoblast, almost complete filling of syncytial nests by foetal nuclei, penetration of allantoic capillaries and almost final apposition of maternal and foetal blood-streams.

Fig. 19.—Placental area showing section through a gland. Note disappearance of gland epithelium, the enlargement of the gland lumen at the apex and the tendency to form lateral slits in the plasmodium. This section shows well the intruding leucocytes of the large mononucleate type. The plasmodiblast shows a particularly noteworthy giant cell, pigment patches, and ingested material.

Fig. 20.—Section of placental area towards the margin. Cytoblast is intact. The nests are well filled with plasmodiblast nuclei.

Fig. 21.—Section of placental area at the margin. For description see text.

PLATE 11.

FIGS. 22–27, PERAMELES GUNNI, 6·6 mm. FIG. 28, PERAMELES OBESULA, 7 mm. FIG. 29, PERAMELES OBESULA, 6·1 mm.

Fig. 22.—Section showing structure of the bilaminar omphalopleure. Note the extreme vacuolation of the ectoderm cells.

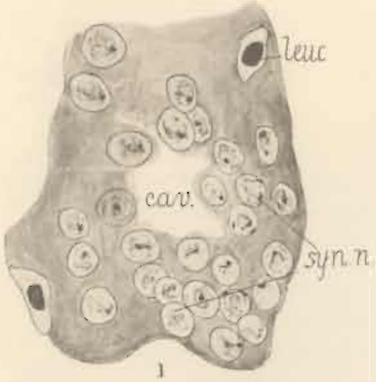
Fig. 23.—The yolk-sac circulation. For description see text.

Fig. 24.—Diagram showing the relation of the embryo to its membranes.

Figs. 25, 26, and 27.—Pigment-bearing cells: fig. 25 of the serosa, fig. 26 from the connective tissue, and fig. 27 from the trophoblast.

Fig. 28.—Section through the mouth of a gland.

Fig. 29.—Shows the relation of a plasmodial proliferation to a group of syncytial nuclei.



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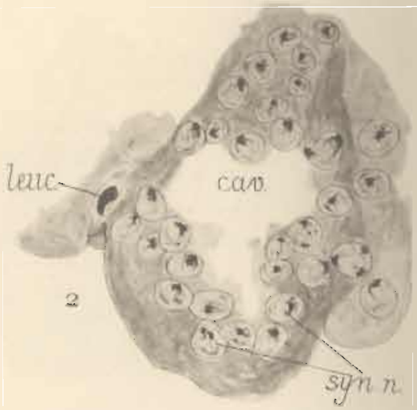

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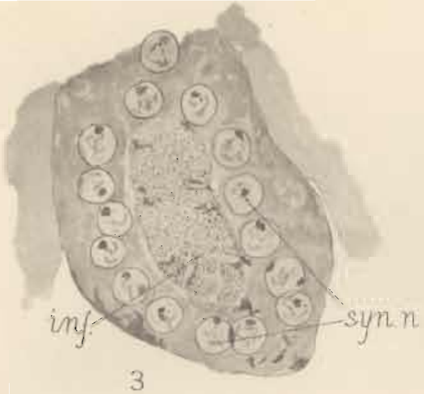

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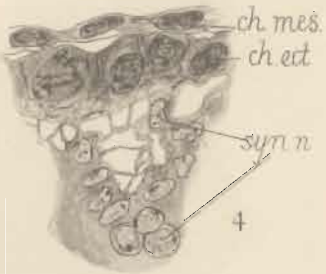

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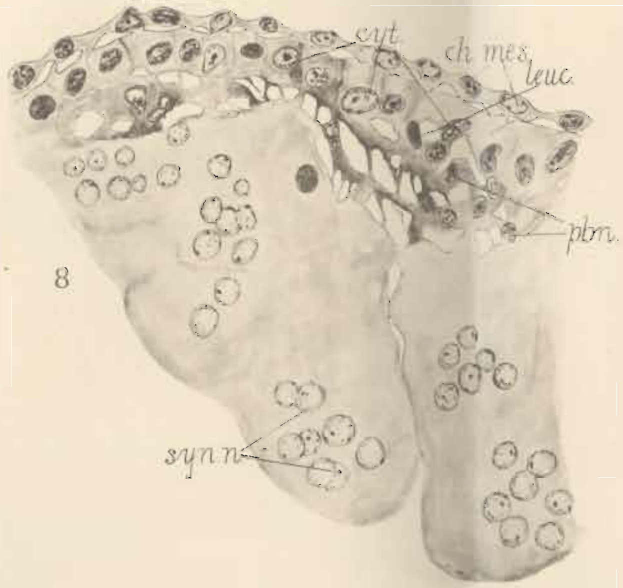

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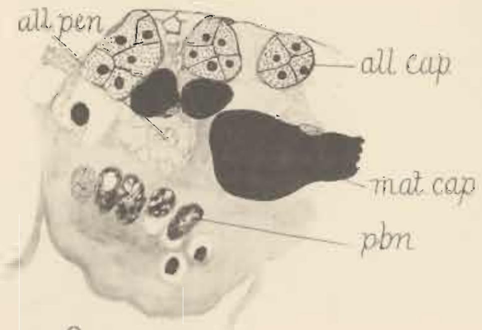

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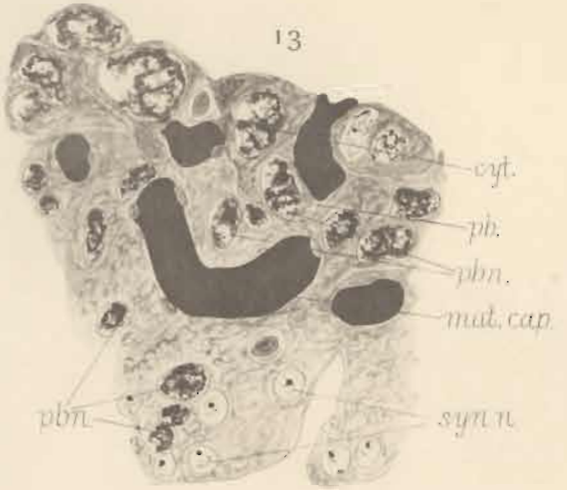
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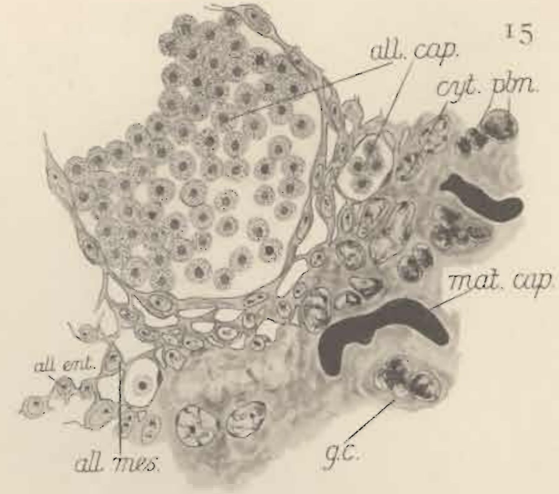
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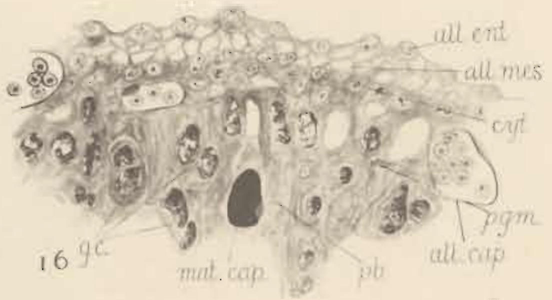
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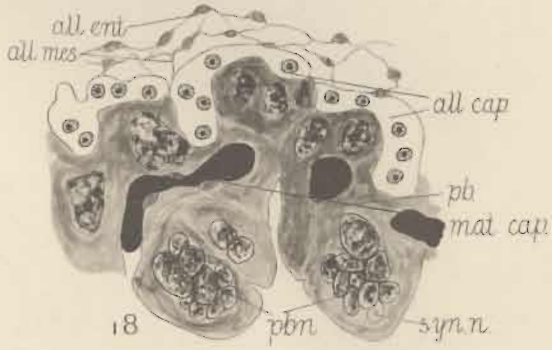
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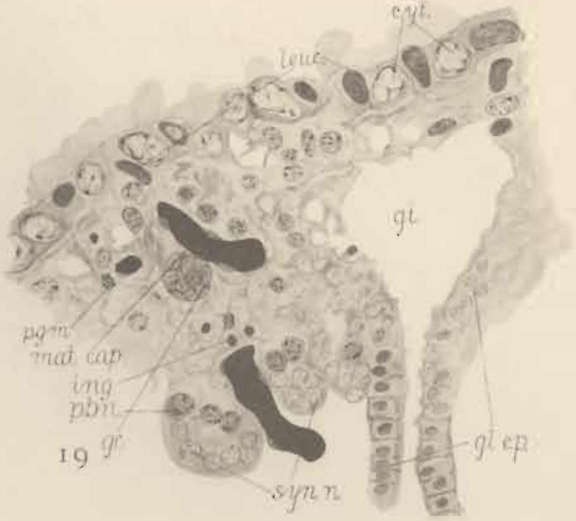
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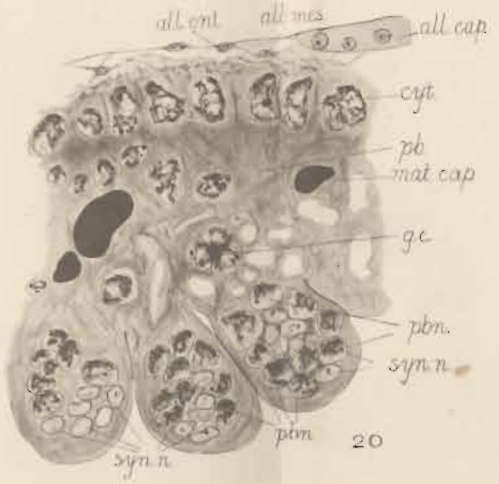
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