

AMNIOGENESIS

BY

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At the seventh International Zoological Congress at Boston, HUBRECHT (1907) delivered an address "On the foetal membranes of the Vertebrates". In this lecture he expounded his revolutionary ideas about the origin of the foetal membranes and the phylogenetic history of the vertebrates in general. HUBRECHT was of the opinion that in the ancestors of the Mammalia there was only one embryonic membrane, and that has been retained as the the trophoblast. Its original function was to protect the larva, but when, in the early mammals, intra-uterine development was established, this membrane assumed the function of feeding the embryo as we see in our present day mammals. In order to function properly a connection was then established with the embryonic vascular system, this connection became the allantois.

According to HUBRECHT the ancestors of the mammals had not the large, yolk-laden eggs as found in reptiles and birds, but they were viviparous animals with small, holoblastic eggs. From such an early viviparous ancestor, the reptiles and birds might have developed as a side branch, when the small vesicular blastocyst accumulated more and more yolk in its yolk-sac, which made it possible for viviparity to be replaced by oviparity. HUBRECHT appeals to the palaeontologists to find these hypothetical Promammalia.

The palaeontologists have found them, but the mammalian ancestors are not amphibian-like animals, as HUBRECHT supposed them to be, but the reptilian *Therocephalia*. DE LANGE (1923), who accepted HUBRECHT's view in a somewhat milder form, therefore concludes that these *Therocephalia* were viviparous animals with small eggs. In view of what we know about reptiles, recent and extinct, this supposition is totally unwarranted.

Concerning the amnion, HUBRECHT was of the opinion that this was developed independently from the trophoblast, as an additional membrane in order to surround the embryo by a water cushion for further protection. One might ask, why was it then developed at all? The embryo was already surrounded by the exocoelomic cavity filled with fluid, so it had the protection of a water cushion; and, after all, the amnion is only a thin membrane sepa-

rating two fluid-filled cavities. However, as the amnion had arisen independently from the other membranes, HUBRECHT concluded that amniogenesis through cavitation, as it is shown in man, is the original way, or, maybe, the process of folding up of the embryonic disc, as exemplified by *Erinaceus*, is the most original form of amnion formation. Anyhow, the formation of the amnion and chorion simultaneously by folding, as seen in reptiles, birds and many mammals, is considered by HUBRECHT to be a secondary way of amniogenesis. On the other hand, a hypothesis which would separate the phylogeny of the chorion and of the amnion would thus, in itself, appear to be more acceptable, and might prove to be a better guide to the understanding of those mammals that have no amnion folds. The formation of the amnion as a closed cavity should be more primitive than by folding, and the trophoblast must be older than the amnion.

HILL's contention is exactly the opposite of HUBRECHT's in all aspects. HILL (1932) is of the opinion that the mammals have originated from oviparous reptiles, with large yolk-laden eggs; the Monotremes still have such eggs. Becoming viviparous, the large amount of yolk was superfluous, it was greatly reduced in the Marsupials, and, as several features of the mammalian development show, the small eggs of the monodelphic mammals are derived from the large eggs of the reptiles in accordance with the acquisition of intra-uterine development. Therefore, the closed method of amnion formation is a secondary feature, probably induced by the precocious attachment of the covering trophoblast to the uterine lining. HILL regards the acquisition of an amnio-embryonic vesicle, and the correlated closed method of amnion formation, as a purely secondary adaptive phenomenon, by no means primitive, as HUBRECHT maintained, but highly specialised. Amniogenesis by folding, in the reptilian fashion, is, according to HILL, the original form in mammals also.

I am in full agreement with HILL that the early development of all Mammalia can only be readily explained by accepting the view that they originally had large, yolk-laden eggs, like their reptilian ancestors. The question arises, however, in how far does this affect the formation of the amnion in the monodelphic mammals.

Amniogenesis in mammals can only be understood if linked up with the formation of the other embryonic membranes. The fundamental mistake of HUBRECHT was that he considered the amnion to be a later addition, acquired by the mammalian ancestors independently from the trophoblast.

Of the two membranes, the amnion and the chorion, the latter is undoubtedly the most important from a functional point of view, where ever these membranes occur. I cannot accept the often-repeated statement that the amnion is there to provide the embryo with a water cushion, and I must confess that the function of this always very delicate, a-vascular membrane is not clear to me. Yet its retention in all reptiles, birds and mammals, without exception, indicates that it has to perform some indispensable function. Any-

how, amniogenesis can only be understood in relation to the formation of the chorion.

In a previous publication (1946) I have tried to explain that the monodelphic mammals, with the yolk, have lost the reptilian yolk-sac covered by the extra-embryonic ectoderm. In reptiles and birds this extra-embryonic ectoderm is enormous in extent in comparison with the embryonic shield; in the Monotremes it is still very large; in the Marsupials it is reduced to half of the surface of the embryonic vesicle, and in the monodelphic mammals it has disappeared altogether. The mammalian trophoblast, therefore, does not correspond to the yolk ectoderm of the *Sauropsida*. In these animals the amniotic folds clearly arise within the limits of the original embryonic shield, and in Marsupials there is no doubt that these folds are formed by the embryonic half of the blastocyst. The outer layer of the fold, the chorion, although it is directly continued into the yolk-ectoderm, originally forms a part of the embryonic shield. When in the *Monodelphia* the yolk-ectoderm is completely lost, the reptilian chorion forms the whole outer surface of the young embryo. I use here the word "reptilian" purposely, because the chorion of the monodelphic mammals, as defined by MOSSMAN (1937), is more limited in extent, and does not include the ectoderm covering the mammalian yolk-sac, yet this ectoderm of the mammalian yolk-sac was originally part of the chorion of the reptiles.

When in reptiles and birds the amniotic folds are completely closed, which is achieved at a rather late period of development, the chorion, and the yolk-sac ectoderm continuous with it, are separated from the amnion and the embryonic disc. In the monodelphic mammals this separation is completed at a very early stage. The original chorion of the *Sauropsida*, and of the *Marsupialia* as well, becomes the trophoblast of the *Monodelphia* that surrounds the rest of the embryo on all sides and is completely separated from it. This early separation, no doubt, is effected because the embryo, having lost its yolk and yolk-sac, is compelled at a very early stage to absorb food from the outside. Consequently the embryo, lying in the uterus surrounded by its food, at once forms an organ for the absorption of that food and it uses the original chorion for this purpose, which, to a limited extent, already performs that function in reptiles. Although the trophoblast of the higher mammals corresponds to the reptilian chorion, as a trophoblast it does not exist in the reptiles and birds, and not even in the marsupials; in the Amphibia there is not even a trace of it; it is a new organ, formed in the *Monodelphia* in connection with the intra-uterine development.

In order to understand the amniogenesis of the mammals we first have to be clear about the fact that the original chorion, in the form of the trophoblast, is separated from the rest of the embryo, including the presumptive amnion, at a very early stage of development. In all *Monodelphia* the embryonic knot includes in principle not only the embryo itself, but also the amnion and other

membranes, except the surface layer of ectoderm or the trophoblast. There are some exceptions, to be true, some mesodermal and entodermal cells may be delaminated from the trophoblast later on, but this only shows that the separation of the trophoblast from the rest of the embryo took place before the differentiation of the tissues was finally effected. As far as the ectoderm of the embryo and of the amnion is concerned, the separation from the chorionic ectoderm appears to be complete once the trophoblast is formed, with one possible exception; according to HEUSER and STREETER (1941) the amniogenic cells are split off, or delaminated, from the trophoblast cells and not from the node cells in the macaque, but it is difficult to discern with certainty in their figures that the amniogenic cells are in the process of being delaminated from the trophoblastic wall.

With this possible exception, in all monodelphic mammals the trophoblast or chorion is completely separated from the ectoderm, including that of the amnion, at an early stage, and this trophoblast surrounds the rest of the embryo on all sides uninterrupted.

Once the chorion is established, the amnion can be formed generally in three ways: — In the first place the amniogenic cells can be separated from the embryonic ectoderm by the process of cavitation; in the centre of the embryonic knot a cavity appears, the roof of which becomes the amnion ectoderm, and the floor the embryonic ectoderm. In the second place the knot can re-establish contact with the chorion, and, either by breaking open the chorion, or by the disappearance of the chorion above it, become intercalated between the chorionic cells, whereupon the amnion is formed by folding, as in reptiles and birds. In the third place the knot can flatten out underneath the trophoblast, and then roll up to form an amnio-embryonic vesicle. The question arises, which of these is the original method amongst the *Monodelphia*.

Taking all *Amniota* into consideration, I fully agree with HILL that the amnion formation by folding is the ancestral method, but it is another matter within the group of *Monodelphia* with their early formed trophoblast. Here HILL is again of the opinion that the second method, mentioned above, is the primitive one, whereas HUBRECHT considers the first or maybe the third as the more original.

To answer this question, it does not help at all to consider which method prevails in the most primitive *Monodelphia*, that are comprised in the old order of the Insectivora, because all three methods are followed amongst them. Amnion formation by cavitation occurs in the *Centetidae* (GOETZ, 1938), and in *Elephantulus* (VAN DER HORST, 1944), the method by folding was found in *Tupaia* (HUBRECHT, 1895), in the *Chrysochloridae* (VAN DER HORST, 1948), in *Talpa* (VERNHOUT, 1894), and in *Sorex* (HUBRECHT, 1893). Rolling up of the embryonic disc underneath the trophoblast is the fashion followed by *Erinaceus* (HUBRECHT, 1889).

Neither is it of much avail to take other mammals into consideration, which are considered to be more or less primitive; in bats, as a rule the amnion is formed by cavitation (KOHLEBRUGGE, 1913, HAMLETT, 1935, e.o.); also in *Galeopithecus* (HUBRECHT, 1919); *Procavia* (STURGESS, 1948), and the *Anthroproidea*. The method of folding, on the other hand, can be observed in *Tarsius* (HUBRECHT, 1902), and in the *Lemuroidea* (HILL, 1932).

Until recently, *Erinaceus* was the only animal known in which the amnion is formed by the process of folding up of the edges of the embryonic disc underneath the trophoblast. However, WIMSATT (1944) found that in the bat, *Myotis lucifugus*, a primary amniotic cavity is formed by cavitation within the embryonic cell mass, its roof representing the primitive amnion. Later on, during the formation of the embryonic disc, the primitive amnion degenerates, and for a time the overlying syncytiotrophoblast comprises the roof of the amniotic cavity. The definitive amnion is formed then by the upgrowth of the peripheral portions of the embryonic disc, thus in the same manner as in *Erinaceus*. *Myotis* gives evidence that the *Erinaceus* method of amnion formation is not primitive, but is acquired secondarily.

So we have to decide which of the two, amnion formation by folding or by cavitation, is the more primitive method in the monodelphic mammals.

According to HILL, the closed method of amnion formation was probably induced by the precocious attachment of the covering trophoblast to the uterine lining. I cannot agree with this statement. In my opinion, the closed method of amnion formation was induced by the precocious differentiation of the trophoblast or chorion. By this early differentiation the amniotic epithelium is severed from the chorion, and is contained in the embryonic ectoblast. This is the case in all *Monodelphia*.

A separation of the amniotic and embryonic ectoderm can now be effected in two different ways:

The method of amnion formation by folding is indirect: First the original continuity between the chorion and the amnion has to be re-established, and only then can the amnion folds be formed. This ancestral method seems to be so ingrained in all *Amniota*, including the *Monodelphia*, that the latter revert to it whenever this is possible, even when they are already on the way to form the amnion by cavitation. SANSOM (1937) found that in the Indian musk shrew a primary amniotic cavity appears in the embryonic knot at an early stage, but subsequently the trophoblastic cavity, and the roof of the amnio-embryonic cavity, disappear, thus the embryonic shield is exposed and intercalated in the trophoblast, and then the definitive amnion is formed by folding. Whenever the early attachment of the covering trophoblast to the uterine lining is not an impediment, the reversal to the ancestral method seems to result. In the very primitive *Chrysochloridae* the embryo attaches itself to the lateral wall of the uterus at an early stage, but the embryonic knot is situated at the mesometrial, or the antimesometrial side, so the early attach-

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ment has no direct influence on the amnion formation, and this is done by folding (VAN DER HORST, 1948). In *Sorex* (HUBRECHT, 1893) and in *Talpa* (VERNHOUT, 1894) the place of implantation coincides with the position of the knot, but the reversal to the folding method is initiated before the embryo implants itself. As a consequence the early placenta has the form of a ring, and pari passu with the closure of the amnion folds, the placenta extends towards the centre. In these animals the open method of amnion formation resulted in a belated attachment of the trophoblast to the uterine lining.

On the other hand, the direct method of amnion formation is by cavitation. Often no histological difference between the amniotic and embryonic cells can be observed for some time, when the primary amniotic cavity has already been formed, as in *Hemicentetes* (GOETZ, 1938), in *Galeopithecus* (HUBRECHT, 1919), *Procavia* (STURGESS, 1948) and in *Elephantulus* (VAN DER HORST, 1944). This indicates that, following the chorion, the amnion is separated from the embryogenic cells at a very early stage. I agree with HILL that this method of amnion formation is a purely secondary, adaptive phenomenon, and not primitive but specialised, provided that all *Amniota* are taken into consideration. However, the *Monodelphia*, with their intra-uterine development and precocious formation of the trophoblast, are specialised. The reversion to the original method of amnion formation, should now be considered as a secondary feature, and the new method by cavitation as primary.

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