Zoology. — "*On the placentation of Sciurus vulgaris.*" By Dr. F. MULLER. (Communicated by Prof. A. A. W. HUBRECHT).

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I. The very earliest stages. The ovule of Sciurus undergoes its first developmental stages in the oviduct. Meanwhile the bicornuate uterus has prepared itself for the reception of the ovule: underneath the single layer of epithelium the mucosa, which mesometrially remains very thin, has become very strongly thickened, so that an excentrical T-shaped slit is left open, the transverse part of which lies closest to the mesometrium. A special arrangement for the attachment of the ovules can nowhere be detected; a subepithelial zone is found to be richer in nuclei, however, than the loose connective tissue, separating this layer from the muscularis.

II. Pre-placentary stages (From the arrival of the ovule in the uterus until the first formation of the allantoid placenta).

The ovules fix themselves in varying numbers, to the right generally more than to the left, at about equal distances on the anti-mesometral (i.e. anti-placentary) uterine wall; they are fixed with their vegetative poles. A pellucid zone is absent, on the other hand the ovule becomes surrounded by a mass, formed from glandular secretions of cellular origin from the uterine wall.

The ovules grow pretty quickly, for the greater part by dilatation of the umbilical vesicle, which in these stages still forms the principal part of the ovule. It is remarkable that the area vasculosa remains so small, so that only entoderm and trophoblast form the wall of the germinal vesicle over the greater part of the umbilical vesicle.

The uterine wall shows intense activity during this stage. Many processes take place here in rapid succession and simultaneously. They all start from the spot where the ovule has settled, and from this point extend in all directions, successively reaching the spaces of the uterine horn, left open between the fixations of the ovules, as also the mesometrally situated parts; all these processes begin sub-epithelially, gradually penetrating deeper and deeper. These successive processes thus gradually give rise to dish-shaped layers of varying structure, surrounding the ovule at the anti-mesometral side and the character of which is most sharply pronounced in the points that are at the greatest distance from the mesometrium. By the extension of the anti-mesometral part of the long end of the

T-shaped slit, a broadening is brought about here, which, progressing more and more in the mesometral direction, finally produces a space, the cross-section of which presents a shape like that of a cone, truncated mesometrally by the old transverse part of the T, and bordered anti-mesometrally by a circular segment corresponding to the umbilical vesicle. The ovular chambers, formed in this way, have originated as the result of growth and extension of the anti-mesometral uterine wall, as a consequence of which the parts of the horn that connect them, are implanted at the mesometral side of the foetal chambers and at the same time are bent in this direction. The proliferation in the stroma tissue, beginning in the sub-epithelial layer, squeezes the mouths of the glands asunder. Later the epithelium in these latter degenerates, the walls agglutinate, finally only remnants of glands are found in the more normal stroma under the muscularis.

The processes by which the first formation of dish-shaped layers takes place (the existence of which is always of a relatively short duration, however) are the oedematous imbibition of the tissue and disintegration of cell-elements, accompanying the proliferation of the sub-epithelial multinuclear zone, the final result being a system of cavities, separated by thin cell-partitions and filled with the products of oedema and disintegration of tissue. This layer is externally surrounded by layers which form the transition to the still normal, although proliferating tissue, while at the opening they are more and more separated by products of a later process.

Very remarkable is the appearance at this time of "giant cells", plasma-lumps of different size, which assume a dark colour and contain many giant nuclei with a large nucleolus. Continuous layers or more isolated groups are found as individual differences, apparently. They lie mostly superficially, often connected with the trophoblast, not with the vessels. At first sight one would feel inclined to regard them as the foetal "suction roots" of the trophoblast, described for Spermophilus by REJSEK. Since all transitions are found between the mucosa tissue and these elements (in some cases even the transition having been followed up); since, moreover, they are found on the ovule, in course of being dissolved in the surrounding mass; since, on the other hand, in no case an origin from the trophoblast could be made probable, the giant cells are for this and other reasons in my opinion to be considered as a degenerative maternal formation, as a symplasm. They cannot be identified with the "monster cells" of MINOT, SCHOENFELD and others, described for the rabbit, since these elements are also found in Sciurus, only much later. Finally com-

parative-anatomical considerations plead for my opinion (see below).

The uterine epithelium gradually disappears in all places where the germinal vesicle is in contact with it. There is never question of proliferation now.

Relatively soon already (even with a very extensive material the transitions are difficult to follow) a second stage sets in, in which by proliferation of the stroma cells, beginning from the surface, a dish is formed of cubical cells with granulated plasm (decidua), which extends more and more, while the above described cavities disappear, probably by resorption under influence of the pressure. In the mean time the decidua cells at the surface undergo further alterations and are resorbed (very likely by the umbilical vesicle, since in this and in the cells of the wall a similar substance can be found), so that a fine meshy texture is formed by the peripheral part of the cells remaining; by the pressure this meshy texture is compressed to a thin layer of lamellar structure, which in its youngest parts still shows the meshes. Vessels are not or scarcely found in the decidua. The separation between the decidua and the little or not changed subdecidual tissue outside it, is the limit to which the differentiating processes in the wall have progressed, at the same time approximately the limit, marking how far the agglutination of the germinal vesicle with the wall has advanced; it may therefore be called "differentiation limit".

At this time the above described giant cells become fewer and fewer in number, have an increasingly degenerate appearance and soon disappear altogether. At the mesometral side especially by proliferation of the epithelium an increase in number and size of crypts takes place (not of glands).

A second period in these pre-placentary stages is characterised for the ovule by the origin of the amnion etc. The growing embryo seeks place in an impression of the upper part of the umbilical vesicle, which becomes more and more accentuated. At the umbilical vesicle begins, starting again at the part that is most distant from the embryo, an outgrowth of the trophoblast cells with their nuclei, which process also continually advances towards the embryonic pole.

Characteristic for this stage are different processes beginning on the ovule as well as on the uterus in equatorial bands above the differentiation limit, and from here also proceeding mesometrally on all sides. For the ovule these processes consist in a proliferation of the trophoblast which will later cover the outer layer of the amnion fold; irregularly placed, dark, polygonal cells with distinct borders appear; on the surface of the trophoblast small cell-heaps rise every-

where. On the corresponding spot of the uterine wall a progressive process sets in; first: formation of crypts by proliferation of epithelium; at the same time thickening of the intermediate stroma; later by this process a ring has been formed, which everywhere projects a little into the lumen above the differentiation limit, dividing the cavity of the foetal chamber into a mesometrally situated placentary part, and an omphaloid part situated below it, while by this arrangement the meanwhile completed diplotrophoblast (chorion) with its very strongly thickened layer of trophoblast bulges out. The hyperplastic stroma of the projecting ring is everywhere well provided with crypts.

In the meantime this proliferation process has been closely followed by a regressive one; the epithelium begins to degenerate, first at the surface, later deeper and deeper in the formed crypts; plasm and nuclei become darker, more homogeneous, smaller; later the pycnotic nuclei dissolve in the plasm and a mass is formed, epithelial symplasm, in which finally greater and smaller vacuoles are evenly distributed. Everywhere short ramifications of epithelium have penetrated into the stroma, which soon degenerate. Also the stroma itself undergoes similar alterations later.

Wherever this degeneration has somewhat advanced, a third process sets in, likewise extending as a band: the thickened trophoblast penetrates with its ramifications some distance into the crypts, later also between these into the degenerated mass. Here and there the foetal mass thereby changes, after its elements have become enlarged and paler, into a syncytium, the nuclei of which contrast well with those of the maternal symplasm. In this connecting ring the syncytium soon disappears again; extension causes the trophoblast with its hollow ramifications, penetrating into the crypts, to become a single layer once more; exactly here the area vasculosa still remains for a time in connection with the trophoblast: everything pleads, in my opinion, for the hypothesis that this has to be considered as a rudiment, namely of an omphaloid placentation (Sorex, which among the Insectivora stands nearest the Rodents, shows a distinct omphaloid placentation).

The products of the crypts and glands, transsudates and symplasm masses, are shed over the cupola of the diplotrophoblast and probably are resorbed by this latter.

The vessels in the meantime proliferate strongly in the wall of the mesometral part of the foetal chamber between the crypts, which has remained unchanged yet; their wall consists as before of simple endothelium, without a perivascular sheath.

III. Placentary stages (After the beginning of the formation of the allantoid placenta). In the omphaloid part of the foetal cavity the wall is more and more attenuated by extension and resorption of tissue, although the layers may be recognised as before. The increase in size of the trophoblast cells of the umbilical vesicle, which had set in formerly, now leads to the formation of true "monster cells", the cellular body of which often shows concentric rings and other peculiarities, while the big nucleus often lies like a crescent round a vacuole. This process comes nearer and nearer the mesometrally situated formations. The entoderm, covering these monster cells, is very narrow and small-celled; where it covers the area vasculosa, it consists on the other hand of cubical, strong cells. In the umbilical vesicle a coagulated mass is always present. The large embryo more and more invaginates the upper part of the umbilical vesicle. Between the monstercells and the entoderm a sort of cuticle develops.

The processes, extending in equatorial bands, continally advance towards the mesometral pole of the foetal chamber, also in the partitions of the foetal chambers, so that they are more and more incorporated by these latter. In this manner extremely complicated pictures are formed, especially in cross-sections.

The dilatation now affects very strongly as well the placentary part of the foetal chambers as their mutual connecting pieces, so that the omphaloid part becomes smaller and smaller, while the formerly existing comb-shaped division between them disappears.

The progressive process finally reaches the mesometral pole of the placentary space and continually advances further into the connecting pieces of the foetal chambers: the still intact part of the wall, which at first had the shape of a cupola, later assumes the form of an 8, finally reduced to two round planes, which by the proliferation are more and more limited to the connecting pieces. The progressive process now forms crypts, which in other places are narrow and deep, but in the place of the placenta are broad and wide by dilatation and excessive proliferation of the stroma. The epithelium has many layers, its surface still rises everywhere in papillae. In the stroma not all the cells reach their full development as decidua cells simultaneously, so that a peculiar reticulated aspect is produced. Also the vessels increase.

In this soil now the degenerative process occurs, again advancing centripetally towards the mesometral pole. The epithelium becomes a symplasm, exactly like that described above, but this time more abundant and, everywhere covering the trophoblast. In the stroma

a conjunctival symplasm is formed, and, as was the case in the hyperplastic process, not everywhere simultaneously, so that small partitions of symplasm still surround more healthy groups. Outside the placentary trophoblast all this goes on until a single mass of epithelial and conjunctival symplasm is formed; within reach of the placentary trophoblast, however, the trophoblast has already penetrated before that time. During this degeneration also vessels are opened, so that extravasates are not rare now and altered blood is found against the trophoblast.

The third process by which the trophoblast is connected with the uterine wall, consists in the formation of hollow, one-layered invaginations in the crypts, which trophablast papillae are covered by caps of symplasm; between the crypts the trophoblast is extremely thin by extension, often irrecognisable, also when later the formation of giant cells had advanced thus far: these latter are then very long and narrow.

Finally the placentary trophoblast (which now forms if it were the keystone of the cupola of the diplotrophoblast and consists of a distinct basal layer of cells of one cell thickness and an often 20 cells thick layer above it) now lays itself everywhere against the papillae of the mesometral cupola of the placentary foetal chamber cavity, which papillae are in progress of being degenerated; the trophoblast papillae are likewise still covered by the symplasm, when between them this has already been resorbed.

Next comes the formation of a foetal syncytium from the superficial layers of the thickened placentary trophoblast, the process beginning above and centrally and proceeding centrifugally downwards; the nuclei which at first were dark and small, become larger and clearer, contain one big nucleolus and are clearly distinct from all maternal elements. This syncytium everywhere penetrates into the maternal tissue in strands, so that an intimate interweaving of maternal and foetal tissue results, proceeding centripetally into the papilla. Then everywhere "vacuoles" are formed in this mass (probably now for the first time at the expense of maternal tissue), which, when they become larger, bend the basal trophoblast layer (cytotrophoblast) inwards and finally fill with maternal blood. The allantois has meanwhile penetrated into the trophoblast papillae and is divided into small lobes by the growth of pairs of bulges of the cytotrophoblast. Foetal vessels soon penetrate freely into each lobule.

The primordium of the placenta as a whole has no round shape, the edges facing the connecting pieces of the foetal chambers are

concave to these sides, corresponding to the shape of the surface, here occupied by the progressive and regressive processes.

Gradually all the maternal tissue is replaced by foetal, so that finally the papillae which at first were entirely maternal, have become entirely foetal. Now the "vacuoles", surrounded by foetal syncytium dilate further (also grow at the expense of a foetal symplasm which now forms everywhere) and subdivide, a process, accompanied by constantly increasing separation of the allantoic villi by these cavities, containing maternal blood; the final result is that papilla-shaped lobes with secondary lateral lobules are formed, all separated by allantois-strands with foetal vessels; these are surrounded by the cytotrophoblast, which in its turn surrounds the "vacuoles" (now identical with cavities, containing maternal blood), enclosed by a layer of syncytium which at first is broad, later becomes gradually narrower. The placenta, originated in this way rests on a substructure of maternal tissue, composed of the same elements as formerly (decidua, etc.); the decidua-cells often grow out strongly, while the border between foetal and maternal tissue is in many places marked by a narrow streak of symplasm. The formation of trophoblastic giant cells gradually reaches also the supra-placentary parts, so that here also the enormous cells (later often free) lie in the maternal tissue.

Outside the placenta a stage soon is reached in which the progressive and regressive processes, described above, have attained their extreme limit. Superficially all has been changed into symplasm, only in the depth decidua-cells still exist, which meanwhile, since the degeneration does not reach to this depth, have become pretty large. The parts of the mucosa spared by these processes, are only the mucous membrane of the dilated connecting pieces, now entirely incorporated in the foetal chambers and whose mucosa, attenuated by extension, only possesses crypts still, that are squeezed flat, and a rather thick epithelium which for a part turns into symplasm. Against all these extra-placentary parts lies the extra-placentary trophoblast, now consisting entirely of giant cells which at present often get loose and then lie freely amid the decidua.

The embryo has, during its further growth, found place in the umbilical vesicle which gradually has become entirely invaginated and whose walls almost touch each other. The edges of the bowl, thus originated and containing the embryo, are not formed by the sinus terminalis; this latter lies further down in the inner wall. The small space in the umbilical vesicle is still filled with coagulating masses, while the entoderm, covering the area vasculosa, which now

often forms papillae, has still a very healthy appearance. The outer wall of this bowl never disappears. In its further growth the placenta reaches the edges of the bowl of the umbilical vesicle, later still it grows into it and coalesces with the inner wall: the sinus terminalis then lies halfway the thickness of the placenta, while a fold of the endoderm seems to have been incorporated into it.

In the last period of pregnancy, from the above described parts, left free by the progressive and regressive processes, epithelium grows between the degenerated and the normal part of the mucosa, perhaps joins with the meanwhile proliferating glandular remains in the depth: the umbilical vesicle is lifted off from the mucosa. Somewhat later this begins also all round the placenta, so that at the end of pregnancy this organ is more or less stalked and after parturition the greater part of the uterine wall is already provided with a new epithelium.

Comparative considerations. Among Rodents the investigation of the times at which various processes and organs of the ovule (not of the foetus) are found, leads to the following series: Sciurus — Lepus — Arvicola — Meriones — Mus — Cavia, in which the first has retained the most primitive forms, Lepus in many respects forms à transition to the last, in which more and more by new processes coming to the fore, the old, primitive ones are supplanted, mixed up and altered, in a word become nearly irrecognisable. Of this latter fact the study of the literature on the relation of ovule and uterus in Rodents, gives sufficient evidence; it also appears here how great a support is afforded by a comparative anatomical investigation; even, that various problems cannot be solved without its assistance.

The progression appears clearly in the pecularities of the umbilical vesicle in the various animals: in all the upper part is invaginated into the lower, with Sciurus not until late, with Cavia the process is among the first; the distal wall always remains with Sciurus, with Lepus it disappears late, with Cavia already quite at the beginning; the endoderm covers the inner wall already very early in Sciurus, very late in Mus, never entirely in Cavia.

In the same order the antimesometral fixation and the allantoid placenta occur earlier and together with these the trophoblast thickening, which causes them. It is exactly the remarkable pre-placentary processes which have been so carefully studied with Mus and Cavia, which by this replacing present the greatest difficulties.

With all Rodents the vegetative ovular pole becomes connected

with the anti-mesometral wall of the uterus. This connection only ends in Sciurus towards parturition, in Mus and Cavia already very early, in Lepus at an intermediate stage, by epithelium being pushed underneath from the connecting pieces of the foetal chambers.

In this fixation the umbilical vesicle is surrounded by proliferating mucosa tissue which later degenerates and is dissolved and resorbed by the ovule. The epithelium soon disappears after slight progressive changes, the stroma changes into decidua by very strong proliferation which in Mus, Cavia, etc. rises as reflexa round the ovule, corresponding with the smallness of the umbilical vesicle and consequently of the ovule. In accordance with an existing inclination, in the order of the above mentioned series, to replace nutrition by stroma products by maternal blood, the vascularisation of the decidua is very small in the squirrel, very strong in Cavia and correspondingly the extravasates, surrounding the ovule are very rare in Sciurus, common and abundant in Mus and Cavia.

In these processes in Sciurus maternal giant cells appear (symplasm) and later foetal ones, when the former have disappeared. In Lepus SCHOENFELD and others found the foetal giant cells (monster cells) already in earlier stages, in accordance with our series; all the cells then occurring are by him considered as foetal; probably, however, the maternal cells occur at the same stage and part of the described cells are of maternal, symplasmatic origin. With Mus both were found and distinguished by JENKINSON at much earlier stages, KOLSTER did not see the foetal ones, DUVAL not the maternal ones. So they must occur still earlier in Cavia; the foetal ones are then probably the proliferating "Gegenpolcellen" of v. SPEE, which perforate the zone at the vegetative pole; the maternal ones correspond to the products of the processes in the "Implantationshof" of v. SPEE. Also the disappearance of these formations takes place at an increasing rate (By all this it becomes clearer still that the comparison of Cavia and man by v. SPEE, which already from a phylogenetic point of view is hazardous, must be received with caution).

In the light of the comparative investigation these foetal "monster cells" may be considered as rudiments of an organ which was strongly developed in the ancestors of the Rodents.

In Sciurus the mass surrounding the ovule ("coagulum") consists especially of tissue products; these become less prominent in the order of the series and are replaced by blood.

Of the omphaloid placentation, which in Sciurus is already rudimentary, not much can be expected in the other members, although the study (until now neglected) of the morpho logy of the extra-

the Wolffian duct grows forth and takes its course archwise through the mesorchium in the testicle. (Fig. 4 *w. g.*). Here is brought about in one place (Dasyurus) a connection with the future spermatic tubes, which are still present in the stadium of solid cords of cells.

The mesonephridial tubules disappear almost quite, so that at a certain stadium (Dasyurus viverrinus 53 m.m.) the Woffian duct, strongly grown forth in length, runs twisting through the mass of tissue, which must be considered as the epididymis, without any appearance of tubules in the form of the coni vasculosi.

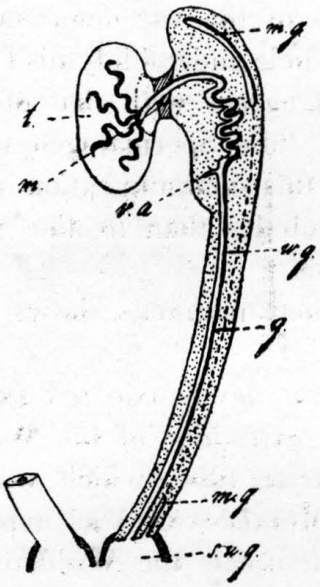

Fig. 4.

Relation of the genital ducts to the testicle.

t. Testicle.
m. Mesorchium.
m.g. Remnants of the Müllerian duct.
w.g. Wolffian duct (vas deferens).
g.s. Genital cord.
s.u.g. Sinus uro-genitalis.
v.a. Vas aberrans.

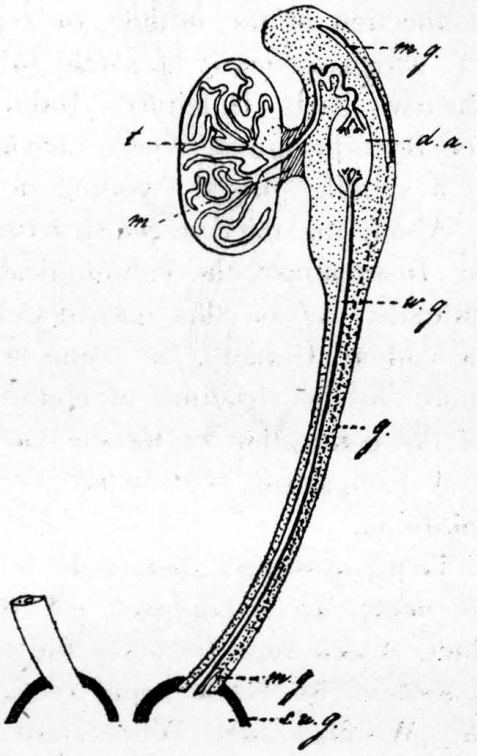

Fig. 5.

Relation of the genital duct to the testicle.

t. Testicle.
m. Mesorchium.
m.g. Remnants of the Müllerian duct.
d.a. Glandule part in the epididymis.
w.g. Wolffian duct (vas deferens).
g.s. Genital cord.
s.u.g. Sinus uro-genitalis.

Meanwhile the Müllerian duct is for the greater part reduced. The cranial extremity remains as a remnant of the duct either beginning with an ostium abdominale or not, and ending caudally blind in the epididymis tissue.