Zoology. — *The placentation of Tupaia javanica.* By C. J. VAN DER HORST. (Hubrecht Embryological Laboratory and University of the Witwatersrand.) (Communicated by Prof. CHR. P. RAVEN.)

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In 1899 HUBRECHT published a description of the placenta of Tupaia javanica. Although small additions to this description were made by DE LANGE and NIERSTRASZ (1932) and again by DE LANGE (1933), these authors fully accepted HUBRECHT's explanation of the placental structure. At the time of HUBRECHT's publication, GROSSER's (1909) classification of the various placentas of mammals was still unknown, but in 1933 DE LANGE classified the placenta of Tupaia as being haemo-chorial and this was in agreement with HUBRECHT's description.

When I had an opportunity to visit the HUBRECHT Laboratory I was anxious to study the placenta of Tupaia. Not only were all facilities for doing so given to me, but I was presented also with a number of uteri of Tupaia in various stages of pregnancy. These were later on sectioned in Johannesburg, where I could study them more at leisure.

When studying the placentation of Elephantulus (VAN DER HORST, 1950) it occurred to me that, from a morphological point of view, the placenta of Tupaia could be different from that of Elephantulus. And, as the placenta of Elephantulus fundamentally corresponds to that of Insectivores like Erinaceus and the Soricidae and also to that of the Primates like Tarsius and the monkeys, it would be of even more interest to establish the relationship of the placenta of Tupaia. In these Insectivores and Primates the placenta is either mesometrial or antimesometrial, or either dorsal or ventral in position and, what is even more important, it is formed either at the same side as the embryonic node or at the opposite side. If it is remembered that in monkeys two placentas are often present at opposite sides and that Elephantulus, with the placenta and embryo both at the mesometrial pole, forms a rudimentary placenta opposite the permanent one, then this difference appears to be of no great importance. In Tupaia, however, the embryo is directed towards the antimesometrial side, but the two placentas are formed in the lateral walls of the uterus. This might be an indication that the placenta of Tupaia is a structure totally different from that of other Insectivores or Primates. As we will see in the following pages, it really is.

As the results of my observations deviate in several, and sometimes important, respects from HUBRECHT's description, I will endeavour to give a complete description of the whole process of placentation in Tupaia as far as the available material allows, indicating, by the way, where I cannot agree with HUBRECHT.

The youngest stage, at my disposal, contains an embryo in the stage of a bilaminar blastocyst, lying free in the uterine lumen near the place where soon it would have fixed itself to the lateral walls of the uterus (fig. 1). Here, as HUBRECHT also mentions, the implantation sites are

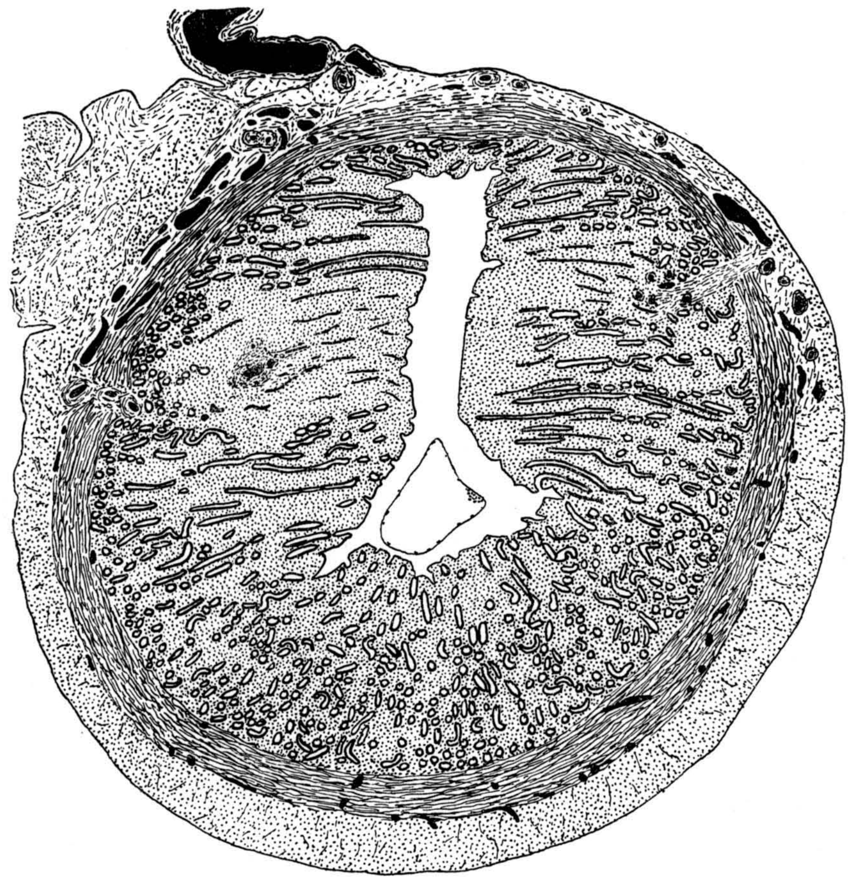

Fig. 1. Cross section of the uterus with an embryo in the early blastocyst stage, before it has attached itself to the uterine wall. × 30.

already clearly indicated before the embryo comes in direct contact with them. It is, therefore, likely that this is the only place along the length of each uterine horn where an embryo can implant. In other words, in Tupaia, like in Elephantulus, implantation is strictly localised and orientated. The implantation sites are richly vascularised; the circular musculature is even interrupted where the bloodvessels enter the endometrium. These vessels, the arteries as well as the veins, branch repeatedly after their entrance into the endometrium.

An obvious characteristic of the implantation sites is the absence of glands. HUBRECHT says that, here near the surface, the glands have become reduced (rückgebildet) and that they remain only in the deeper part of the mucosa. This statement is not quite correct. Along the whole circumference of the uterus the glands are very numerous in the deeper part of the mucosa facing the circular musculature. These glands usually continue as irregularly curved tubes through the more superficial region of the mucosa, to open anywhere in the uterine lumen. This, at the level of implantation, is particularly clear in the antimesometrial wall (fig. 1). In the lateral walls, however, the glandular tubes are deflected and form, on the whole, a simple curve round the implantation sites. Therefore, the glands here can often be seen in sections as long and nearly straight tubes surrounding the implantation area. The glands, seen below the implantation site, therefore are not reduced, nor do they end blindly, but they go in a wide swoop round the implantation area on their way to the uterine lumen.

When the embryo has developed 12 pairs of somites the two lateral placentas are well established (fig. 2) and their structure is revealed (fig. 3). Only the very first beginning of the allantois may be discerned. The yolksac, which is now trilaminar, is alone in contact with the inner

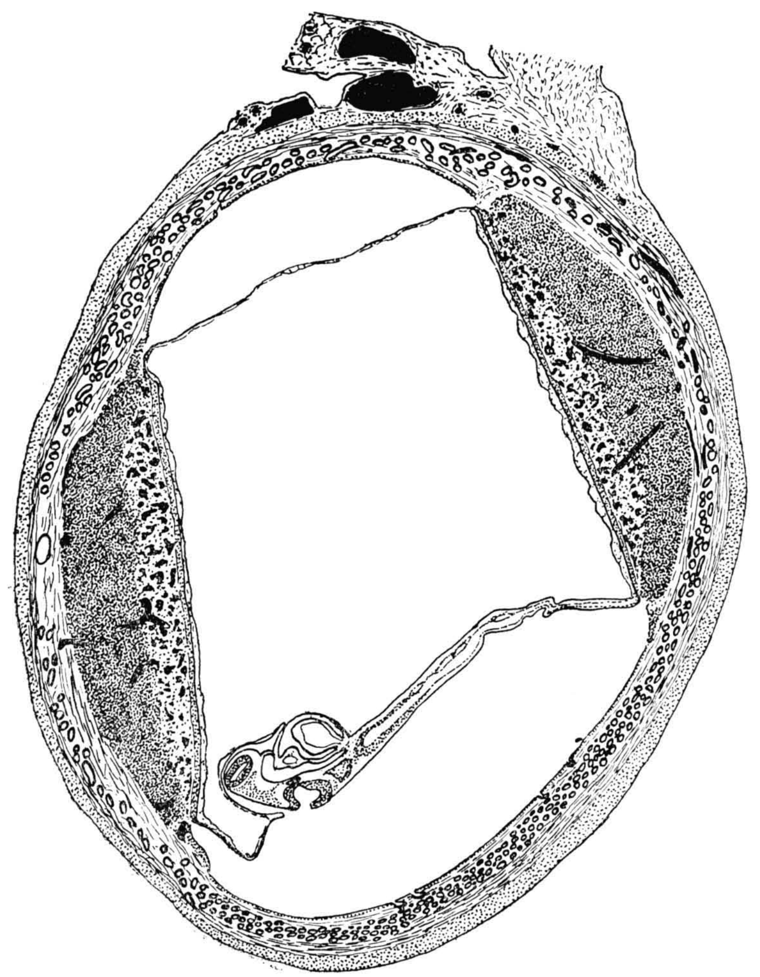

Fig. 2. Cross section of the uterus containing an embryo with 12 somites. × 20.

surface of the maternal part of the placenta and extends over the whole of it. Facing the muscularis, all round the uterus, the stroma consists of a layer of rather dense fibrous tissue in which the coiled deeper parts of the glands are embedded. Underneath the placenta there are relatively few

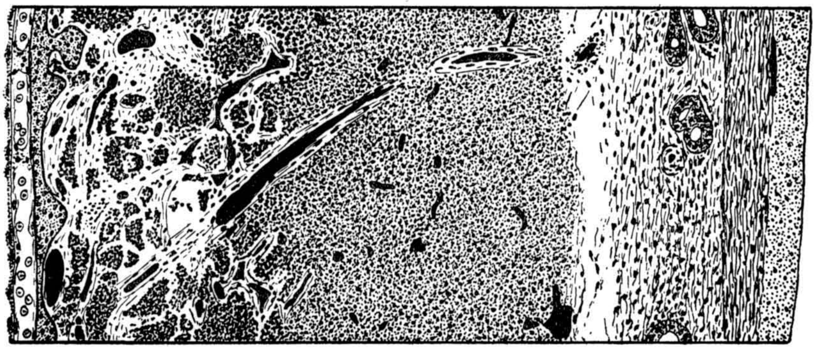

Fig. 3. Part of a cross section of the placenta of the same specimen as shown in fig. 2. The section is the next one to that shown in fig. 2. × 100.

glands, which may be caused by the stretching of the uterine wall. These glands can be traced to the circumference of the circular placentas, where they open into the uterine lumen. Underneath the placenta, at the inside of the layer of glands, the fibres become very delicate and few in number; in the rest of the uterine wall the thicker layer of glands extends nearly to the uterine epithelium.

At this stage the placentas are very obvious structures. The maternal part, called by DE LANGE (1933) the "Plazentarscheiben", is derived from the vascular tissue, without glands, the "Plazentarleisten" of DE LANGE, that can be observed before the attachment of the embryo (fig. 1). For the greater part these plaques consist of a very dense tissue, crowded with nuclei. The number of nuclei steadily increases as is shown by the great number of mitotic figures in the deeper region. It is obvious that a vigorous growth of this layer takes place. However, whereas it grows at the peripheral side, this layer is dissolved and digested on the inside. About in the middle of this layer the darkly stained nuclei congregate in larger or smaller clusters. The individual nuclei can still be discerned at this level, but more to the inside the nuclei break up and only a dark irregular mass of chromatic substance is left. Between these clusters of nuclei, or what remains of them, the tissue is very open with only a few scattered and apparently healthy nuclei, many bloodvessels are embedded in this tissue, the trophospongia of HUBRECHT.

On the inside this placenta is covered by the trilaminar yolksac. The trophoblast can be seen penetrating into the maternal tissue with short blunt protruberances, but the mesoderm, richly supplied with omphaloidean vessels, does not penetrate at all.

Concerning the bloodvessels, many arteries can be seen penetrating into the placental disc from the muscularis. These arteries, giving off several branches at all levels into the dense layer, pursue on the whole a straight course, until they reach the necrotic mass near the inner aspect of the placenta. Here they branch up into smaller vessels. A rich network of small vessels can be observed in the necrotic tissue, where they are more obvious, and in the densely nucleated tissue, where these vessels can be seen only with some difficulty. The larger veins assemble the maternal blood near the peripheral surface. Therefore, on the whole the maternal blood flows through the placenta from the inner, embryonic, side towards the periphery. The straight course of these arteries, and the fact that they give off small side branches at any level, shows that they are certainly not coiled arteries. As coiled arteries are so conspicuous a feature in the uterus of Elephantulus during early pregnancy, their absence in Tupaia is noteworthy, the more so as coiled arteries also occur in the Centetidae and in Erinaceus amongst the Insectivores and in monkeys, apes and man amongst the Primates.

In the next stage available, the length of the embryo, unfortunately, could not be measured. The allantois is in the process of spreading out over the inner surface of the placenta, here replacing the yolksac. Here and there the allantoic mesoderm, carrying embryonic bloodvessels, starts to penetrate into the placenta as blunt processes (fig. 4). It is obvious

Fig. 4. Part of a cross section of the placenta at the time when the allantois is spreading out over it. × 100.

that at this stage the densely nucleated tissue, forming the peripheral part of the placenta, has considerably decreased in thickness as compared with the earlier stage, and that notwithstanding the vigorous mitotic activity of the earlier stage. Even at this stage several nuclear divisions could be observed, indicating that active growth of this layer continues. But at a greater rate than it is formed, this layer disintegrates at its inner aspect. The dark masses of breaking down nuclei with the upen spaces between them, now occupy about the middle third of the placenta. The trophoblast has penetrated deeper and evidently absorbs the disintegrating mass. Often it is difficult to define the surface of the trophoblast exactly, because in the first place here and there some healthy maternal cells are left and in the second place the trophoblast is often less dense where it penetrates deepest (fig. 5).

HUBRECHT also described this increasing thickness of the trophoblast at the expense of the maternal trophospongia. According to him the

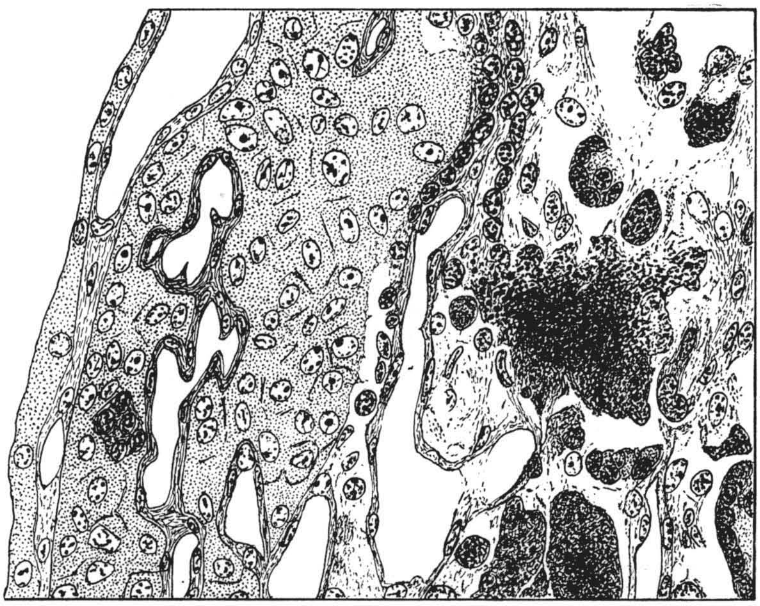

Fig. 5. A small part of the same placenta as shown in fig. 4, near the edge of the allantois. × 500.

deepest penetrations are formed by plasmoditrophoblast, whereas the cytotrophoblast is found nearer the embryonic side. It may be true that at places, where the trophoblast has penetrated deepest, the cell membranes are absent; if so then these areas are of very limited dimensions and only transitory in nature. The greater part of the trophoblast definitely is cellular (fig. 5). On the other hand, I could not observe amitotic nuclear division in the trophoblast, which was mentioned by HUBRECHT. All nuclear divisions in the trophoblast are mitotic ones. According to HUBRECHT the trophoblast cells, upon making contact with the uterine epithelium, increase in size and their nuclei become larger; he also speaks about megalocaryocytes. The trophoblastic nuclei are often somewhat larger than the nuclei of the maternal tissue, but giant cells, which are so obvious in Elephantulus, as well as in several other mammals, do not occur in Tupaia in neither the maternal nor the embryonic tissues and at no stage of the development.

The process of penetration of the trophoblast continues in the following stages that could be studied. When the embryo is 7.14 mm long, the trophoblast, well supplied now with embryonic bloodvessels, occupies more than half the thickness of the placenta. Only a thin layer of the densely nucleated maternal tissue is left at the outer surface, otherwise the whole peripheral part of the placenta consists of the necrotic trophospongia. In the next stage the embryo, in sections, is 11.34 mm long. Some isolated patches of the healthy maternal tissue are left at the periphery of the placenta and the trophospongia is reduced to a narrow layer. Otherwise the whole placenta consists of trophoblastic tissue with a network of allantoic vessels. When the embryo is 17.40 mm long, hardly any necrotic trophospongia is left.

It may be worth mentioning, by the way, that the embryonic erythrocytes are still nucleated when the embryo is 11.34 mm long, but that they have lost their nuclei at the 17.40 mm stage. There may be some relation between the final establishment of the placenta and the loss of these nuclei.

Of major importance is the fate of the maternal bloodvessels in the placenta. HUBRECHT says that the numerous maternal capillaries become surrounded by the proliferating trophoblast that penetrates into the trophospongia. After some time the endothelium of these maternal vessels should disintegrate, as was described by NOLF in Vespertilio. In this way the maternal blood should penetrate into the system of lacunae inside the trophoblast. DE LANGE (1933) accordingly classifies the placenta of Tupaia as a haemo-chorial one. It has been mentioned already that the placental arteries, upon leaving the muscularis, penetrate, in a nearly straight course, through the whole thickness of the placenta, although side branches are given off at different levels. This fact considerably facilitates the observation of these maternal vessels. It is easy to trace the course of an artery from the muscularis to deep in the placenta. As can be expected the endothelium is present in the densely nucleated, healthy, zone of the placenta and it is continuous with the endothelium of the same artery in the muscularis. In the decaying trophospongia the necrosis affects the densely nucleated tissue only, but the endothelium of the vessels is left intact (fig. 5). The endothelial nuclei have a healthy appearance. This endothelium again is continuous with that of the main arteries that penetrate into the placenta. Even the cells, immediately surrounding the side branches and further vessels of a capillary size, prove to be more resistant than the main mass of maternal tissue, although the nuclei become more pycnotic upon the approach of the trophoblast, and finally these cells succumb to the digestive influence of the trophoblast. But the endothelium of the maternal vessels remains intact even when these vessels are finally completely surrounded by the trophoblast (fig. 5). Even at later stages, when the densely nucleated tissue and finally the trophospongia have completely disappeared, the continuity of the endothelium of the radial arteries with that of the capillary network could be established without any doubt (fig. 6). Therefore, the placenta of Tupaia is not of the haemo-chorial but of the endothelio-chorial type, according to GROSSER's classification.

The dual placenta of Tupaia is different from that of Elephantulus and of Insectivores like Erinaceus and the Soricidae, on the one hand, as well as from the placenta of Tarsius and the monkeys, on the other hand, in several respects. It differs in its general position, being located in the lateral walls of the uterus, whereas the embryo itself faces the antimesomet-

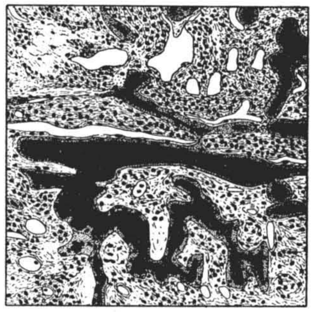

Fig. 6. A small part of the placenta associated with an embryo, at least 28 mm. long. A placental artery is shown in the centre. The maternal blood is black, the embryonic blood white. × 200.

rial pole. It differs in its histological structure, being an endothelio-chorial placenta. And finally the whole mechanism of feeding the embryo is different. In Elephantulus and the other Insectivores and Primates, enumerated above, the embryo is fed by an exchange of substances between the maternal and embryonic blood. In Tupaia the mother builds up a highly nutritive substance in the form of the densely nucleated tissue and this is digested by the embryo. There will be, of course, an exchange between the embryonic and maternal blood as well, but one gets the impression that the decaying trophospongia is the main source of food for the developing embryo.

It is generally accepted nowadays that Tupaia is closely related to the Primates, in particular to the Lemuroidea. The systematic position of the Macroscelididae, however, is held to be more dubious; if they are supposed to be related at all to the Primates, then this should be through their affinities to Tupaia. I discussed the pros and cons of this taxonomic problem in more detail elsewhere (VAN DER HORST, 1950). It is remarkable that, concerning the placenta in all its aspects, Elephantulus closely agrees with the Primates. In particular the early placenta of Tarsius structurally is hardly different from that of Elephantulus, although Tarsius has the secondary placenta of the monkeys and Elephantulus the primary one with only a rudiment of the secondary. This haemo-chorial placenta is a characteristic common to several Insectivores and Primates. Tupaia has lost this placenta and in this respect it agrees with the Lemuroidea. But Tupaia differs from the Lemuroidea in having a double endothelio-chorial placenta. The latter have a well-developed epithelio-chorial placenta with often complicated interdigitations of the maternal and embryonic tissues.

In Tupaia the uterine glands remain functional at least for a long time, if not during the whole period of pregnancy. In the oldest stage available, with an embryo at least 28 mm long, the glands do not show any sign of diminished activity and a capillary network can often be seen surrounding them. In Elephantulus, at this stage, the glands have long since disappeared. In the Lemuroidea, with their epithelio-chorial placenta, the growing embryo is fed by glandular secretion. In Tupaia the uterine glands also feed the embryo, although they supply only part of the necessary nutriment. But in contrast to the Lemuroidea, no interdigitations of the uterine and embryonic tissues have been elaborated; both the uterine epithelium and the trophoblast have a smooth surface where they are in close approximation to each other. This contact can only be considered as an epithelio-chorial placenta of a primitive type. It can be added that it is a yolksac placenta, as is clearly shown by the layer of very large entodermal cells that are separated from the trophoblast by a solid mesoderm layer. In the area of the endothelio-chorial placentas the yolksac is replaced by the allantois, but the latter does not extend further, nor does even the extra-embryonic coelom penetrate between the entoderm of the yolksac and the trophoblast in the region outside the endothelio-chorial placentas.

It may be concluded that Tupaia lost the original haemo-chorial placenta found in other Insectivores and Primates. The nutrition of the embryo by the uterine glands gained in importance as compared with Elephantulus but it did not reach the same level of perfection as in the Lemuroidea, where the uterine glands are the only source of food for the embryo. In Tupaia a double endothelio-chorial placenta has been evolved instead, a type of placenta which otherwise does not occur in the Insectivores or Primates.

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