

**Is the Trophoblast of Hypoblastic Origin as
Assheton will have it?**

By

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With 7 Text-figures.

IN the 'Quarterly Journal of Microscopical Science' there has lately appeared (vol. 54, part 2) an article by my friend Assheton, in which he points out certain objections which he feels inclined to raise against some of the views that were developed by me in a contribution to the fifty-third volume of this Journal, entitled "Early Ontogenetic Phenomena in Mammals."

Although I regret that he has not seen his way to comply with the invitation which I addressed to my fellow-embryologists in October, 1901 (it was published on p. 5 of my article on "Tarsius" in the 'Verh. Kon. Akad. v. Wetenschappen te Amsterdam,' vol. viii, No. 6, 1902), and which was intended to minimise printed disputes, where personal inspection of the preparations might bring about consensus of opinion, still, I accept his challenge (*loc. cit.*, p. 221), and will now "discuss more fully the difficulties which have arisen in the minds of some who are unable to accept (my) theoretical conceptions." In doing so I wish to remind my readers that I am not going to treat all the objections raised by Assheton one by one. Many of them will remain *sub lite* until new facts have been discovered, settling the point in dispute either one way or the other. I will on this occasion

restrict myself to a point of very fundamental importance on which Assheton's and my own views are diametrically opposed to each other, ever since 1898. If the new facts which I bring forward in this paper should be convincing enough to change the minds of those who feel inclined—following Assheton's example—to look upon the trophoblast as hypoblastic, I have no doubt that my proposal to exclude from the phylogeny of Eutherian mammals any ancestor who deposited megalecithal eggs, like the Sauropsids and the Ornithodelphia, will find a more easy acceptance on their part.

Assheton's reasons for considering the trophoblast as an essentially entodermal foetal envelope were first developed in 1898, in his article on "The Segmentation of the Ovum of the Sheep" ('Quart. Journ. Micr. Sci.,' vol. 41). Plate 18 of that article presents us with a series of diagrams most delicately shaded in red and blue, which were meant to explain the mutual relations of trophoblast, epiblast, and hypoblast in ten different genera of mammals, and to compare them with the Sauropsidan arrangement.

These diagrams have not found favour with later authors on this subject, and have been taken no notice of in Hertwig's extensive 'Entwicklungsgeschichte,' in three volumes. At that time I refrained from entering into any polemical discussion, considering that later observations would show the untenability of Assheton's ingenious but unsatisfactory generalisation. In writing his latest article Assheton has, however, allowed himself to come too strongly under the influence of his own hypothesis of twelve years' standing. I see no necessity for entering upon any detailed discussion concerning the numerous and different arguments which have led other embryologists as well as myself to reject that hypothesis of Assheton's now that new facts have come to light concerning the very earliest segmentation stages of *Galeopithecus*. This very archaic genus may be looked upon as a derelict representative of a group that in earlier geological epochs gave rise to the modern bats. There are

certain points of agreement between its early development and that of Pteropus, whilst Leche's anatomical work ('Kgl. Svenska Vet. Akad. Handl.,' Bd. xxi, 1886) upon Galeopithecus points in the same direction. Of this genus I have now in my possession several series of sections made through segmentation phases, some of which I have here figured.

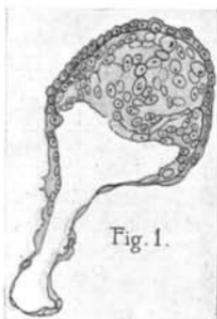
These sections leave no doubt that the trophoblast of Galeopithecus originates by delamination at as early an age as the two- and four-cell segmentation stage, and render it utterly futile to try and explain the Galeopithecus trophoblast as "due to an overflow of the yolk or hypoblast cells over the epiblastic rudiment" (Assheton, l.c., p. 228).

If we look more closely at the three stages of Galeopithecus here figured and begin with the one that is the furthest developed (Text-fig. 1), we find full coincidence with a similar stage described by Assheton for the sheep (l.c., 1898, Pl. 16, figs. 14, 15), by Keibel for the stag ('Arch. f. Anat. and Phys. Anat. Abt.,' 1902, p. 292), by Weyssse ('Proc. Amer. Acad.,' vol. xxx, p. 283) for the pig, by van Beneden for the rabbit and bat ('Archives de Biologie,' vol. i), by myself for the hedgehog, for the shrew ('Quart. Journ. Micr. Sci.,' vol. 30, Pl. 17: vol. 31, Pls. 36, 37), for Tupaja, for Tarsius ('Verh. Akad. Wetensch. Amsterdam,' vol. iv, 1895, Pls. 1, 2; vol. viii, 1902, Pls. 1, 2), and for Nycticebus ('Keibel's Normentafeln,' 1907), as well as by other embryologists for various other mammals. This is the common starting-point in which there is a trophoblast and an embryonic knob with a cavity below it, and in which a hypoblast is not as yet distinctly developed, although just beginning to make its first appearance. It should be borne in mind that this very stage is thus characteristic for genera of mammals so diverse as those mentioned above. The way in which Assheton attempts to prove from yet earlier stages of the sheep that the outer trophoblastic layer is in reality a derivate of the hypoblast appears to me to be so pre-eminently artificial (c.f. l.c. his figures 9-14) and the argumentation so weak, that I must ascribe to a similar

incredulity on van Beneden's part that this latter author in the important article which appeared one year later than Assheton's ('Anat. Anzeiger,' 1899, p. 305), does not take the slightest notice of the English author's view that the trophoblast (van Beneden's "couche enveloppante") should be looked upon as an entodermal derivate.

If we now return to Text-fig. 1 of this paper and inquire how this stage in the ontogeny of Galeopithecus has been reached,

TEXT-FIG. 1.



Section of a blastocyst of Galeopithecus with embryonic knob and enveloping trophoblast, just before the establishment of the continuous hypoblast.

we see that it has been preceded by the stages of which Text-figs. 2 and 3 are the representatives.

In Text-fig. 2 the centre of the different sections is occupied by comparatively large nuclei, evidently belonging to a central group of cells—the mother cells of the embryonic knob. Outside this embryonic knob and forming the peripheral layer in these sections is protoplasm in which distinct cell-boundaries are not visible, but in which a certain number of nuclei (smaller than those of the embryonic knob) clearly indicate that in the live blastocyst a peripheral cell-layer was differentiated in addition to the embryonic knob.

Going back yet one stage earlier, in which the two first cleavage-cells are just on the point of splitting up into four (as is distinctly indicated by the karyokinetic figures of fig. 3 C), we notice, besides the two cleavage-cells, three polar bodies of comparative large size, as they are known for mammals in general. Moreover, at the periphery of the two cleavage-cells we find separate nuclei, indicating the very first origin by an early delamination process of the cells which in Text-figs. 2 and 3 constitute the continuous layer of trophoblast.

TEXT-FIG. 2.

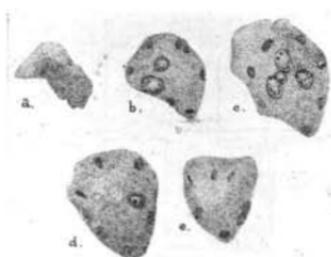


Fig. 2.

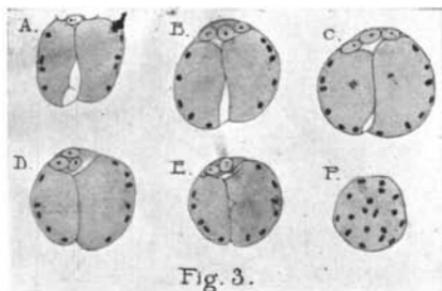
Galeopithecus. A series of five sections through a stage of cleavage preceding the blastocyst of Text-fig. 1. Embryonic knob with bigger nuclei contrasting with the smaller peripheral trophoblast-nuclei. No central space as yet developed.

In the mammalian genera hitherto examined with respect to the origin of the trophoblastic layer (Tarsius, Tupaja, rabbit, sheep, pig, stag, dog, mouse, guinea-pig, etc.), the trophoblast undoubtedly makes its appearance at a somewhat later stage of cleavage, or rather the distinction in the morula stage between the mother-cells of the embryonic knob and those of the trophoblast is not so soon evident as it is in Galeopithecus. However, the karyokinetic processes by which in this latter genus the trophoblastic nuclei separate from the segmentation nucleus (which in its turn owes its

origin to the union of the male and female pronucleus) at so early a moment are not revealed by my preparations, and we cannot for the present come to any sound conclusion as to which of the two modes of formation of the trophoblast is the more archaic one.

Recognising that the definite answer to this question can only be given when a number of new observations will be at our disposal, I may still be allowed to call attention to the fact that in *Galeopithecus* the spot where the polar bodies are

TEXT FIG. 3.



Galeopithecus. A series of six sections through a cleavage stage just preceding the formation of the second pair of cleavage-cells. In *C* karyokinetic figures indicate this. In *B-E* the polar bodies are visible. Apparent trophoblast nuclei are situated peripherally.

applied against the egg (see Text-fig. 3 *B-E*) remains without trophoblast nuclei somewhat longer than other parts of the egg's surface. The question presents itself—supposing the process is more primitive in *Galeopithecus*—whether this particularity might have led (in such mammalian genera that should be considered as phylogenetically younger) to the arrangement which has induced van Beneden, Duval, and Assheton (in his later publications) to consider the cleavage-process of those mammals as revealing epibolic characteristics. In case this question will later have to be answered in the

affirmative, the so-called blastopore which van Beneden (1875) described in the rabbit's morula-stage might correspond to the spot referred to in the three figures (Text-fig. 3 B—D), where the polar bodies lie.

Having thus shown that Assheton's hypothesis of the hypoblastic nature of the trophoblast is irreconcilable with the phenomena in *Galeopithecus*, I emphatically repeat my conclusion that we are not justified in accepting it for any other vertebrate. He himself will admit that, such being the case, the comparison of the trophoblast of mammals with the "deckschicht" of fishes comes to the foreground with increased validity.

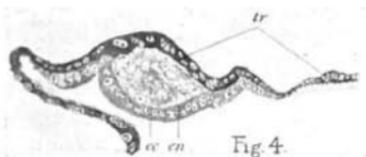
I have already stated above that it is not my intention in this paper to follow Assheton's criticism step by step. A more extensive article on the ontogeny of *Galeopithecus* will appear in the course of this year. I shall there find occasion to reply more fully to other parts of Assheton's criticism. There is, however, one point on which I feel bound to apologise, viz. that I have not allowed enough space for the recognition of the fact that my cephalo- and notogenesis had already been partly forestalled in several of Assheton's papers, and had by him been termed proto- and deutero-genesis. I ought to have particularly mentioned these names in my paper of 1908. Still, I must maintain my terminology now that Assheton himself states (l. c., p. 240) that his and my names "signify a different interpretation," and now that he maintains that mine "does not represent the actual facts." As matters stand I feel that the important issue which is at the base of the whole question of gastrulation in vertebrates (very fully treated in Keibel's contribution to vol. x of the 'Ergebnisse der Anatomie und Entwicklungsgeschichte,' but since then looked upon in a somewhat different light after his and my own short papers in the 'Quart. Journ. Micr. Sci.' [vol. 49] and in the 'Anat. Anzeiger' [vol. xxvi] had appeared) renders any polemics about the nomenclature that should be adhered to untimely. Very numerous investigations

are yet necessary, and will undoubtedly soon be undertaken, before we dispose of the comparative material which is necessary for settling this important point in Vertebrate ontogeny, and for finally deciding which nomenclature ought to be adhered to. I gladly leave the latter decision to others, but would not let this paper see the light without recognising that until lately I have not sufficiently been aware that Assheton already in 1894 expressed opinions to which Keibel and myself have come along other roads, and which, though far from identical, still overlap each other in many respects.

APPENDIX.

While this paper was in the press, attempts were made by

TEXT-FIG. 4.



Part of a section through the blastocyst of *Manis*. The ectoderm (*ec.*) and endoderm (*en.*) of the embryonic knob are transversely cut. The trophoblast cells appear darker in this figure.

me to ascertain whether other genera of mammals might perhaps exist which furnish evidence concerning the early phases of the trophoblast that might further corroborate the facts such as they are presented by *Galeopithecus*. I was all the more anxious to obtain information concerning the earliest stages of the scaled ant-eater (*Manis*), as, by a regrettable *lapsus calami*, which disfigures both the English and the German version of my "early ontogenetic phenomena in mammals, etc.," a gastrula stage of *Manis* is erroneously attributed to *Galeopithecus*.

It is fig. 18 on Pl. C, in vol. 53 of the 'Quart. Journ. of Micr. Sci.,' and fig. 46 in the German publication. I here

reproduce the misnamed figure of this early *Manis*, and have since had the good fortune of obtaining sections of yet earlier cleavage stages of the same animal.

Sections of early blastocysts of two specimens of *Manis* are

TEXT-FIGS. 5A AND B.

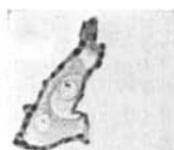


Fig. 5a.



Fig. 5b.

Two consecutive sections of very early blastocysts of *Manis*, which show what is presumably the earliest trophoblastic covering of the mother-cells of the embryonic knob.

reproduced here in Text-figs. 5 a, b, and 6. The stage of Text-fig. 5 is presumably a two-cell, the other (as far as I can follow it up in the consecutive sections of the series) a four-cell cleavage stage (purposely but incorrectly not counting the

TEXT-FIG. 6.



Fig. 6.

Another section through another blastocyst of the same genus in the same stage.

trophoblast cells as such). In both the differentiation between the mother cells of the embryonic knob on the one hand, and the already so much more numerous trophoblast cells, leads to the inevitable conclusion that the phenomenon of the separation of the larval trophoblast from the remaining

embryonic cells takes place at quite as early a moment as we have above described it for *Galeopithecus*, and that also in *Manis* it is perfectly excluded to look upon the trophoblast cells as hypoblastic. And so the early *Manis* may be joined to the early *Galeopithecus* as fatal to Assheton's interpretation of the trophoblast. I cannot yet say with certainty, but I have reason to believe that also in the very young hedgehog similar peculiarities occur.

At the same time it is very suggestive that the quaint and aberrant mode in which the trophoblast cells of *Galeopithecus* and *Manis* arise offers so many points of mutual resemblance between these two genera, and differ not inconsiderably from what we find in Primates, Rodents and Carnivores.

Later investigations will have to decide whether the phenomenon, as it presents itself in *Galeopithecus* and *Manis*, is one of precocious segregation.