Early developmental stages of Manis Javanica Desm.

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G. J. VAN OORDT.

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PROEFSCHRIFT

TER VERKRIJGING VAN DEN GRAAD VAN DOCTOR IN DE PLANT- EN DIERKUNDE AAN DE RIJKS-UNIVERSITEIT TE UTRECHT, OP GEZAG VAN DEN RECTOR MAGNIFICUS Dr. W. VOGELSANG, HOOG-LEERAAR IN DE FACULTEIT DER LETTEREN EN WIJSBEGEERTE, VOLGENS BESLUIT VAN DEN SENAAT DER UNIVERSITEIT TEGEN DE BEDEN-KINGEN VAN DE FACULTEIT DER WIS- EN NATUUR-KUNDE TE VERDEDIGEN OP VRIJDAG 18 MAART 1921, DES NAMIDDAGS TE 4 UUR, DOOR

GREGORIUS JOHANNES VAN OORDT,

GEBOREN TE ARNHEM.

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MEDEDEELINGEN UIT HET EMBRYOLOGISCH INSTITUUT
VAN HET HUBRECHT-FONDS
N°. III

EARLY DEVELOPMENTAL STAGES OF MANIS JAVANICA DESM.

BY

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EARLY DEVELOPMENTAL STAGES OF MANIS JAVANICA DESM.

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INTRODUCTION.

In the comprehensive collections of embryological material, brought together by the late Prof. Dr. A. A. W. HUBRECHT in the end of the last and the beginning of this century, a great number of pregnant uteri and embryos of *Manis javanica* DESM. are to be found. These *Manis*-specimens, not yet being investigated, (HUBRECHT himself published only some short communications on *Manis*) Dr. DAN. DE LANGE JR., Director of the HUBRECHT-Laboratory at Utrecht, put a part of the collection of *Manis*-embryos at my disposal in order to examine the early developmental stages.

There are very few publications on the early ontogenetic processes in *Manis*. Some contributions to the knowledge of placentation have been published. Only a few communications of HUBRECHT, treating early developmental stages, are known to me. A concise description of the placenta was that of SHARPEY (1864) in HUXLEY's „Elements of comparative Anatomy", which was elaborated by TURNER by means of investigations on the same object. In 1878 follows the not quite correct description of the placenta by ANDERSON.

The development of certain organs (scales, mammary gland, hand-skeleton) and the placentation of *Manis javanica* and *Manis tricuspis*, were described in detail by M. WEBER (1891). The youngest embryo of *Manis javanica* having a length of 8.25 mm., no observations could be made on the first developmental stages.

RESINK (1903) too mentions something about the placentation after an examination of the preparations of WEBER.

First HUBRECHT gave a short communication on *Manis javanica* in his „Spolia nemoris" (1894), in which were reproduced (figs. 42—45) two older embryos with foetal membranes.

In HUBRECHT's paper entitled „Early ontogenetic phenomena in Mammals etc." (1908) we also find some observations on *Manis*. In the first

place a reproduction of an embryonic knob with hypoblast is given (plate C, fig. 18, erroneously attributed to *Galeopithecus*) and later on HUBRECHT mentions (pp. 32 and 34) that the „protochordal plate" and the „annular zone of proliferation" are also well developed in *Manis*. On p. 114 a few notes on the placentation follow.

In 1910 the above-mentioned, misnamed figure is reproduced and also some sketches are given of young segmental stages (figs. 5*a*, 5*b* and 6) to explain his opinion, at that time, on the origin of the trophoblast. This view is repeated in his posthumous work on the early developmental stages and placentation of *Galeopithecus* (1919) From the above it follows that the early development and the placentation of the only genus known of the Pholidota have been insufficiently investigated.

Material and methods.

One hundred and eighty uteri of *Manis javanica*, which HUBRECHT received between 1891 and 1902 from different regions of the Malayan Archipelago, are to be found in the HUBRECHT-Laboratory.

They were collected by different persons. Not all uteri are pregnant. Of about 106 this could be ascertained, most of the others being virginal or puerperal. The uteri, with ovary and oviduct, were fixed in toto in picro-sulphuric acid (KLEINENBERG's mixture)[1]) immediately after death.

After having been transferred into alcohol 90 %, the uteri were forwarded to the Zoölogical Laboratory of the University of Utrecht and examined here.

In the year 1907 (and earlier) those oviducts and uteri were sectioned, which, judging from the state of the corpus luteum, probably contained very early developmental stages. After staining and embedding in paraffin, the whole oviduct and the uterus were sectioned and examined till the ovum was found. Also some older embryos, visible macroscopically, were sectioned.

In the beginning of this investigation (September 1918), only a few

[1]) In the catalogue this is not always mentioned, but in HUBRECHT's „Spolia nemoris" (1894) it is stated (p. 82).

embryos (N°. 64 and 68) and early developmental stages (N°. 151, and 164) needed to be stained and sectioned.

A number of other oviducts and uteri were examined, but nothing was found in them. All sections of the *Manis*-embryos have a thickness of 10 μ. The figures reproduced are drawn at a relatively high magnification (the reconstructions excepted) with the aid of the camera lucida or of the large projection-apparatus of ZEISS, which is to be found in the Anatomical Laboratory of the University of Utrecht [1]). Most of the drawings are greatly reduced in reproduction.

As is known, *Manis* brings forth only one young at a time. *Manis* has an uterus bicornis with a relatively short uterus-body. The ovaries are lying free. Sometimes both horns of the pregnant uterus are swollen. It then seems, as if we have to do with a case of twins, but this is not so, the foetal membranes of the embryo extending in both horns (to compare M. WEBER 1891, fig. 49).

As is mentioned the HUBRECHT-Laboratory (in which all the collections of the late Prof. HUBRECHT are to be found) possesses about 106 pregnant *Manis*-uteri. Three uteri, however, contain no embryo, though there are rests of the blastocyst. For this reason the collection of *Manis*-embryos is, so far as I can ascertain, composed of 103 specimens. A relatively small part of them consists of early stages (to compare table 1).

It was to be expected a priori that this series would not be complete. A few cleavage-stages are present. The formation of the hypoblast can be traced in two specimens. Two embryonic areas are in the primitive streak-stage. Embryos, in which a relatively small number of somites is formed, are well represented.

It is not my intention to describe these different ova one after the other, according to age. On the contrary, after description of the early segmentation-stages, the origin of the hypoblast will be treated in the above-mentioned ova. After a concise description of the surface-views of the embryonic areas, a chapter will follow, in which the formation of the mesoblast will be traced, and finally the origin and development of the notochord will be described in detail.

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[1]) I wish to express my thanks to Prof. A. J. P. VAN DEN BROEK, whose kindness enabled me to use this apparatus.

TABLE 1.

Cat. N°.	Stage	Plane of sectioning	Collected at	Year	Fixation	Stained in
51	unsegmented ovum in oviduct.		Benkajang (West-Borneo)	?	picro-sulphuric acid	haemalun.
164	" " " "		Muntok (Banka)	1900	"	paracarmine.
171	3-cell stage " "		" "	1901	"	iron-carmalun.
44	4-cell stage " "		Solok (Sumatra)	1891	"	haemalun.
87	blastocyst with embryonic knob in utero.....		Djember (Java)	1892	"	"
113	blastocyst with embryonic knob in utero.....		Benkajang (West-Borneo)	?	"	iron-carmalun.
151	primitive streak-stage.....	transverse	Muntok (Banka)	1899	"	iron-haematoxylin-eosin.
180	" " "	sagittal	" "	1900	"	iron-carmalun.
83	4 à 5 pairs of somites.....	sagittal	Moeara Laboe (Sumatra)	1891	"	"
64	8 à 9 " " "	transverse	Benkajang (West-Borneo)	?	"	haemalun.
32	10 à 11 " " "	"	Padang Pandjang (Sumatra)	1891	"	iron-carmalun.
89	13 " " "	"	Benkajang (West-Borneo)	?	"	"
31	13 à 14 " " "	"	Banka	1891	"	"
108	13 " " "	sagittal	Benkajang (West-Borneo)	?	"	"
68	16 " " "	transverse	" "	?	"	iron-haematoxylin-eosin.
53	18 " " "	"	" "	?	"	iron-carmalun.
	Moreover:					
38	rests of blastocyst present, no embryo.	—	Buitenzorg (Java)	1891		
109		—	Benkajang (West-Borneo)	?		
179		—	Muntok (Banka)	?		

Then a theoretical part will follow, in which the so-called process of gastrulation in Mammals (and in Vertebrates in general) will be treated and after this a comparative part, in which the literature will be discussed, so far as necessary in connection with the descriptive part.

I wish to express my sincere thanks to Prof. Dr. H. F. NIERSTRASZ for his many critical remarks in reading the manuscript and to Dr. DAN. DE LANGE Jr., Director of the HUBRECHT-Laboratory, not only for his kindness in putting the *Manis*-embryos at my disposal, but also for his usefull help and valuable advice during this investigation.

Terminology.

Before describing the different developmental stages, I will first give a survey of the terms to be used in this investigation. In the literature dealing with the early ontogenetic phenomena in Mammals, so many different names are used, especially in connection with the formation of the germinal layers and the so-called process of gastrulation, that it is certainly recommendable to begin with inserting a list, arranged in such a way that the reader will know at once, what standpoint is taken by the author with regard to the different questions. In the following list I will use the detailed survey of „Termini technici" given by C. RABL in his paper on „EDOUARD VAN BENEDEN" (1915), especially there, where synonyms are mentioned. Terms generally used or self-evident will be omitted in order to be concise.

Gastrula, gastrulation. Strictly speaking, HAECKEL's definition („Gastraeatheorie" 1874) is not applicable to Mammals, because I think that in the didermic blastocyst of Mammals (consisting of epiblast and hypoblast) no primitive mouth in HAECKEL's sense is formed. In the other Vertebrates too, except in *Amphioxus*, no gastrulation can be distinguished, because in the didermic embryonic stages no aperture, the blastopore, communicates with the exterior. The formation of the gut has not come to an end after the formation of the hypoblast in Vertebrates. The alimentary tract consists of archenteron, formed during protogenesis and of metenteron, formed during deuterogenesis (ASSHETON 1905, 1909).

Epiblast, the outer layer of the didermic blastocyst, not yet differentiated. An embryonic and extra-embryonic epiblast (= trophoblast) can be distinguished. Epiblast = ectoderm.

Hypoblast, the inner layer of the didermic blastocyst, generally formed by delamination (immigration in Marsupials, HILL 1910, HARTMAN 1919,) out of the embryonic knob. It forms the lining of the archenteron. Synonyms: „Dotterentoblast" (BONNET e. g. 1920), lecithophore (VAN BENEDEN), paraderm (VON KUPFFER 1882), caenogenetisches entoderm (WENCKEBACH 1891), subgerminal layer. An extra-embryonic hypoblast also occurs.

Mesoblast, the tissue lying between epiblast and hypoblast and formed in several places of the germdisc.

Epi-, meso- and hypoblast are topographical conceptions.

Blastocyst, the generally bladder-shaped stage, consisting of trophoblast and embryonic knob, in Mammals, excepting Monotremata. Synonymous with blastodermic vesicle.

Trophoblast (HUBRECHT 1888), the part of the blastocyst, not participating in the formation of the embryo. Except for the part lying on the embryonic knob, (the so-called RAUBER-layer (RAUBER 1875)) it is an organ, which takes part in the nutrition of the embryo. Therefore DUVAL calls it ectoplacenta. Trophoblast = couche enveloppante (VAN BENEDEN 1899*b*).

Embryonic knob (*Embryonalknoten*), HUBRECHT 1890, the inner mass of cells in the wall of the blastocyst. The germdisc will derive from it. It is generally covered by a part of the trophoblast, which disappears later on and is called RAUBER-layer (RAUBER 1875), when distinguishable from the rest of the trophoblast.

Germdisc, *embryonic shield*, *embryonic area*, the part of the blastocyst, derived from the embryonic knob, when the latter stretches, comes to lie superficially and consequently is not entyped. After anlage of the medullary groove and somites the embryo proper is formed.

The *blastocyst-cavity* (blastocoel (HUXLEY), subgerminal cavity) originates from the compact morula by absorption of fluid from the uterus-lumen. After formation of the hypoblast it becomes entirely or partly the umbilical vesicle or yolksac, from which the archenteron derives.

Archenteron, the anterior part of the gut of the Vertebrates, formed during protogenesis, its lining being formed by the hypoblast (= Dotterentoblast BONNET). It is called „Ergänzungshöhle" by BONNET (1920).

Metenteron, the posterior part of the gut, connecting itself in all

Vertebrates with the archenteron, and formed by overgrowth and ingrowth of cells at the dorsal deuteroporic lip (= blastoporic lip of earlier authors) during deuterogenesis. It is the lumen in the head-process of the Amniotes.

A *blastopore* occurs, in the sense, which HAECKEL attached to it („Urmund" in „Gastraeatheorie" 1874), only in *Amphioxus* amongst Vertebrates. It is not developed in Craniota (= „blastopore virtuel", BRACHET 1902), because here the metenteron, not the archenteron, communicates with the exterior by the *deuteropore* or *somatopore*, the latter being still often called blastopore.

Protogenesis (ASSHETON 1905, 1909) the first developmental process of the embryo in which, among others, the archenteron is formed („the production of a radial symmetry, due to growth from one centre involving gastrulation", ASSHETON 1909, p. 240). The *cephalogenesis* of HUBRECHT and BRACHET's *acrogenesis* and *cephalogenesis* (1914) taken together express about the same.

Deuterogenesis (ASSHETON 1905, 1909), the following second developmental phase of the embryo, in which, among others, the metenteron is formed by overgrowth and ingrowth (invagination) of cells (= *notogenesis* HUBRECHT and *cormogenesis* BRACHET (1914)). „Deuterogenesis is growth in length, bringing about bilateral symmetry", (ASSHETON 1909, p. 240).

Headprocess of the primitive streak (KOELLIKER 1879) = Kopffortsatz of the German authors (also called *chorda-anlage*), the name used in the descriptive part of this investigation for the homologue of the wall of the metenteron in Mammals (Birds and Reptiles). It is a prolongation of the primitive streak in the direction of the future head-region of the embryo. The part of it, formed in later stages in the region of the primitive streak and from it, I call, for simplicity's sake, headprocess too. Synonyms: Urdarmstrang (BONNET 1920), Mesodermsäckchen (O. HERTWIG 1903), wall of the archenteron, Urdarm, etc.

Lieberkühn-canal. (LIEBERKÜHN 1882) the name used in the descriptive part of this investigation for the metenteron (= canal archentérique v. BENEDEN 1912, Urdarmlumen, also chordal canal (the latter *not* used by me in this sense), sometimes called neurenteric canal, which is not correct, because this canal in Anamnia is found in

older stages, then forming the connection between medullary tube and posterior part of the gut, and lying behind the anal plate.

Hensen-knob (HENSEN 1876) = Gastrulaknoten or Primitivknoten (BONNET 1889), protochordal wedge (HUBRECHT 1890, 1902), the thickened part of the headprocess, where it originates from the primitive streak.

Primitive streak (PANDER 1817, VON BAER 1837), the term used in the descriptive part for the proliferation of cells, lying in the median line in the posterior region of the embryonic shield. The greater part of the mesoblast originates from it; the hypoblast sometimes fuses secondarily with it. From the primitive streak, during the growth in length of the embryo, the organs are formed from before backwards. It is the homologue of the changed deuteroporic lips of the Anammia (blastoporic lips in the old sense) and is called by HIS „Achsenstrang", by BONNET „Urmundleiste" or „Gastrulaleiste".

Primitive groove, the longitudinal groove in the middle of the primitive streak (= „Gastrulagrube" BONNET).

Chordal plate, the stage of the chorda-anlage, which develops out of the headprocess after incorporation of the latter in the hypoblast, and which occupies the roof of the primitive gut as a broad, flat plate. After separation from the hypoblast, the definite *notochord* is formed.

Prochordal plate (= protochordal plate, HUBRECHT 1890 and later, Ergänzungsplatte, BONNET 1901, 1920, Endodermale Zwischenplatte, v. DAVIDOFF 1899), the thickened plate of the hypoblast, lying in front of the anterior end of the headprocess, which fuses later on with it. The cephalic mesoblast originates from it. According to BONNET the anterior part of the notochord, the hypoblastic layer of the primary pharyngeal membrane and the cephalic mesoblast are derived from it. According to HUBRECHT and HEAPE (1883) only cephalic mesoblast and the anterior part of the notochord are derived from it.

Annular zone of proliferation (HUBRECHT 1890), the peripherally situated, ring-shaped part of the hypoblast, from which the peripheral mesoblast originates. It is called by BONNET „Mesoblasthof" (sheep 1884), by KOLLMANN 1884: „Randkeim" or „Akroblast".

Cephalic mesoblast and *peripheral mesoblast* are two of the four parts composing the mesoblast, both originating from the hypoblast, respec-

tively from the prochordal plate and the annular zone of proliferation. Both are protogenetic and are called by SCHLATER primary mesoblast (1907).

Peristomal and gastral mesoblast, the two other, deuterogenetic mesoblast-parts, respectively formed out of the deuteroporic lips (= blastoporic lips in the old sense, primitive streak of the Amniotes) and metenteron-wall (= headprocess). They are also called axial mesoblast, and by SCHLATER (1907) secondary mesoblast.

Chordal canal, the canal situated in the part of the headprocess, which is formed from the primitive streak, when the embryonic shield becomes longer (the „Primitiefstreifenteil des Chordas" of the German authors). It moves caudalward during the growth in length of the embryo. It is probably to be homologized with the neurenteric canal of the Anamnia.

Archamnion-cavity, the cavity occurring in some Mammals, from which the amnion-cavity is derived directly. This is called by BONNET a „Schizamnion", contrary to „Faltenamnion", formed by means of folding.

DESCRIPTIVE PART.

I. *Early segmentation-stages.*

In the collection of *Manis*-embryos there are, besides the cleavage stages, two unfertilized or unsegmented eggs, indicated in the catalogue by N°. 51 and N°. 164, both situated in the oviduct.

The one N°. 51 (fig. 1, plate I) occupies the centre of the right oviduct. It has a diameter of ± 70 μ, and is surrounded by a great number of dark-stained nuclei. These nuclei are also to be seen on the egg-membrane. I have not been able to find the nucleus of the ovum. The large nucleus (*n'*) in the centre of the egg cannot be considered the nucleus of this cell, but is, like all the other nuclei reproduced in fig. 1, one of the follicle-cells, which pass into the oviduct with the ovum, when the follicle bursts. In describing the cleavage stages, I will speak at greater length about the signification of this covering. It is not at all remarkable that the nucleus of the egg-cell is not visible. In most of the early stages of Mammals the nucleus is scantily provided with chromatin, due to the chromatin-emission; gradually the nuclei of the blastomeres become richer in chromatin and owing to this, can be stained more deeply. The nuclei of the follicle-epithelium are well stained. Only a few of them lie against the egg-membrane, most of them at some distance of the ovum.

The ovum N°. 164 being fixed badly, I omit a further description.

The cleavage stage is represented by two specimens. The younger of the two is reproduced in fig. 2, plate I. This ovum, lying in the left oviduct, is probably a three-cell stage: in the successive sections only three nuclei are visible. The lower part of the ovum shows one nucleus in each of the two sections, at a distance of about 30 μ from each other, which points to two cells. Between both nuclei a cell-membrane is not to be found, probably owing to the membrane running parallel with the sectional plane.

The third nucleus is present in the upper part of the ovum. This one is not oval in shape like the other nuclei, but oblong and contains two dark-stained bodies. The suppositon is obvious that this cell will soon divide, and that this ovum is passing from the three- to the four-cell stage. The nuclei of the corona radiata, lying close round the ovum, are not very deeply stained. Fig. 2 shows about 13 of these nuclei. The small nucleus, apparently lying in the upper part of the ovum, also belongs to the corona radiata as the surface of the cell has been cut. The largest diameter of this ovum is about 72 μ measured without the corona radiata.

Besides the ovum and the covering enveloping it, fig. 2 also shows a fold of the oviduct-wall to indicate that the egg is lying close against this wall in a state of preservation. Naturally this does not imply that the ovum lies here in living condition too.

The four-cell stage is represented in the collection by one specimen (N°. 44), of which two sections are reproduced (figs. 3 and 4). All four blastomeres are visible in both figures, but of the four nuclei only three are to be found in the different sections. However, the cell-membranes of these four cells are so clearly visible that it is certain that we have to do with a four-cell stage. In contradiction to the preceding ovum, this is preserved and stained much better.

The nuclei of the corona radiata, surrounding the egg in great numbers, are clearly visible. From the figures it is obvious that the ovum is not spherical, which is most probably the case in living state. This egg too lies in the left oviduct, close to the wall. The oviduct-epithelium of this *Manis*-embryo N°. 44 is as distinctly ciliated as in the embryo first described. Whether this ciliated epithelium moves the ovum in the direction of the uterus or whether this is caused by a fluid-mass, propelled by the peristalsis of the oviduct-wall (SOBOTTA 1914), cannot be traced.

The largest diameter of this ovum is somewhat more than that of the preceding one and is $\pm$ 90 μ.

The sections, reproduced in figs. 2, 3 and 4, are the same HUBRECHT (1910) sketched. He intended to prove that the nuclei, to be found at the periphery of the ovum, belong to the egg-cell itself. They were supposed to represent the nuclei of the future trophoblastic covering, which in *Manis* and also in *Galeopithecus* was

supposed to originate very prematurely out of the nuclei of the ovum. As de Lange remarks in a note in the posthumous publication of Hubrecht (1919), the number of trophoblastic nuclei decreases very considerably in the successive developmental stages. This could not be the case if they were future trophoblastic nuclei, as the number would then increase.

I have re-examined these *Galeopithecus*-preparations in the Hubrecht-Laboratory and quite agree with this opinion. Moreover the great difference in the structure of the nuclei of the circumference and centre points to the fact that these bodies cannot be of the same origin. In *Manis* too, the same was supposed to be the case.

Next to the ovum, represented in fig. 1, there is a great number of nuclei, which cannot have originated out of the nucleus of the egg-cell, the latter not having divided as yet. They cannot belong to the ovum and are undoubtedly discus-nuclei, which have been expelled from the ovary together with the ovum and which have both passed into the oviduct. The greater part of these nuclei probably belongs to the discus proligerus; a small part is situated against the circumference of the ovum and must be considered the corona radiata.

In the three-cell stage (fig. 2) the number of nuclei of the corona radiata in each section has been reduced to twelve or thirteen, whilst the discus-cells have disappeared here. In the four-cell stage (figs. 3 and 4) the nuclei of the corona radiata or cumulus proligerus, form a distinct layer around the egg-cell in the same manner. Their number is greater here and amounts to about 40 à 50 in each section.

From the following considerations it is evident that I am entitled to the opinion that these nuclei really belong to the follicle-epithelium.

In the first place, I have measured the different sizes of the nuclei of the ovum, and of the covering of the ovum in the oviduct of *Manis* and *Galeopithecus* and compared them with the sizes of the nuclei of the follicle-epithelium in the ovary of both species (table 2).

From this list, in which average numbers are indicated, it follows that the nuclei of the follicle-epithelium of the ovary are about as large as the nuclei of the covering around the ova, situated in the oviduct. This is the case in *Manis* as well as in *Galeopithecus*. In both species the nuclei of the cleavage cells having, as indicated, a

TABLE 2.

Catalogue N°.	Size of nuclei of follicle-epithelium in ovary.	Size of nuclei of covering of ovum in oviduct.	Size of nuclei of cleavage-cell.	Size of trophoblastic nuclei.	Size of nuclei of embryonic knob.
Manis N°. 51.	± 6 μ	6—7 μ	nucleus lacks		
„ N°. 171. . . .	5—6 μ	5 μ	16 μ		
„ N°. 44.	6 μ	6 μ	15 μ and 16 μ		
Galeopithecus N°. 5	6—7 μ	6 μ	nuclei not visible		
„ N°. 56	6—8 μ	6—7 μ	14 μ, 16 μ, 18 μ		
Manis N°. 87. . . .				8—9 μ	9—10 μ.

diameter three times as large as that of the nuclei of the follicle-epithelium, are of about the same size.

In the second place, there is a distinct limit between outer layer and ovum, from which it follows that these are different things. Moreover there is a marked difference in staining and appearance of both nuclei. The nuclei of the covering are well provided with chromatin and consequently much darker than the nuclei of the blastomeres, which are larger, more inflated and less stained. The follicle being composed principally of a discus and much liquor folliculi, it is easy to understand that at the moment of the bursting of the follicle, both ovum and a part of the discus are expelled from the ovary and pass into the oviduct. Here these cells are probably used for the nutrition of the developing ovum. The smaller number of the nuclei in the younger egg (N°. 171), compared with that in the slightly older four-cell stage (N°. 44), need not be a proof of HUBRECHT's supposition, as the consumption of the follicle-cells naturally shows individual differences in different ova.

Finally I must point to the fact that, if HUBRECHT's supposition were true, it would not be easy to explain what follows. In early stages the nuclei, which HUBRECHT supposed to be future trophoblastic nuclei, are much smaller, than those of the ova. Later on, however, when the trophoblast has been formed as a distinct layer and represents the wall of the blastocyst, the nuclei of the embryonic knob and trophoblast are of about the same size. In the above list the sizes

of the trophoblastic nuclei and of the nuclei of the embryonic knob of *Manis* N°. 87 have been indicated. The difference is apparent and wants an explanation, I cannot give.

We can conclude by saying that the nuclei, which HUBRECHT supposed to be future trophoblastic nuclei in *Manis* and *Galeopithecus*, are nuclei of the follicle-epithelium, which, in the stadia above described, still remain around the ova. What led HUBRECHT to this opinion on the origin of the trophoblast is not easy to understand, especially as he has described and reproduced under this name in 1895 and 1912 young ova with a follicle-epithelium.

The material lacking in the collection, I cannot trace the origin of the trophoblast.

On comparing the figures in HUBRECHT 1910 and the above sections (figs. 2, 3 and 4) one will observe that the ovum, reproduced by me in figs. 3 and 4, is considered a two-cell stage by HUBRECHT (HUBRECHT 1910, figs. 5*a* and 5*b*) and that the ovum, sketched in fig. 2 (HUBRECHT 1910, fig. 6, p. 593), is taken to be a four-cell stage by him. On comparing carefully the successive sections of both ova, I concluded from the above-mentioned data that the former represents a four-cell and the latter a three-cell stage.

II. *Origin of the hypoblast.*

The origin of the trophoblast, the layer forming the outer covering of the blastocyst and taking an important part in the nutrition of the embryo later on, cannot be traced, the material lacking in the collection.

Both ova, succeeding those described in the preceding chapter in age, possess a well-developed embryonic knob. Moreover the formation of the hypoblast is distinctly visible in these eggs. (N°. 87 and 113).

Figs. 5 and 6, plate I represent sections of the younger stage (N°. 87). The blastocyst, lying free in the uterine lumen, is strongly compressed in preservation. Both embryonic knob and trophoblast are clearly distinguishable. The trophoblast, forming a rather thick layer, covers the embryonic knob. The nuclei of the trophoblast are more deeply stained and more compactly arranged. Hence it is easy to distinguish this inner mass of cells and its covering. The same was observed by BONNET (1897) in the dog and BAUMEISTER (1913) in *Erinaceus*.

Fig. 5 gives a section through the centre of the embryonic knob. In this section no hypoblast is visible but in the next one (having 10 μ thickness) this is the case. Here the embryonic knob is divided (fig. 6) distinctly into two layers and has become the embryonic epiblast and formed a very thin lower layer, the hypoblast, by delamination. Between epiblast and hypoblast a distinct slit is visible. The thickness of these two layers taken together is about the same, as that of the embryonic knob. From this it follows that the delamination has begun on one side of the embryonic knob and is not quite finished as yet. In this stage, the hypoblast as such covers only a part of the embryonic knob and is visible as a very much flattened layer. The length of the embryonic anlage is $\pm$ 75 μ, the thickness (without the trophoblastic covering) $\pm$ 28 μ.

In the blastocyst, reproduced in the figs. 7 and 8, plate I, the hypoblast has grown round the inside of the trophoblast and formed a completely closed sac, its lining consisting of flattened epithelium-cells. The hypoblast, lying against the embryonic knob, consists of much taller cells. Fig. 7 shows a schema of this stage, the situation in the uterine lumen also being indicated. In fig. 8 is reproduced the middle part of fig. 7, much more enlarged and more accurately drawn.

The large blastocyst with its wide lumen is lying free in the uterus. It has been compressed in preservation, but not as strongly as the preceding one. In one place it has burst, probably during fixation. The outer layer of the blastocyst is formed by the trophoblast, which also covers the embryonic knob. The trophoblast has about the same thickness as that in N°. 87; some small differences can be distinguished: around the embryonic disc it is slightly thinner. The hypoblast, which lacks nowhere and is very thin in the extra-embryonic part of the vesicle, becomes much thicker with tall and cubical cells beneath the embryonic knob [1]). The epiblast is found in the embryonic disc between trophoblast and hypoblast. The nuclei in this layer are more numerous than in the other parts of the blastocyst, the protoplasm showing a somewhat vacuolized structure. The nuclei of the trophoblast and hypoblast, which are more clearly visible, are more numerous.

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[1]) This BONNET also observed in the dog (1897).

A „schizamnion"-cavity cannot be distinguished in either of these blastocysts. Neither are there any indications of this blastocyst having been in contact with the uterine wall. *Manis* possesses a makromphalic blastocyst as can be seen in fig. 7 and fig. 20, and it is believed that in this stage contact with the uterine epithelium is out of the question as yet, the blastocyst on the contrary still floating free in the uterine lumen.

The largest diameter of the embryonic disc of N°. 113 is ± 125 μ, the thickness (without the trophoblast) ± 70 μ. The blastocyst described is the same of which HUBRECHT 1908, plate C, fig. 18 reproduced the embryonic part. In the plate-description the name *Galeopithecus* was erroneously attributed to this stage, and this was corrected in a following communication (HUBRECHT 1910, p. 592).

In both blastocysts described the trophoblast covers the embryonic knob. In the germdisc, succeeding in age, a primitive streak has been formed. Then the embryonic epiblast passes peripherally into the trophoblast, which consequently no longer covers it. How the trophoblast disappears from the embryonic disc, I cannot trace, the stages lacking. This is a pity as opinions differ on this point.

In the primitive streak-stages, to be described in subsequent pages, the hypoblast lies as an uninterrupted layer under the primitive streak. Secondarily in older embryos, possessing 8—18 pairs of somites epi-, meso- and hypoblast are intimately fused in the cranial part of the primitive streak, in the mid-axis of the embryo.

HEAPE has figured this as early as 1883. The description of the development of the notochord, however, must be compared to see this.

Here we must still mention that the thickness of the hypoblast, extending as a continuous layer along the inside of the primitive streak-stages, can be very different in the same embryo. This is apparent in the description of N°. 180 (p. 22).

Beneath the posterior part of the primitive streak (to compare fig. 29 plate III) the hypoblast forms a distinct epithelium, composed of large cubical cells. Gradually it decreases in thickness and becomes a very flat layer beneath the headprocess. HUBER also figured this in his recent paper on the development of the notochord in *Cavia* (1918, fig. 4). Before the anterior end of the headprocess the hypoblast suddenly becomes a thickened plate, composed of two or three strata of cells. This plate,

the prochordal plate, will be discussed more fully later on. To summarize, we have seen that in *Manis* the hypoblast is derived from the embryonic knob by delamination. This is in agreement with the observations in almost all other Mammals. Only in Marsupials it was observed lately by HILL (1910) and HARTMAN (1919) that the anlage of the hypoblast is formed by immigration of some few „entoderm-mothercells" out of the embryonic knob.

After delamination the hypoblast grows round the inside of the whole blastocyst and here forms against the trophoblast a closed sac, the umbilical vesicle, out of a part of which the archenteron is formed.

III. *Description of surface-views of the embryonic shields.*

Fig. 9, plate II (embryo N°. 180) represents the ventral side of one of the two primitive streak-stages. From the sections (to compare next chapter) it follows that this embryonic area is slightly older than N°. 151, which strongly resembles it in shape (fig. 21, plate III). The shield is pear-shaped; the white spot indicated, is the place, where the headprocess originates from the primitive streak. The dorsal side of this embryo could not be reproduced, for it is covered by the uterine wall, with which the trophoblast has fused in some places.

Embryo N°. 83 is shown in fig. 10, plate II. It is a sole-shaped embryonic shield, possessing 4 pairs of somites with a 5th pair forming. The anterior pair of somites is not sharply outlined in front, as it is in connection with the mesoblast here, which appears from sagittal sections through the embryo. The medullary groove is still quite open. Neither the brain nor the pleuro-pericardial space is to be seen. The gastral mesoblast (to compare the chapter on mesoblast-formation), from which the somites are formed, is traceable cranialward, where it fuses with the so-called cephalic mesoblast; this is visible in the sections only. Around the anlage of the embryo proper a well-developed area vasculosa is to be seen.

Figs. 11 and 12, plate I represent a sole-shaped embryonic shield (embryo N°. 64), possessing eight pairs of somites with a 9th pair forming. The anlage of the brain is visible. The pleuro-pericardial space is to be seen in sections only. The medullary groove is still open. A pro-amnion is not formed. (Fig. 11 dorsal, fig. 12 ventral view).

Fig. 13, plate II (embryo N°. 32) shows an embryonic area with 10 pairs of somites and an 11th forming. The pro-amnion is clearly visible, the pleuro-pericardial space is not to be seen. The brain-anlage and a beginning of the head-bend are distinct. The medullary groove is partly closed.

Embryo N°. 89 possesses 13 pairs of somites and is reproduced in fig. 14, plate II. The pleuro-pericardial space and the brain-anlage are clearly visible. The medullary groove is closed for the greater part.

About the same can be observed in the slightly older embryo N°. 31, in which a 14th pair of somites is forming (fig. 15, plate II). In neither of the embryos a pro-amnion is visible (figs. 14 and 15), but this is the case in the younger embryo N°. 32 and is also to be seen in the transverse sections of N°. 31. The sketches having been drawn before 1907, I cannot ascertain whether the pro-amnion is pushed aside by the artist, in order to set off the embryo.

Fig. 16, plate II (embryo N°. 108) shows a distinct head-bend. The embryo, in which 13 pairs of somites have been formed, is sketched from the ventral side.

Embryo N°. 68 possesses a well-developed head (fig. 18, plate I). The heart and the brain are clearly visible in fig. 17 (dorsal view) and fig. 18 (ventral view); 16 pairs of somites have been formed. The medullary tube is still open posteriorly. A pro-amnion is distinctly perceptible.

Embryo N°. 53 with 18 pairs of somites (fig. 19, plate II) shows a distinct brain. Pro-amnion and head-bend are easily distinguishable.

IV. *Development of the mesoblast.*

The origin and development of the mesoblast can be well traced in two *Manis*-embryos, both in the primitive streak-stage. The one (N°. 151) is almost cross-, the other (N°. 180) sagitally sectioned. The former is slightly younger than the latter. A schema of this younger *Manis*-stage presents fig. 21, plate III. This reconstruction has been made in such way that, at a magnification of 100, the breadths of different organs of each section of the germdisc were set out on millimetre-paper. The thickness of the sections being 10 μ, every millimetre agrees with one section. As a fixed point the middle of each

section of the embryonic area was taken. To obtain a survey of the different organs and of the germdisc itself in situ, this schema suffices. The sections, which will be discussed in subsequent pages, are indicated.

The length of this embryonic shield is about 720 μ (72 sections of 10 μ each), the largest breadth $\pm$ 800 μ. As is shown in the reproduction, the embryonic area is pear-shaped, the primitive streak (*p.s.*) occupying the hinder part. Somewhat more in the direction of the future head-region a tongue-shaped organ originates out of the primitive streak in cranial direction. It is the headprocess of the primitive streak, which will be called headprocess for the present. This name means that the primitive streak forms a process in the direction of the future head-region. As will soon be apparent in the description of the different sections, the hypoblast covers the whole inner face of the blastocyst just as in the younger *Manis*-stage N°. 113. Fig. 20, plate I is a reproduction of a section through the whole, strongly folded blastocyst, partly lying against the uterine wall. The embryonic anlage is very small, compared with the large extra-embryonic part.

The hypoblast forms two thickened parts, both indicated in fig. 21, plate III. One of them, lying just before the anterior end of the headprocess has been called protochordal plate by HUBRECHT. This name indicates that a part of the notochord develops out of this plate. Another part of this organ derives from the protochordal wedge (HUBRECHT 1890 = HENSEN-knob, HENSEN 1876). This thickened part of the hypoblast must be called prochordal plate, if we suppose that the notochord reaches to this point and fuses with it, but that no elements originate from it to form the anterior part of the notochord.

Whether this opinion is right or wrong, I will not decide here; I will treat this question later on (pp. 42 and 43), when discussing the development of the notochord. The second thickened part of the hypoblast is to be found in the periphery of the embryonic shield and is called annular zone of proliferation by HUBRECHT (1890). Out of it the peripheral mesoblast will develop.

On studying the cross-sections, (figs. 22—28, plate III) I can observe the following facts.

The section, reproduced in fig. 22, passes through the posterior part of the primitive streak. In the mid-axis, on the upper side, a small shallow

pit is found. (Fig. 21 must be compared for this and following sections). This is taken to be a remnant of the primitive groove, which has now disappeared for the greater part. Out of the primitive streak mesoblast-cells proliferate. This part of the mesoblast is called peristomal mesoblast in connection with the theoretical signification attached to the primitive streak. On the right and on the left the primitive streak passes into the trophoblast, which has disappeared from the upper side of the embryonic area. The inner face of the germdisc is formed by a layer of hypoblast, equally thick everywhere, with round nuclei.

Somewhat more cranialward the epiblast rises above the level of the trophoblast (fig. 23). In this and following figures the epiblastic tissue has been drawn but partly: for simplicity's sake it has been dotted. This section too passes through the primitive streak, which also forms peristomal mesoblast here. The hypoblast also lies as an uninterrupted layer at the inner side of the germdisc, and is slightly thinner in the middle only.

The following section sketched (the 16th from the first section described) passes through the front part of the primitive streak (fig. 24). The anterior part of the primitive streak and the posterior part of the headprocess are strongly thickened in this region of the embryo. This place has been called HENSEN-knob (HENSEN 1876) or protochordal wedge (HUBRECHT 1890) or „Primitivknoten" (BONNET 1889). The HENSEN-knob is also covered by the hypoblast. Caudad from this place, over the whole length of the primitive streak, the peristomal mesoblast is formed. It spreads laterally from the primitive streak between epiblast and hypoblast.

Fig. 25 represents a section through the headprocess, which is developed slightly in this embryo. In this region the epiblast of the embryonic area forms no mesoblast; it is a continuous layer, composed of tall columnar cells. Between headprocess and epiblast, there is a distinct limiting membrane. The headprocess extends cephalad as a wedge-shaped or tongue-shaped organ between epiblast and hypoblast. On the left side of the section, just against the border of the germdisc, the hypoblast shows a tickened place [1]).

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[1]) On comparing the figs. 22—28 and 21 it will have been noticed that what is on the left in the cross-sections is on the right in the schema (fig. 21). This comes out clearly in the asymmetric

In fig. 26 too, which is a section through the cranial end of the headprocess, this thickening lies only on the left side of the reproduction. This is a part of the annular zone of proliferation (HUBRECHT 1890), which, however, does not proliferate as yet in this part of the embryonic area.

This is the case in the section, represented in fig. 27, where the annular zone is found on the left as well as on the right. Here, on the left, we see the very first mesoblast-formation, on the right this process has not yet begun. It is called the peripheral mesoblast (BONNET 1920, p. 111), because it is formed at the periphery of the germdisc. In the centre of the embryonic shield the hypoblast still shows another thickened part, situated exactly in front of the cranial border of the headprocess and called *pro*chordal plate for that reason. In *Manis* this plate has an oval shape and is of about a breadth of 330 μ and 220 μ length. The annular zone is still better visible in the anterior part of the area. Here it forms a ring around the anterior part of the embryonic shield (figs. 28 and 21) and in different places distinctly produces the peripheral mesoblast. In the same section the anterior part of the prochordal plate is shown. Here the prochordal plate is connected with the annular zone (fig. 21) and so, in the mid-axis of the embryo, the hypoblast is thickened from the cranial point of the headprocess to the anterior margin of the embryonic area. In this younger primitive streak-stage the hypoblast is principally thickened in the anterior part of the shield. In some places the mesoblast is formed from the hypoblast, viz. the annular zone of proliferation. This is not yet the case in the prochordal plate.

BONNET has also described HUBRECHT's protochordal plate in other Mammals and calls it „Ergänzungsplatte", because, according to him, the anterior part of the notochord, the mesoblast in the future front-part of the head (the so-called cephalic mesoblast), a part of the fore-gut and the inner layer of the primary pharyngeal membrane are derived from it (BONNET 1920, p. 100).

In the older embryo, N°. 180, also a primitive streak-stage, the

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figs. 25 and 26, where this proliferating place (*a.z.*) is shown on the left side of the sections, whilst according to fig. 21 this is the case on the right. This is easy to explain, however, because the embryonic area has been cut and fixed to slides from before backwards, so that what is on the left in the figures, is on the right in reality.

mesoblast-formation has been going on for some time. This is visible in fig. 29, plate III giving a survey of this embryo, which is very well sectioned in the sagittal plane. The section passes through the mid-axis of the embryo, of which the convex side lies closest to the uterine wall. The primitive streak extends from the left to the point marked by an *; the embryonic epiblast, clearly separated from the tissue lying beneath, occupies the region between the primitive streak and the trophoblast (on the right). The wall of the blastocyst is torn off just in front of the embryonic area, probably in the preparing of the embryo. The place of attachment, however, is evident. Along the whole length of the primitive streak the peristomal mesoblast is formed. Near the * the headprocess develops out of the primitive streak in the direction of the future head-region and extends between epiblast and hypoblast, gradually becoming thinner. The hypoblast is visible as a very thin layer beneath this organ. Under the cranial part of the headprocess it is composed of a few flattened cells. Exactly in front of the anterior end of the headprocess, it suddenly passes into a thickened plate, the prochordal plate, with nuclei in two or three strata. Before the germdisc the prochordal plate passes into the extra-embryonic hypoblast, present in a single layer of cubical cells. In this stage the prochordal plate reaches to the anterior margin of the germdisc. Behind the embryo the extra-embryonic hypoblast is nearly as thick as in front of the embryonic area. However, beneath the posterior part of the primitive streak, it gets much thicker, generally remaining a single layer of cells, gradually becoming thinner more in front. In this embryo too the prochordal plate produces no cephalic mesoblast as yet.

Fig. 30, plate III shows the middle part of fig. 29 greatly enlarged. It represents the anterior part of the primitive streak, the part, where the headprocess originates. A thickened „HENSEN-Knob" is not present. The headprocess extends in a cranial direction, the peristomal mesoblast spreads laterally from the primitive streak. The hypoblast is a thin flat layer of cells with few nuclei.

Figs. 31 and 32, plate III represent parts of sagittal sections through the same germdisc. Fig. 31 shows a section of the prochordal plate and the anterior part of the headprocess. This is not a section exactly through the mid-axis of the embryonic area. The epiblast forms a layer with three or four strata of nuclei, distinctly separated from the

underlying tissue. Here the thin cranial part of the headprocess can be observed, extending between epiblast and hypoblast. The hypoblast under this part of the headprocess forms a very thin layer with few nuclei and passes suddenly into the thickened prochordal plate, just in front of the headprocess. Beneath the anterior margin of the embryonic area the prochordal plate passes into the extra-embryonic hypoblast, consisting of a single layer of cubical cells.

The formation of the mesoblast out of the prochordal plate has started nowhere.

The peripheral mesoblast develops in this embryo. Fig. 32, plate III shows the origin of this mesoblast behind the caudal region of the germdisc. In the embryo, described in preceding pages (N°. 151, also a primitive streak-stage), we have found the early development of the peristomal mesoblast in the anterior part of the germdisc. Here, however, the annular zone of proliferation has extended along thep eriphery of the embryonic area, meeting behind the primitive streak. Here we see a distinct mesoblast-formation, just outside the border of the germdisc. In fig. 32 the mesoblast-cells proliferate out of the hypoblast. Moreover the mitotic figure (*m*) indicates the direction, in which the cleavage takes place. In other sections these figures are more numerous. The direction of cleavage is the same in all. More anterior (under the posterior border of the germdisc) the peripheral mesoblast gradually passes into the embryonic peristomal mesoblast; a limit cannot be observed. The peripheral mesoblast, originated from the hypoblast and the peristomal mesoblast, originated from the primitive streak, form one sheet of cells. The peripheral mesoblast is also found at the sides of the germdisc, but here the formation has stopped for the greater part.

Out of the sides of the headprocess mesoblast also develops, spreading peripherally between epiblast and hypoblast. This is the mesoblast from which the somites, pronephros, wall of the coelomic cavity etc. derive in the anterior part of the embryo later on. It is called gastral mesoblast, in connection with the theoretical signification of the headprocess.

This also fuses with the peripheral mesoblast, developed at the borders of the germdisc, and passes into the peristomal mesoblast caudalward. So we see that a continuous sheet of mesoblast-cells is

extending in this primitive streak-stage between epiblast and hypoblast. On comparing the germdiscs N°. 151 and 180, we see that, taking into consideration that N°. 180 is somewhat older than N°. 151, the mesoblast in the former is much more developed. In the younger the peripheral mesoblast has been formed but for a small part, the annular zone of proliferation extending in the future cranial part of the disc only. In the older germdisc, however, the formation of the peripheral mesoblast has progressed further and in some places, especially at the anterior borders of the embryonic area, it has come to an end. Behind the posterior part of the embryo, outside the germdisc, this development still continues in a place, where in the younger embryo there is as yet no trace of the annular zone. The headprocess is more developed in the older embryo than in the younger, and is tongue-shaped in both shields, extending between epiblast and hypoblast. The hypoblast in N°. 151, as yet a rather thick layer of epithelium-cells, has become in N°. 180 a very flat layer under the headprocess. It seems that the hypoblast, pushed away by the developing headprocess, has become a flat epithelium with few nuclei. In both embryos the prochordal plate lies just in front of the anterior end of the headprocess. In the younger germdisc this plate is connected with the part of the annular zone, lying in front. In this case the hypoblast is thickened from the cranial point of the headprocess to the anterior border of the germdisc. In N°. 180 the same is the case, the annular zone having fused intimately with the prochordal plate. No limit is visible between these parts. For convenience sake I have called prochordal plate the whole thickened part of the hypoblast, lying in front of the headprocess in the second primitive streak-stage described. The anterior part of the prochordal plate passes directly into the other parts of the zone. The embryo being cut in the sagittal plane, it is not easy to see this connection.

In tracing the further development of the mesoblast in *Manis*, we lack several stages, for the embryo succeeding in age is much more developed, possessing four pairs of somites with a 5th forming and a medullary groove. However, this stage is of great importance, because it shows a very distinct formation of cephalic mesoblast. This embryonic shield has been cut very well in the sagittal plane. Fig. 33, plate IV shows a schema of a section through the mid-axis of it. We see

that the peristomal mesoblast still originates from the primitive streak. At the cranial side of the embryo the cephalic mesoblast develops out of a very distinctly thickened part of the hypoblast, the prochordal plate. The chorda-anlage originates from the headprocess, which is strongly thickened in its caudal part, the HENSEN-Knob. The chorda-anlage is incorporated in the hypoblast and the so-called chordal plate occupies the roof of the future gut-lining. This chordal plate passes into the prochordal plate, composed of two or three layers of cells. The cephalic mesoblast clearly develops out of this plate (to compare fig. 34, plate IV, in which the cranial part of the chordal plate and the prochordal plate are greatly enlarged). It spreads as a broad sheet of cells between epiblast and hypoblast to the anterior border of the embryonic shield, and has reached a considerable extension. This marked formation of cephalic mesoblast takes place, when at least four pairs of somites have been formed. It develops remarkably late in *Manis*; BONNET e.g. finds (1901) that a distinct cephalic mesoblast-formation takes place in young primitive streak-stages of the dog.

The chordal plate originates from the HENSEN-Knob. Along the whole length of the chordal plate the mesoblast is connected with it at both sides. Near the primitive streak this connection is clearly visible; more cranially it is difficult to trace, the embryo being cut in the sagittal plane and the mesoblast being connected with the sides of the chordal plate. In some places it is easy to see it, when we carefully compare the consecutive sections.

This gastral mesoblast, out of which 4 pairs of somites with a 5th pair forming have originated, extends to the anterior part and the periphery of the embryonic shield. Caudalward it passes into the peristomal mesoblast and from these two parts most organs of mesoblastic origin are derived. Behind the anterior part of the head-region, in which the cephalic mesoblast is formed, the gastral mesoblast is connected with the cephalic mesoblast. The latter is also composed partly of peripheral mesoblast, and in other regions of the embryonic area the gastral mesoblast or the peristomal mesoblast is fused with the peripheral mesoblast. So we see that a continuous layer of mesoblast-cells, developed out of primitive streak and headprocess, as well as out of the hypoblast, extends between epiblast and hypoblast. So the mesoblast of *Manis* develops in several regions of the embryo.

With the name mesoblast is indicated only the fact that this layer lies between epiblast and hypoblast. It is a topographical term and does not signify that all parts composing it, are of the same origin.

In the *Manis*-embryo, possessing 4 pairs of somites with a 5th forming, (this is still connected with the undifferentiated mesoblast) a fore-gut has not yet been formed. This is the case, however, in embryo N°. 64, which possesses 8 pairs of somites with a 9th forming, and succeeds the foregoing in age. In an other embryo, N°. 32, with 10 à 11 pairs of somites, a well-developed fore-gut is present. A primary pharyngeal membrane, however, is still absent. This oral plate, the place where the hypoblast lies close against the epiblast and where afterwards the mouth is formed, is derived from the anterior part of the prochordal plate, which follows from the investigations of BONNET (1901). Behind this pharyngeal membrane we must look for a place, where the hypoblast and mesoblast are connected and indeed in the embryo N°. 64 with 8 à 9 pairs of somites there is still an intimate connection between the cephalic mesoblast and the sides of the fore-gut. In a *Manis*-embryo with 10 à 11 pairs of somites it is impossible to ascertain this with certainty. It then seems that the connection of cephalic mesoblast and hypoblast exists no longer, for in older embryos with 13, 16 and 18 pairs of somites I cannot find it.

In embryos with 8 à 9 and 10 à 11 pairs of somites (N°. 64 and 32) the gastral mesoblast is connected just behind the fore-gut with the sides of the chordal plate, which is incorporated in the hypoblast here. Superficially seen (fig. 36, plate IV) the mesoblast seems to be connected with the hypoblast. This is not the case, however. The broad, thin layer, extending beneath the medullary groove, is the chordal plate, which can be stated with certainty, when we trace this plate caudalward. This will be apparent from the description of the development of the notochord. At the borders this chordal plate passes into the hypoblast, the gastral mesoblast being connected with this plate here.

In the region of the somites this is no longer the case, but caudalward, in the neighbourhood of the primitive streak, the mesoblast has not yet separated from the headprocess, there to be found, and so mesoblast and chorda-anlage are still connected with each other here. As the primitive streak shortens caudalward (the embryonic organs originating from it) the zone, where chorda-anlage (i. e. head-

process) and mesoblast are connected, recedes still more. At the place, where formerly the peristomal mesoblast was formed, now the chorda-anlage, developed out of the primitive streak („Primitivstreifenteil des Chordas") is connected with mesoblast, derived from it as well. We must still call it peripheral mesoblast and so, in the caudal region of older embryos, the chorda-anlage, derived from the primitive streak, and peripheral mesoblast are connected. For this the chapter on the development of the notochord must be compared.

Summary.

The mesoblast of *Manis* originates in the primitive streak and the headprocess, as well as in the hypoblast. The primitive streak ventrally and laterally forms over the whole length the *peristomal mesoblast.* This is principally to be found in the posterior region of the embryo. Out of the sides of the headprocess the *gastral mesoblast* develops, when the formation of the peristomal mesoblast has already begun. Later on, when the chordal plate is formed out of the headprocess, this mesoblast is still connected with the borders of this plate, separating from it in a succeeding stage. Caudalward the gastral mesoblast fuses with the peristomal mesoblast. At about the same time that the peristomal mesoblast formation starts, the *peripheral mesoblast* originates from a ring-shaped part of the hypoblast, the annular zone of proliferation. This formation begins in the future head-region of the embryo, gradually moving backward along the borders of the germdisc. The formation of the peripheral mesoblast, coming to an end in the cranial part, still takes place behind the caudal part of the primitive streak, outside the embryonic area. In the anterior region of the germdisc another thickening in the hypoblast develops, just in front of the cranial end of the headprocess. This is the prochordal plate, which fuses in the mid-axis of the embryo with the part of the annular zone, lying still more in front. In the older primitive streak-stage described, both parts form one intimate plate from the cranial end of the headprocess to the anterior border of the germdisc. From this plate the *cephalic mesoblast* originates, which has absorbed the peripheral mesoblast formed out of the anterior part of the annular zone. This cephalic mesoblast forms only the mesoblast of the anterior

head-region. The rest of the mesoblast of the head must be considered gastral mesoblast. Before we can speak of a head proper, this cephalic mesoblast is already fused with the gastral mesoblast, lying posteriorly. During the development of the cephalic mesoblast in *Manis*, the anterior somites and wall of the coelomic cavity are derived from the gastral mesoblast. The rest of the peripheral mesoblast also fuses in the different regions of the area with the cephalic mesoblast, gastral mesoblast or peristomal mesoblast. So a continuous layer of cells, the *mesoblast*, originated from the primitive streak, from the sides of the headprocess and from the two parts of the hypoblast (prochordal plate and annular zone of proliferation) is formed between epiblast and hypoblast.

The formation of the peripheral mesoblast comes to an end first. Then, in the primitive streak-stage, the cephalic mesoblast is not yet formed. In an embryo, possessing 4 pairs of somites with a 5th forming, the development of it is in full progress and is to be observed in an embryo with 8 à 9 pairs of somites. In embryos with 10 and more pairs of somites this is no longer possible. The mesoblast is for a long time connected with the chorda-anlage in regions, where no somites have as yet been formed out of the mesoblast. This is the case at the cranial part of the chordal plate (gastral mesoblast, fig. 36, plate IV) and at the posterior part of the chorda-anlage, near the primitive streak (peristomal mesoblast and the so-called „Primitivstreifenteil des Chordas"). The peristomal mesoblast is formed first of all mesoblast-parts in the primitive streak and originates from it as long as the primitive streak exists, the latter being the organ, from which the embryonic organs develop from before backward.

V. *Origin and development of the notochord.*

In the above description of the development of the mesoblast, the origin of the notochord has been frequently discussed. The youngest germdisc, in which a headprocess, from which the chorda-anlage will develop, is to be found, is embryo N°. 151. In this embryonic area (cf. the reconstruction fig. 21 and the cross-sections figs. 24, 25 and 26, plate III) the headprocess still has the appearance of a tongue-shaped organ, which extends cranialward from the anterior part of the

primitive streak between epi- and hypoblast. From the headprocess originate the gastral mesoblast, the chorda-anlage and perhaps elements of the future gut-lining as RABL (1915) supposes.

In connection with the theoretical signification attributed to this organ it ought to be called metenteron, but for the present I will keep the more usual and meaningless name headprocess.

The part of the primitive streak, from which the headprocess originates, will be indicated by the name of HENSEN-knob (HENSEN 1876), when this part is strongly thickened. HUBRECHT called it protochordal wedge, but often erroneously included the headprocess. The transverse sections (figs. 24, 25, and 26) pass respectively through the anterior part of the primitive streak (the HENSEN-knob), through the middle part of the headprocess and through the most cephalic end of the headprocess.

In fig. 24 there is no limiting membrane visible between the epiblastic part of the primitive streak and the headprocess, originating from it. The HENSEN-knob causes the hypoblast to project ventrally. In both of the other sections (figs. 25 and 26) the headprocess is distinctly separated from epiblast and hypoblast, and extends between these germlayers, as far as a thickened part of the hypoblast, the prochordal plate. The headprocess has a length of about 150 μ and a breadth of $\pm$ 190 μ at its base. It is remarkable that in this young primitive streak-stage no mesoblast develops as yet out of the sides of the headprocess. This is the case, however, in the slightly older germdisc, also in the primitive streak-stage and sectioned in the sagittal plane (N°. 180, figs. 29—32). Here the headprocess is considerably longer ($\pm$ 500 μ). The origin of the gastral mesoblast is clearly visible, when we compare the successive sections. It spreads in the form of two wings from the sides of the caudal and middle part of the headprocess. From the figs. 29 and 30 it follows that the headprocess extends from the cranial part of the primitive streak, between epiblast and hypoblast. Here a HENSEN-knob is not as clearly visible as in the younger embryo N°. 151 and in the older embryo N°. 83.

Besides the gastral mesoblast originating from the headprocess in the embryonic shield, this area is also distinguishable by an other peculiarity from N°. 151. In this embryonic area the hypoblast extends as a continuous layer beneath the headprocess and primitive

streak, and is equally thick everywhere. In N°. 180, however, the hypoblast becomes much thinner under the headprocess and is reduced to a flat epithelium with few nuclei. It gradually becomes higher under the primitive streak. Just in front of the anterior end of the headprocess, it suddenly forms a thickened plate with nuclei arranged in two or three strata: the prochordal plate. The hypoblast lacks nowhere at the ventral side of the embryonic shield. This is the case, however, in older stages, where for some time the chordal plate forms the roof of the future gut. In neither of the *Manis*-embryos discussed, in which a free headprocess is found between epiblast and hypoblast, a so-called LIEBERKÜHN-canal is to be found. This canal LIEBERKÜHN described for the first time in Mammals *(Cavia)* in 1882. It is the homologue of the metenteron of the Anamnia and, when formed completely, penetrates the whole length of the headprocess and opens ventrally into the archenteron, the cavity enclosed by the hypoblast.

The embryo next in age to the two germdiscs described, is N°. 83 with 4 pairs of somites and a 5th pair forming. Fig. 33, plate IV shows a schema of the mid-sagittal plane of this embryo. Here the headprocess also originates from the cephalic part of the primitive streak, forming a distinct HENSEN-knob. The posterior part of the embryonic shield is somewhat curved, hence the section does not pass through the mid-axis here. This is the case, however, in the greatly enlarged section, reproduced in fig. 35. As is evident from figs. 33 and 35, the ventral part of the embryo has been torn from the dorsal part. Between primitive streak and headprocess, between the medullary groove and the chordal plate, an artificial space is to be found.

The HENSEN-knob is clearly visible. Behind it, beneath the primitive streak, lies the hypoblast, gradually becoming thinner and disappearing completely under the HENSEN-knob. In the section reproduced, two lumina are found in the HENSEN-knob. Neither is artificial, for both possess a regularly formed wall, and the numerous cell-membranes of the larger of the two are radially arranged. Both occupy the center of the HENSEN-knob, but they are not connected. An opening into the primitive streak or into the archenteron is not to be found. In some adjoining sections a few similar lumina are to be seen, but most of them are narrower than the largest of the lumina reproduced. These lumina, also to be observed in older stages,

are no rests of the LIEBERKÜHN-canal, according to me, but more homologous with the rudimentary neurenteric passage of the Anamnia. The LIEBERKÜHN-canal indicates only the canal, which originally is to be found in the headprocess, the homologue of the metenteron of the Anamnia. When the embryo grows in length, a new part of the headprocess is derived from the primitive streak, the original part having incorporated itself in the hypoblast. The older the embryo becomes, the longer the chorda-anlage grows. The part of the headprocess, formed out of the primitive streak, moves more and more caudalward, and the lumina to be found here do the same. The neurenteric passage of the Anamnia connects the posterior part of the medullary tube with the primitive gut. It also moves caudalward, during the growth in length of the embryo, whilst the headprocess, homologous with the metenteron, in a younger stage grows through invagination in a cranial direction. For this reason the lumina, just described, and others, still to be described in older stages, must be homologized with the neurenteric passage of the Anamnia and not with the LIEBERKÜHN-canal or metenteron. I call it chordal canal, because in Mammals the notochord principally originates out of the headprocess, in which these lumina are to be found.

The HENSEN-knob passes cranialward into the chorda-anlage, which is not covered by hypoblast ventrally. Here the chorda-anlage forms the roof of the future alimentary tract. So the chorda-anlage is incorporated in the hypoblast. In this study this developmental stage of the notochord will be called chordal plate. Later on, when the chordal plate has separated from the hypoblast, the notochord proper is formed.

The chordal plate continues caudalward in a layer, in which the nuclei are more numerous than in the other part of the HENSEN-knob. During the longitudinal growth of the embryo, the chordal plate is derived from the dorsal part of the headprocess, the gastral mesoblast from the sides. In front of the HENSEN-knob, the chordal plate is still rather thick, consisting of tall columnar cells. It becomes much more flattened cranialward (fig. 34). In the cephalic part of the chordal plate it is difficult or impossible to trace a difference with the hypoblast, when we compare the sagittal sections. Still more in front the chordal plate suddenly passes into the thickened

prochordal plate. A limiting membrane between these parts is not traceable; the anterior part of the chordal plate is directly continuous with this part of the hypoblast. If we did not know from younger stages that the headprocess reaches as far as the prochordal plate there, we could not know that in this stage the chordal plate passes into the prochordal plate. In older cross-sectioned stages we can also trace the chordal plate, incorporated in the hypoblast, up to a region far advanced in the head of the embryo.

In this embryo the gastral mesoblast is still connected with the chordal plate (cf. p. 25). There is no question of the edges of the split hypoblast trying to fuse with each other, neither in this nor in other embryos (with about 8, 10 and in a few cases 13 pairs of somites).

The embryo N°. 64 has 8 pairs of somites with a 9th pair forming. A fore-gut, having a length of ± 80 μ, is formed; the head-bend has not taken place and consequently no pharyngeal membrane is to be found. The sides of the fore-gut are still connected with the cephalic mesoblast in some places. Here we still find a remnant of the prochordal plate in the wall of the fore-gut.

Behind the fore-gut we see that the roof of the future alimentary tract is distinctly thinner than the adjoining hypoblast. With the borders of this plate the mesoblast is connected. It is evident that here we see the cranial part of the chordal plate, incorporated in the hypoblast and that the front part of the gastral mesoblast is connected with it (fig. 36, plate IV). From this region we can trace the chorda-anlage backward, but it must be observed that the chordal plate as such is not distinctly visible everywhere. In the anterior part there is a connection between gastral mesoblast and chordal plate in several places. More caudalward, at the level of the first somites, hardly any difference is to be seen between chordal plate and hypoblast. Here the chordal plate is but slightly thinner than the hypoblast. Gradually the difference between both parts becomes more marked (fig. 37). Under the medullary groove a broad plate extends, which is a little thicker than the hypoblast and easily distinguishable from it. On the right the hypoblast is torn from the chordal plate, the connection, however, is clear. A similar view shows fig. 38, drawn after a section through the eighth pair of somites. The chordal plate being higher, the dif-

ference with the hypoblast is more distinct. The borders of the chordal plate are still directly continuous with the hypoblast. The chordal plate possesses numerous cells and is clearly distinguishable from the hypoblast, which contains less nuclei. In the preceding pages (embryonic shields N°. 151 and 180) we have seen that the hypoblast in primitive streak-stages still extends under the headprocess. This is no longer the case here, the headprocess having fused with the underlying hypoblast: the ventral side of the headprocess cleaves and spreads in a flat plate. The underlying hypoblast is split and is pushed aside by the borders of the chordal plate, which is now developed out of the headprocess. In the sections described the borders of the chordal plate are directly continuous with the cleft hypoblast: the incorporation of the hypoblast is quite finished here. More caudalward, however, all possible stages of incorporation can be traced in the same embryo.

The chordal plate in fig. 39 is still higher than is the case in an anterior part. Here it does not pass directly into the hypoblast: the latter extends slightly under the borders of the chordal plate. The latter is not as broad as in more cephalic parts: the incorporation is not quite finished here. A little more caudalward the hypoblast extends beneath a considerable part of the chorda-anlage and has just been cleft. The chorda-anlage is both higher and broader than in the preceding sections. The undifferentiated mesoblast is not connected with the chorda-anlage, which is the case in the section reproduced in fig. 41. Here the connection between both parts is distinct, though they were torn from each other, when the embryo was sectioned. An incorporation of the chorda-anlage in the hypoblast has not yet taken place. The connection of the mesoblast and the headprocess gradually becomes more intimate, the headprocess being distinguishable, because it has not fused with the epiblast of the primitive streak and because the nuclei in the headprocess are more numerous than in the mesoblast-wings. The place, where the headprocess originates from the primitive streak, lies somewhat more caudalward and here we also see, how the hypoblast in the mid-axis of the embryo is secondarily fused with the primitive streak-tissue. To obtain a general view of the organs in this posterior part of the shield in the embryos just described and still to be described, I have made a schema of

the mid-sagittal section of the medulla, chorda-anlage and primitive streak of each embryo. To this end the heights of hypoblast, chorda-anlage, and medulla were measured in the center of each cross-section at a magnification of 100. As a fixed horizontal line the ventral side of the embryo was taken and above it the measures, found in each section, were set out on millimetre-paper. The ventral part of this region of the embryo being almost flat, we may use this method here. For the head-region, however, it will not do; therefore the method was used only for the posterior part of the embryo. Figs. 62—67, plate VI show these sections through the mid-sagittal plane of the different embryos. Moreover, to indicate the situation of the somites, their circumferences and also the upper face of the medullary groove are projected on this sagittal plane. Finally the sections described are indicated.

Fig. 62 presents a schema of the posterior part of embryo N°. 64. On the right we see the primitive streak, out of which the medullary groove and the headprocess originate cranialward. Like the primitive streak the headprocess is still covered by hypoblast on its ventral side: more in front the chordal plate, incorporated in the hypoblast, is formed out of the headprocess. The hypoblast has secondarily fused with the primitive streak-tissue, lying above it just behind the place, where the headprocess develops out of the primitive streak. Laterally the three layers, epi-, meso- and hypoblast are easy to distinguish. In older stages the same occurs and on comparing the different stages, we see that this zone moves caudalward, at the same time that the primitive streak shortens in order to form the embryonic organs.

The *Manis*-embryo N°. 32 has 10 pairs of somites with an 11th forming. The medullary groove is closed along a great distance, but is still open in the cranial and caudal part. A fore-gut is traceable along a length of $\pm$ 130 μ, a primary pharyngeal membrane is not found as yet. The wall of the fore-gut is of the same thickness everywhere. I can nowhere find any indications of the chorda being incorporated in the hypoblast here. The wall of the fore-gut is no longer connected with the cephalic mesoblast, which develops out of the prochordal plate. Just behind the fore-gut, there is no chordal plate but, when we trace the sections backward, it soon appears. Only in a very few places a connection of the gastral mesoblast with the borders of the

chordal plate is to be found. In most cases a distinct limiting membrane is noted and consequently these parts are connected no longer. The chordal plate is clearly distinguishable from the hypoblast and is very broad under the undifferentiated mesoblast of the head-region. More backward it becomes less broad, but in the region of the first somites it has the shape, figured in fig. 42, a section through the 5th pair of somites. Here the chordal plate is much thinner than the hypoblast lying on both sides. On the right the hypoblast is not quite pushed away by the incorporating chordal plate. On the left, however, the chordal plate, without being covered by the hypoblast, is quite incorporated and passes into the hypoblast. It seems, as if the edges of the cleft hypoblast are compressed by the incorporating chordal plate. On the left and on the right the primitive aortae are to be found.

In the region of the somites VI—XI the very broad and flat chordal plate becomes less broad and taller. Moreover, the edges of the hypoblast are extending distinctly beneath the borders of the chordal plate: the chordal plate is in the very act of incorporating in the hypoblast. Here the chordal plate becomes taller than the hypoblast. In the region of the undifferentiated mesoblast, from which the somites will develop, the situation is still about the same. Fig. 43 shows a rather broad and high chordal plate, the hypoblast extending beneath the borders of it. A little more caudalward the hypoblast is present as an uninterrupted layer under the headprocess. Fig. 44 represents the transitional stage: only a small part of the ventral side of the headprocess is incorporated in the hypoblast in this place. Moreover, the mesoblast is connected with the headprocess here, which was not yet the case in the preceding section described (fig. 43). In the centre of the headprocess a lumen is visible. This is not only present in one section, but traceable in 13 consecutive sections ($\pm$ 130 μ). As will be evident from the description of the other embryos, one or more similar canals occur in the headprocess. Sometimes two lumina are present in the same section of the headprocess. This is the case in front of the canal just described. The nuclei and cell-membranes are arranged radially around the lumen; this being also the case, when the lumen appears double. Though I intend to discuss the signification of these lumina in subsequent pages, I remark here that the chordal canal must be considered the homologue of the

canalis neurentericus of the Anamnia. In *Manis* it is present in embryos with 4—18 pairs of somites.

On tracing the headprocess of N°. 32 still more caudalward, we find about the same as in the embryo just described. Here the hypoblast is forming an uninterrupted layer beneath the headprocess. A schema of this part of the embryo is to be found in fig. 63, reconstructed in the same manner as fig. 62 (*Manis* N°. 64). In the region, where the headprocess originates from the primitive streak, the hypoblast is secondarily fused with the tissue of the primitive streak (laterally the three layers are distinguishable). More caudalward the hypoblast is visible as a distinct continuous layer at the ventral side of the primitive streak.

The fore-gut of embryo N°. 89 with 13 pairs of somites has a length of ± 470 μ. The chorda-anlage cannot be found in the most cranial part of it along a distance of 18 sections (± 180 μ). From this point, however, we can trace the chordal plate, incorporated in the hypoblast, caudalward. A primary pharyngeal membrane is present, traceable along a distance of 160 à 170 μ.

One of the first sections, in which the cranial part of the chordal plate is to be found, is reproduced in fig. 45, plate V. Beneath the brain-wall lies a flat plate, which rises distinctly above the level of the hypoblast, into which it passes. The wall of the fore-gut is rather thick here, partly due to the embryo being cut tangentially. A separation of the notochord from the hypoblast has not yet taken place in this region, but in the slightly older embryo N°. 31 the first indications of this process are to be found.

As follows from fig. 45 the difference between the chordal plate and the hypoblast, in which the former is incorporated, is not very marked as yet. Gradually this becomes more distinct, however, and in the region of the first somites we see that the chordal plate, incorporating in the hypoblast, is a rather thin plate. (Fig. 46 a section through the 4th and fig. 47 a section through the 5th pair of somites). Here we see that the hypoblast is compressed, evidently by pressure of the borders of the incorporating chordal plate, for more peripherally the hypoblast has its usual thickness. In N°. 32 (fig. 42) the hypoblast was also thickened in a slight degree. More caudally we see that the chordal plate becomes taller than the hypoblast.

Slightly further caudalward of the 13th and last pair of somites we find a region, where the headprocess has not yet incorporated in the hypoblast and here, in the cranial part of the headprocess, a distinct chordal canal is to be found, which opens ventrally into the cavity, enclosed by the hypoblast (archenteron). Figs. 48 and 49 portray a distinct view of this chordal canal, traceable along a distance of ± 140 μ (= 14 sections). It has a diameter of ± 17 μ. Fig. 49, plate V shows the cranial part of this canal, where the headprocess is about to spread, in order to form the chordal plate, pushing aside the cleft hypoblast. When the embryo was sectioned, these edges were torn from the chordal plate-borders. Two sections further cranialward the chordal canal has opened into the archenteron and here we find a distinct chordal plate (fig. 48). On tracing the headprocess caudalward (cf. figs. 50, plate V and 64, plate VI), we see that the mesoblast is not connected with the headprocess, this being the case a little further on. In most sections caudalward of the chordal canal just described, a narrow excentric lumen is to be found. This is not necessarily the case, however; often no lumen can be observed in the sections through the headprocess. Sometimes the lumen appears double, and around it the cell-membranes and nuclei are also radially arranged (fig. 50). In all sections the headprocess is broader and less high than in a more cranial part. Both lumina are traceable caudalward in 8 sections (= 80 μ), then again fusing to a single lumen, which is to be found along a distance of 110 μ in the center of the headprocess, and then again appearing double along a distance of 40 μ (4 sections). Further caudalward a single lumen occurs either to the left or to the right, forming short canals in two or three sections. In this region the mesoblast is still connected with the headprocess and a little further caudalward the transition of the headprocess to the primitive streak takes place. Here too in the mid-axis a region is to be found, where the hypoblast fuses intimately with the primitive streak-tissue. This zone lies slightly more backward (fig. 64).

N°. 31 is a *Manis*-embryo possessing 13 pairs of somites with a 14th pair forming. Consequently it is slightly older than N°. 89. The fore-gut has a greater length, being ± 560 μ long. The roof of it is formed for the greater part by the chorda-anlage, which is not to be found in the cranial part along a distance of ± 130 μ. A

primary pharyngeal membrane is visible in 10 sections. This represents a length of at least 100 μ (cf. p. 42). A connection between the wall of the fore-gut and the cephalic mesoblast is no more to be found. Fig. 51, plate V shows one of the very first sections, in which the chorda-anlage is visible. Beneath the medulla in the hypoblast we find a clearly perceptible thickening, which undoubtedly belongs to the cephalic part of the chorda-anlage. The hypoblast is rather thick here, partly due to the lining of the fore-gut being cut tangentially. The chordal plate passes into the hypoblast, no distinct limit being found. It makes the impression, as if the notochord is in the very first act of separating from the hypoblast. This process is much clearer a few sections further caudalward (fig. 52). Here the notochord (a chordal plate exists no longer, as it has become notochord) has a markedly cylindrical shape, both hypoblast-edges being almost fused. The separation of the notochord from the hypoblast has nearly come to an end. In each section of the notochord very few nuclei are visible. Mesoblast and notochord are separated by a distinct membrane.

Further caudalward the notochord loses its cylindrical shape. In the region of the first somites a real chordal plate is visible. At first it has a small size, but gradually it gets the shape as is shown in fig. 53, drawn after a section through the 9th pair of somites. On the left the chordal plate is no longer covered by the underlying hypoblast, on the right, however, this is still the case. The edges of the cleft hypoblast are compressed by the spreading chordal plate, but in a less degree than in embryo N°. 89.

Caudalward the chordal plate increases in height and breadth. A little behind the 13th pair of somites the chordal plate is arched (fig. 54, plate V). One section further the hypoblast extends beneath the whole headprocess. From this point to the primitive streak the headprocess shows cell-membranes and nuclei radially arranged around one or two lumina. Even virtual openings are to be found. Sometimes such a lumen suddenly becomes larger, and disappears in the following section. The lumen, reproduced in fig. 55, is one of the larger lumina. It is traceable in three sections (= 30 μ). The whole headprocess shows several parts of this chordal canal. A first indication of the formation of this canal we find in the cells, which are arranged around a virtual

lumen. Then a real lumen appears, which is to be found in the centre or to the left or to the right in the headprocess. Out of this chordal canal the ventral dehiscence of the headprocess takes place, in order to form the chordal plate. A schema of the caudal part of embryo N°. 31 is shown in fig. 65, plate VI. Two parts of the chordal canal are indicated, only these occupying the centre of the sections. To the left and to the right of the section figured, many lumina are to be found. The hypoblast in this embryo has also fused with the primitive streak-tissue in the mid-axis.

A third *Manis*-embryo, also with 13 pairs of somites, is n°. 108, which is cut nearly in the sagittal plane, the chorda-anlage thus being sectioned obliquely. The cranial part of the notochord is reproduced in fig. 56, plate VI. The chordal plate occupies the roof of the fore-gut, and ends, covered by the hypoblast, in the mesoblast of the head. As is evident from fig. 56 the primary pharyngeal membrane is cut tangentially and has a length of about 160 μ. The cephalic part of the lining of the fore-gut is distinctly thickened, a connection of the mesoblast with this part is not to be found here. In the caudal region, we also find a headprocess, the hypoblast extending beneath it, and here also in the mid-axis the hypoblast fuses intimately with the primitive streak.

Embryo N°. 68 differs little from the other embryos, as to the chorda-anlage. I portray only a sagittal schema and a transverse section (figs. 66 and 57, plate VI). The sagittal schema shows a very short headprocess in comparison with others. A very distinct chordal canal with wide lumen is to be found (fig. 66). Here some other peculiarities follow: a primary pharyngeal membrane is present, as well as a fore-gut, traceable in 57 sections (at least 570 μ). The chorda-anlage can be followed up cranialward along a great distance and is not present in 5 sections of the roof of the fore-gut only. It must be taken into consideration that the fore-gut has been sectioned tangentially; so the part, in which no chorda-anlage is to be found, has a length of more than 50 μ. For the same reason the primary pharyngeal membrane visible in 8 sections, has a length of more than 80 μ.

The notochord has distinctly separated from the wall of the fore-gut further caudalward. No figures of this are given, because in the embryo with 18 pairs of somites (N°. 53) this process, which has

started in an embryo with 13 à 14 pairs of somites (N°. 31) has progressed further and is more distinct here. The hypoblast is compressed by the chordal plate, incorporating in it (cf. N°. 89). Caudalward we see a clearly perceptible chordal canal in the headprocess. The largest diameter of the wide canal is ± 24 μ (fig. 57); the cell-membranes and nuclei are arranged radially. The canal is very long, being traceable in 26 sections (± 260 μ) and opens ventrally into the future alimentary tract. On the left and on the right a few lumina are visible in the headprocess. Close behind the chordal canal reproduced, the headprocess originates from the primitive streak, and in this region the hypoblast is again intimately fused with the primitive streak-tissue.

The last embryo to be described for the development of the notochord is N°. 53 with 18 pairs of somites. It is an important stage, because the notochord has separated from the hypoblast in this embryo along a great distance.

Fore-gut and primary pharyngeal membrane are well developed and respectively traceable in 96 and 14 sections (= at the least 960 and 140 μ). The chorda-anlage extends far cranialward, the wall of the fore-gut being free of it in 6 sections. It is remarkable that the cranial part of the chorda-anlage, represented in fig. 58, plate VI is still incorporated in the hypoblast, whilst parts of it further caudalward have already quite separated from the hypoblast. This figure (58) is drawn after the 4th section, in which the chorda-anlage is traceable from before backwards. The separation of the notochord has already started here. The wall of the fore-gut is rather thick here, consisting of about two layers of cells. Fig. 59 shows the 14th section caudad of the section, reproduced in fig. 58. Here the notochord has completely separated from the hypoblast, extending beneath it. The notochord is slightly compressed here, the hypoblast a little thickened.

Further caudalward the notochord becomes more high than broad, but at the level of the greater part of the somites it is cylindrical. This is shown in fig. 60. It is noticeable that the diameter of the notochord is much larger and here more nuclei are to be found than in the parts situated further cranially.

The place, where in younger stages the chordal plate was incorporated in the hypoblast, can still be indicated, for here, beneath the

notochord, the hypoblast is thinner than quite near it. In younger stages we have met with the compressed edges of the hypoblast; it seems that the hypoblast has coalesced from these parts.

From the 11th pair of somites caudalward the chordal plate is still slightly incorporated in the hypoblast, as is evident from fig. 61, a section through the 13th pair of somites. The chordal plate is still a flat organ. This we can see only in the region of the somites XI—XV (cf. fig. 67), for beneath the last somites the chorda-anlage is completely covered by the hypoblast. On comparing figs. 66 and 67, we see how the part in front of the primitive streak, covered by the hypoblast, has become much larger: in an embryo with 16 pairs of somites (N°. 68) the incorporation of the headprocess comes to and end, for in the slightly older embryo N°. 53 with 18 pairs of somites the hypoblast extends as an uninterrupted layer along a great distance under the chorda-anlage. From the head-region of the embryo the closure of the hypoblast beneath the notochord takes place caudalward. So we see that only along a short distance (the region of 5 pairs of somites) the chordal plate is still partly incorporated in the hypoblast (the most anterior part of the chorda-anlage excepted). The incorporation of the head-process in the hypoblast takes place no longer at a certain moment (probably when the stage of 16 pairs of somites is reached); then the hypoblast remains intact.

Though the incorporation of the headprocess in the hypoblast is out of the question in this part, yet lumina are formed in the head-process here. Properly speaking, we cannot use the name headprocess i.e. the prolongation of the primitive streak in the direction of the future head-region. The organ we are discussing now, has formed out of the primitive streak in situ, which shortens during the longitudinal growth of the embryo. Consequently the headprocess in this part of the embryo is no outgrowth of the original primitive streak. Three of the lumina, above mentioned, are indicated in the sagittal section (fig. 67).

With the description of the development of the notochord in this embryo we will end. In still older embryos the notochord has entirely separated from the hypoblast and lies free between medullary tube and alimentary tract. Cranially it is always much thinner than caudally, where it remains broad and tall with compactly arranged cells for a long time.

Two important questions must be treated now:

1°. Does the anterior region of the notochord originate from the prochordal plate?

2°. Do elements out of the metenteron-wall, i.e. headprocess, pass into the definite lining of the gut or is the latter formed only by the hypoblast, which, as we have seen, develops out of the embryonic knob by delamination?

BONNET answers the first question in the affirmative (like HUBRECHT 1890) and concludes this (1901) amongst others from the following facts. In dog-embryos of different stages he traced the place of the anterior limit of the chorda-anlage and found that in embryos with 10 pairs of somites the chordal plate passes into the thickened part of the hypoblast, which he calls „Ergänzungsplatte" (= protochordal plate HUBRECHT). In an embryo with 16 pairs of somites the most cranial part of the notochord reaches to quite near the anterior point of the fore-gut. He points out that the notochord gradually grows cranialward and concludes that this added part is formed by the „Ergänzungsplatte" (to compare also the comparative part, p. 79). Of course he can trace this best in embryos sagitally sectioned.

I can ascertain the following data in the *Manis*-embryos, which are cross-sectioned for the greater part. In primitive streak-stages the headprocess extends exactly as far as the posterior part of the prochordal plate. After incorporation in the hypoblast the chordal plate passes directly into this plate (cf. the sagittal sections of N°. 83, figs. 33, 34, and the description of embryo N°. 64). The *Manis*-embryo with 10 completely formed pairs of somites (N°. 32) possesses a gut-lining free from chorda-anlage along a distance of $\pm$ 280 μ (measured from the cranial point of the fore-gut). From this point the chordal plate is traceable caudalward. The older the embryo, the further the cranial limit of the chorda-anlage reaches, which is apparent from table 3.

It must be noted that the cranial part of the fore-gut is curved ventrally and is consequently sectioned in a more or less tangential plane. Hence the measures, which are calculated from the number of sections, are too small, especially in the older embryos, in which the head-bend is more distinct than in younger ones. This is marked by a).

Table 3.

Cat. N°.	32	89	31	108 sagittally sectioned.	68	53
Number of pairs of somites..	10 à 11	13	13 à 14	13	16	18
Length of the part of the fore-gut without chorda-anlage	300 μ	$\pm$ 180 μ	$>$ 130	200 μ	$>$ 50 μ	$>$ 60 μ

The measure of the embryo sagittally sectioned (N°. 108) is correct (fig. 56, plate VI), which corresponds to the measure calculated for the embryo N°. 89.

These data are indications for the opinion that the notochord in older stages reaches further cranialward than in younger ones. They do not prove that the prochordal plate participates in the formation of the anterior part of the notochord. Moreover, it is possible that the anterior part of the notochord grows cranialward out of the chordal plate itself, without being formed out of the prochordal plate.

In the *Manis*-embryos, however, something else is to be observed.

We start from the younger stages, where the chordal plate gradually passes into the prochordal plate and is quite incorporated in the hypoblast. In the embryo N°. 31, the anterior part of the chorda-anlage is still distinctly incorporated in the lining of the fore-gut, a beginning of separation from the hypoblast being visible, however, further caudalward (cf. figs. 51 and 52, plate V). The difference is not very marked here, but in the embryo N°. 68 with 16 pairs of somites and especially in the embryo N°. 53 with 18 pairs of somites, the cranial part is distinctly incorporated in the hypoblast, whilst the hypoblast-edges have coalesced under the notochord more caudalward (figs. 58, 59 and 60, plate VI). Consequently the anterior part of the notochord separates later from the hypoblast.

On connecting these two observations — the growth of the anterior part of the notochord in cranial direction and the later separation from the hypoblast of the same part — I must admit that they support the opinion of BONNET and HUBRECHT, but they do not prove

it. Both observations can just as well confirm the view that the anterior part of the chorda-anlage grows out of the chordal plate. On considering them in mutual relation, I come to the conclusion that they certainly are a support for the view of BONNET. I do not use the name protochordal plate to indicate this part of the hypoblast, because it is not proved that the anterior part of the notochord arises from it. I used the name *prochordal plate*, because in young stages it is lying just before the headprocess, out of which the chordal plate originates. —

The second question, whether there is a transition of cells from the headprocess to the future lining of the alimentary tract, is answered in the affirmative by RABL (1915). Counting the number of cells in a cross-section of a broad chordal plate at a fixed point — the first pair of somites — of a rabbit-embryo, and counting in the same region later on the number of cells in a section of the notochord, he found that in the former case each section of the chordal plate possesses a much larger number of cells than in the latter. In some cases only half the number of cells is found. From the chordal plate, developing out of the headprocess (or archenteron-wall in RABL's terminology), cells have disappeared and, according to RABL, these cells have passed into the hypoblast, the lining of the future alimentary tract. RABL even thinks that the greater part of the gut-lining derives from cells of the headprocess. As will be discussed more fully in the theoretical part, it is my opinion (agreeing with ASSHETON) that the headprocess of the Amniotes is the homologue of the metenteron of the Anamnia, the part of the gut, formed by overgrowth and ingrowth (invagination) of cells at the „deuteroporic" lips. The distinct metenteron of the Anamnia is reduced in size in the Amniotes, the wall of the metenteron being a prolongation of the primitive streak (= the changed „deuteropore" of the Anamnia) in Birds and Mammals. In this organ, the headprocess, is to be found in many cases a longitudinal canal, the metenteron or LIEBERKÜHN-canal. In both, Anamnia and Amniotes, the wall of the metenteron fuses with that part of the gut-lining, which has originated from cells, formed in most cases by delamination of the embryonic knob, and called by me hypoblast for short. A communication between the archenteron, the cavity enclosed by the hypoblast, and the metenteron is esta-

blished. In Anamnia the posterior part of the gut is formed by a large part of the metenteron; consequently we can expect that this is also the case in Amniotes.

RABL tries to prove the transition of cells of the headprocess to the definite gut-lining as indicated above. Against this the following facts must be objected:

1°. STRAHL (1916) is of opinion that these cells in the live embryo, in which all cells are movable with respect to each other, can pass as well into the mesoblast, generally lying in close connection with the notochord. STRAHL does not prove this, mentions it only against RABL's view.

2°. It is not certain that these cells of the chordal plate take part in the formation of the gut-lining. KEIBEL (1916) argues that it is very well possible that, whilst the notochord separates from the hypoblast, these cells move in such a way that they become situated behind each other, contributing in this manner to the growth in length of the notochord (KEIBEL 1889, 1916, „Scheinwachstum" of the notochord).

As we have observed, *Manis* has a very broad chordal plate in many cases, sometimes even with as many nuclei as RABL describes for the rabbit. In later stages in *Manis* we also find a cylindrical notochord in the same region of the embryo, where the broad chordal plate was to be found. In most cases every section of the notochord consists of much less cells than the sections of the chordal plate (cf. figs. 36, 37, 38, 42, plate IV and 59, 60, plate VI). Here a table is given, in which is indicated the number of nuclei from chordal plate and notochord in sections through different somites (table 4).

Table 4.

N°. of embryo.	Som. I	Som. IV	Som. VIII
64 (8 à 9 som.)..	13	15	24
32 (10 à 11 som.).	15	17	26
53 (18 som.).....	7	12	17

From this table it follows that RABL's observations in the rabbit are corroborated in *Manis*. [In connection with this, it is to be noted that KEIBEL described (1889) cross-sections of the notochord with only two nuclei (*Cavia*)]. These observations are not a proof of RABL's opinion, according to me, for though I do not believe that STRAHL's supposition that in the live embryo cells of the chordal plate pass into the mesoblast, is right, I think that KEIBEL's opinion is not improbable. The data mentioned above may even support it. It is also in favour of this opinion that in stages in which the notochord is no longer incorporated in the hypoblast, the cranial and older part always has a small diameter with few nuclei, while the caudal and younger part has a large diameter with numerous compactly arranged cells. It seems as if in this younger part of the notochord, the cells are not yet situated behind each other. KEIBEL's „Scheinwachstum" will still take place here. I believe that consequently the observations in *Manis* are in favour of KEIBEL's opinion and that RABL's view must be rejected. It is probable that the formation of the metenteron of the Anamnia is reduced in the Amniotes to such an extent that the headprocess no longer participates in the formation of the future alimentary tract. It would even be very difficult to trace elements passing from the headprocess into the lining of the gut, for it is almost impossible to ascertain the transition of elements from one organ to another, when these organs are intimately fused. So I believe that the second question (p. 42) must be answered in the negative.

I must still point to some facts, which become clearer, when we compare the different stages. In doing so it is evident that the primitive streak is a centre of growth, from which the headprocess and also the chorda-anlage (derived from the headprocess), are formed. We can distinguish a part of the headprocess, with which the mesoblast is connected, and a part, where this is the case no longer, the latter lying more cranially. Both parts move caudalward, just as the chordal canal, formed in it. More cranialward a short transitional part follows and then the chordal plate extends a great distance, being incorporated in the hypoblast and thus forming the roof of the future alimentary tract. In stages, in which about 13 pairs of somites are formed, the chordal plate begins to separate from the hypoblast. Before the chordal plate a fifth part of the chorda-anlage follows, separating from the

hypoblast later on. It is not certain, whether this part is formed by the so-called chordal plate.

The notochord can be divided into a part, formed out of the head-process (the metenteron of the Anamnia) and a part, lying caudally of this and formed out of the primitive streak, the homologue of the changed deuteroporic lips. Perhaps an anterior, accessory part can be distinguished, but it is not certain, whether this is formed out of the chordal plate itself, or out of the prochordal plate. When the latter is the case, and this seems probable, it would be formed from the lining of the archenteron.

Summary.

In *Manis* a tongue-shaped organ, the so-called headprocess, develops out of the cranial part of the primitive streak and grows cranialward between epiblast and hypoblast. The place, where it is formed out of the primitive streak, is generally swollen and is then called HENSEN-knob.

The headprocess reaches as far as a thickened part of the hypoblast, the prochordal plate. The hypoblast, extending beneath the headprocess and primitive streak, is originally equally thick everywhere, but gradually, before the headprocess fuses with it, the hypoblast becomes thinner in the mid-axis. From the sides of the headprocess the gastral mesoblast is formed, out of which the somites, lying in the cephalic region of the embryo, derive.

The anterior border of the headprocess fuses with the prochordal plate and at the same time the headprocess incorporates in the hypoblast, and then the chordal plate is formed out of it. The incorporation of the chorda-anlage occurs in *Manis* as follows. A lumen, the chordal canal, appears in the centre. (A LIEBERKÜHN-canal, observed in the original headprocess in other Mammals, I did not find in the *Manis*-embryos at my disposal). Then the wall, lying beneath the canal, splits, the hypoblast is cleft here, and the chorda-anlage spreads, laterally pushing the hypoblast, which process is visible, as the edges of the cleft hypoblast are distinctly compressed.

The parts of the chordal canal can extend along a great distance, but usually they are only traceable in a few sections. They can open

cranially into the cavity enclosed by the hypoblast. The largest and most distinct canals occur in the mid-axis of the headprocess, but they can also be observed on the left or on the right, and in many cases, even on both sides. Generally the lumina, which appear double, are also traceable along some distance. They are all considered the homologue of the canalis neurentericus of the Anamnia. The cell-membranes and nuclei are radially arranged around these canals.

The incorporation of the chorda-anlage in the hypoblast takes place from before backward. When the chordal plate is entirely incorporated in the cranial region of the embryo (i.e. when the borders of the chordal plate pass into the hypoblast, which then does not extend beneath it) the incorporation still continues more caudalward. Then all transitional stages of this process are traceable in the same embryo.

There are indications of the anterior part of the notochord being formed by the prochordal plate, a thickened part of the hypoblast. If this anterior part grew independently out of the chordal plate, already incorporated in the hypoblast, these observations could be made as well.

Originally the length of the headprocess is not considerable. Later on it derives from the primitive streak, which shortens from before backwards. This posterior part also incorporates in the hypoblast. This is still the case in an embryo with 13 pairs of somites and a 14th pair forming. In an embryo with 16 pairs of somites the incorporation comes to an end, and in an embryo with 18 pairs of somites the notochord is for the greater part separated from the hypoblast. Then in the region of only 5 pairs of somites and in the most anterior part of the chorda-anlage this is not the case. Still further caudalward a part of the chorda-anlage, which does not incorporate in the hypoblast, is formed out of the primitive streak. Here too parts of the chordal canal are to be found. On comparing younger and older embryos, we see that the chordal canal moves caudalward, just as the neurenteric passage of the Anamnia.

In an embryo, possessing 8 pairs of somites and a 9th forming, the gastral mesoblast is still connected with the anterior part of the chordal plate. In the posterior part of the headprocess the mesoblast is always connected with it.

In younger stages, in which the chordal plate not yet separates from the hypoblast, 4 zones of the chorda-anlage are to be found.

They are from back to front: 1°. The part just formed out of the primitive streak, connected with the mesoblast. 2°. The part, no longer connected with the mesoblast. These parts are both covered by the hypoblast; parts of the chordal canal can be observed in them. 3°. A transitional zone. 4°. The chordal plate incorporated in the hypoblast. Later on the chordal plate separates from the hypoblast, the most anterior part of the chorda-anlage separating still later.

Originally the hypoblast extends as an uninterrupted layer under the primitive streak. Secondarily in the mid-axis, in the anterior part of the primitive streak, the hypoblast fuses intimately with the tissue of the primitive streak. This place moves caudalward, when the primitive streak shortens from before backward. Laterally epiblast, mesoblast and hypoblast are distinctly separated in this region of the embryo.

It could not be proved that cells of the headprocess pass into the lining of the archenteron. It is probable that the cells of the chorda-anlage place themselves behind each other, contributing in this way to the growth in length of the notochord. This is probably the explanation of the fact that in young embryos a very broad chordal plate, consisting of numerous cells, occurs, while later on in the same region of the embryo the diameter of the notochord is small, each section containing few cells. In embryos, in which the chorda has separated from the hypoblast a long time ago, and lies free between medullary tube and gut, the anterior part is always thinner than the posterior part, having been formed out of the primitive streak last. This observation might also be in favour of this view.

THE SO-CALLED PROCESS OF GASTRULATION IN MAMMALS.

Before discussing more fully the so-called process of gastrulation in Mammals, I must make some general remarks on gastrulation. HAECKEL, who formulated the idea „gastrula", defined it („Gastraea-theorie", 1874, p. 15) as „einen einaxigen ungegliederten hohlen Körper ohne Anhänge, dessen einfache Höhle (Urdarm) sich an einem Pole der Axe durch eine Mündung (Urmund) öffnet und dessen Körperwand aus zwei Zellenschichten oder Blättern besteht: Entoderm oder Gastralblatt und Exoderm oder Dermalblatt." The gastrula is formed in many different ways. The formation of it can take place by means of invagination, delamination, epiboly etc. A two-layered embryo is always formed with epiblast on the outside (= exoderm) and hypoblast on the inside (= entoderm). Both layers enclose a cavity, which communicates with the exterior by an aperture, the blastopore or primitive mouth (= Urmund). At present HAECKEL's view that the process of invagination is the more primitive is still opposed to RAY LANKESTER's view that the process of delamination is the more primitive, which is nothing but a matter of taste, according to me. The blastopore, formed by invagination, is the natural consequence of the process of invagination itself, whilst in the two-layered stage, formed by delamination, the blastopore secondarily opens into the exterior.

In the descriptive part we have seen how in *Manis* the hypoblast is formed out of the embryonic knob by delamination and how afterwards it grows round the inside of the trophoblast and finally forms a closed sac. The formation of the hypoblast by delamination has been observed in a great number of Mammals. Lately, however, (HILL 1910, HARTMAN 1919) a somewhat different process is described in Marsupials, viz. „immigration" (p. 17).

In the embryonic knob (epiblast or hypoblast) an opening is not

to be found in *Manis*. Yet in the literature on the first developmental stages in Mammals some cases are communicated, from which it would be evident that in the two-layered germdisc a true blastopore is to be found.

In the first place SELENKA (1886) mentions that in blastocysts of *Didelphys virginiana* (in three cases out of eight), he has observed an opening in both germ-layers. At the inside of the opening a coagulation is found. Later on, neither opening nor coagulation are visible.

KEIBEL too (1889, p. 53) has found in the rabbit a place, where „ein Ubertreten von Zellen aus der oberen in die untere Schicht (scheint) statt zu finden." Later on, he could never find similar formations.

HEAPE (1883) figures somewhat older stages of the mole, in which „mesoblast-cells" are developing. A spot is visible, where the hypoblast passes into the epiblast. Here there is an indication of a „blastopore", but no communication of the blastocyst-cavity with the exterior is to be found. HEAPE himself considers it to be an early LIEBERKÜHN-canal. Here the very first anlage of the headprocess is to be observed, for to the right of the „blastopore" (fig. 31) a row of cells is visible, extending cranialward between epiblast and hypoblast. These cells are called „mesoblast-cells" by HEAPE, but evidently they are the anlage of the headprocess.

In young shields of the dog (1897), BONNET has found openings, penetrating the epiblast, but not the hypoblast. According to him, they are not artificial. BONNET utters his opinion with great reservation, however, for according to him, it is not at all certain that they can be compared to the openings, described by SELENKA or by HEAPE.

Finally, HUBRECHT describes different „blastopores" (1890 *Sorex*, 1902 *Tarsius* and *Erinaceus*, cf. also HUBRECHT 1908 and 1912). In *Sorex*, in the posterior part of the embryo (showing amongst others a prochordal plate, a thick layer of epiblast and a distinct primitive streak, from which the mesoblast develops), he finds a rudimentary „blastopore", not quite penetrating the germdisc. Usually no „blastopore" is to be observed in *Tarsius* (1902), but in one case HUBRECHT mentions a deep pit in front of the primitive streak (consequently, the embryo is not a two-layered germdisc). According to HUBRECHT this pit is a rudimentary blastopore. Young *Erinaceus*-stages (HUBRECHT 1902, 1908, 1912), however, often show a distinct canal in the pos-

terior part of the germdisc. In the figures, sketched by him (1902: figs. 8 and 9, plate XII, 1908: fig. 53, 1912: figs. 46*a* and 46*b*) only epiblast and hypoblast are to be seen, the „blastopore" penetrating both. In the sections of the *Erinaceus*-embryos (which I have examined), between epiblast and hypoblast cells are distinctly visible, however, which have disappeared as a distinct layer in the reproduction.

Must these cases be considered examples of blastopores, where hypoblast and epiblast pass into each other? I do not think so. In many cases, I take this „blastopore" to be the LIEBERKÜHN-canal, of which I hope to explain the theoretical signification in subsequent pages (p. 53, sqq.). In *Tarsius* and *Sorex* the „blastopore" must certainly not be regarded as such, the reasons being, 1°. that the stage of the embryo, in which a great part of the mesoblast, a primitive streak and a prochordal plate have originated, is too far developed; 2°. that the place of these openings or pits lies just in front of the primitive streak (in the HENSEN-knob); 3°. the presence of a headprocess in *Erinaceus*, which is not reproduced well in the figure of HUBRECHT.

This became evident after a renewed examination of the sections, present in the HUBRECHT-Laboratory at Utrecht. Also for *Erinaceus* I think that this opinion holds good. The germdiscs (though HUBRECHT reproduced but two sections with a „blastopore", yet openings occur in many blastocysts) are much younger, however, than those of *Tarsius* and *Sorex*, also described by him. Between epiblast and hypoblast a row of cells is to be found — not reproduced as a distinct layer — lying in the embryo, figured in 1902, 1908, and 1912, to the right of the opening. These cells are the first anlage of the headprocess, according to me. The primitive streak lies (in the figure) above the opening, representing the LIEBERKÜHN-canal, which has developed early in *Erinaceus*. (Perhaps this is due to the very early attachment of the embryo to the uterine wall.)

HEAPE's figure is to be interpreted in the same way. This investigator thinks, he has to do with an early „neurenteric canal" (= LIEBERKÜHN-canal). To the right of the opening HEAPE's figure (1883, fig. 31) shows a headprocess, to the left a distinct primitive streak.

HARTMAN examined (1919) a great number of young blastocysts of the opossum (*Didelphys virginiana*), but he never found the ope-

ning, described by SELENKA in 1886, which according to HARTMAN must be considered the place, where a mother-cell of the hypoblast migrates from the epiblast („immigration"). Consequently, in this case we must reject the view that the opening, described by SELENKA, is a blastopore.

Finally KEIBEL and BONNET are so reserved in their opinions on the theoretical signification of the openings, described by them that we need not consider them at greater length.

So it has not at all been proved that a blastopore is found in the didermic germdisc of Mammals, and it is certain that in many cases the opening described is a short LIEBERKÜHN-canal, which has opened into the blastocyst-cavity. If this is true, HAECKEL's definition of the gastrulation is not applicable to Mammals. Hence we cannot speak of a Mammalian gastrula.

Now we will proceed to a discussion on the formation of the alimentary tract in Vertebrates. As a starting point the holoblastic ova, found in Amphibia (Dipnoi, Ganoids and *Petromyzon*) are chosen, these representing phenomena easy to explain, as they are microlecithal.

In Amphibia we observe the following facts in the main. After the formation of the blastula a process begins, called „clivage gastruléen" by BRACHET (1902). After this process (the separation of the micromeres and macromeres by a slit) the macromeres spread over the inside of the blastula-cavity and consequently the wall in this part of the embryo then consists of two layers, epiblast and hypoblast.

From the place, which was always designated by the name blastopore, but must be called deuteropore, an overgrowth and ingrowth of cells take place simultaneously, the result being a cavity, the roof of which is formed by micromeres. This cavity, formed by invagination, fuses with the lumen, formed out of the blastocoel during „clivage gastruléen". Like ASSHETON, I will call the latter *archenteron*, the former *metenteron*. Moreover, ASSHETON accepts two developmental phases, the *protogenesis*, during which the archenteron is formed in a radially symmetrical larva, and the *deuterogenesis*, during which the growth in length of the embryo is established.

The archenteron originates directly out of the blastocoel; it then fuses with the metenteron and both form the primitive gut.

From the wall of the metenteron the notochord and a great part

of the mesoblast derive. After separation of the notochord from the metenteron-wall, the latter closes beneath it.

In *Amphioxus* both developmental phases are also present; during protogenesis a radially symmetrical gastrula is formed by invagination and consists of two layers, epi- and hypoblast enclosing an archenteron. The blastocoel, however, disappears. During deuterogenesis complicated processes take place (CERFONTAINE 1906) with the result that the archenteron is enlarged caudalward by a metenteron. In the bilaterally symmetrical larva notochord and mesoblast develop.

In the megalecithal and meroblastic ova of the Selachii a blastocoel is to be found between germdisc and yolk-mass. Here the hypoblast is formed from the germdisc by delamination. As in most of the Amphibia (*Triton* excepted) the blastocoel becomes the archenteron. The way, in which the hypoblast is formed, is different, however, in Amphibia and Selachii: in the latter by delamination, in the former by „clivage gastruléen." A process now starts, which is very distinct in Selachii, viz. the formation of the metenteron by invagination. Here archenteron and metenteron also fuse and form the primitive gut. The deuterogenetic part of the gut-lining, however, forms the greater part of it. Probably this is in connection with the extension of the hypoblast over the bulky yolk-mass. A consequence of this process is that the formation of the trunk anticipates the formation of the head. From the roof of the metenteron, the notochord and a part of the mesoblast develop.

The deuteroporic lips are easy to distinguish in the groups discussed. In Amniotes they have disappeared as such. The primitive streak of the Amniotes, however, can be homologized with the deuteroporic lips of the Anamnia. RAUBER („Primitivrinne und Urmund", Morph. Jahrb. II, 1876) was the first to hold the opinion that the primitive streak of the Amniota is formed by intimate fusing of the sides of the elongated deuteropore of the Anamnia. Later on, BALFOUR (1880) independent of him expressed the same view. BONNET (1920) is one of the warm adherents of this theory. In Anamnia a great part of the mesoblast is formed out of the sides of the deuteropore, in Amniota out of the fused sides of the elongated deuteropore, the primitive streak. In both groups an organ develops in front of the deuteroporic lips by invagination. From this organ the notochord and a considerable part of the mesoblast originate.

In Reptiles we also see that the hypoblast separates from the germdisc by delamination during protogenesis. The cavity, situated between hypoblast and yolk-mass, is called archenteron here too. The metenteron is formed by invagination. The cell-mass, in which the metenteron occurs, grows cranialward between epiblast and hypoblast and is called headprocess. Hence the headprocess is the homologue of the metenteron-wall of the Anamnia. The metenteron communicates by an aperture with the exterior; it is the rest of the deuteropore. In Reptiles archenteron and metenteron fuse: the wall of the metenteron connects itself with the archenteron-wall (= hypoblast) and the metenteron opens into the archenteron. In Reptiles too the notochord is formed out of the roof of the metenteron and a great part of the mesoblast out of its sides. The definite gut-lining consists principally of hypoblast. The metenteron-wall forms but a small part of it; the production of notochord and mesoblast is the chief function of the metenteron-wall now. Moreover, it is difficult to trace, whether cells of the metenteron-wall pass into the lining of the alimentary tract.

In Birds the metenteron is still more reduced. Though a lumen is sometimes to be observed (generally called canalis neurentericus — GASSER 1878 —), yet in most cases a so-called headprocess without lumen is found, extending from the primitive streak in the direction of the future head-region. The headprocess fuses with the protogenetic hypoblast, formed from the germdisc by delamination. In Birds it is still more difficult to trace, whether cells of the metenteron-wall participate in the formation of the future gut-lining.

Reptiles, Birds and Monotremata are all megalecithal forms. The processes above described are about equal in these groups.

The other Mammals, which are microlecithal, (in Marsupials the yolkbody is extruded in early cleavage-stages, cf. HILL 1910, 1918) show the same organs and developmental phases.

Starting from the two-layered developmental stage, we soon see that, in the posterior part of the germdisc, the primitive streak develops, which is a thickened part of the epiblast, and from which mesoblast proliferates. In front we see the HENSEN-knob and at the posterior border of the shield the caudal knob. These two parts are the homologues of the dorsal and ventral deuteroporic lips, the primitive streak the homologue of the closed elongated deuteropore of the Anamnia. In a

true deuteropore the epiblast and hypoblast pass into each other; this does not take place, however, in the primitive streak. From the HENSEN-knob the headprocess grows out in a cranial direction. In most cases a canal appears in the headprocess. The headprocess must be considered the homologue of the metenteron-wall of the Anamnia, the lumen in it the metenteron.

The canal in the headprocess was first found by LIEBERKÜHN in Mammals (*Cavia*, 1882) and is still often called LIEBERKÜHN-canal. LIEBERKÜHN himself designated it by the name chordal canal. Very often the name neurenteric passage or canalis neurentericus is used.

For some time the headprocess extends freely between epiblast and hypoblast, which has been formed from the embryonic knob by delamination („immigration" in Marsupials) and encloses the umbilical vesicle, from which a part of the gut will derive.

Consequently in Mammals a part of the blastocyst-cavity becomes the archenteron.

Then the headprocess fuses with the wall of the archenteron, after having formed mesoblast out of its sides. The chorda-anlage is then said to have incorporated in the hypoblast: the chordal plate occupies the roof of the primitive gut for some time. After that the notochord separates from the hypoblast, which closes again beneath it. Probably the headprocess is not participating in the formation of the lining of the alimentary tract in Mammals (cf. the descriptive part pp. 44—46). Here the metenteron-wall is distinctly present, but the metenteron has been greatly reduced in size. In the megalecithal ova of Selachii, Reptiles and Birds, the archenteron is naturely small; in Mammals, the Monotremata excepted, it is very large. The part, which the metenteron-wall takes in the formation of the definite gut-lining, becomes less and less important. In Anamnia the dorsal and ventral deuteroporic lips form a growth-centrum, in Amniota, the primitive streak also forms one, by the activity of which the embryo grows in length.

In Vertebrates the archenteron-wall is generally formed by delamination; a delamination-blastopore is never found in the different groups. In Mammals, I think that such a blastopore does not occur either (cf. pp. 51—53).

As we shall see, the mesoblast is derived both from the protogenetic and from the deuterogenetic hypoblast (i.e. headprocess) and

primitive streak in different Mammals. The names given (in the descriptive part) to the four parts of the mesoblast are partly *topographical* terms (*cephalic* mesoblast, *peripheral* mesoblast, both protogenetic) and partly terms in connection with the theoretical signification of the organs, from which they develop. These two deuterogenetic mesoblast parts are called *gastral* mesoblast, because it originates from the metenteron-wall and *peristomal* mesoblast, because it is formed out of the wall around the stoma i.e. deuteropore.

The above survey gives a comparative morphological description of the same ontogenetical processes in Vertebrates. It most resembles the opinion of ASSHETON, who has given a clear account of it in 1907 and 1909. For some peculiarities I made use of BONNET's view, which he states in the last edition of his „Lehrbuch".

In proceeding to a concise description of the different opinions of the authors on the so-called gastrulation-process and on the formation of the primitive gut in Mammals, we must mention in the first place RAUBER, who, as early as 1876, homologized the primitive streak of the Amniotes and the „blastopore" (i.e. deuteropore) of the Anamnia. Independent of him, BALFOUR compares the primitive groove of the Amniotes to the primitive mouth of the Anamnia. VAN BENEDEN comes to agree with this opinion in 1886. He then thinks that the LIEBERKÜHN-canal (very marked in *Vespertilio murinus*) is homologous with the »gastrula"in-growth of the Amphibia. Consequently he calls the LIEBERKÜHN-canal archenteron.

At that time it was already known that the hypoblast, which participates in the formation of the gut-lining, originates from the embryonic knob by delamination before the invagination of the archenteron. It was thought that the principal part of the gut-epithelium was formed by „precocious segregation", though a part of it was invaginated (this being indicated in the name archenteron). The early origin of the hypoblast was connected with the increase in yolk in Amniotes and with the secondary loss of it in Mammals.

Gradually many investigators began to say that the gastrulation of the Vertebrates (especially of the Amniotes) occurs in two phases. In the first phase the hypoblast is formed by delamination, in the second phase the material for the notochord and mesoblast finds its way into

the interior of the embryo. So later on, two sharply opposed opinions are found side by side. The one holds that the invagination of the metenteron is the most important process of gastrulation (RABL, SOBOTTA, GREIL), the other that the gastrula of the Mammals is the two-layered germdisc and that the invagination process has nothing to do with gastrulation (HUBRECHT, KEIBEL).

Formerly HUBRECHT and KEIBEL accepted a gastrulation in two phases in Mammals, called caenogenetic and palingenetic phase by the former and first and second gastrulation-phase by the latter.

In 1902, however HUBRECHT changed his point of view, which he explained more fully in 1905. From that time onwards he thinks that only the first phase is gastrulation and that the second phase has nothing to do with it. The second phase is a process, to which he gives the name of notogenesis in connection with LWOFF's opinion (1894). Only in *Amphioxus* the gastrula is formed by invagination, in all other Vertebrates by delamination. When the delamination-gastrula is formed i.e. when „an intestinal entoderm is differentiated as against an integumentary ectoderm" (HUBRECHT 1905, p. 408) the second phase of development begins. The gastrulation (= cephalogenesis) is followed by a process, which starts with the formation of notochord and mesoblast (notogenesis). The radially symmetrical larva has become bilaterally symmetrical.

Properly speaking, the terms cephalogenesis and notogenesis do not express what happens in reality. As HUBRECHT himself remarks, only the most anterior portion of the head is formed during cephalogenesis and moreover trunk-segments enter into the composition of the head. For that reason, I have used in the above ASSHETON's terms protogenesis and deuterogenesis, which are almost identical with HUBRECHT's cephalo- and notogenesis.

Like HUBRECHT, KEIBEL too accepts (since 1905) a definition of gastrulation, applicable to the Invertebrates as well as to the Vertebrates. This definition, however, does not agree with the definition of HAECKEL 1874, who defines the gastrula as a didermic stage *with* a blastopore. HUBRECHT's definition includes all ways in which the inner layer, the hypoblast, is formed (1905, p. 408).

HUBRECHT does not indicate the LIEBERKÜHN-canal and the head-process by the names formerly usually used. The primitive streak does

not represent a changed blastopore (in the old sense) of the Anamnia. The stomodaeum and the oral slit of the Actinia correspond, according to HUBRECHT, to the notochord and the primitive groove of the Amniotes (HUBRECHT 1905, p. 412).

We have seen (pp. 51, 52) that HUBRECHT thought to have found a blastopore in several Mammals, investigated by him, and that it is my opinion that this opening is not a real blastopore, but that it is the metenteron-lumen (LIEBERKÜHN-canal), which has opened into the archenteron and is in communication with the exterior. Consequently, his „blastopore" is not a „cephalogenetic" but a „notogenetic" formation.

The view, elaborated by ASSHETON (1894*b*, 1905, 1909) I have treated for the greater part in preceding pages (pp. 53—57). Here I will mention only the principal facts, especially in connection with HUBRECHT's theory. Since 1894 ASSHETON accepts two growth-centra in the embryo (primary and secondary growth-centra, 1894*b*). Later on he distinguished (1905), as well as HUBRECHT, two developmental phases, protogenesis and deuterogenesis, which, however, are not identical with cephalo- and notogenesis: during protogenesis the anterior part of the embryo is formed. The embryo now is in the „gastrula"-stage, according to ASSHETON and has a radially symmetrical shape. In his phylogenetical speculations HUBRECHT supposes that the aboral side of this gastrula-stage (both HUBRECHT and ASSHETON think, in this connecton, of the gastrula of the Actinia with a blastopore) forms the ventral side of the older embryo, and that the oral side forms the dorsal side of it (hence HUBRECHT's term notogenesis). According to ASSHETON, however, — who came to his conclusions by actual experimental observations — the aboral side of the gastrula becomes the anterior part of the embryo, and the oral side i.e. the blastopore lips form the posterior part of the embryo by elongation in the direction of the mid-axis of the gastrula (to compare ASSHETON 1909, fig. 2).

During protogenesis the archenteron-wall is formed, either by invagination (*Amphioxus*) or by delamination (most other Vertebrates). During deuterogenesis the metenteron-wall is formed, in what way is of no great importance (by epiboly, invagination and so-called „inflexion" in *Amphioxus*, according to CERFONTAINE (1906), overgrowth and ingrowth of cells in Selachii, Amphibia and Amniotes). It is of importance that by the primary growth-centrum a radially symme-

trical larva is formed (protogenesis), becoming bilaterally symmetrical in a later stage (deuterogenesis). Archenteron and metenteron always fuse. From the roof of the metenteron the notochord originates, from the sides and also from the „blastopore"-lips the mesoblast is formed for the greater part. The metenteron gradually reduces in size in „higher" Vertebrates, the wall of the metenteron and the headprocess are homologous organs. According to ASSHETON, a considerable part of the gut, including the pharynx, and certainly the heart, are protogenetic (1909, p. 244). The notochord is deuterogenetic. ASSHETON is of opinion that no accessory protogenetic part of the notochord is formed, which is the case according to HUBRECHT, BONNET and HEAPE.

BONNET (here I communicate the view out of his „Lehrbuch der Entwicklungsgeschichte", 1920) to explain the gastrulation and the formation of the germinal layers in Amniotes, starts from the same processes in Amphibia.

Gastrulation is never an independent process. It is always connected with other processes, especially with the formation of the notochord and a considerable part of the mesoblast.

In the microlecithal ova of the Amphibia (e.g. *Rana*, *Bufo*, and *Salamandra*) a part of the blastocoel fuses with that part of the future gut, which is formed by invagination from the „blastopore". The part of the gut, derived from the blastocoel, he calls „Ergänzungshöhle", the invaginated part of the gut „Urdarm" (= primitive gut or archenteron).

The wall of the gut is composed of two parts: primitive or „Protentoblast", and „Dotterentoblast" or lining of the „Ergänzungshöhle". The thickened part between both components of the gut is the „Ergänzungsplatte". From the wall of the „Urdarm" the notochord and a part of the mesoblast (gastral mesoblast) originate. Consequently, the formation of the gut-lining, often called gastrulation, is not a simple process. BONNET distinguishes ASSHETON's archenteron and metenteron, but he calls the former „Ergänzungshöhle" and the latter „Urdarm". BONNET is of opinion that his „Urdarm" is the oldest part of the gut; according to ASSHETON this part is formed later, however. I believe that ASSHETON is right: the part of the gut formed by „clivage gastruléen" (BONNET does not mention this name, though the process, described by him, is identical with it) develops

first and must be called archenteron for that reason. Later on the metenteron opens into the archenteron.

The gastrulation-process, as we have seen it in Amphibia, so BONNET continues, is greatly reduced in Amniotes („bis zur Unkenntlichkeit verwischt", BONNET 1920, p. 93). This is in connection with the increase of yolk in Sauropsida and the secondary loss of yolk in Mammals. The „Dotterentoblast" is formed in an early stage by „precocious segregation". This is a phenomenon, which must be called heterochrony, often to be observed in the ontogeny. Hence BONNET considers the formation of both „Dotterentoblast" and „Protentoblast" to belong to the process of gastrulation. In Amphibia both formations occur almost simultaneously. Because BONNET calls the archenteron of ASSHETON „Ergänzungshöhle", it is evident that he considers the formation of the gut-part by invagination the most important phenomenon.

BONNET then describes in Amniotes the same processes we have already mentioned in preceding pages. A few remarks must still be made. In Amniotes the anterior part of the „archenteron"-wall (= metenteron-wall of ASSHETON) fuses with a thickened part of the „Dotterentoblast", the „Ergänzungsplatte". (It is the same plate, which we have called in the descriptive part prochordal plate.) The „Dotterblatt" of the Reptiles and Birds is homologized by him with all the makromeres of the Amphibian blastula. The primitive streak is considered to be the strongly elongated Amphibian „blastopore", of which the sides are fused intimately. In Amphibia already a linear blastopore is to be found. The primitive streak, however, is a changed „blastopore", for in a real „blastopore" epiblast and hypoblast pass into each other and in a primitive streak this is not the case.

In Mammals, having secondarily become yolk-less, an „Ergänzungshöhle" and an „Urdarm" are also to be found and fuse.

BRACHET (1902 and later on) distinguishes in Amphibia a gastrulation-process, divided into two separate phases. His „clivage gastruléen" is the first phase. The wall of the cavity then formed is composed of makromeres. This part of the future gut is the same ASSHETON called archenteron. A „second temps de gastrulation" succeeds the „clivage gastruléen". The blastopore, virtual up till now, becomes real. The „second temps de la gastrulation" is a complicated process, in

which an overgrowth and ingrowth of cells take place. It is to be noted that the part of the gut formed in the *second* phase is called *Arch*enteron by BRACHET. For that reason ASSHETON's name *met*enteron is to be preferred. BRACHET and ASSHETON are of about the same opinion, but they name the organs differently.

In 1915 C. RABL published an elaborate paper on: „EDOUARD VAN BENEDEN und der gegenwärtige Stand der wichtigsten von ihm behandelten Probleme". Besides giving a critical discussion of the different investigations of VAN BENEDEN, he also criticizes the theories of HUBRECHT, KEIBEL, BRACHET and others, and gives own investigations of the development of *Hatteria* and the rabbit, after the process of gastrulation in Vertebrates has been treated.

After the year 1886 VAN BENEDEN is of opinion that the LIEBERKÜHN-canal in Mammals is homologous with the gut of the Amphibia, formed by invagination. The protogenetic hypoblast of ASSHETON is called lecithophore by him and compared to the paraderm in Reptiles (v. KUPFFER, or the „Dotterentoblast" of BONNET). Both layers of the didermic stage of the Amniotes cannot be homologized with the „ectoderm" and „entoderm" of *Amphioxus*, for the formation of these two layers takes place before the process of gastrulation. For that reason the names blastophore and lecithophore are used. The headprocess (= archenteron in VAN BENEDEN's sense) fusing with the lecithophore, the latter does not exist as such any longer and then both parts of the gut-lining are called hypoblast by him. The early formation of the lecithophore is considered by VAN BENEDEN and others to be connected with the early attachment of the embryo to the uterine wall. The lecithophore becomes a nutritive organ and because of this a differentiation of the hypoblast in lecithophore and archenteron-wall takes place.

After the discussion of VAN BENEDEN's investigations, RABL, agreeing with VAN BENEDEN on the whole, proceeds to the description of the different theories of gastrulation in Vertebrates, principally in Mammals. Especially with HUBRECHT's and KEIBEL's opinion he cannot at all agree. In the first place he criticizes HUBRECHT's definition of gastrulation (HUBRECHT 1905, p. 408 and antea p. 58). According to RABL gastrulation is not a process of differentiation, but a process of growth, (RABL 1915, p. 230: „dass also die Gastrulation in erster Linie nicht ein Differenzierungs-, sondern ein Wachstumsprozess ist."), because

it follows from the „cell-lineage" that the lining of the gut is to be found in much earlier stages.

Apart from this, RABL's greatest objection is that according to HUBRECHT and KEIBEL, the gastrulation comes to an end, when the hypoblast has been formed by delamination. RABL thinks that then the formation of the gut-lining has not quite stopped in Mammals. During the process of invagination not only material for notochord and mesoblast finds its way into the interior of the embryo, but also material for the gut-lining is invaginated. During the incorporation of the headprocess in the hypoblast (= lecithophore of VAN BENEDEN), cells of the headprocess pass into the hypoblast. We have seen (pp. 44—46) in what manner RABL, who even thinks that the greater part of the gut-lining derives from the headprocess, tries to prove this, and moreover, how STRAHL (1916) and KEIBEL (1916, p. 9) refute his arguments.

When RABL asks HUBRECHT (1915, p. 255) on what grounds he proves that the gut-lining is derived from the hypoblast, formed by delamination of the embryonic knob, this question can be answered by the following question: What conclusive proof does RABL give of his opinion that such an important part of the gut-lining originates from the headprocess? It is true that HUBRECHT never asked himself, whether cells of the gut-epithelium are formed out of the headprocess, but that such an important part of the lining of the alimentary tract in Mammals arises from the headprocess, as RABL will have it, is altogether improbable.

KEIBEL (1916 and earlier) admits that, besides the formation of the notochord and mesoblast, also a small part of the gut-lining might derive from the headprocess, but he thinks it more probable that this is no longer the case in Mammals. We have seen (pp. 45, 46) that KEIBEL explains, by his so-called „Scheinwachstum", the fact that in younger stages the chordal plate is composed of many cells, and in older stages the notochord contains, in the same region of the embryo, very few cells. HUBRECHT makes a sharp distinction between the processes of gastrulation and of mesoblast- and notochord-formation. The incorporation of the chorda-anlage in the hypoblast and the separation of the notochord from it later on, is never interpreted theoretically by HUBRECHT.

To summarize, we can say that RABL considers the process of invagination of the headprocess the process of gastrulation in Mammals. The early development of the hypoblast in Mammals is explained by him as follows. In Sauropsida, where the yolk-mass of the ovum has increased enormously, a part of the gut-lining is necessary for the nutritive function. For that reason it is formed earlier than the rest of the hypoblast. In Mammals, which have become yolk-less again, a remnant of the former state is still to be found, the hypoblast developing prematurely here. Against the view of RABL it can be objected that he has not traced the formation of the gut in all groups of Vertebrates. In doing so it is evident that in all Vertebrates, in megalecithal as well as in microlecithal or yolk-less groups, the alimentary tract is formed in two parts, the archenteron and metenteron.

I will be concise in discussing the opinions of other investigators.

GREIL too (1914) is a supporter of the „invagination-theory". The hypoblast, called subgerminal layer by him, is not homologous with the hypoblast of *Amphioxus*, formed by invagination. The cells of the germdisc of the meroblastic Amniotes, divided by him into a germinal and a subgerminal layer and a subgerminal syncytium, arise in connection with the yolk-mass. „Was wir bis jetzt erörtert haben, vollzieht sich im Blastulazustände und umfasst Episoden des Ringens des Keimes mit seinem Dotterballaste", (p. 228).

The headprocess is the primitive gut-wall („Urdarm"), homologous with the hypoblast of *Amphioxus*, its formation is the gastrulation. Later on, when the „Urdarm" is reduced in size, the subgerminal layer takes the place of the „Urdarm"-wall as gut-lining. The Mammals are derived from the Sauropsida by loss of yolk. We see that GREIL differs from RABL only in details. According to him the gastrulation is also the invagination of the headprocess.

SOBOTTA, who has investigated the mouse especially, expresses himself in favour of RABL's „Urdarmtheorie" (e.g. 1911). So also JENKINSON (1906, 1913): the paraderm (= hypoblast) is formed by precocious segregation, the headprocess is the homologue of the archenteron of the Amphibia.

DE LANGE (1907, 1912, 1913), who has investigated *Megalobatrachus*, has come to a conclusion, differing little from that of HUBRECHT, BRACHET and ASSHETON.

SCHLATER (1907, 1909) supports HUBRECHT's view on the ground of theoretical considerations and agrees also with his phylogenetical speculations (to compare the comparative part, p. 76).

Other investigators I will pass over in silence, except TRIEPEL (1916, 1917*a*, 1917*b*), who holds an opinion, keeping the mean between HUBRECHT's and RABL's views. All Metazoa pass through a morula-, blastula- and gastrula-stage. In Vertebrates also a chordula-stage must be distinguished, (according to SCHLATER). The gastrula is the „die erste Darmanlage enthaltende(n) Keim der Metazoa" (1917*a*, p. 288). It is of less importance, whether the gastrula develops by invagination, epiboly or delamination. „Die Chordula is durch das Auftreten des die Chorda-anlage enthaltenden Mesoblasts charakteresiert". Because in Primates (and in *Tatusia*, PATTERSON 1912) a part of the mesoblast arises very early in front of the axial organs, the chordula-stage is defined by him as „den eine Chorda-anlage zeigenden dreischichtigen Keim der Chordaten". In *Amphioxus* he distinguishes an „entodermal" chordulation, in other Vertebrates an „ectodermal" chordulation. Whether this distinction is correct or not, I will not decide (cf. CERFONTAINE 1906). The hypoblast, perhaps participating in the formation of the anterior part of the notochord (cf. pp. 42—44) an „ectodermal-entodermal" chordulation must be distinguished in Craniota, to be correct. During the „ectodermal" chordulation, cells, which will form a part of the intestinal epithelium, find their way into the interior of the embryo. These cells are called „Urentoderm" by him.

Many facts are in favour of this opinion. It agrees so obviously with ASSHETON's opinion, however, that the greatest difference is a difference of terminology. For gastrulation and protogenesis, chordulation and deuterogenesis are identical conceptions. As STRAHL (1916) remarks, the gastrulation-problem is partly a problem of denomination. It is due to this that so many investigators, misunderstanding each other's terms, come to totally different conclusions, though the facts observed by them differ slightly.

Summarizing the above theories and opinions, we see in the first place that HUBRECHT and KEIBEL, ASSHETON and BRACHET in the main agree. These investigators accept two developmental phases.

HUBRECHT makes a too sharp distinction between „cephalogenesis" and „notogenesis", according to me. He does not point to the fact that

the headprocess (notogenetic) fuses with the cephalogenetic hypoblast and that this must have a theoretical signification. Moreover, the terms cephalogenesis and notogenesis are not quite correct (cf. p. 58). As we must accept HAECKEL's definition of gastrulation, HUBRECHT's definition cannot be right, because his definition does not mention a blastopore. The opening, described by HUBRECHT as a real blastopore in different Mammals, is not a cephalogenetic, but a notogenetic formation, according to me.

KEIBEL, who agrees with HUBRECHT in the main, also excludes the formation of the notochord and mesoblast from the gastrulation. It is possible, however, that cells of the headprocess pass into the gut-lining, though it is very difficult to trace, whether this is the case in Mammals. The „Scheinwachstum" of the notochord is, in connection with the formation of the notochord out of the chorda-anlage, more probable. KEIBEL does not make such a sharp distinction between cephalogenesis and notogenesis.

I think that ASSHETON's theory, founded on a comparative morphological investigation of the formation of the gut, notochord and mesoblast in all Vertebrates, is the most logical. To both developmental phases he gives names, indicating only that the one comes about earlier than the other (*proto-* and *deutero*-genesis). During protogenesis the archenteron, during deuterogenesis the metenteron develops. Both fuse in all Vertebrates, which explains the incorporation of the headprocess in the hypoblast in Mammals. The metenteron, easy to distinguish in Anamnia, decreases in size in Amniotes, the archenteron becoming more distinct in the latter. The theoretical signification of the primitive streak, headprocess and LIEBERKÜHN-canal is clear, according to his opinion. For that reason I agree with ASSHETON.

BRACHET, who investigated especially Anamnia and whose opinions cannot be treated in detail, because of that, accepts the same developmental phases. By „clivage gastruléen" the acrogenetic part of the embryo is formed. After that, during cephalogenesis, the head is formed as far as the nervus vagus, and in the third place BRACHET distinguishes a cormogenesis, during which trunk and tail are formed. The part of the gut, opening into the exterior by the „blastopore", is the archenteron, according to BRACHET. DE SELYS LONGCHAMPS calls it deutenteron in *Petromyzon*, however (1910).

DE LANGE too distinguishes a cephalo- or protogenesis, a somato- or deuterogenesis, and moreover an uro- or tritogenesis. In the first phase, the head is formed as far as the auditory vesicle, the parachordal mesoblast excepted. In the second phase the anterior part of the trunk, and the parachordal mesoblast of the head is developed, and in the third phase the other parts of trunk and tail are formed.

A distinct survey of the different opinions of HUBRECHT, BRACHET, ASSHETON and DE LANGE is to be found in DE LANGE (1912) and BRACHET (1914).

Though the data, given by BONNET, agree with the above, he does not mention two different developmental phases in the embryo. The formation of both „Protentoblast" and „Dotterentoblast" belongs to the gastrulation, though the „Dotterentoblast" is formed in an earlier stage by precocious segregation. This occurs in connection with the yolk-increase in Sauropsida and the secondary yolk-loss in Mammals. The invagination, the formation of the headprocess, is the most important process of gastrulation. According to me, BONNET attaches too little importance to the fact that the „Dotterentoblast" (= hypoblast) is formed so early in *all* Vertebrates. BONNET, in his explanation of the facts, agrees with the so-called invagination gastrula- or „Urdarm"-theory, of which RABL and VAN BENEDEN are the chief supporters. The invagination of the headprocess is, according to this theory, the gastrulation-process.

According to RABL, the hypoblast derived from the embryonic knob by delamination, even takes an inconsiderable part in the formation of the gut-lining of the Mammals. Gastrulation and invagination are identical. Consequently the opinions of HUBRECHT and RABL are sharply opposed. RABL attaches little importance to the protogenetic hypoblast in connection with the gastrulation; neither does HUBRECHT call attention to the headprocess and the incorporation of the chorda-anlage in, and the separation of the notochord from the hypoblast. RABL gives an explanation of the fact that the chorda-anlage incorporates in the hypoblast (p. 44).

Starting from the definition of HAECKEL, we have seen that it is applicable to none of the processes, which take place during the ontogeny of the Vertebrates.

The invagination of the metenteron is no gastrulation, because it takes place in a two-layered stage, already existing.

The process of delamination, by which the future wall of the archenteron is formed, is no gastrulation either, because I believe that no primitive mouth is formed. Only in *Amphioxus* amongst Vertebrates an invagination-gastrula is found. Consequently a process of gastrulation does not take place in Craniotes.

Protogenesis, cephalogenesis and acrogenesis are not identical with gastrulation.

We have also seen that the gut of the Vertebrates consists of a protogenetic (archenteron) and a deuterogenetic part (metenteron).

In „lower" Vertebrates the formation of the metenteron is most important, with regard to the formation of the alimentary tract. In Amniotes the most important part is formed by delamination (archenteron). In Mammals the last process is so momentous that during the invagination of the headprocess probably no cells of the gut-lining find their way into the interior of the embryo.

COMPARATIVE PART.

The number of investigations on the first developmental stages of Mammals having greatly increased, it is impossible to give a complete survey of the literature existing. This is not necessary either, as here I will only treat facts in connection with the investig tions, above described.

I intend to give a survey arranged as much as possible after the organs. Only the more recent investigators will be treated in detail, in order to be concise. In this part the hypoblast is not mentioned, because it has been discussed fully in the chapters on the origin of the hypoblast and the gastrulation of the Mammals. What must be said about the primitive streak, is to be found in the descriptive and comparative parts on the development of the notochord.

I. *Early segmentation-stages.*

In 1875 Ed. van Beneden investigated the segmentation of the Mammalian ovum in detail. It was lready known that this ovum is holoblastic. Van Beneden now found that the two first blastomeres have a different size in the rabbit and supposed that the epiblast and hypoblast become separated from each other, when the two first cleavage-cells arise. Besides differing in size, the blastomeres are differently stained. The „epiblast"-cell, the larger one, cleaves before the „hypoblast"-cell and consequently a three-cell stage is often to be found (to compare also Van Beneden et Julin 1880, on the bat). In the four-cell stage the line connecting the centres of the „epiblast"-cells is at right angles to the line, connecting the centres of the „hypoblast"-cells. Then a change takes place. One „hypoblast"-cell passes into the centre, surrounded on one side by four „epiblast"- and on the other by three „hypoblast"-cells. The

segmentation of the „epiblast"-cells anticipating the segmentation of the „hypoblast"-cells, later on a so-called *metagastrula* is formed, differing from the real gastrula in this only that a lumen is not to be found. The place, where the „hypoblast"-cells lie superficially was supposed to be the blastopore.

Later on VAN BENEDEN (v. BENEDEN et JULIN 1884), in consequence of KOELLIKERS investigations (e. g. 1882), came to the conclusion that in such early stages the hypoblast could not be present as a germ-layer and that a blastopore could not be found either. Then the theoretical signification of the metagastrula was rejected by him.

HEAPE, however, found that the segmentation of the ovum of the mole is irregular. A „metagastrula" is present, but the layers, composing it, are not derived from two different cleavage-cells. Out of the interior layer epi- and hypoblast are supposed to develop.

Differences in size and staining in the blastomeres could not be found in the rabbit, sheep, and pig by ASSHETON (1894*a*, 1898*a*, 1898*b*), in the hedge-hog by KEIBEL (1888) and mouse by TAFANI (1889) and SOBOTTA (1895).

SOBOTTA explained the phenomenon often observed that one of the two cleavage-cells is larger than the other. He found that a blastomere about to divide, increases in size, that its plasma is then less deeply stained and that the larger cell cleaves before the other. For that reason three-cell stages are often to be found [to compare also KUNSEMÜLLER (1906, *Erinaceus*) and HARTMAN (1919, *Didelphys*)].

In *Manis* a distinct difference in size of the cleavage-cells is not present (figs. 2, 3 and 4, plate I), but I have found a three-cell stage in the oviduct.

The young *Manis*-ova are all covered by follicle-cells. This is also described for the mole (HEAPE 1883, mature eggs); SOBOTTA found (1895) that the ovum of the mouse passing into the oviduct, is still surrounded by the corona radiata. In two-cell stages these cells have disappeared. HUBER observed (1915) in the rat unsegmented ova in the oviduct, surrounded by a few follicle-cells, and about the same is found in the ferret (ROBINSON 1903) and hedgehog (KEIBEL 1888 and KUNSEMÜLLER 1906). In *Manis* the cells of the corona radiata surround the ovum for a considerable time; the cells of the discus proligerus are to be observed in an unsegmented ovum.

In *Galeopithecus* too, these cells can be distinguished. In the descriptive part I have already mentioned that HUBRECHT (1910, 1919) supposed that the nuclei of them were future trophoblastic nuclei (cf. the descriptive part pp. 12—14).

R. HERTWIG mentions (1903) that in many cases the zona pellucida still surrounds the ovum, after the cells of the corona radiata have disappeared. All kinds of differences can be observed. Now the zona surrounds the unsegmented egg only (*Sorex*, *Tupaja*, HUBRECHT 1890 and 1895), now this is the case only in very early stages (*Tarsius*, HUBRECHT 1902) or not at all. In *Manis* I could not find a zona pellucida anywhere. Some authors think (for example VAN BENEDEN) that acids dissolve the zona pellucida, but this was not confirmed by others (SOBOTTA). The *Manis*-embryos were preserved in picro-sulphuric acid.

The stage, in which the young embryo passes into the uterus, is also very different. HUBRECHT (1902) found *Tarsius*-ova with 48—64 cells still in the oviduct, whilst KEIBEL (1888) observed two-cell stages of *Erinaceus* close to the uterus. The three- and four-cell stages of *Manis* lie in the oviduct. The oviducts, being sectioned in 1907 and earlier, are only kept partly; so I cannot trace in what part of the oviduct these ova are to be found.

The succeeding *Manis*-stage, in which trophoblast and embryonic knob are developed, is to be found in the uterus.

II. *Trophoblast and embryonic knob.*

BISCHOFF already observed the difference between the blastocyst-wall and embryonic knob (1842-1854) and traced the development of the compact mulberry-stage to the hollow blastocyst.

In the years between 1875 and 1884 a dispute arose between VAN BENEDEN and KOELLIKER, principally concerning the interpretation of the embryonic knob. VAN BENEDEN thought (1875, 1880) that the outer layer of the embryonic knob represents the definite epiblast, that the inner layer is the hypoblast (visible already in the meta-gastrula) and that the tissue between these layers must be considered the mesoblast.

Independently, RAUBER (1875) found that the outer layer (called

RAUBER-layer after him) disappears later on and consequently does not participate in the formation of the embryonic epiblast. It is a part of the trophoblast. The investigations of KOELLIKER (1879, 1882) proved that VAN BENEDEN was wrong. The layer below the RAUBER-layer is the embryonic epiblast, and not the mesoblast, this arising in a later stage. VAN BENEDEN came to agree with this opinion in 1884 (VAN BENEDEN et JULIN 1884).

The blastocyst with its inner mass of cells is found in all kinds of Mammals. HUBRECHT called the part of the blastocyst, not participating in the formation of the embryo, trophoblast (1888) and the inner cell-mass embryonic knob (1890).

HUBRECHT (1902, 1908) distinguishes the trophoblast and the embryonic knob in the first segmentation-stages, principally by different reactions against staining reagents (*Tupaja*, 1895) or different position of the cleavage-cells. He interprets a figure given by SELENKA in the following way (HUBRECHT 1908, plate A, fig. 4): he regards the central cell as the mother-cell of the embryonic knob, and the peripheral cells as the trophoblastic cells. HILL, however, has shown (1918) that the larger cells (the central cell too) are trophoblastic cells and that the smaller ones are the mother-cells of the embryonic knob. Consequently in Marsupials the trophoblast never covers the embryonic anlage.

According to RAUBER and KOELLIKER the cells of the RAUBER-layer disappear, whereas HEAPE (*Talpa*, 1883) and BALFOUR (rabbit, 1881 p. 220) hold that these cells are absorbed by the definite epiblast.

O. HERTWIG (1903) feels inclined to compare the embryonic knob to the germdisc of the macrolecithal eggs of the Monotremata, Birds and Reptiles, the trophoblast then being homologous with the unsegmented part of these ova.

The usual opinion that the trophoblast is epiblastic, is denied by ASSHETON (1898*a*). He thinks that the trophoblast is of hypoblastic origin. Like VAN BENEDEN (1899*b*), he accepts two different layers in the morula, but the part VAN BENEDEN calls epiblast is called hypoblast by ASSHETON and the reverse. A clear survey of the opinions of ASSHETON and of VAN BENEDEN (and also of HUBRECHT's opinion before 1910) is to be found in ASSHETON 1908, fig. 11, 12 and 13. HUBRECHT's last opinion I have treated on pp. 12—14.

III. *Mesoblast.*

We have seen that in *Manis* the mesoblast originates from several places of the embryonic shield viz. partly out of the primitive streak and headprocess, partly out of the hypoblast.

All investigators agree about the origin of the mesoblast from the primitive streak and headprocess in Mammals. Different names are used for these two mesoblast-parts, which can be united under the name axial mesoblast. The fact that so many names are used, is due to the different theoretical significations attached to the primitive streak and headprocess by the authors. To give an example: HUBRECHT (1908) calls the mesoblast, derived from the primitive streak, ventral mesoblast, RABL (1889, 1915) and BONNET (1920) call it peristomal mesoblast to express that this mesoblast-part is formed around the stoma (= the elongated blastopore, with which the primitive streak can be homologized).

Opinions, however, differ very much about the question, whether this mesoblast is partly formed out of the hypoblast in Mammals.

TSUKAGUCHI (1912) gives a survey of the investigators, who deny that it is formed out of the hypoblast and are of opinion that the mesoblast (the headprocess is also considered by most of these investigators to belong to the mesoblast) is formed exclusively from the epiblast.

RABL does not agree with different other authors, who think that a part of the mesoblast can develop from the protogenetic hypoblast. The protogenetic mesoblast is formed in different Mammals. The two parts of the hypoblast participating in its formation are the prochordal plate and the annular zone of proliferation.

The prochordal plate [protochordal plate (HUBRECHT)] is first found by HUBRECHT in *Sorex* (1890). According to him the anterior part of the notochord is derived from it. Later on HUBRECHT also figured the plate in *Tarsius* (1902) and mentioned it in *Erinaceus* (in which BAUMEISTER (1913) did not find it), *Gymnura*, *Tupaja* and *Manis* (1908). In most cases the origin of the cephalic mesoblast from this plate is evident. Usually it arises earlier than in *Manis*, where I have found the plate in primitive streak-stages, but where a distinct cephalic mesoblast-formation takes place in an embryo, in which 4 à 5 pairs

of somites have developed. In *Galeopithecus* too, HUBRECHT has described (1919) a protochordal plate. Probably the part, described as such by HUBRECHT, belongs to the annular zone of proliferation, however, the protochordal plate being present in a more cranial part of the hypoblast (cf. the remark of DE LANGE in HUBRECHT 1919, p. 18). By BONNET the prochordal plate is described under the name „Ergänzungsplatte".

The mesoblast described by HEAPE in the mole (1883), by SELENKA (1882) in the mouse and by ROBINSON (1892) in the mouse and rat, as originating from the hypoblast, probably is cephalic mesoblast too. The correctness of these observations, however, is often called in question. In the mouse and the rat recent investigators as SOBOTTA (1911) and HUBER (1915) could not confirm it.

ASSHETON (1894*b*) observed that the cephalic mesoblast develops from a thickened part of the hypoblast in the rabbit. NEWMAN and PATTERSON (1910) describe a protochordal plate, which gives rise to the cephalic mesoblast in *Tatusia*. Not only in Mammals the cephalic mesoblast is observed. To give some examples: BALFOUR and DEIGHTON (1882) observed it in the chick; BRACHET (1914) in *Chrysemys* and DE LANGE (1913) in *Megalobatrachus*. Moreover BRACHET and DE LANGE distinguish a prochordal plate (plaque prochordale).

Many authors, however, deny the existence of a prochordal plate, participating in the formation of the cephalic mesoblast. According to RABL (1915, p. 227) this plate is „Teil der unteren Keimschicht mit dem sich später das Vorderende des Kopffortsatzes verbindet"; on p. 239, however, „die Protochordalplatte ist das vorderste Ende des im Lecithophor vorgeschobenen Kopffortsatzes oder Urdarmsäckchens; das daraus hervorgehende Mesoderm ist also „gastrales" in dem erwähnten Sinne. Dieser Teil des gastralen Mesoderms bleibt weitaus am längsten mit dem Entoderm in Verbindung und löst sich erst sehr spät von ihm ab." Yet a real cephalic mesoblast exists, as is evident from the observations made by HUBRECHT and BONNET.

In *Manis* the existence of it cannot be called in question. When we examine only the *Manis*-embryo N°. 83, where the chordal plate passes into the prochordal plate, from which the cephalic mesoblast arises, this observation seems to be in favour of RABL's opinion. In the younger stages (151 and 180), however, we see that RABL's opinion

cannot be correct, because here a prochordal plate is present too, with which the chorda-anlage has not yet fused.

In sagittal sections of young rabbit-areas (RABL 1915, fig. 8, plate V and figs. 1 and 2, plate VI), we see in front of the anterior border of the headprocess some mesoblast-cells, distinctly connected with the hypoblast. RABL supposed these cells to originate from the headprocess. The figures, however, may be interpreted also in such a way that the cells must be regarded as developing directly from the hypoblast.

Thickened parts of the hypoblast just in front of the headprocess are often figured e.g. by CARIUS (1888), who was the first to draw attention to this part in the rabbit and considered it to be the primary pharyngeal membrane. In the sections figured by him, just in front of this thickened part, with which the chorda-anlage is connected, lie some mesoblast-cells, out of which the pericard is formed in later stages. The great difference between his figures and mine is that in mine (figs. 33 and 34), the cephalic mesoblast is distinctly connected with the prochordal plate, and that this is not the case with the primary pharyngeal membrane of CARIUS.

KEIBEL describes a primary pharyngeal membrane in *Cavia* (1889), the mesoblast being separated from it here too. The sagittal sections, figured by VAN BENEDEN (*Vespertilio murinus*, 1888, 1912) and HUBER (*Cavia*, 1918) show the same. TSUKAGUCHI describes how in the goat (1912) the notochord ends in an indifferent „Mesoderm-entodermmassa". NEWMAN and PATTERSON (1910) figure a protochordal plate in *Tatusia*, the same being described by WILSON and HILL (1907) in *Ornithorhynchus* (here, however, formed out of the headprocess), by STRAHL (1914, 1916) and GROSSER (*Homo*, 1913).

These observations all differ very much. They can be divided into 1°. observations, from which it is evident that the cephalic mesoblast, giving rise to the formation of the pericard amongst others, originates from the prochordal plate [dog, *Sorex*, *Tarsius*, *Manis*, mole, rabbit (ASSHETON)]; 2°. observations pointing to the fact that in front of a primary pharyngeal membrane mesoblast-cells are to be found (probably also giving rise to the pericard), not connected with this thickened part of the hypoblast. These cells are considered to be the most anterior cells of the gastral mesoblast, formed out of the headprocess [rabbit (CARIUS), *Cavia*, *Vespertilio murinus*, etc.].

Opinions also differ about the existence of the annular zone of proliferation and the peripheral mesoblast originating from it.

In 1890 this zone is described by HUBRECHT in *Sorex* and later on in *Tarsius* (1902) and *Galeopithecus* (1919). In 1884 BONNET had already figured (in the sheep) the so-called „Mesoblasthof," also a peripheral, ringshaped hypoblast-thickening, from which extra-embryonic mesoblast develops principally. KEIBEL, however, tracing the mesoblast-formation in the sheep too (1894), could not confirm BONNET's observations.

Neither in *Cavia* and the rabbit, nor in the pig (1891) KEIBEL could find the peripheral mesoblast. It is described, however, by ROBINSON (1892) in the mouse and rat. KOLLMANN (1884) gave an example of the existence of the peripheral mesoblast in Birds, describing the so-called „Randwulst" in the chick.

We must not forget that according to BRANCA (1912) the connection between mesoblast and hypoblast can be secondary.

The peripheral mesoblast originates partly out of the region of the embryonic shield, lying in front of the primitive streak.

TSUKAGUCHI (1912) shows that in the goat and (1913) in the rabbit, in front of the primitive streak, mesoblast-cells develop out of the epiblast. He denies the origin of the mesoblast from the hypoblast.

BAUMEISTER (1913) finds mesoblast in *Erinaceus* at the anterior border of the shield, but shows that these cells are in connection with the mesoblast, derived from the primitive streak.

The statements above described, concerning the existence of a peripheral mesoblast, are confirmed in *Manis*. Here the peripheral mesoblast is in connection with the anterior part of the prochordal plate. This is also stated by HUBRECHT (1908, p. 33) in other Mammals. The ring-shaped zone, producing the peripheral mesoblast, extends along the borders of the germdisc.

When it was discovered that both in Vertebrates and Invertebrates the mesoblast is developed in many different ways, some investigators rejected the dogma of the specificity of the germ-layers. They came to consider the mesoblast a topographical layer.

SCHLATER (1907) does not agree with this conception of the mesoblast. The fact that the mesoblast in Vertebrates is formed out of different sources, is interpreted phylogenetically by him. In his explanation, he

starts from the very early development of the mesoblast in Primates, before primitive streak and headprocess are visible. This primitive three-layered stage of the Vertebrates corresponds to a so-called mesenchymula-stage, which all Metazoa, the Coelenterata excepted, have passed through, according to him. In Vertebrates a new organ, the axial skeleton, makes its appearance; the Chordata-type has originated and in connection with this, new mesoblast, the secondary mesoblast or axial mesoblast, is formed. The phylogenetic stage, which all Vertebrates have passed through, is called *chordula* by SCHLATER, in connection with the appearance of the notochord. The mesoblast formed in the mesenchymula (protogenesis corresponds phylogenetically to the formation of the mesenchymula) is to be found in a few cases only and among them SCHLATER reckons all cases, in which the mesoblast is formed in an earlier stage than the primitive streak-stage and out of other sources than primitive streak and headprocess. Examples of this are the early mesoblast-formation in Primates *(Homo, Semnopithecus, Tarsius)* and also in *Tatusia* (PATTERSON 1912) and *Galeopithecus* (HUBRECHT 1919). In *Manis* the peripheral mesoblast, originating in primitive streak-stages, and the cephalic mesoblast, developing still later, must be considered the primary mesoblast, the gastral and peristomal mesoblast the axial or secondary mesoblast, according to SCHLATER.

Phylogenetically speaking, cephalic and pheripheral mesoblast belong to the mesenchymula, gastral and peristomal mesoblast to the chordula.

RABL denies the existence of cephalic and of peripheral mesoblast. Only the axial mesoblast, formed in close relation with the process of invagination is to be found, according to RABL. In the chapter „Lehre von den Keimblättern", in his „Handbuch" 1903, O. HERTWIG does not mention either the peripheral or the cephalic mesoblast (cf. also his „Lehrbuch" 1915).

The authors, who accept the existence of the peripheral mesoblast agree about its derivatives. According to both HUBRECHT (1908) and VAN BENEDEN (1912) the bloodvessels and the blood of the umbilical vesicle are derived from the hypoblast or „lécitophore". KOLLMANN regards (1884) the „Randkeim" or „Akroblast" as a „Bindegewebsblutkeim". RÜCKERT agrees with this view (1906) and TRIEPEL (1916) accepts it.

To sum up: the protogenetic as well as the deuterogenetic part

of the future gut-lining can produce mesoblast. As a result a sheet of cells is formed between epiblast and hypoblast, in the embryonic as well as in the extra-embryonic part of the blastocyst, This layer I have called mesoblast for short.

IV. *Notochord.*

It is clear that the question, to which „germinal layer" the notochord belongs, is answered in many different ways, so many opinions on the gastrulation-process existing.

HUBRECHT, for example, holds that the notochord originates from the epiblast and hypoblast, others consider the headprocess and notochord to belong to the mesoblast, derived from the epiblast, whereas RABL; SOBOTTA and BONNET think that the notochord is hypoblastic.

O. HERTWIG (1903) accepts, besides the germ-layers, a separate „chordal layer". In *Manis* there are indications, but no proof that the anterior part of the notochord is formed from the wall of the archenteron. It is clear that the posterior part of the notochord is not formed, like the principal part out of the metenteron-wall, but out of the primitive streak, which we considered to be homologous with the changed deuteropore of the Anamnia.

We have mentioned BONNET's, HUBRECHT's and also HEAPE's opinion that the so-called protochordal plate or „Ergänzungsplatte" forms not only the cephalic mesoblast, but also the anterior part of the notochord. JENKINSON (1906, 1913) agrees with this opinion.

It is not clear to me, what were the grounds of HUBRECHT's opinion that the protochordal plate forms a part of the notochord. In his paper, in which the name protochordal plate is first used (*Sorex* 1890), this name is given to the thickening „developed in situ as part of the hypoblast" (p. 501) and later, p. 508 he states: „part of this patch will develop into the anterior portion of the notochord. For this reason I will call it the protochordal plate". As far as I can trace, he never proved this. He says only (p. 509) „that the formation of a protochordal plate has preceded the appearance of the very first indications of a gastrula-ridge (primitive streak)" and on p. 512, after he has described the protochordal plate and the development of the cephalic mesoblast, he mentions that the size of the proto-

chordal plate decreases and that then „the front portion of the notochord folds off, this being the last phase in the developmental phenomenon of what we have called the protochordal plate. This has been figured by HEAPE for the mole and is not further entered upon in this paper" (to compare also ASSHETON 1909).

Consequently he quite agrees with HEAPE (1883) and BONNET (1889), who stated that the cephalic mesoblast forms out of the thickened part of the hypoblast and who found that later on the notochord develops here. HEAPE did not prove either that this part of the notochord did not arise from the anterior part of the headprocess.

In 1889 BONNET had already shown that in the sheep the anterior part of the notochord originates from the hypoblast. In the dog, however, he was able to prove it (cf. antea p. 42). He recognized the „Ergänzungsplatte", not only because it produces the cephalic mesoblast, but also because it possesses peculiar chromatophile granules. In older stages he saw that the notochord proceeds more and more cranialward and observed also that this anterior part of the notochord is formed out of a part of the hypoblast, in which these granules are also to be found. This hypoblast-part must be considered a remnant of the „Ergänzungsplatte." In dog-embryos with 10 pairs of somites the chordal plate passes into the „Ergänzungsplatte", in embryos with 16 pairs of somites the notochord, separated from the hypoblast, is found as far as the primary pharyngeal membrane which, as it also possesses these granules, must also be considered a derivative of the „Ergänzungsplatte".

In the descriptive part we have seen that in *Manis* the chordal plate forms out of the headprocess after incorporating in the hypoblast. The headprocess, extending cranialward between epiblast and hypoblast, does not receive cells from either of these layers, as they are clearly separated from it.

About the length of the primitive streak opinions differ. KEIBEL for example (pig, 1893, 1895) thinks that it originally reaches as far as the anterior border of the embryonic shield and becomes gradually shorter, the headprocess simultaneously developing caudalward.

In 1915 RABL figured a great number of embryonic areas of the rabbit, from which it is clear that in the rabbit at least the primitive streak gradually becomes longer up to a certain stage, but

never reaches the anterior border of the shield. (HUBER's observation (1918) of the first anlage of the headprocess in *Cavia*, does not agree with the opinion of KEIBEL either, the primitive streak being short here.). When the primitive streak has reached its greatest length a headprocess is already formed and the primitive streak then gradually shortens from before backwards. During this process, the posterior part of the headprocess (the so-called „Primitivstreifenteil des Chordas") forms out of the primitive streak.

In a recent investigation on the first developmental stages in Mammals (TSUKAGUCHI on the goat, 1912) the author describes four primitive streak-stages, in which the primitive streak reaches a length of 75 % of the germdisc and the headprocess extends as far as the anterior border of the shield. A lumen is not always to be found in the headprocess; discontinuous canals and slits are described. The anterior part of the headprocess passes into an „indifferente Mesoderm-entodermmassa," compared by the author to BONNET's „Ergänzungsplatte". Stages with free headprocess (found in the pig, rabbit, *Cavia*, *Vespertilio* and also in *Manis*) TSUKAGUCHI has not found in the goat.

The notochord is described in detail in an older embryo with 5 pairs of somites. He distinguishes five different zones from back to front.

1°. The part in front of the primitive streak, lately formed out of it (the so-called „Primitivstreifenteil des Chordas"). It is distinctly separated from the epiblast and is no longer connected with the mesoblast on both sides. Canals are partly visible.

2°. An „Umbildungszône", differentiated distinctly in a dorsal and a ventral part with a canal lying between. Like zone I, it is covered by the hypoblast. It passes gradually into

3°. the chordal plate, incorporated in the hypoblast. During the process of incorporation the ventral mass of the chorda-anlage, described in 2°, has spread, the hypoblast being cleft simultaneously. The chordal plate is principally formed out of the dorsal cells, mentioned under 2°; the ventral cells are used for the growth in length of the notochord by „Zellumlagerung" (= „Scheinwachstum", KEIBEL 1889). This zone is very long. The growth in length of the notochord is possible, because the „Primitivstreifenteil des Chordas" (zone I) is very thick.

4°. A short zone, in which the notochord is separated from the hypoblast; a secondary chordal canal is found here (cf. KEIBEL 1889).

5°. a still shorter zone, which is not yet separated from the hypoblast, and passes into the same „Mesoderm-entodermmassa", in which the headprocess of the primitive streak-stage ended. TSUKAGUCHI cannot decide, whether this part of the notochord develops from the hypoblast here, or whether this zone is formed out of cells derived from the headprocess.

In young *Manis*-embryos, in which the separation of the notochord has not progressed very far, we can distinguish about the same zones. Yet there are differences. The „Primitivstreifenteil des Chordas", also present in *Manis*, is connected with the mesoblast, this not being the case in the goat. In front of this zone a second region is to be found, no longer connected with the mesoblast. In *Manis* these two zones, both covered by the hypoblast, contain many discontinuous lumina, some times forming long canals. Like TSUKAGUCHI I think that these lumina are not homologous with the metenteron-lumen, but with the canalis neurentericus of the Anamnia. In some few embryos described, this chordal canal opens into the future gut and just in front of this place the chorda-anlage passes into the third zone: the chordal plate, incorporated in the hypoblast. Consequently the transitional zone (TSUKAGUCHI's second zone) is very short.

In *Manis* I could not find a secondary chordal canal. TSUKAGUCHI's 4th and 5th zone are present in *Manis*.

The LIEBERKÜHN-canal or metenteron-lumen is found in the headprocess in many Mammals. In *Manis*, the pig (KEIBEL 1895), the dog (BONNET 1901) it probably does not occur. The „blastopore" described by HUBRECHT in *Erinaceus* (1902, 1908, 1912) must be considered a LIEBERKÜHN-canal. [By BAUMEISTER it was not found in *Erinaceus* (1913), but PETERMANN observed it here (1907)]. It is possible that in these cases the stages, in which a LIEBERKÜHN-canal occurs, lack.

The primary chordal canal (KEIBEL 1889) is described in all kinds of Mammals [e. g. dog with 8 à 9 pairs of somites (BONNET 1901), rabbit with 11 pairs of somites (CARIUS 1888), and 7 à 8 pairs of somites (STRAHL 1886)].

Probably it is often mistaken for the LIEBERKÜHN-canal, appearing in the headprocess proper in primitive streak-stages. The terminology of these canals is very confusing (cf. p. 7). Some authors take the canalis neurentericus to be the caudal rest of the LIEBERKÜHN-

canal (e.g. GROSSER 1913, STRAHL 1916), which moves caudalward. Moreover TRIEPEL (1914, 1917*a*) thinks that the presence of the canalis neurentericus is a convergent phenomenon in Vertebrates.

These few examples may suffice to show that the problem of the relation of metenteron and canalis neurentericus has not yet been solved.

STRAHL (1916) distinguishes a posterior „Ergänzungsstück" of the notochord, formed out of the primitive streak. The same is stated by BONNET (1901), who also mentions (1920) that this part of the notochord is always solid, and sometimes shows some small traces of canals. In a *Manis*-embryo with 18 pairs of somites, however, we can still observe a canalized part of the notochord, which will never incorporate in the hypoblast.

RABL (1915) does not lay stress on the part of the notochord, derived from the primitive streak. Yet the development of the notochord in the rabbit is treated elaborately by him. He thinks that the headprocess participates in the formation of the gutlining (cf. antea, p. 44). According to me this is not proved. From his sections it further follows that in rabbit-embryos with 12 pairs of somites the notochord has for the greater part separated from the hypoblast. This is the case in *Manis*, when 18 pairs of somites are formed. In rabbit-embryos with 12 pairs of somites the anterior part of the chorda-anlage is still connected with the hypoblast. From his description of the chorda-anlage of an embryo with 6 à 7 pairs of somites (RABL 1915, pp. 433—434), I will still mention the following facts. A chordal canal is not described. The chordal plate becomes flatter and flatter and gradually contains less cells. At the level of the 7th pair of somites the chordal plate-section consists of 7 nuclei. According to RABL all cells of the chordal plate have then passed into the gut-epithelium. Still more in front the chordal plate is no longer distinguishable from the hypoblast and it then passes into an „interepitheliale Zellplatte (über), aus der nach rechts und links auch jetzt noch, wie übrigens auch später, das Mesoderm mächtig hervorsprosst. Hier bleibt also das Mesoblast am längsten mit der dorsalen Darmwand in genetischem Zusammenhang". [Here we have a clear description of the prochordal plate, of which he denied the existence in preceding pages (pp. 227 and 239)]. „Leider lässt sich von allen diesen Dingen, von denen sehr wenig bekannt ist, ohne zahlreiche

Abbildungen keine gute, klare Beschreibung geben; von einer grösseren Zahl von Abbildungen wollte ich aber absehen, da der Gegenstand nicht unmittelbar zum Thema der Abhandlung gehört". And also on p. 419 he mentions this plate, in describing the hypoblast in primitive streak-stages: „Sodann folgt unter dem Vorderende der Hirnplatte eine dicke Strecke der unteren Zelllage. Von dieser Strecke sieht man auf Querschnitten rechts und links Mesoderm hervorsprossen. Später wird dieses Epithel noch dicker, und die Zellwucherung mächtiger. Die Strecke entspricht der von REX bei der Ente und Möwe beschriebenen interepithelialen Zellmasse des Entoderms", and this one must also be compared to the prochordal plate.

Recently HUBER (1918) has elaborately traced the anlage and morphogenesis of the notochord in *Cavia*. He describes 1°. primitive streak-stages with the first anlage of the headprocess, originating from a comparatively short primitive streak; 2°. a primary pharyngeal membrane, in front of which mesoblast-cells occur (the anterior cells of the mesoblast-wings developing from the primitive streak, cf. antea p. 76); 3°. a LIEBERKÜHN-canal, opening into the cavity, enclosed by the hypoblast, by different apertures. When the headprocess has become a chordal plate, the hypoblast still covers this plate in different places. This was not observed in *Manis*.

The oldest stage, in which the chordal plate is still entirely incorporated in the hypoblast in *Cavia*, is a stage, in which no somites are to be found. In *Manis* this is still the case in an embryo with 13 pairs of somites. The separation of the notochord takes place in two ways in *Cavia* (also according to KEIBEL 1889).

1°. By undergrowth of the hypoblast-edges. A flat notochord is then formed, which becomes cylindrical later on. 2°. By infolding of the borders of the chordal plate, by which a cylindrical notochord is directly formed. In both cases a secondary chordal canal can be formed. These two methods of separation HUBER could find in the same *Cavia*-embryo. In *Manis* only the second method (infolding) could be observed.

HUBER does not mention that in the part of the notochord, originating out of the primitive streak last (zone I and II) lumina occur. He mentions only the presence of a LIEBERKÜHN-canal in the headprocess.

In *Manis* we have observed that the chordal canal appears double in many cases. Sometimes (e.g. *Cavia*, v. SPEE 1888 and HUBER

1918) such a formation is also observed in the headprocess, before the formation of somites. Evidently a double metenteron is found here, in these young stages.

The appearance of a double metenteron as well as of a double chordal canal requires an explanation. According to me, the appearance of the canals (single or double) is connected with the mechanical function of the incorporating organ, in which they occur. The LIEBERKÜHN-canal and the chordal canal are two phylogenetically different formations (metenteron and canalis neurentericus), but the ontogenetical function of them is the same: i. e. the incorporation in the hypoblast, and this occurs by spreading of the wall of both canals. In both cases this can come about from a single or a double canal.

Before concluding this survey of the literature, treating the developmental stages of the notochord, I will state:

BONNET's „Ergänzungsplatte" probably occurs also in *Manis*. Because I cannot prove that the anterior part of the notochord is formed out of this protogenetic part of the hypoblast, I have called this plate prochordal plate.

A following part of the notochord develops from the headprocess proper, in which a canal appears in many Mammals. In *Manis* I could not find this canal. This part of the notochord is derived from the metenteron-wall.

The posterior part of the notochord is the „Primitivstreifenteil des Chordas", the „Zuwachsstück" of BONNET. It is formed out of the primitive streak. In this part a distinct canal appears in many cases in *Manis*. The lumina in it made many authors think: „Der Chordakanal rückt in den hinteren Theil der Keimscheibe" (LIEBERKÜHN 1882, p. 421) or „dass der Chorda-kanal sich in den Primitivstreifen hinein fortsetzt" (BONNET 1901, p. 267). This is supposed to happen after the formation of the first pair of somites.

Though I dare not state that this opinion is wrong, yet I believe that these lumina can be more successfully compared to the canalis neurenticus of the Anamnia. The LIEBERKÜHN-canal and these canals cannot be considered the same lumina. However, there is a close relation between these formations, as well as between metenteron and canalis neurentericus in Anamnia.

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DESCRIPTION OF PLATES.

List of abbreviations.

a. pr. = aorta primitiva.
a. z. = annular zone of proliferation.
Br. = brain.
ch. = notochord.
ch. can. = chordal canal.
ch. p. = chordal plate.
cor. rad. = corona radiata.
disc. prol. = discus proligerus.
e. k. = embryonic knob.
end. = heart-endothelium.
ep. = epiblast.
H. k. = HENSEN-knob.
h. p. = headprocess.
hyp. = hypoblast.
ex. hyp. = extra-embryonic hypoblast.
med. = medulla.
med. gr. = medullary groove.
mes. = mesoblast.
ceph. mes. = cephalic mesoblast.
gast. mes. = gastral mesoblast.
per. mes. = peripheral mesoblast.
p.s. mes. = peristomal mesoblast.
ov. ep. = oviduct-epithelium.
p.c. = pericardium.
ph. m. = pharyngeal membrane.
p. g. = primitive groove.
p. p. = prochordal plate.
p. s. = primitive streak.
som. I etc. = first etc. pair of somites.
tr. = trophoblast.
ut. = uterine wall.

Explanation of figures.

PLATES I AND II.

Fig. 1. A section through an ovum not yet divided. The discus proligerus and the corona radiata, surrounding the ovum, are visible. The nucleus is not found (*n'*, a nucleus of the corona radiata). The ovum lies in the oviduct. × 410.
HUBRECHT-Lab. Cat. N°. *Manis*, 51 *b* III, 5.

Fig. 2. A section of a three-cell stage, surrounded by corona radiata and lying against the ciliated oviduct-epithelium. One nucleus of the cleavage-cells is visible (*n*). × 525.
HUBRECHT-Lab. Cat. N°. *Manis*, 171 *e* III, 11.

Fig. 3. A section of a four-cell stage, surrounded by corona radiata and lying close against the oviduct-epithelium. Three cleavage cell-nuclei are visible. × 525.
HUBRECHT-Lab. Cat. N°. *Manis*, 44 c_1 II, 8.

Fig. 4. A section of the same stage. Two nuclei are visible. × 525.
HUBRECHT-Lab. Cat. N°. *Manis*, 44 c_1 II, 9.

Fig. 5. A section through a strongly compressed blastocyst, lying in utero. The wall of the blastocyst consists of the trophoblast only, with the embryonic knob at one place. In this section no hypoblast is visible. × 220.
HUBRECHT-Lab. Cat. N°. *Manis*, 87 *g* II, 5.

Fig. 6. A section through the same blastocyst. The first anlage of the hypoblast is visible. × 325.
HUBRECHT-Lab. Cat. N°. *Manis*, 87 *g* II, 4.

Fig. 7. Schema of folded blastocyst with embryonic knob, consisting of epiblast and hypoblast and still covered by the trophoblast. The hypoblast has spread over the whole inside of the trophoblast. The uterine wall is indicated. × 90.
HUBRECHT-Lab. Cat. N°. *Manis*, 113 d_2 II, 6.

Fig. 8. Embryonic knob and part of the same blastocyst. Epiblast with few nuclei, hypoblast beneath it with many tall or cubical nuclei; extra-embryonic hypoblast consisting of flattened cells. × 225.

Figs. 9 *and* 10 *(plate II),* 11 *and* 12 *(plate I),* 13—16 *(plate II),* 17 *and* 18 *(plate I) and* 19 *(plate II) show surface-views of the embryonic shields (cf. pp.* 17 *and* 18*).*

Fig. 9. A primitive streak-stage. *Manis*, N°. 180. × 10.

Fig. 10. Embryo with 4 à 5 pairs of somites. *Manis*, N°. 83. × 10.

Figs. 11 and 12. „ „ 8 à 9 „ „ „ „ N°. 64. × 10.
Fig. 11 dorsal, fig. 12 ventral view.

Fig. 13. Embryo with 10 à 11 pairs of somites. *Manis*, N°. 32. × 10.

Fig. 14. „ „ 13 „ „ „ „ N°. 89. × 10.

Fig. 15. „ „ 13 à 14 „ „ „ „ N°. 31. × 10.

Fig. 16. „ „ 13 „ „ „ „ N°. 108. × 10.

Figs. 17 and 18. „ „ 16 „ „ „ „ N°. 68. × 13.
Fig. 17 dorsal, fig. 18 ventral view.

Fig. 19. Embryo with 18 pairs of somites. *Manis*, N°. 53 × 10.

Fig. 20. *(plate I) and figs.* 21—28 *(plate III) represent the primitive streak-stage N°.* 151.

Fig. 20. General view of a section through the strongly folded blastocyst, lying against the uterine wall. Extra-embryonic hypoblast consisting of flattened cells. *Emb.*, embryo. × 20.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_{11} I, 6.

PLATE III.

Fig. 21. Reconstruction of this stage. Embryonic shield pearshaped, in which are indicated: primitive streak with rest of primitive groove, headprocess, prochordal plate and annular zone of proliferation. The situation of the sections figs. 22—28 is indicated. × 50.

Figs. 22—28. *Cross-sections through the embryonic shield, and indicated in fig.* 21.

Fig. 22. The 8th section (counting from the posterior border) through the primitive streak, from which the peristomal mesoblast originates. The probable remnant of the primitive groove is shown. The hypoblast forms a continuous layer with columnar cells under the primitive streak. The epiblast passes laterally into the trophoblast. × 191.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_9 III, 5.

Fig. 23. A section (9 sections in front of that, shown in fig. 22) through the primitive streak, covered by the hypoblast. The embryonic shield rises above the level of the trophoblast. × 191.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_{10} I, 6.

Fig. 24. The 7th section in front of that, shown in fig. 23, passing through the HENSEN-knob. Here the hypoblast forms an uninterrupted layer too. × 194.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_{10} II, 5.

Fig. 25. The 10th section in front of that, shown in fig. 24, passing through the headprocess. To the left the annular zone of proliferation. × 198.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_{10} III, 7.

Fig. 26. The 5th section in front of that, shown in fig. 25, passing through the anterior border of the headprocess. The annular zone is visible on the left, on the right not yet. × 200.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_{11} I, 4.

Fig. 27. The 6th section in front of that, shown in fig. 26, passing through the prochordal plate. The annular zone is visible on the left as well as on the right. The very first formation of the peripheral mesoblast is to be seen (at both sides a mitotic figure). × 200.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_{11} II, 2.

Fig. 28. A section through the prochordal plate and annular zone, with distinct peripheral mesoblast-formation, 7 sections in front of that, shown in fig. 27. × 200.

HUBRECHT-Lab. Cat. N°. *Manis*, 151 a_{12} I, 1.

Figs. 29—32. *Sagittal sections through the primitive streak-stage N°.* 180.

Fig. 29. Schema of the median section. The primitive streak from the caudal border (on the left) to *. Here the headprocess extends cephalad between epiblast and hypoblast, as far as the prochordal plate. The peristomal mesoblast originates from the primitive streak. The hypoblast in the posterior part of the embryonic shield is a layer composed of cubical cells, gradually becoming thinner in the anterior part. Just in front of the prochordal plate the wall of the blastocyst is torn off. × 96.

HUBRECHT-Lab. Cat. N°. *Manis*, 180 a_2 II, 14.

Fig. 30. Enlarged middle part of fig. 29. The much flattened hypoblast is to be noted. × 265.

Fig. 31. Enlarged cephalic part of the same germdisc, showing the headprocess and the prochordal plate. × 345.

HUBRECHT-Lab. Cat. N°. *Manis*, 180a_2 II, 1.

Fig. 32. Formation of the peripheral mesoblast out of the extra-embryonic hypoblast (annular zone of proliferation) behind the embryonic shield. The mitotic figure *(m)* is to be noted. The trophoblast lies against the uterine wall, fused with it in some places (this is not visible in the figure). On the right between primitive streak and hypoblast the peristomal mesoblast is to be seen. × 280.

HUBRECHT-Lab. Cat. N°. *Manis*, 180a_2 I, 10.

PLATE IV.

Figs. 33—35. *Sagittal sections through the embryo N°.* 83, *possessing* 4 *pairs of somites with a* 5th *pair forming.*

Fig. 33. Schema of the mid-sagittal section. The peristomal mesoblast is formed out of the primitive streak. The posterior part of the chorda-anlage is torn from the primitive streak. The hypoblast is present beneath the primitive streak, but disappears (in the section) under the HENSEN-knob, from which the chordal plate, incorporated in the hypoblast, extends. In the anterior part of the embryo the chordal plate passes directly into the prochordal plate, from which originates the cephalic mesoblast as a sheet of cells between epiblast and hypoblast. A pleuropericardial space has not been formed as yet. × 50.

HUBRECHT-Lab. Cat. N°. *Manis*, 83a_2 IX, 9.

Fig. 34. The cephalic region of the same embryo A part of the medullary groove, a part of the chordal plate and the prochordal plate and the cephalic mesoblast are visible. × 210.

HUBRECHT-Lab. Cat. N°. *Manis*, 83a_2 IX, 8.

Fig. 35. The HENSEN-knob with the lumina (= chordal canal) in it, greatly enlarged. Cranialward the chordal plate. × 210.

HUBRECHT-Lab. Cat. N°. *Manis*, 83a_2 IX, 12.

Figs. 36.—41. *Cross-sections, passing through the chorda-anlage of embryo N°.* 64 *with* 8 *à* 9 *pairs of somites (cf. fig.* 62*).*

Fig. 36. A section through the anterior part of the broad chordal plate, quite incorporated in the hypoblast. The undifferentiated mesoblast still connected with the chordal plate-borders. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 64 a_1 IV, 8.

Fig. 37. A section through the 7th pair of somites. The broad chordal plate passes into the hypoblast, which is torn from it on the right. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 64 a_2 IV, 28.

Fig. 38. A section through the 8th pair of somites. The chordal plate is slightly higher and incorporated in the hypoblast. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 64 a_3 I, 4.

Fig. 39. A section through the undifferentiated mesoblast. The chordal plate, higher than the hypoblast, is not quite incorporated in it. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 64 a_3 III, 11.

Fig. 40. A section still further caudalward, showing the very first beginning of the incorporation of the chordal plate in the hypoblast. × 342.

HUBRECHT-Lab. Cat. N°. *Manis*, 64 a_3 III, 18.

Fig. 41. The headprocess, not yet incorporated in the hypoblast, is connected with the undifferentiated mesoblast: the connection in the section being broken, however. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 64 a_3 IV, 3.

Figs. 42—44. *Cross-sections through the chorda-anlage of embryo N°.* 32 *with* 10 *à* 11 *pairs of somites, (cf. fig.* 63*).*

Fig. 42. A section through the 5th pair of somites, showing a very broad chordal plate, not quite incorporated in the hypoblast on the right side. The hypoblast is slightly compressed against the borders of the chordal plate. On the left and on the right the primitive aortae. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 32 a_2 VI, 15.

Fig. 43. A section through the undifferentiated mesoblast. Distinct broad and tall chordal plate; the hypoblast extending under the borders of it. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 32 a_4 II, 7.

PLATE V.

Fig. 44. A section through the headprocess, with chordal canal and connected with the mesoblast. Very first beginning of the incorporation of the chorda-anlage in the hypoblast. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 32 a_4 III, 11.

Figs. 45—50. *Cross-sections through the embryo N°.* 89 *with* 13 *pairs of somites (cf. fig.* 64*).*

Fig. 45. A section through the undifferentiated mesoblast of the head and anterior part of the chordal plate, incorporated in the hypoblast, into which it passes directly. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 89 a_2 VII, 11.

Fig. 46. A section through the 4th pair of somites. The chordal plate is not very broad, and incorporated in the hypoblast, the latter being strongly compressed by the borders of the chordal plate. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 89 a_3 VI, 10.

Fig. 47. A section through the 5th pair of somites, much resembling fig. 46. The hypoblast much compressed here too. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 89 a_4 II, 7.

Fig. 48. A section through the undifferentiated mesoblast just in front of the chorda-anlage, not yet incorporated. The chordal canal (cf. fig. 49) has opened into the cavity, enclosed by the hypoblast (archenteron). Beginning of the incorporation of the chordal plate in the hypoblast. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 89 a_6 I, 10.

Fig. 49. A section through the anterior part of the chordal canal (cf. fig. 64). The hypoblast extending under the borders of the arched chordal plate; this connection, however, being broken. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 89 a_6 I, 12.

Fig. 50. A section through the headprocess with chordal canal, appearing double. The hypoblast extends beneath it. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 89 a_6 III, 11.

Figs. 51—55. Cross-sections through the chorda-anlage of embryo N°. 31 with 13 à 14 pairs of somites (cf. fig. 65).

Fig. 51. Anterior part of the chorda-anlage, not yet separated from the hypoblast. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 31 a_2 V, 11.

Fig. 52. A little further caudalward the notochord is almost completely separated from the hypoblast; only a very small part is not yet covered by the hypoblast. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 31 a_2 VIII, 15.

Fig. 53. A section through the 9th pair of somites. Chordal plate with hypoblast, slightly thickened under its borders. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 31 a_3 VIII, 14.

Fig. 54. A section through the 14th pair of somites, which is forming. The chordal plate is incorporating in the hypoblast, which extends under the greater part of it. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 31 a_4 VI, 6.

Fig. 55. A section through the headprocess with chordal canal and connected with the undifferentiated mesoblast. × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 31 a_6 II, 9.

PLATE VI.

Fig. 56. Sagittal section through the fore-gut of embryo N°. 108, with 13 pairs of somites. The chordal plate forms the roof of the primitive gut, except in the most cephalic part, where it ends in the mesoblast. The pharyngeal membrane is cut tangentially. The heart-endothelium and the pericardial space are visible. The hypoblast surrounding the anterior part of the fore-gut is distinctly thickened. × 200.

HUBRECHT-Lab. Cat. N°. *Manis*, 108 a_4 II, 6.

Fig. 57. A section through the well-developed chordal-canal in the headprocess of the embryo N°. 68 with 16 pairs of somites. The undifferentiated mesoblast is not connected with the headprocess (cf. fig. 66.). × 340.

HUBRECHT-Lab. Cat. N°. *Manis*, 68 a_6 II, 6.

Figs. 58—61. *Cross-sections through the chorda-anlage of embryo N°.* 53 *with* 18 *pairs of somites (cf. fig.* 67*)*

Fig. 58. A section through the anterior part of the chorda-anlage, not yet separated from the hypoblast. × 340.
HUBRECHT-Lab. Cat. N°. *Manis*, 53 a_1 V, 2.

Fig. 59. The 14th section caudalward of that, shown in fig. 58. The notochord is quite separated from the hypoblast, which extends beneath it and is somewhat thinner here. × 340.
HUBRECHT-Lab. Cat. N°. *Manis*, 53 a_1 V, 16.

Fig. 60. A section through the 8th pair of somites, showing a large cylindrical notochord. The hypoblast is somewhat thinner beneath it. × 340.
HUBRECHT-Lab. Cat. N°. *Manis*, 53 a_3 III, 15.

Fig. 61. A section through the 13th pair of somites. The notochord is not yet quite separated from the hypoblast. × 340.
HUBRECHT-Lab. Cat. N°. *Manis*, 53 a_4 II, 13.

Figs. 62—67. *Reconstructed sagittal sections through the mid-axis of the hinder part of the chorda-anlage of the embryos N°.* 64, 32, 89, 31, 68, *and* 53.

The circumference of the somites, projected on the mid-sagittal plane is indicated by a — — — line, the upper face of the medullary groove, projected in the same way, marked by a —.—.— line. Primitive streak and medulla are dotted, the chorda-anlage hatched and the hypoblast is black. The sections described are indicated. All figs. × 67, except fig. 68 (× 50).

Fig. 62. A section through embryo N°. 64 with 8 à 9 pairs of somites. Chordal plate and headprocess, covered by hypoblast, are visible. In the anterior part of the primitive streak a region is visible, in which the hypoblast is fused intimately with the primitive streak.

Fig. 63. A section through embryo N°. 32 with 10 à 11 pairs of somites. A short narrow chordal canal is visible in the head-process. The hypoblast in the anterior part of the primitive streak as in embryo N°. 64.

Fig. 64. A section through embryo N°. 89 with 13 pairs of somites. Two parts of the chordal canal are visible in the headprocess. The anterior part opens into the archenteron. Hypoblast fused with primitive streak somewhat more caudalward.

Fig. 65. A section through embryo N°. 31 with 13 à 14 pairs of somites. Two small parts of the chordal canal are visible.

Fig. 66. A section through embryo N°. 68 with 16 pairs of somites. A very distinct and long chordal canal is visible, which opens into the archenteron.

Fig. 67. A section through embryo N°. 53 with 18 pairs of somites. The notochord has separated along a great distance from the hypoblast, except in the region of the somites XI—XV. Three small parts of the chordal canal are to be seen in the head-process.

STELLINGEN.

I. Een gastrula s.str. komt bij de Vertebraten niet voor.

II. De darm van alle Vertebraten wordt gevormd door vereeniging van het archenteron en het metenteron.

III. De „entodermalen Wanderzellen", welke V. DANCHAKOFF waarnam in de bloedvaten van jeugdige kip-embryonen (Anat. Hefte, Bd. 37, 1908), zijn primaire geslachtscellen.

IV. De secundaire geslachtskenmerken staan onder den invloed van hormonen, die afgescheiden worden door de interstitieele cellen van de geslachtsklier (z.g. puberteitsklier).

V. In de elementen van den plastischen spiertonus bestaat ook de mogelijkheid van een tonische contractie, die o.a. opgewekt wordt door het regelende centrum.

VI. De kleine kernen, door HUBRECHT in zich klievende Zoogdiereieren (*Galeopithecus*, *Manis*) gevonden, zijn geen toekomstige trophoblastkernen, zooals HUBRECHT meende, doch follikelepitheelkernen.

VII. *Oxyuris equi* (SCHRANK) en *Oxyuris mastigodes* NITSCH zijn zelfstandige soorten.

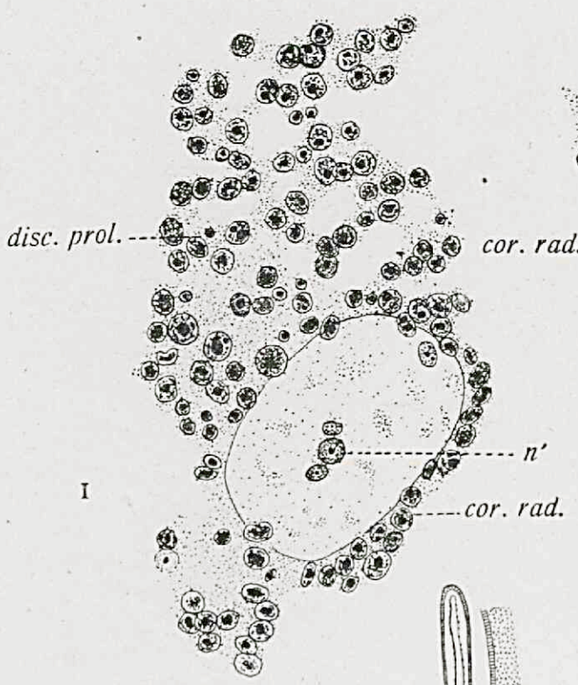
VIII. Het melksap beschermt de planten tegen diervraat.

IX. Het verdient aanbeveling de Aristolochiaceae als Polycarpicae te beschouwen.

X. De Rotatorien en de Nematoden kunnen van een zelfden stamvorm afgeleid worden.

XI. De onderzoekingen van HERIBERT NILSSON (Hereditas, Bd. I, 1920) maken het zeer waarschijnlijk, dat de wetten van MENDEL ook voor *Oenothera Lamarckiana* geldig zijn.

XII. De Monograptidae zijn verwant met *Rhabdopleura* (A. SCHEPOTIEFF, Neues Jahrbuch für Mineralogie, Bd. II, 1905).



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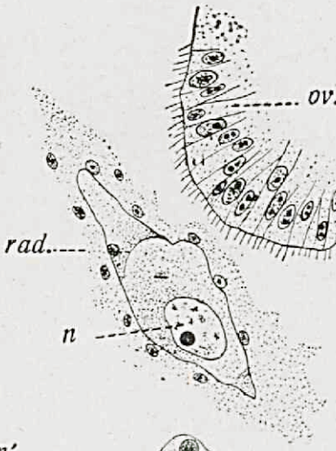

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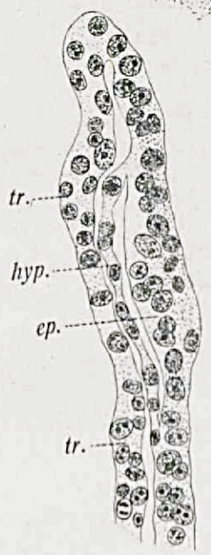

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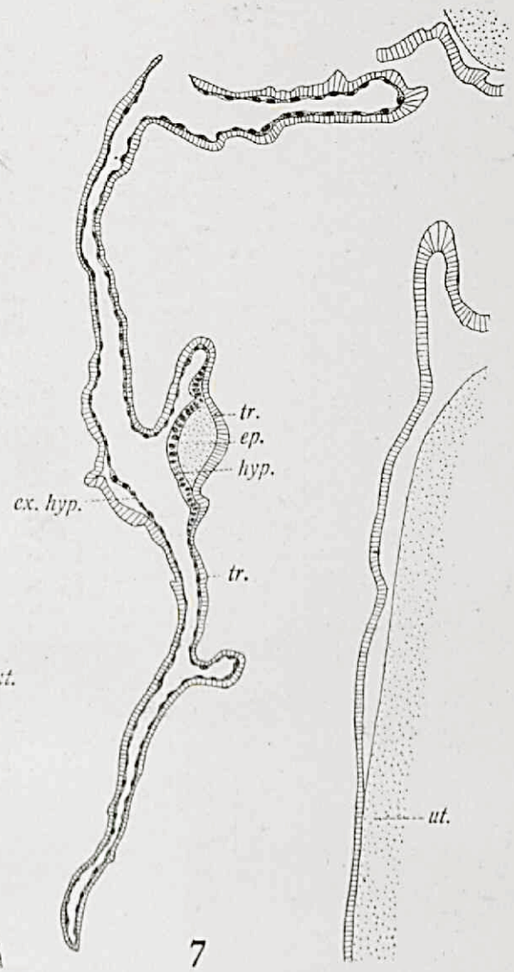

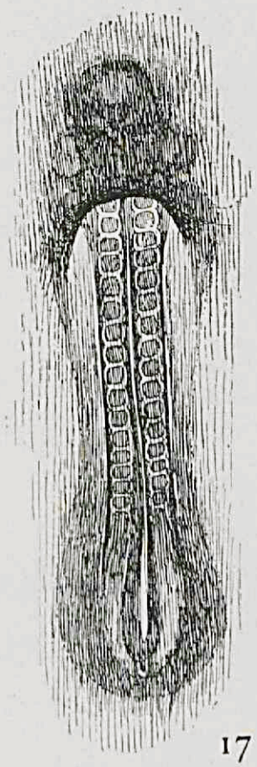

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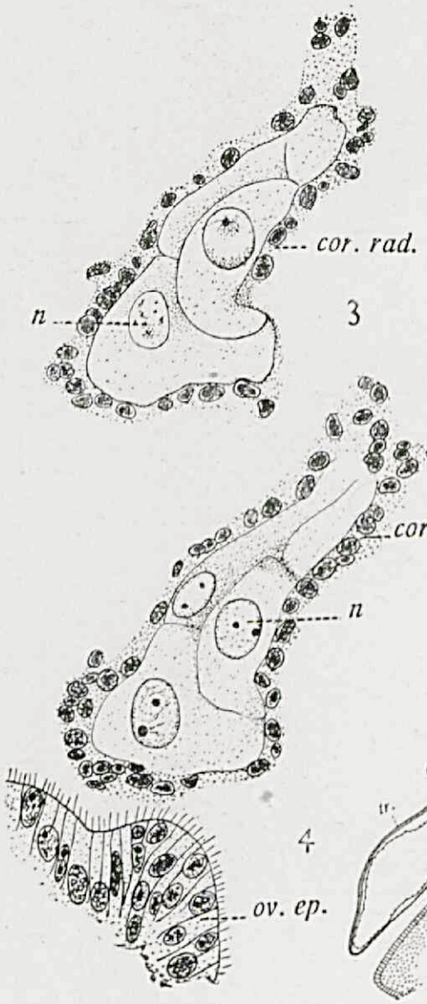

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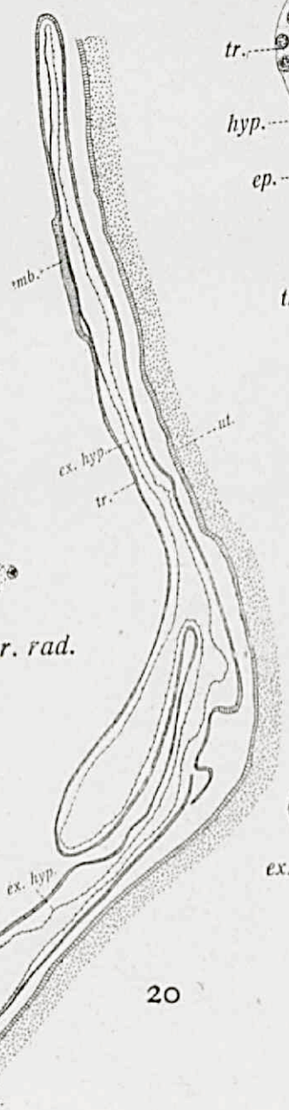

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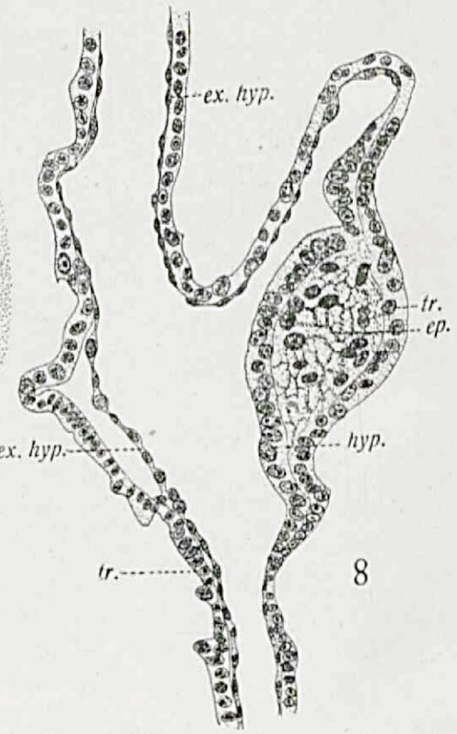

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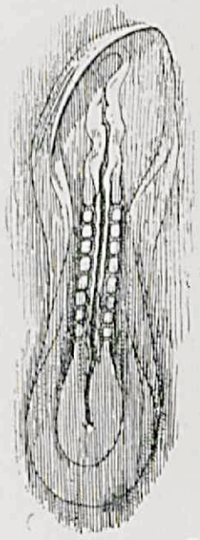

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Figs. 1—9, van Oordt del.; figs. 11, 12, 17, 18, J. Prijs del.

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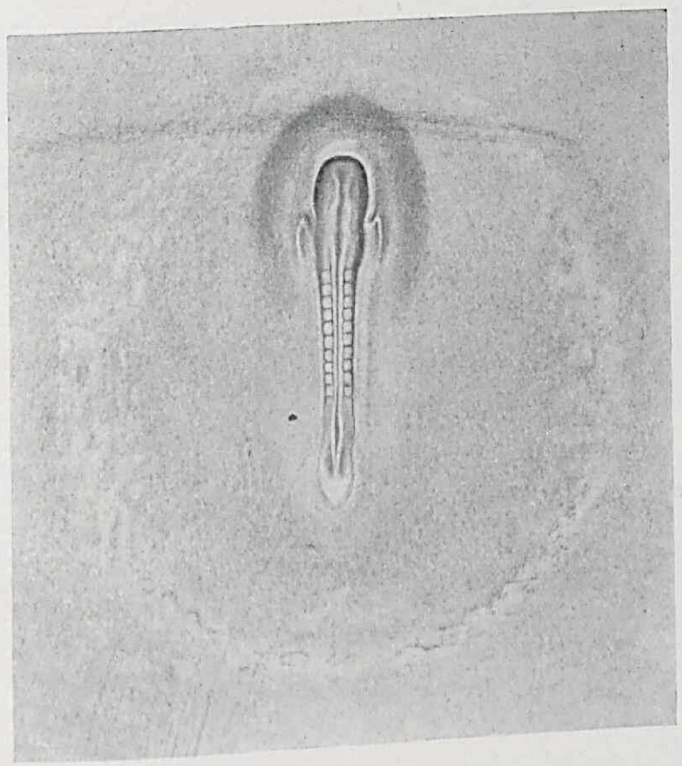

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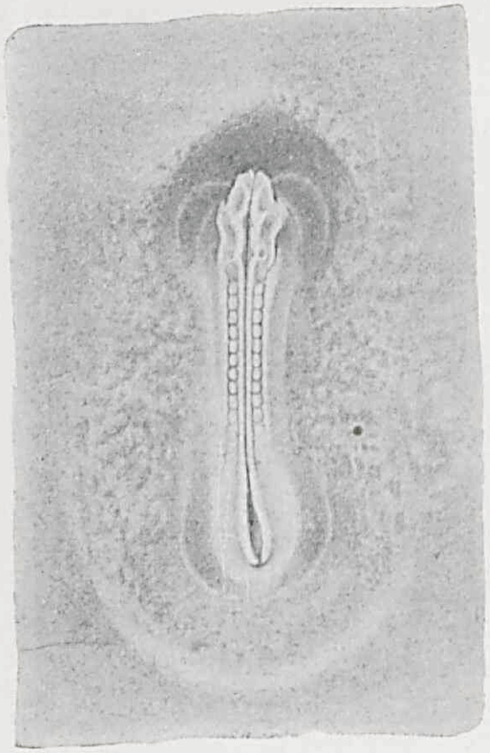

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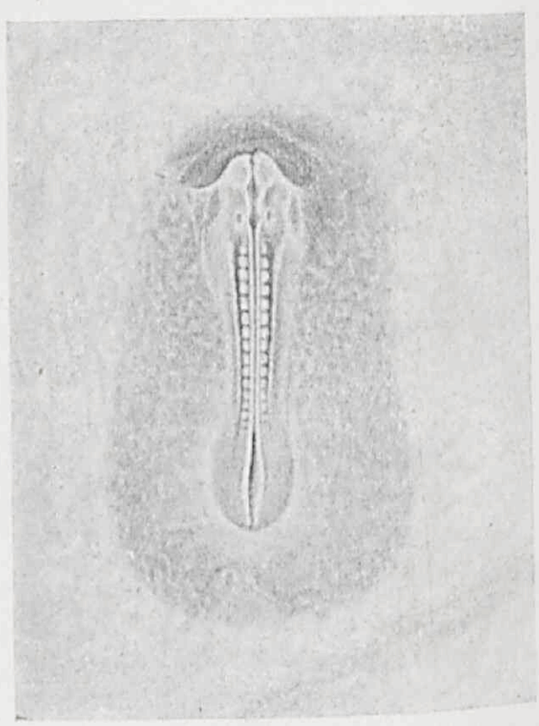

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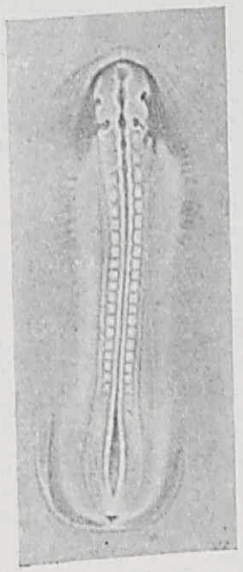

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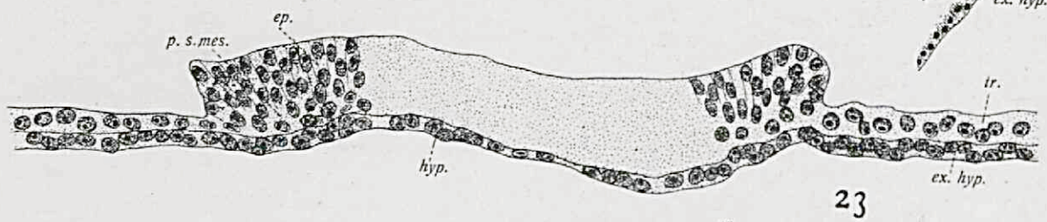

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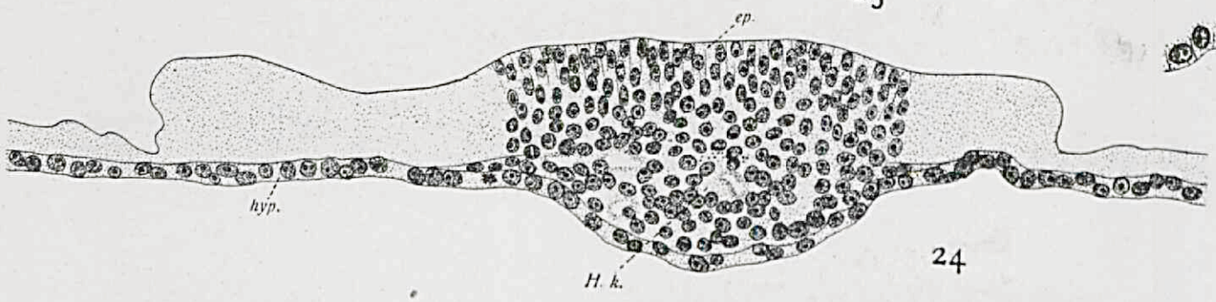

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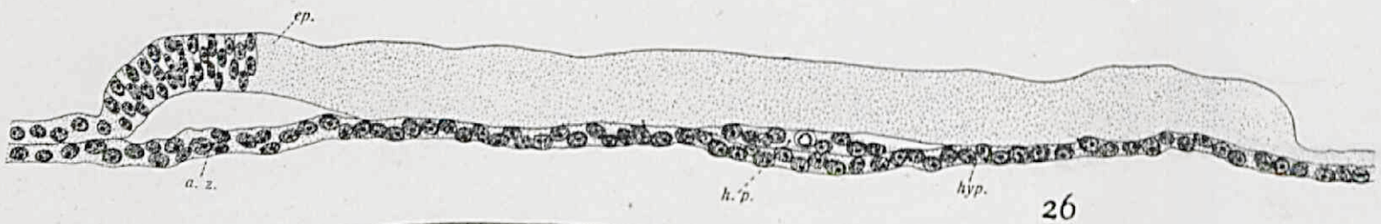

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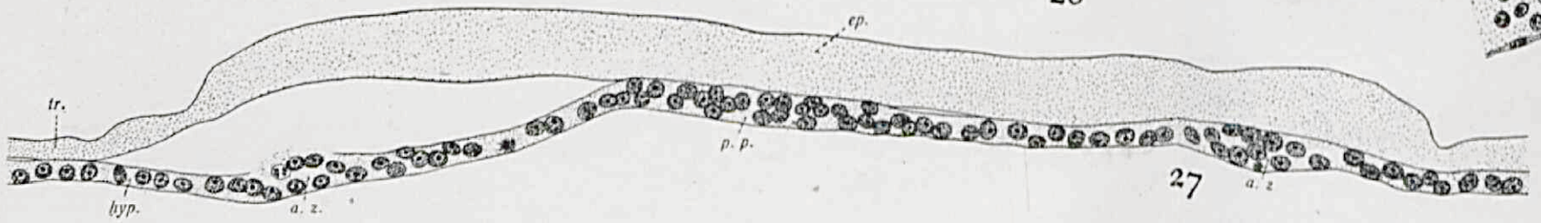

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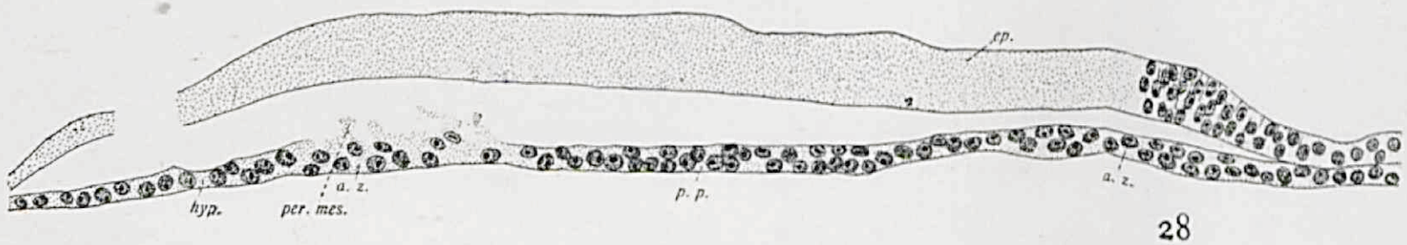

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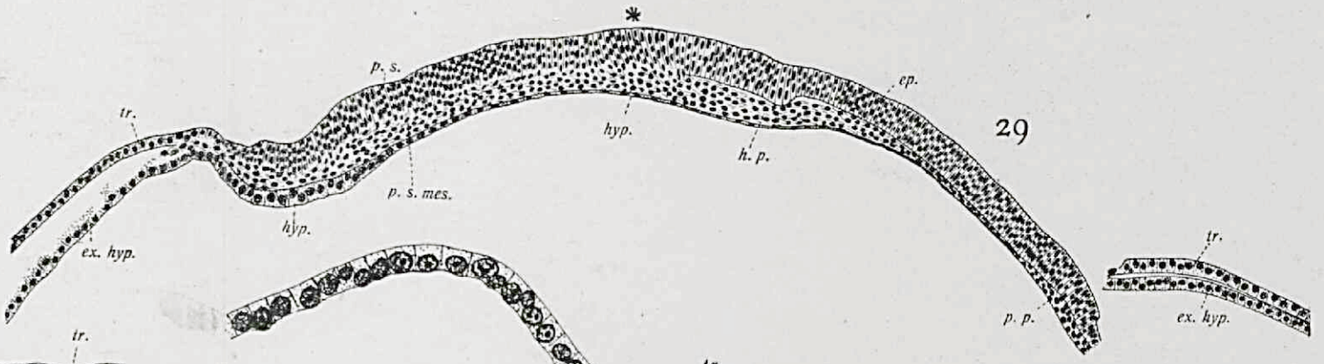

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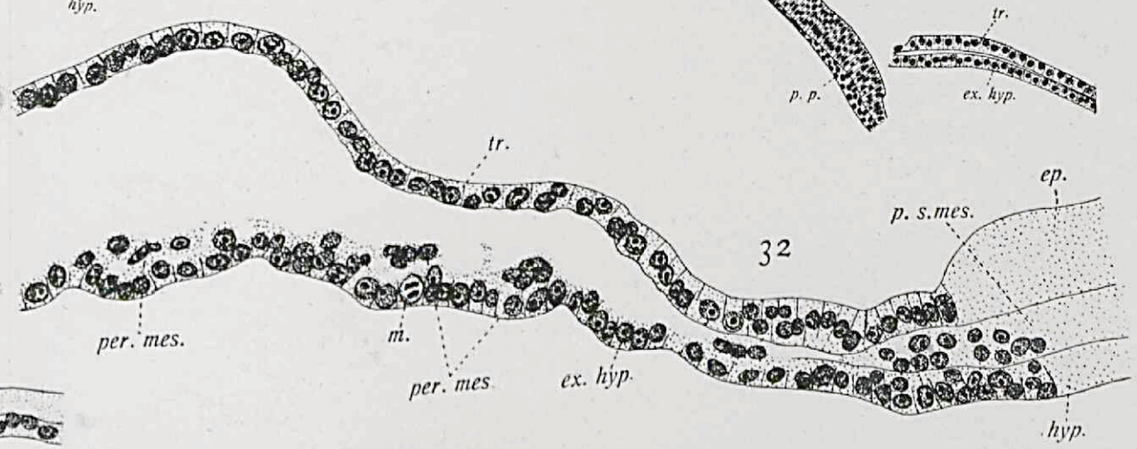

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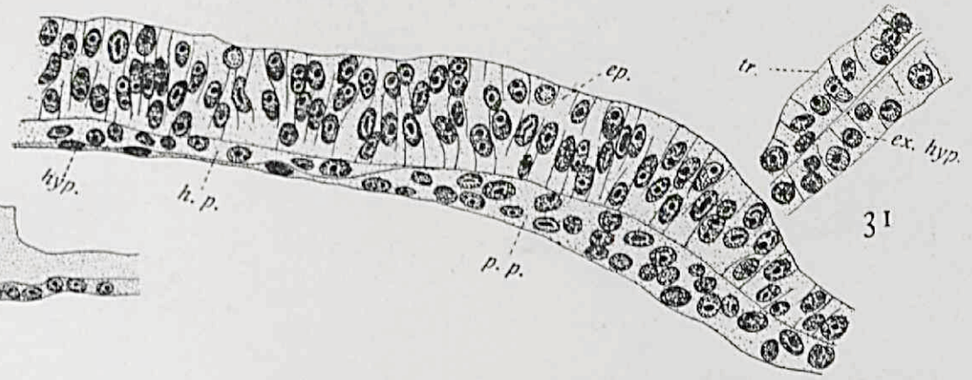

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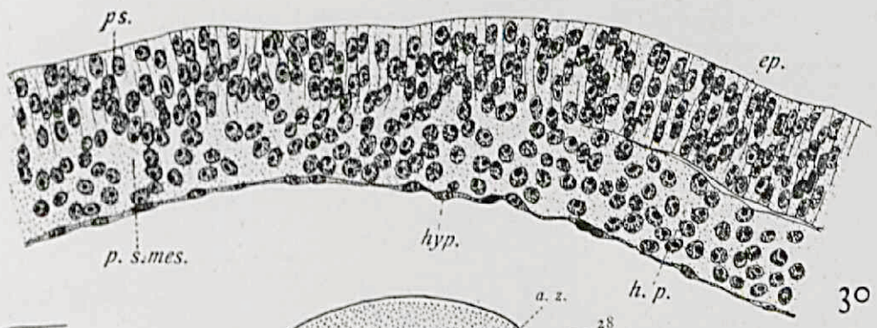

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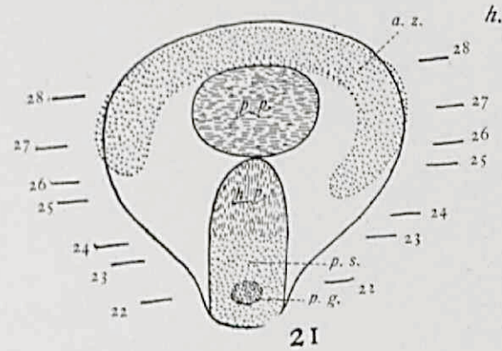

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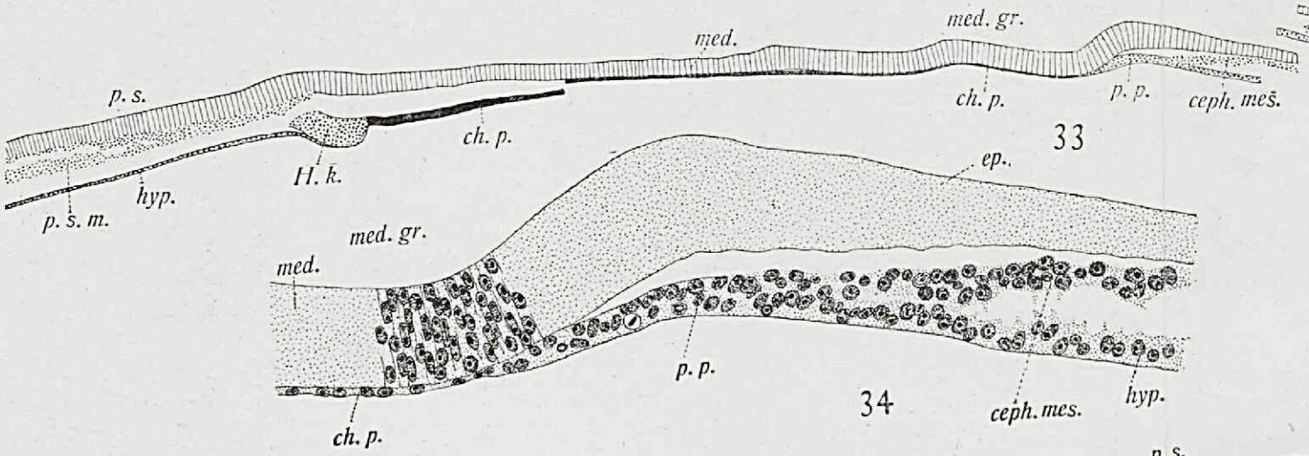

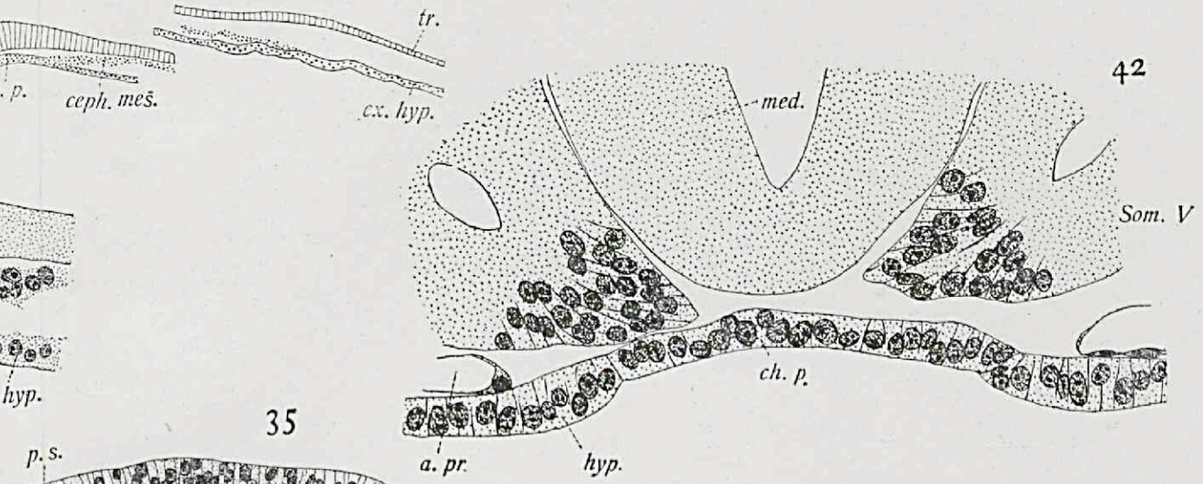

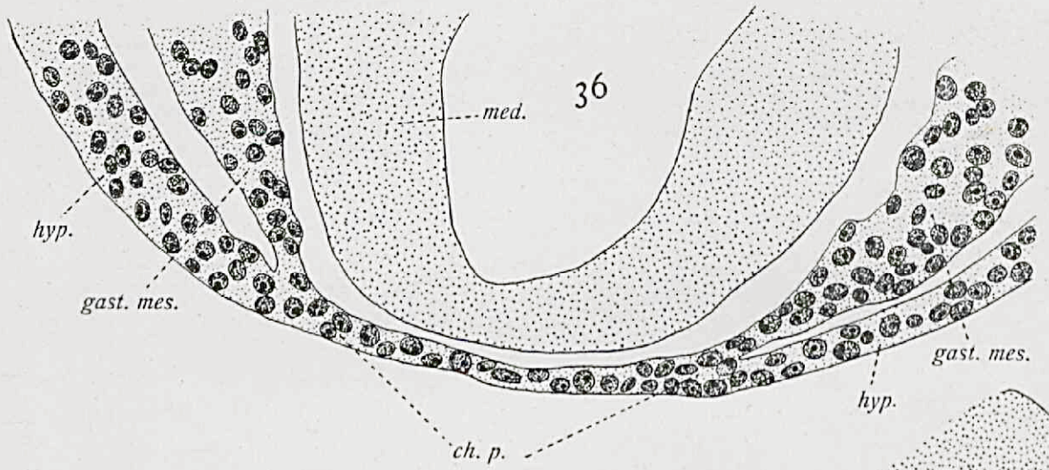

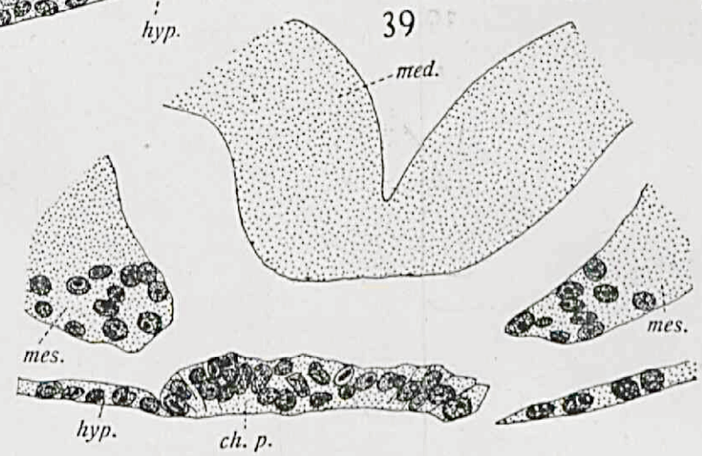

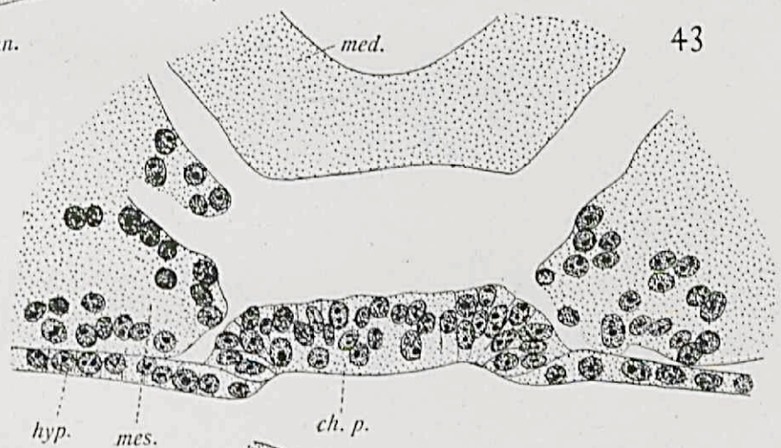

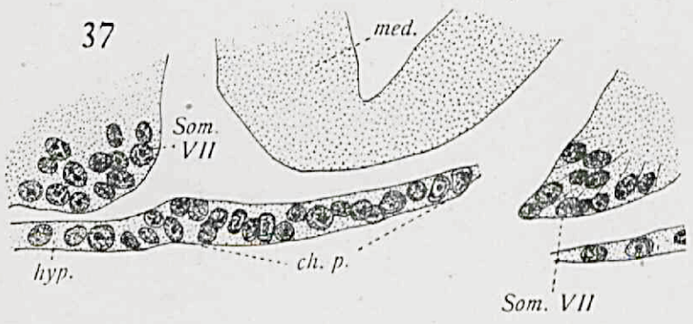

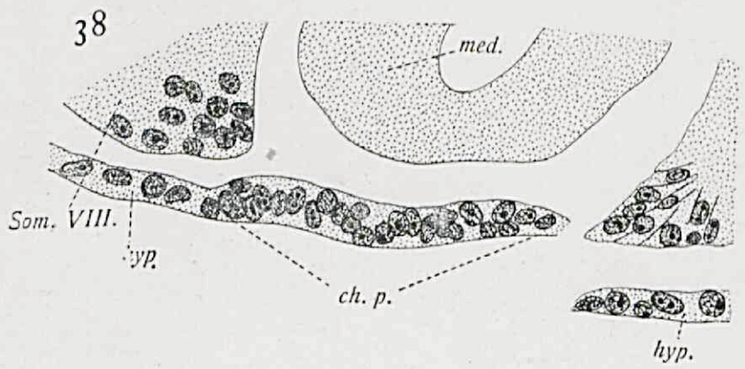

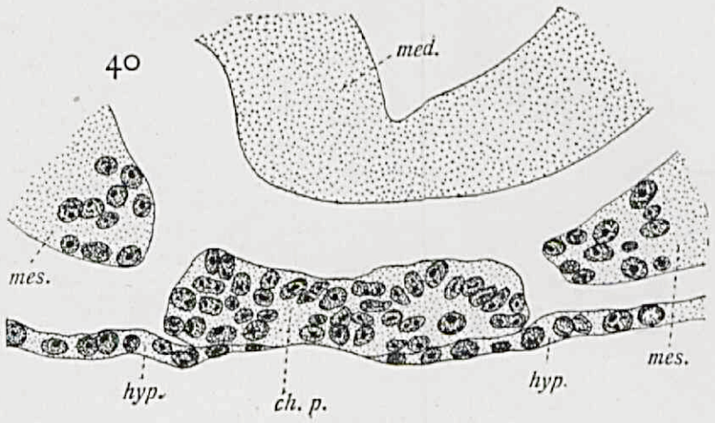

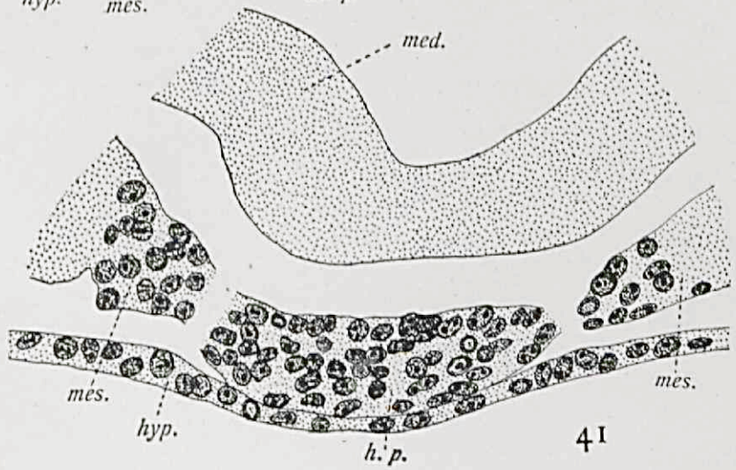

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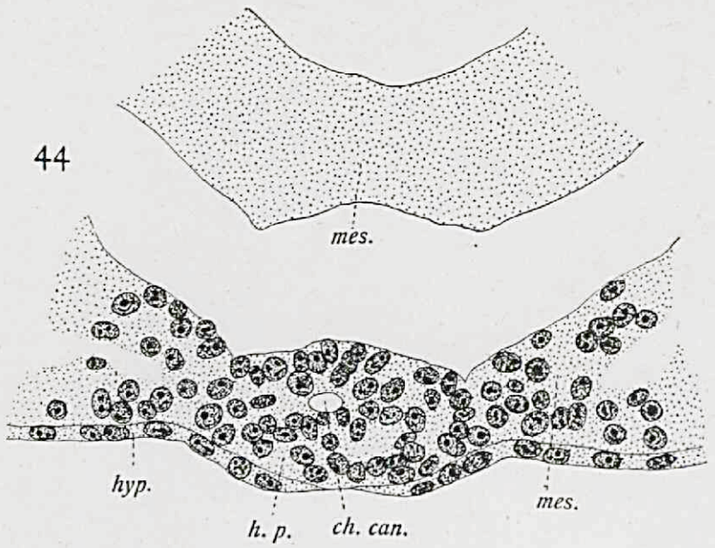

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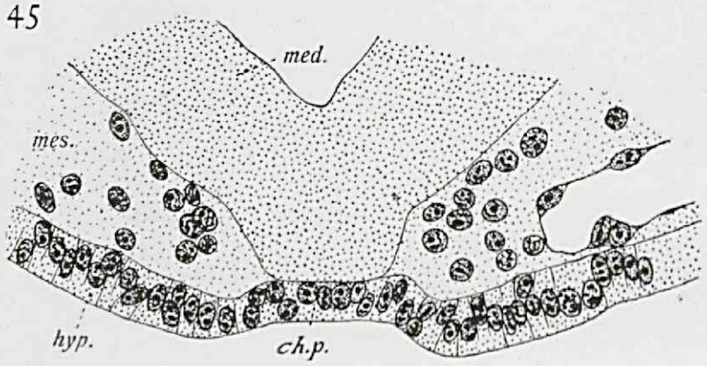

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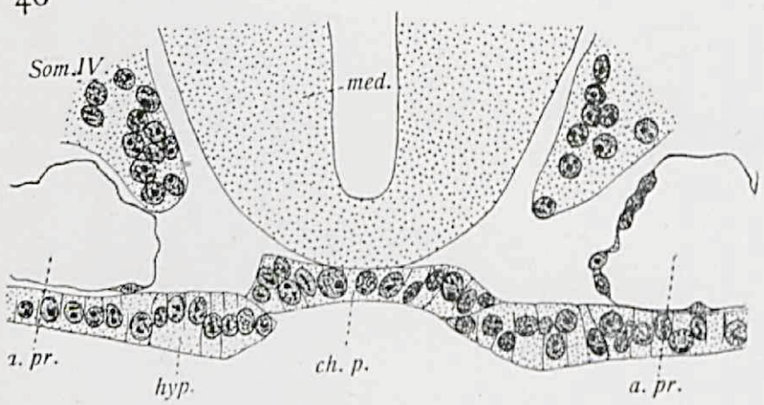

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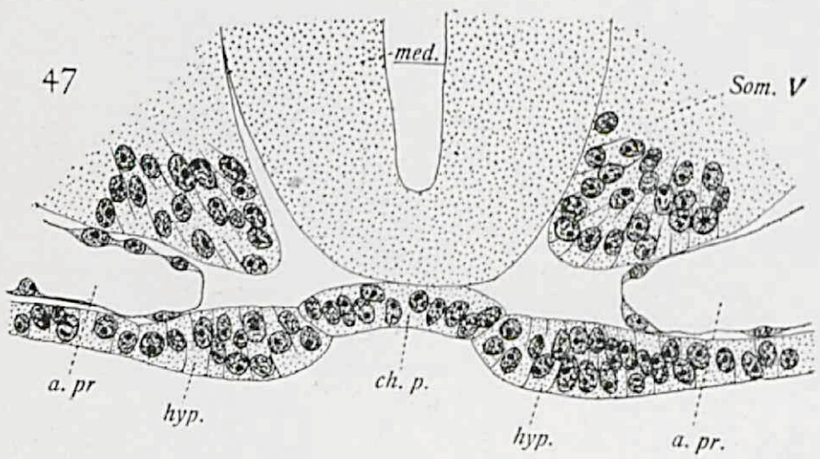

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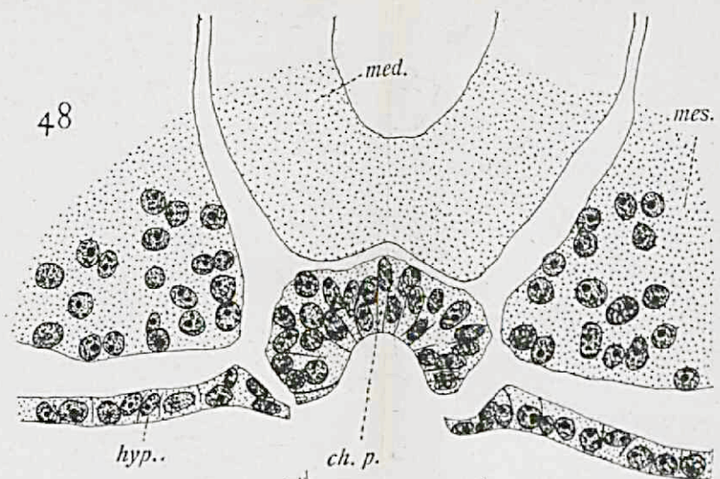

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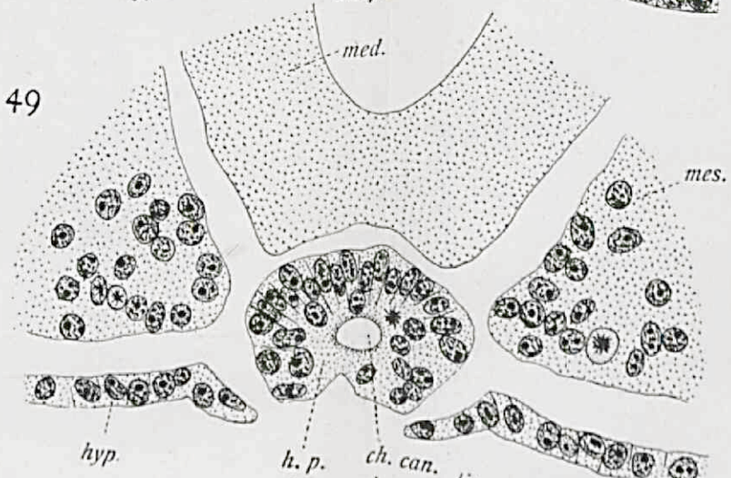

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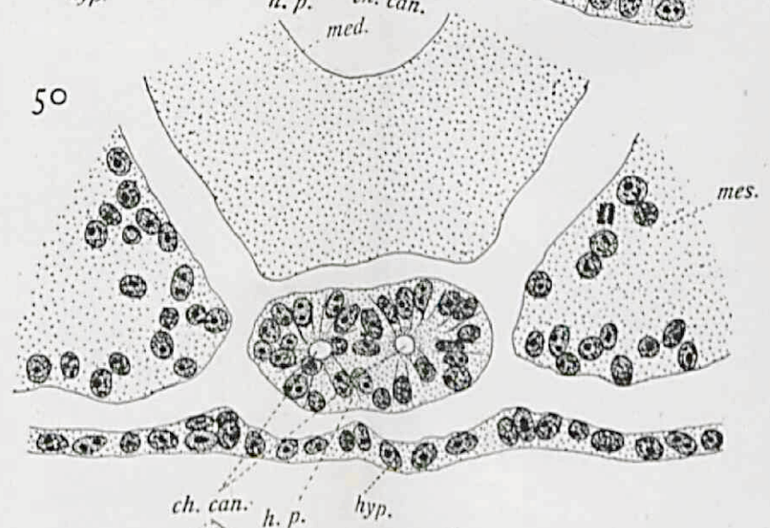

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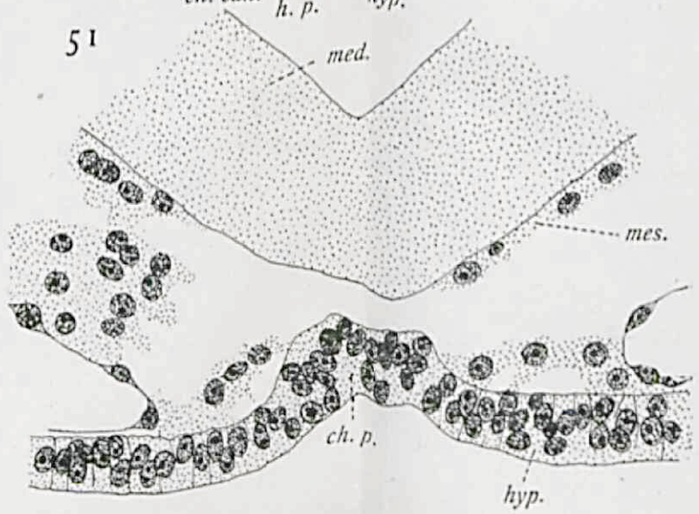

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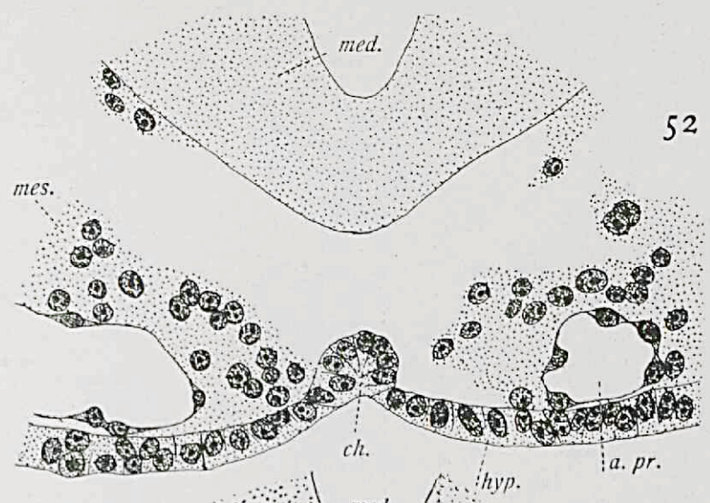

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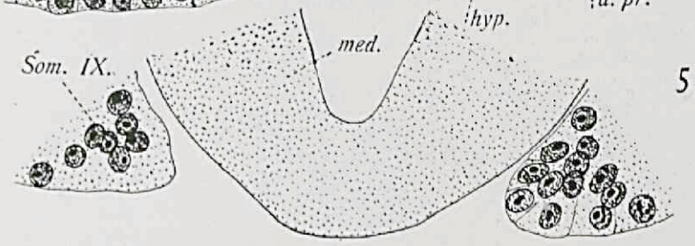

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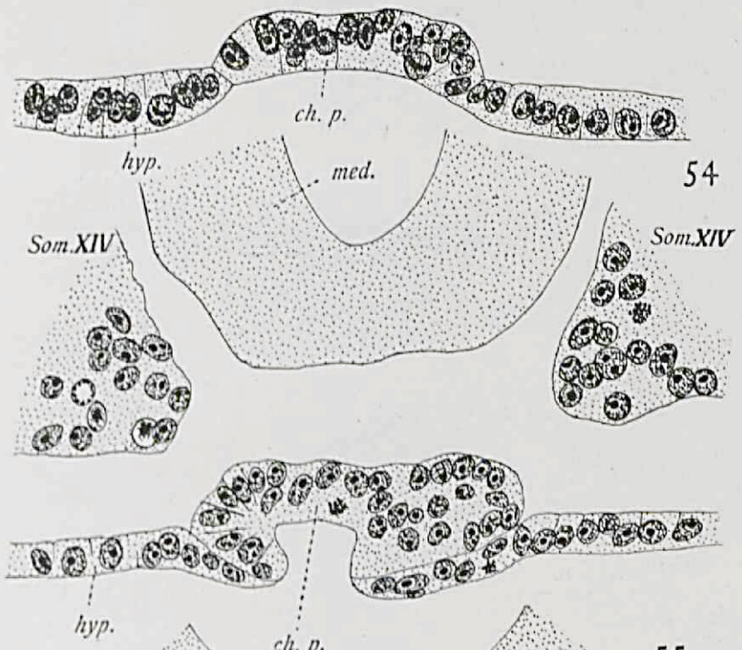

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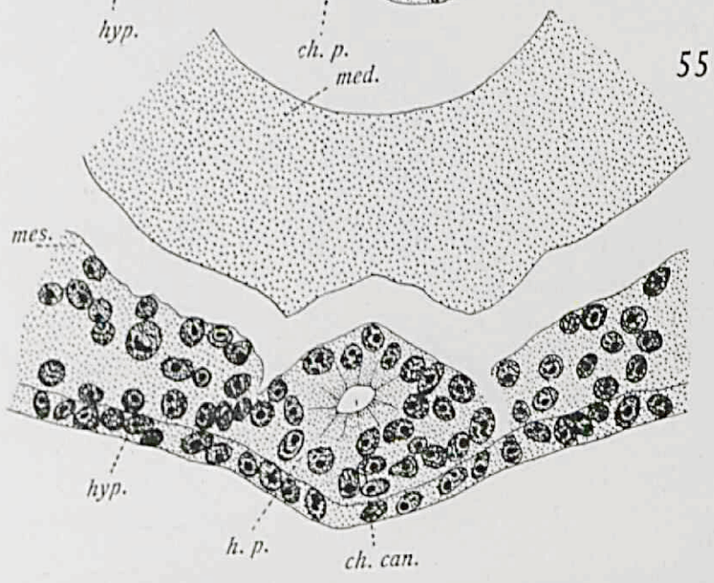

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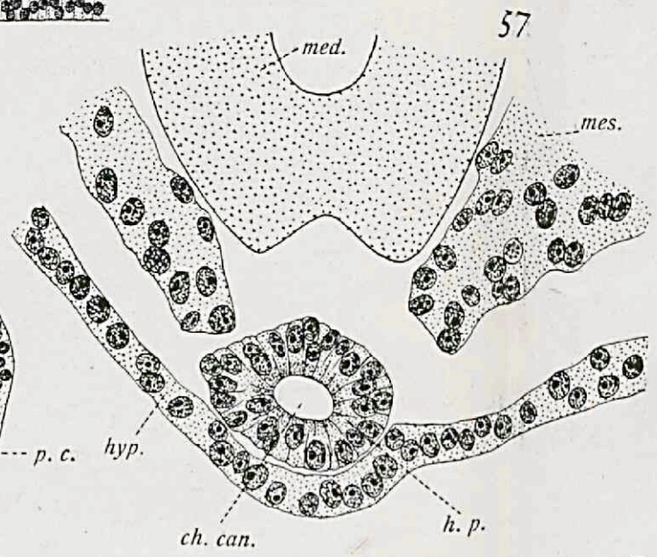

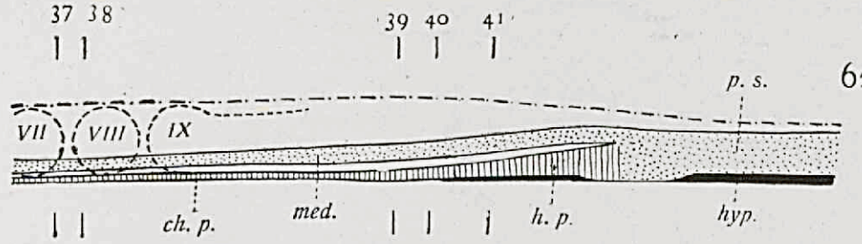

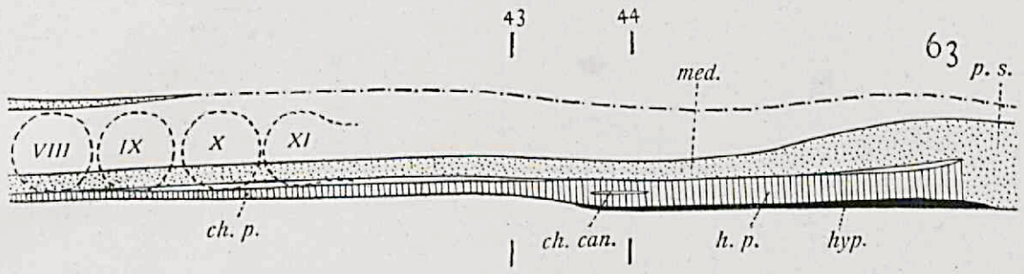

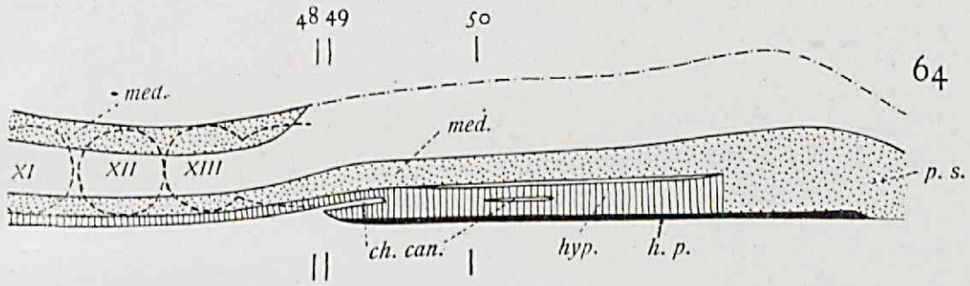

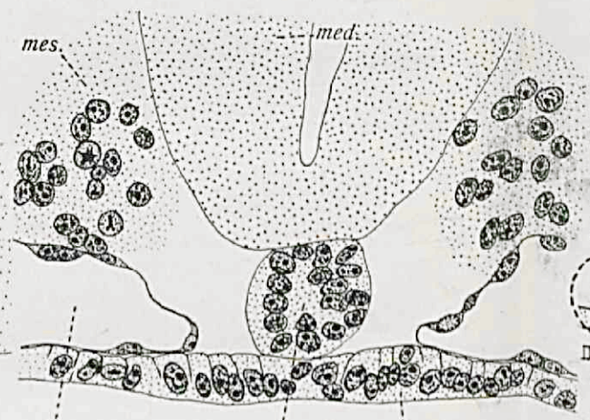

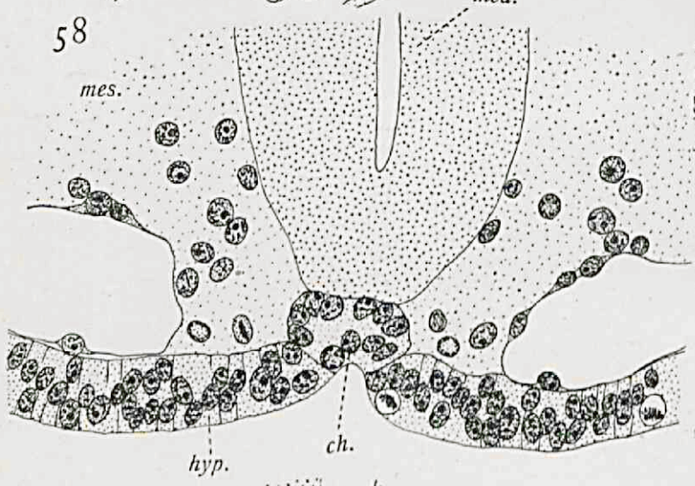

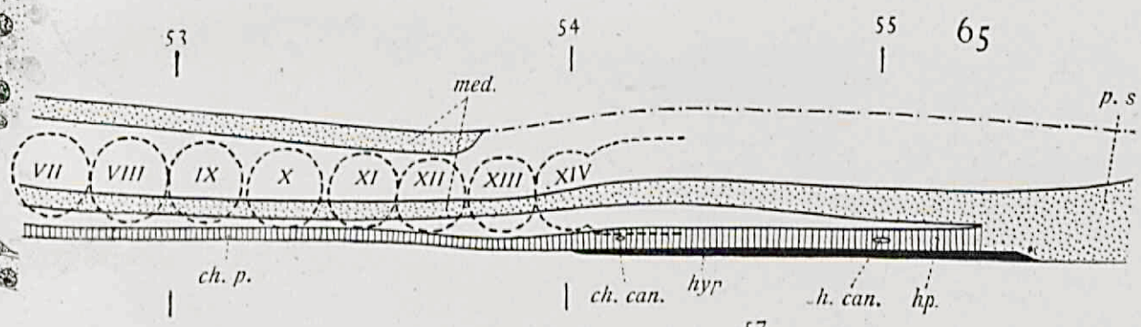

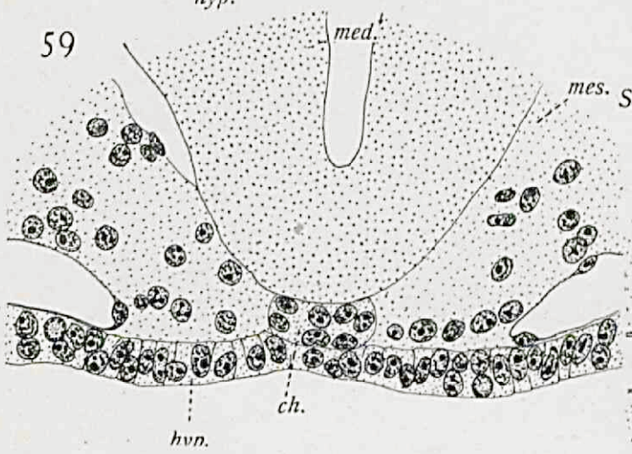

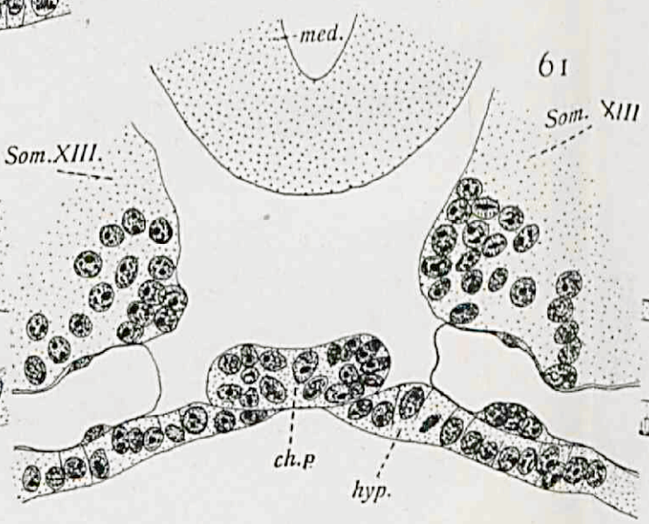

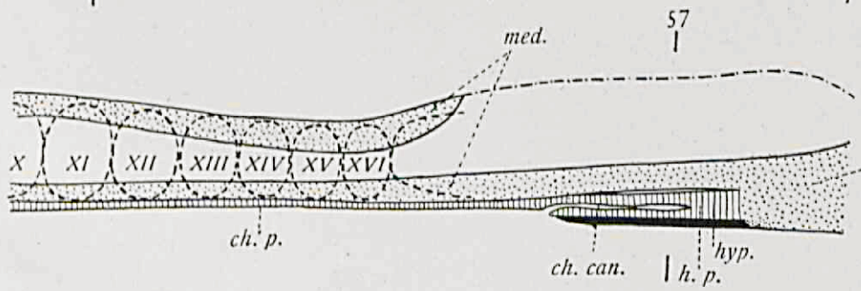

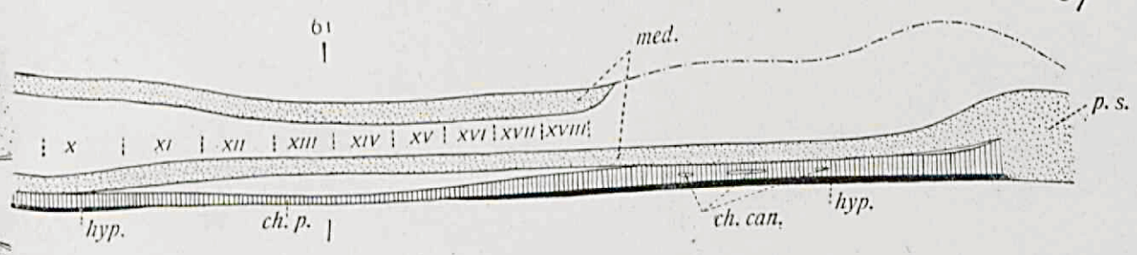

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