Zoology. — *"On the relation of the anus to the blastopore and on the origin of the tail in vertebrates"*. By Dr. H. C. DELSMAN. (Communicated by Prof. J. BOEKE).

(Communicated in the meeting of Feb. 24, 1917).

Both the foregoing communications (May 27 and November 25, 1916) being mainly dedicated to the mode of contraction of the blastopore border of amphibians, in this third one I should like to give some facts and considerations concerning the ultimate fate of the blastopore and its relation to the anus.

The statements made by the numerous investigators on this subject are so divergent that it must be very difficult for any one who cannot judge from personal experience to form a sound opinion. I will try to show that the application of the principles of my theory on the origin of vertebrates will once more serve to furnish us with the solution of an old problem which — especially by GROBBEN's (1900) classification of the animal kingdom — has been resuscitated. In the first place the different views and results of former investigators may be very briefly reviewed. We will confine ourselves mainly to the amphibian egg, in which a relation between anus and blastopore was for the first time noticed. Anurans and Urodelans will be treated separately, because, as I can confirm from my own investigations on *Rana esculenta* and *Amblystoma tigrinum*, these two groups in the relation of the anus to the blastopore exhibit a notable difference. We will begin with that group, on which the first observations were made, the Anurans.

BALFOUR (1881) in his Text-book gives a description of the origin of the anus, based mainly on the figures of GOETTE (1875) for *Bombinator igneus* and his own investigations on *Rana temporaria*, where the anus breaks through somewhat earlier than appears to be the case in toads generally. The blastopore passes into the neurenteric canal and the anus eventually arises at the bottom of a diverticulum of the alimentary tract, which meets an invagination of the skin. Perforation according to GOETTE's well-known representation of a longitudinal section in *Bombinator* only occurs when the growth of the tail is well advanced, in *Rana temporaria* according to BALFOUR somewhat earlier.

SPENCER (1885), on the contrary, comes to the conclusion that the blastopore in *Rana temporaria* remains open and passes directly into the anus. The blastopore is not enclosed by the medullary folds, and thus there is no neurenteric canal. The first conclusion is shared

by DURHAM (1886), but secondarily, according to the latter, a neurenteric canal is formed, independent of the blastopore. KUPFFER (1887), dealing with the same subject, comes to the conclusion that the blastopore remains open as the anus; so, too, PERENYI (1888).

SCHANZ (1887) also operated on *Rana temporaria*, together with *Triton*. In *Rana* he concludes that the medullary folds rather close over the blastopore, that there is indeed a neurenteric canal, though the lumen is not evident, and that the anus arises by perforation at the bottom of a little groove behind it. As regards the facts SIDEBOTHAM (1888) quite agrees with him. According to him BALFOUR's description is the right one, he too sees in sections the "diverticulum from the hind end of the mesenteron, dipping down towards a distinct pit in the epiblast below the blastopore and quite separate from it". Eventually perforation ensues. Similarly by MORGAN (1890) in *Rana halecina* and *Bufo lentiginosus* the anus is seen to arise at the bottom of a little groove in the ectoderm behind the blastopore.

GOETTE (1890) after a renewed investigation on *Bombinator igneus* and some other Anurans reaches the conclusion that the anterior half of the slit-like blastopore is transformed into the neurenteric canal, the posterior half into the anus. Yet in *Pelobates* he claims that this posterior half first closes and that the anus is formed only later.

As is apparent from the foregoing, during this period nearly every year brought forth a new investigation on this subject. In 1890 that of ERLANGER on *Rana esculenta* appeared; in 1891 that of ROBINSON and ASSHETON on *Rana temporaria*; in the same year a small treatise by ERLANGER in reply to some observations made by the two English critics on his work. All agree however that in both cases the anus arises by perforation.

In later years the fate of the blastopore is alluded to only in a few investigations, e.g. by BLES (1905), who for *Xenopus laevis*, and by SEEMANN (1907), who for *Alytes obstetricans* shows that the blastopore is not enclosed by the medullary folds and passes directly into the anus, there being accordingly no neurenteric canal.

Most of the investigators who have paid special attention to the question thus come to the conclusion (which after my own examination of *Rana esculenta* I can support without reservation) that the anus arises by perforation a little distance behind the blastopore, which is transformed into the neurenteric canal. A short description may be given here in addition to the figures for *Rana esculenta*.

After the yolk-plug has disappeared from the surface the blastopore presents itself as a short longitudinal split (textfig. 1*a*). A median

section through this egg is reproduced in Fig. 1 of the plate. In a

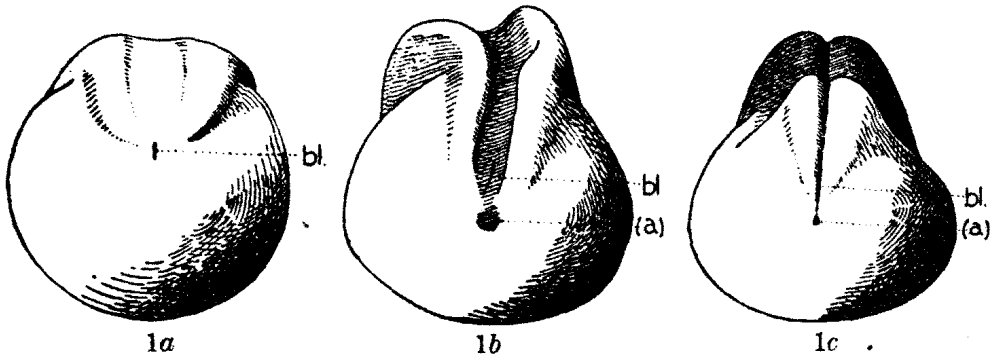


Fig. 1. Three eggs of *Rana esculenta* during the closure of the medullary folds (*a*) anal pit. *bl.* blastopore.

similar longitudinal series one succeeds better than might be expected in getting the blastopore as an opening (*bl.*), though of course this is only the case in one or two sections. The ventral blastopore lip is well developed and includes between itself and the yolkmass in the archenteron the anal diverticulum (Afterdarm, *a.d.*), which however is nothing but the intersection of a circular incision surrounding the mass of yolk-cells.

In a somewhat further advanced stage appears on the surface of the egg (textfig. 1*b*) behind the slit-like blastopore a shallow impression in the ectoderm (*a*), also clearly visible in a longitudinal section, as in fig. 2 of the plate. Underneath this impression a thickening of the ectoderm occurs, of which the beginning is already visible in fig. 1 (*). Opposite the invagination of the ectoderm a similar one is found in the entoderm at the bottom of the anal diverticulum.

In an egg as represented in textfig. 1*c* we see at the bottom of the shallow invagination of the ectoderm mentioned above a little pit, as yet not very deep, from which a still more shallow groove, the anal groove, runs forward to the blastopore-slit. The longitudinal section of this egg is given in fig. 3 of the plate. It bears a close relation to fig. 2, the anal membrane however has become thinner.

In a slightly further advanced stage, not represented here, the greatest part of the slit-like blastopore has been overgrown by the medullary folds, only at the hindmost extremity is there still a litlle opening, from which the anal groove runs to the anal pit. This anal groove, with a deeper depression at its anterior (rest blastopore) and at its posterior end (anal pit) appears to have been confused by several authors with the slit-like blastopore of fig. 1*a* and *b*,

which they accordingly imagine to have closed in the middle by coalescence of the opposite borders, leaving only a passage at the anterior and at the rear end, the future neurenteric canal and the anus, while the rudiment of the tail arises as a double knob at the right and the left side of the place of coalescence, these knobs fusing afterwards over the middle of the blastopore. Thus ZIEGLER (1892) in his little article on the surface-views of *Rana*-embryos writes: "Etwas später sieht man an Stelle des Spaltes eine Rinne, welche vorn in den Canalis neurentericus, hinten in die Aftergrube übergeht; es sind nämlich jetzt die seitlichen Blastoporuslippen median zur Vereinigung gekommen". In the same way things are represented by HERTWIG in his Lehrbuch. Already a close examination of surface views however teaches us that the anal groove is not at all identical with the slit-like blastopore, but that its anterior end coincides with the rear end of the latter. The study of median sections excludes every possibility of doubt. In the present article I could not insert any more some figures of a surface-view and of median sections of this stage, in a more detailed account elsewhere I will do so.

The step to fig. 4 (plate) seems fairly large, yet this is only apparent. Already in fig. 3 we see the cerebral plate curving in. Especially notable is the opposition between the praechordal cerebral plate and the epichordal medullary plate, which as a matter of fact in this stage is no longer a flat plate, but curved into a groove between the medullary folds. Fig. 3 however is realized only in one or two sections, which are exactly median, to the right or the left side immediately one of the medullary folds is intersected, as indicated in fig. 3 with a dotted line. A paramedian section in this series thus offers a much greater resemblance to fig. 4 where the medullary folds have coalesced than the median one of fig. 3.

Fig. 4 is also of interest in that here apparently for the first time the neuropore in Anurans is represented. In his treatise on "Die Morphogenie des Centralnervensystems" in HERTWIG's Handbuch, KUPFFER (1906) says in regard to Anurans: "Der Neuroporus ist im letzten Momente vor seinem Schlusse noch nicht zur Beoachtung gekommen"; neither in investigations published since is there anything to be found on this subject. KUPFFER accordingly only represents a longitudinal section of a somewhat further advanced stage than in my fig. 2 and further stages later than my fig. 4, where the place of the neuropore is still recognisable by the presence of a conical thickening of the ectoderm or of a recessus neuroporicus in the anterior wall of the brain vesicle. It is evident that the curving backward of the transverse cerebral fold plays as great a role in

the closing of the cerebral plate as the overgrowth of the lateral ridges.

There is yet another circumstance I should like to emphasize. Not only the ectoderm of the cerebral plate but also that which is situated in front of the transverse cerebral fold and which according to my theory is equivalent to that part of the apical plate of the Annelid trochophore which in Craniotes is not incorporated into the cerebral plate, is considerably thickened, and as for example in fig. 1 (*pr. cer.*) it exhibits an equally clear separation between the upper and lower layers of the ectoderm as the cerebral plate. Also in fig. 2 this agreement between cerebral plate and the part of the apical plate in front of it, which we might call the praecerebral part is evident. In the course of further development, however, a difference between the two parts of the apical plate evidences itself. In the cerebral, just as in the medullary plate, an intimate union of the upper and lower layers occurs, the demarcation between them disappears, and the upper layer, as ASSHETON (1909) has already observed, is incorporated in the wall of the brain and the medullary canal. In the praecerebral part of the apical plate however the coherence between the upper and lower layers becomes less and less, which no doubt is connected with the circumstance that this part of the ectoderm has to overgrow the cerebral plate. The lower layer finally lies as a compact cell-mass under the upper layer, which acts as ectoderm, and quite dissociated from it (fig. 4 *pr. cer.*). Judging from KUPFFER's (1906) figures of the later stages, it is this cell-mass which moving under the brain vesicle, ultimately gives rise to the hypophysis. A possible relation between the origin of the hypophysis and the animal pole in vertebrates would no doubt be worth closer examination.

If now we revert to the bottom of the body we see that here too the median sections of figs. 3 and 4 differ more from each other than paramedian ones do. The anus has broken through, the ventral blastopore lip accordingly seems to have vanished at once. The blastopore itself has been overgrown by the medullary folds. In the posterior part of the medullary tube the latter have applied themselves so closely one to the other, that the lumen of the tube is not continued between them and only a virtual neurenteric canal can be spoken of. Later, judging from the diagrams of other investigators, a lumen seems to reappear and thus a real neurenteric canal. SIDEBOTHAM and ERLANGER give diagrams of median sections of eggs in which the anus is just on the point of breaking through. From the study of whole eggs it appears quite evident that the medullary folds unite

over the blastopore and that somewhat behind it at the bottom of the little depression indicated in fig. 1*c* (text) the anus breaks through.

I should like to emphasize a peculiarity which has only been pointed out by ERLANGER (1890), especially in relation to what we shall find in Urodelans. In the short time that passes between the stages of fig. 1 and fig. 3, the distance between blastopore and future anus diminishes a little; in other words, if we take the place of the future anus as a fixed point, the slit-like blastopore moves a little backwards towards it. So the ventral blastopore lip in median sections is not only getting thinner owing to the appearance of the groove between blastopore and anus, but also somewhat shorter. To this point we will revert later.

Let us pass now to the Urodelans. Characteristic in the early stages of development is here the little extension of the ventral ectoderm and the strong development of the dorsal parts, the foundation of the embryo accordingly encircling the egg over considerably more than 180°. This peculiarity the Urodelans have in common with the Dipnoans and Petromyzontes, of which the earliest stages of development, externally as well as in sections, exhibit a striking similarity to those of Urodelans.

According to SCOTT and OSBORNE (1879) the blastopore of *Triton* is overgrown by the medullary folds and becomes the neurenteric canal. SEDGWICK (1884) in his well-known article on the origin of metamerism writes concerning *Triton cristatus*: "in this animal the blastopore appears not to close, but to persist as the anus" and his pupil ALICE JOHNSON (1884) verified this by sections. A neurenteric canal, as described by SCOTT and OSBORNE, was never observed by her. SCHANZ (1887) in *Triton punctatus* comes to the conclusion that the blastopore is constricted in the middle, the anterior opening becoming the neurenteric canal, the posterior opening the anus. HOUSSAY and BATAILLON (1880) on the contrary find in the axolotl: "qu'il n'y pas de canal neurentérique, que le blastopore demeure toujours ouvert et qu'il devient l'anus définitif." Next comes the accurate investigation of MORGAN (1889, 1890) for the axolotl. He too finds that the hindmost part of the blastopore passes into the anus, the anterior part being overgrown by the medullary folds. Since my conclusions are closely akin to those of MORGAN, I will revert to them in detail presently.

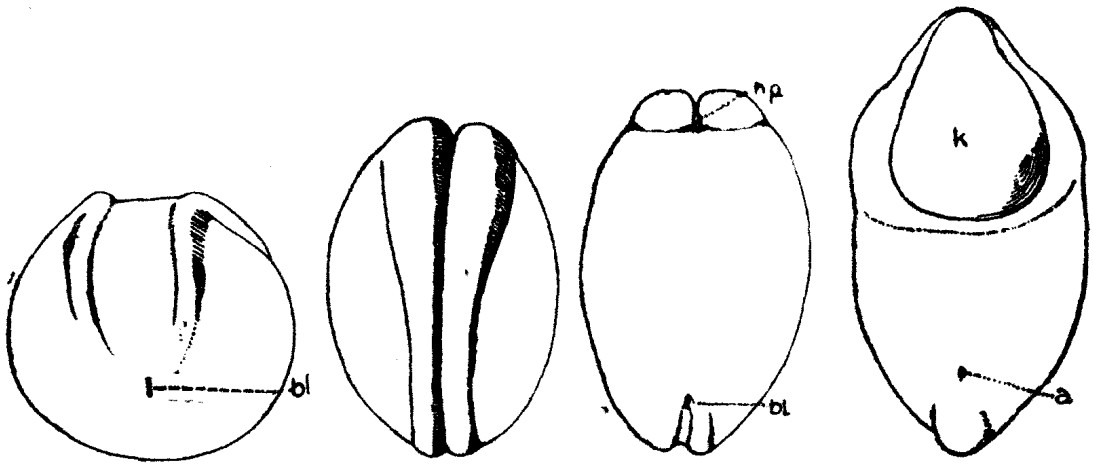
GOETTE (1890) similarly sees in some Anurans (*Triton*, *Siredon*) the rear end of the blastopore pass into the anus.

A few further observations of recent times as to the fate of the blastopore may be touched on, thus those of DE LANGE (1907, 1912)

and ISHIKAWA (1908) concerning *Megalobatrachus maximus*, of KUNITOMO (1911) concerning *Hynobius*, and of SMITH (1912) concerning *Cryptobranchus alleghaniensis*. All agree in this that the hind part of the slit-like blastopore remains open as the anus, the anterior part being overgrown by the medullary folds, except ISHIKAWA, who thinks this course of events to occur only exceptionally, the anus as a rule springing up as an independent formation, which is denied by DE LANGE (1912).

For *Petromyzon* and Dipnoans most investigators hold that either the whole blastopore or its hind end passes into the anus.

My own investigations concerning the axolotl all go to confirm the conclusions already reached by most of my predecessors, viz. that the rear part of the blastopore passes into the anus. If then I give a brief survey of my observations, it is with the express object of emphasizing some few circumstances which were not noticed by former investigators and seem to me of importance in giving a right interpretation.



2*a* 2*b* 2*c* 2*d*

Fig. 2. Three eggs of *Amblystoma tigrinum* during the closure of the medullary folds.

a. seen from behind, *b*. dorsally, *c*. (the same as *b*) and *d*. ventrally. *a*. anus, *bl*. blastopore, *h.p*. cerebral plate, *k*. head.

The stage represented in fig. 2*a* (text) and fig. 5 (plate) corresponds absolutely with that of fig. 1*a* and fig. 1 (plate) for *Rana esculenta*. Here too the medullary folds begin to appear and the blastopore has contracted to a short longitudinal slit. Already in fig. 5 it is evident, how much more the dorsal side is developed than the ventral side, the distance from the animal pole (which according to EYCLESHYMER, 1895, here too is to be found back just in front of the transverse

cerebral fold) to the slit-like blastopore measured ventrally being much less than 180°. In accordance with this the dorsal blastopore lip, as fig. 5 (plate) compared to fig. 1 (plate) shows, and the archenteron are developed very strongly, the ventral blastopore lip and the so-called anal diverticulum very little. Yet both the latter are still easily recognisable and on the outside of the ventral lip, a little distance behind the blastopore, a small depression of the ectoderm (*a*) may even be noted, where the future anus might be expected, if things happened in the same way as in Anurans. Immediately behind that shallow depression we find here again the same thickening of the ectoderm (*) as noted in *Rana* (cf. figs. 1, 2, 3, plate). So there is no fundamental difference, on the contrary agreement in every respect with what we found in *Rana*.

Now in *Rana* we stated that the blastopore, after becoming slit-like, continues to move backward a small distance, approaching the future anus, which manifests itself in longitudinal sections in that the little lip which represents the ventral blastopore border becomes a little shorter. This now we see happening also in somewhat further advanced stages of the axolotl-egg: on sections the ventral lip gets shorter and soon, being here already small, it disappears altogether. In the egg shown in fig. 2*b* and *c* (text) the medullary folds are on the point of coalescing, except at the fore and the rear end. The blastopore still appears as a slit. The longitudinal section (fig. 6) shows that the ventral blastopore lip has nearly disappeared: as a result of the backward movement the rear end of the slit-like blastopore has arrived at the spot where the anus must break through!

Especially interesting is next the egg shown in fig. 2*d*, where the medullary tube has just closed, except at the hindmost extremity, where the anterior part of the slit-like blastopore has just been overgrown by the medullary folds. Whilst in *Rana* the whole blastopore is in this way enclosed, in the axolotl the medullary folds leave an opening over the rear end of the blastopore, which is the anus (*a*).

Only one egg in this stage was found by me among my material. This was cut into longitudinal sections. MORGAN studied a similar egg in transverse sections. I reproduce here the outline of his excellent figures which wholly confirm my way of presenting things. Fig. 3*a* represents a section through the medullary tube just in front of the blastopore. Under it the anal diverticulum has been intersected. The medullary folds just meet. Figs. 3*b* and *c* show the blastopore in its anterior half, as is of course the case in many succeeding sections. The medullary folds meet over the blastopore, the latter

itself constituting the neurenteric canal. Figs. 3*d* and *e* are still further back, the medullary folds are less developed, and leave an

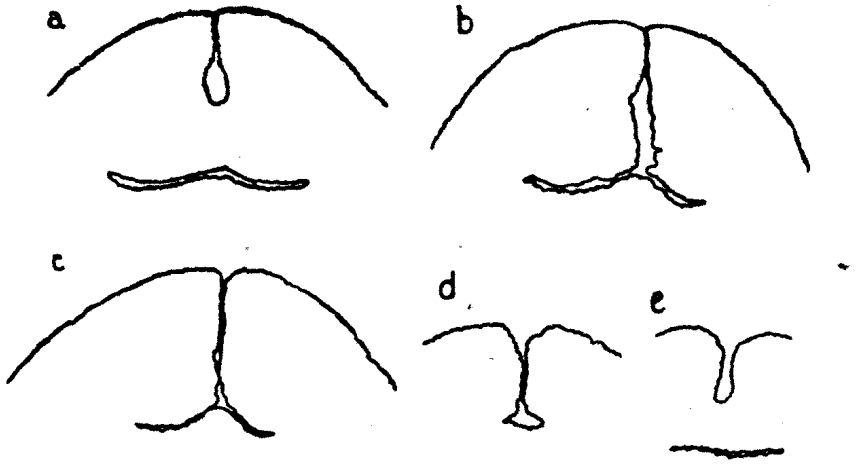


Fig. 3. Transverse sections through the blastopore of an egg of *Amblystoma punctatum*, where the medullary folds just close over it, after MORGAN (1890).

a in front of the blastopore, *b* and *c* through anterior half, *d* and *e* through rear end (anus).

opening, the anus. Comparing my description with that of former investigators it will be noted that, keeping strictly to the facts, I yet present them in a somewhat different way: I do not let the medullary folds finish halfway the length of the blastopore slit, but only in closing leave an opening over the rear end of the blastopore, the anus. Accordingly one can, retracing the medullary canal, not only pass through the neurenteric canal into the archenteron, but also through the anus to the outside, this being nowhere prevented by a coalescence of the two medullary folds across the middle of the blastopore, as many investigators are inclined to assume.

Now in a longitudinal section (fig. 7, plate) the blastopore (*bl.* = *p. neur.*) and the anus (*a*) are easily distinguishable from one another. The blastopore becomes the neurenteric canal or, perhaps better, the neurenteric pore (porus neurentericus), as I prefer to call it hence-forward. Entering the anus, one can pass through the neurenteric pore into the archenteron. The anterior part of the neurenteric pore however becomes — and is already in fig. 7 — virtual, the medullary folds applying themselves behind so closely to one another, that the lumen of the medullary canal is not continued any further between them, as MORGAN has already remarked. Hence the opinion of many investigators that the medullary folds do not reach to the blastopore and that there is no neurenteric canal. The hindmost

part remains open as the internal opening of the anus. The result is really that the hindwall of the hindmost part of the medullary tube is perforated by the anus, which in Anurans arises directly behind it, and this is caused by the circumstance that the neurenteric pore, the former blastopore, in Urodelans has travelled back so far, that its rear end has reached the place where in Anurans the anus breaks through. This is at the same time the solution of the apparent contradiction between Anurans and Urodelans in this respect.

The interpretation which until now has been pretty generally adopted is that of SCHANZ (1887), MORGAN (1890), ERLANGER (1890) and ROBINSON and ASSHETON (1891), who contend that the place where the anus in Anurans breaks through really represents the rear end of the original wide blastopore, which has narrowed down by concrescence of the lateral borders not only at the anterior end, as postulated by HIS's concrescence theory, but also at the posterior end. The longitudinal groove between the blastopore and the anal depression in fig. 1 seemed to be an indication of a raphe. Thus the anus in Amphibia would be closed only temporarily and would not arise as an independent formation. In this way ERLANGER assumed concrescence at the dorsal as well as at the ventral blastopore border, ROBINSON and ASSHETON only at the ventral border. The line of concrescence in both cases is compared to a primitive streak, which, as ROBINSON and ASSHETON in accordance with BALFOUR'S views on this point remark, can be expected only behind the blastopore: wrongly enough the adherents of the doctrine of concrescence call primitive streak the concrescence-seam assumed by them in front of the blastopore. To me it seems that one ought to add that a primitive streak is to be expected only in yolk-laden eggs with a germinal disc or in eggs that are to be derived from yolk-laden ones.

I will not absolutely deny that concrescence ever plays a part in vertebrate gastrulation, especially in yolk-laden eggs. But that its rôle is a much more subordinate one than the well-known doctrine of HIS assumes, seems to me beyond doubt. Even by students of the development of teleosteans, which seemed to afford the most acceptable confirmation of it, HIS' doctrine is rejected, as for example by SUMMER (1904). For amphibians the pricking experiments described in both my former communications have shown that there cannot be any question about the whole dorsal side of the embryonic rudiment arising by concrescence of the blastoporic lips.

It is quite true that in the amphibian egg a fine median line is often seen running from the blastopore forward, which strongly suggests a concrescence-raphe. Only, as ROBINSON and ASSHETON

remark, this line continues to the fore-end of the cerebral-plate, the animal pole, where the blastopore has never been. For concrescence at the hind border of the blastopore still less evidence can be adduced. The groove between the slit-like blastopore and the anal pit does not become gradually longer, as might be expected in this case, the anal pit removing from the ventral border of the blastopore, but on the contrary it only gradually becomes more distinct and at the same time shorter, the blastopore approaching the anal pit. Evidently it is not to be considered as a concrescence-seam, perhaps it may be compared to the groove joining the two impressions made by two fingers pressed near one another into a soft cushion.

Concerning the relation between blastopore and anus in vertebrates three suppositions may be made:

1. there is a primary relation
2. there is no relation
3. there is a secondary relation.

The first supposition mentioned above is now the most widely accepted, even where in Anurans 2. seems to prevail yet it is assumed that this is to be traced back to 1. since what is found in Urodelans must be valid for Anurans. Thus MAURER (1906) in HERTWIG's Handbuch tries to trace back all the results for chordates to 1, though the evidence adduced is not always equally convincing. Already in *Amphioxus* no relation between the anus and the blastopore has as yet been discovered.

The possibility of 1. is in no way excluded by my theory, which derives chordates in opposition to GROBBEN from Protostomia, as long as the possibility of a relation between the anus and the blastopore in the latter group exists, as might be expected from SEDGWICK's well-known theory (1884), which derives the mouth and the anus of Bilateria from the anterior and the posterior extremity of a slit-like actinian mouth of which the borders coalesce in the middle. The concrescence-seam joining mouth and anus, which according to this theory should run over the ventral side of annelids, ought to be able to be traced in vertebrates too then in the groove between anus and blastopore, that is in the so-called "Afterrinne", the "primitive streak" of ROBINSON and ASSHETON (see above) — not in the hypothetical concrescence-raphe in front of the blastopore, the "primitive streak" of the theory of concrescence, as LAMEERE (1891) and HUBRECHT (1905) assume in their application of SEDGWICK's theory on Vertebrates. Thus the presence of a primary relation between the anus and the blastopore in Vertebrates would in no way oblige us to derive them with GROBBEN (1908) from the Deuterostomia, as

long as the possibility of a similar relation in Protostomia exists.

However the theory of SEDGWICK finds in the development of Protostomia just as little support as I hope to show is the case in Tritostomia (Vertebrates). A process of so fundamental phylogenetical significance as assumed by SEDGWICK's theory might be expected to have left more distinct traces in the ontogenetic development than are demonstrated by the most careful research of recent investigators. Again and again we see the anus arise as a new formation, by perforation. In Annelids, where primarily we might expect to find evidence of a common origin of mouth and anus, a direct transformation of the rear end of the blastopore into the anus has never been demonstrated. Even in the primitive *Polygordius*, where as a matter of fact the blastopore is divided into two halves by a median constriction, the posterior opening nevertheless closes and the anus arises by perforation behind the two teloblasts, which lay at the rear end of the blastopore. To me the most probable conception of the origin of the anus seems to be this, that in a larva of the protrochula-type (MÜLLER's larva of Polyclad, pilidium of Nemerteans) the entodermal pouch, which is already turned in a backward direction, has applied itself to the ventral body-wall and is broken through by perforation, in the same way as occurs in Deuterostomia, and that thus the trochophore-larve has originated.

So I think the idea of a primary relation between the anus and the blastopore for Proto- as well as for Tritostomia should be abandoned. The anus in Proto- as well as in Tritostomia arises by perforation, independent of the blastopore.

Of the three above mentioned possibilities regarding the relation of the anus and the blastopore the second then seems to me, both for Proto- and Tritostomia, the right one. The third possibility however we find exemplified in Urodelans and apparently also in Dipnoans and Petromyzontes, which in their early development so closely agree with the former. Let us now invoke the aid of my theory for further interpretation.

According to this theory (DELSMAN, 1913) the vertebrate is to be derived from the Annelid by the stomodaeum growing out backwards so strongly that it extends, as the medullary tube, over the whole length of the soma, and, as we shall see, even further still (formation of the tail!). For the entrance of the stomodaeum into the entodermal part of the gut I propose the name porus cardiacus, this being the former blastopore. Already during the development of Annelids we see this cardiac pore by the lengthening of the stomodaeum travelling backwards into segments situated ever further to

the rear. In Vertebrates this backward movement goes so far that finally the cardiac pore, as neurenteric pore, comes to lie absolutely at the rear extremity of the soma, just in front of the anus. This backward movement is evidently produced by a growing zone which has entered into activity at the inner end of the stomodaeum, round the porus cardiacus and which causes the stomodaeum to extend more and more to the rear. This growing zone I should like to call the periporal growing zone. The longitudinal growth of the soma of Annelids on the contrary is produced by a perianal growing zone. Both these growing zones now exert their influence as I hope to show, in the earliest development of Vertebrates, and things are still further complicated by the fact that the activity of both, ontogenetically anticipated, interferes with the gastrulation. Further researches (pricking experiments, counting of the mitoses) will have to test the correctness of the conclusions reached by the application of the above principles. They are as follows.

The ectoderm, which afterwards has to invest the whole soma, — dorsally too — in a stage as in figs. 1*a* and 2*a* (text) lies principally at the ventral and lateral sides, and only afterwards, by the closing of the medullary tube, extends over the dorsal side as well. The production of this somatic ectoderm now must evidently issue from the perianal growing zone: in the neighbourhood of the future anus, a short distance behind the ventral blastopore lip mitoses may to be expected to be most frequent. When however the blastopore is closed (figs. 1*a*, 2*a*), the rearward extension of this ventral ectoderm comes to an end. If now the perianal growing zone continues to be active, a ring-shaped thickening of the ectoderm round the anal pit will result. This being observed, it appears to me that it is here we have to look for the explanation of the ectodermal thickening, which in the figs. 1, 2 and 3 (plate) we see developing in an increasing degree just under the anal pit (*), and which, as paramedian sections teach us, reach forward, also at the left and the right of it. In the axolotl, where the extension of the ventral ectoderm is so slight, this ectodermal thickening too, though present, is yet of very little importance (5*). The activity of the perianal growing-zone soon afterwards seems to die down and the ectodermal thickening in the ensuing stages gradually disappears again. Somatogenesis has closed simultaneously with gastrulation. If it continued also after the end of the gastrulation, the anus would eventually lie somewhere between the yolk-cell-mass and the extremity of the tail. In fishes this case is pretty generally found. As an example may be mentioned the sturgeon (fig. 5, text), but many teleosteans might

also be mentioned here, in whose larvae the place of the anus varies much and is of importance in determining the species.

Let us now turn to the periporal growing zone, which causes the growing out of the stomodaeum, resp. the medullary tube, resp. the medullary plate, together with the backward movement of the cardiac pore (Annelids), resp. the blastopore, resp. the neurenteric pore (Chordates). Organs or processes that are of much importance for the structure of the adult animal, in ontogeny often appear precociously. In Lamellibranchia e.g. the shell-gland invaginates already during gastrulation, though the latter process phylogenetically is no doubt much older. Thus also the activity of the periporal growing zone, and the backward movement of the cardiac pore associated with it begins very precociously, viz. already during gastrulation, when the future cardiac pore is still the blastopore. The interference of the contraction of the blastoporic rim with the backward movement of the blastopore causes the caudadly excentric closure of the blastopore, which is typical for chordates. The activity of the periporal growing zone, as long as the tubeformation has not set in, results not in the production of a stomodaeal viz. medullary tube, as is the case afterwards during the urogenesis, but provisorily in the formation of the medullary plate. The growing out of the stomodaeum to the medullary tube is thus in its first, somatogenetic part to be imagined projected on a plane, the dorsal plane of the embryo. When the blastopore has narrowed to a slit and the tube-formation sets in in the form of the medullary folds, the caudad wandering of this slit-like blastopore, as stated above, continues nevertheless, truly only over a little distance — indeed in view of the short duration of this stage nothing else could be expected — and so probably with undiminished speed. Further than the anus however this backward movement cannot go, phylogenetically: the stomodaeum of the Annelid, growing out backwards, at last reaches the anus. If now the movement stops a little in front of the anus, there will be no relation whatever between neurenteric pore (blastopore) and anus (fig. 4*a*, text), as we stated in the frog. If the movement continues yet a little further (fig. 4*b*), a secondary relation between neurenteric pore (blastopore) and anus results. [1]) The anus now opens to the exterior through the hindmost extremity of the medullary tube, from the medullary canal one can pass through the anus to the exterior as well as through

[1]) In a longitudinal section as in fig. 4 the constellation at first sight might appear in fig. 4*b* radically different from that in 4*a*. If however one imagines things in space, the agreement between them will be evident.

the neurenteric pore into the archenteron, and from the archenteron through the neurenteric pore and the anus to the exterior. In ontogeny this will result in the medullary folds not closing over

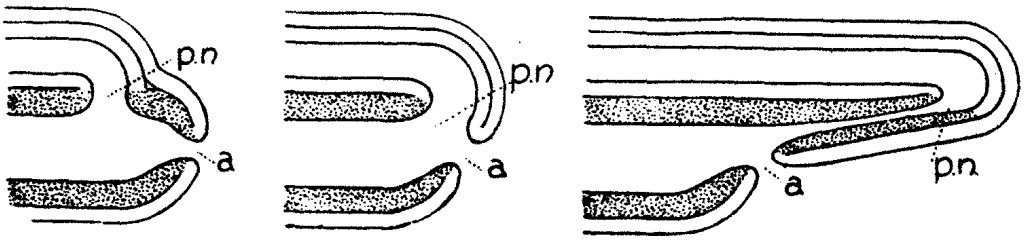


Fig. 4. Diagram of the relation between anus blastopore, and of the tail-forming
a. at the moment of the closure of the neural folds in Anurans,
b. „ „ „ „ „ „ Urodelans.
c. formation of the tail.
a. anus, *p.n.* neurenteric pore; the entoderm is dotted.

the rear end of the slit-like blastopore, but leaving an opening, the anus. Perhaps they will develop slightly at both sides of the rear part of the blastopore under the influence of the formation of the anus at this point, or they may not. If we imagine things very much enlarged and we look through the anus into the interior, we shall see the slit-like blastopore (neurenteric pore) in the distance, though its rear end, under the influence of the formation of the anus, will probably be widened a little. With this conception the facts stated by us in Urodelans so perfectly agree, that it seems hardly possible to doubt the correctness of this interpretation. We see in the axolotl the blastopore move backwards to the place where in Anurans the anus breaks through. We see over that place, that is over the rear end of the blastopore, the medullary folds not, as in Anurans, unite, but leave an opening. We have seen that we can pass from the medullary tube as well through the anus to the exterior as through the neurenteric pore into the archenteron. What makes things later less clear is that the medullary folds caudally so closely apply themselves one to another, that there is no lumen, no medullary canal (fig. 7, plate) — just as in the frog (fig. 4) — and that accordingly as in the frog the neurenteric pore would become virtual, if the rear part did not remain open as the anus. So only the anterior part of the slit becomes virtual, and hence the statement of several authors concerning Urodelans, Dipnoans and Petromyzontes, that the blastopore passes into the anus and a neurenteric canal is wanting, is to be explained. The apparent contrast between Anurans and Urodelans c.s. has thus found a solution. It would

cause us no surprise if in an Anuran a state of things were observed such as in Urodelans seems to be the rule, or the reverse, the difference between them not being fundamental, but only gradual. It would not be impossible that in one species at one time the first,

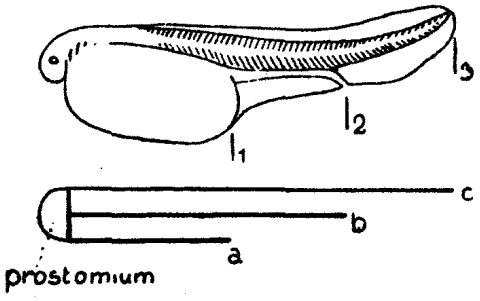


Fig. 5. Larva of the sturgeon after KUPFFER from HERTWIG's Handbuch. 1. limit of the gastrulation, 2. limit of the somatogenesis, 3. limit of the urogenosis. Beneath: Diagram of the interference of the gastrulation (*a*) with the action of the perianal (*b*) and the periporal (*c*) growing zones.

at another the second case might be realized (comp. DE LANGE and ISHIKAWA on *Megalobatrachus !*).

I have spoken above of the caudad movement of the neurenteric pore = blastopore stopping in front of the anus. In reality however there is no question of stopping. Although the anus seems to afford an insurmountable obstacle for the further backward growth of the stomodaeum = medullary tube, the activity of the periporal growing zone has not yet come to an end when the perianal growing zone has stopped working. There being no room however within the soma for further extension, a protuberance of the body wall in front of the anus results, into which the stomodaeum = medullary tube grows out: the tail-knob (fig. 4*c*, text). Thus we see the tail of vertebrates originating by the periporal growing zone continuing its activity after the perianal has stopped. In this way the position of the anus in vertebrates is not terminal, as in Annelids, but at the root of the tail, which overgrows it and which owes its origin simply to the presence of the anus. Phylogenetically we have to imagine that the longitudinal growth of the stomodaeum (medullary tube) surpasses that of the soma, so that the cardiac (neurenteric) pore overtakes the anus and passes it. Just as in Annelids the position of the anus in Vertebrates is terminal in regard to the soma proper, the tail is an outgrowth of the dorsal side of the latter in a backward direction. According to this conception the ventral side of the tail belongs to the dorsal side of the soma. In accordance with this the dorsal unpaired skinfold of the fish- and amphibia-larvae is continued

over the tip and the underside of the tail as far as the anus. The mesoderm originating at the blastopore-border, and evidently being a product of the periporal growing zone, this too takes a considerable part in the tail-formation.

DE LANGE (1912) rightly emphasizes the difference between somatogenesis and urogenesis, though I cannot concur with him in his conceptions on gastrulation and mesoderm formation, as expressed by the words cephalo- and somatogenesis. From the foregoing results it appears that somatogenesis, just as the somatogenesis in Annelids, is produced by the perianal growing zone, which gives rise to the future somatic (not the neural, that is that of the medullary plate) ectoderm of the trunk, which, as long as the medullary plate is open, lies mainly ventrally and at the sides of the egg. Simultaneously, however, with the gastrulation the periporal growing zone is at work, which produces the backward movement of the blastopore and the backward extension of the originally crescentic rudiment of the medullary plate = the rudiment of the medullary tube. And both growing processes are combined with a third one, going on simultaneously: the gastrulation, manifesting itself at the surface in the contraction of the blastopore border.

The urogenesis however sets in after two of these three processes have finished, viz. the gastrulation and the activity of the perianal or somatic growing zone [1]), and accordingly is exclusively the result of the periporal growing zone, which causes an elongation of the medullary tube, disproportional to the length of the soma. The difference between somatogenesis and urogenesis herein finds an explanation. The activity of the periporal growing zone, manifesting itself in the backward movement of the blastopore resp. neurenteric pore, at first interferes with the gastrulation, which causes the backward directed, excentrical closure of the blastopore, then manifests itself in the backward movement of the slit-like blastopore, stated by us above, which stage lasts only a short time), and later in the urogenesis as longitudinal growth of the medullary tube.

There is then no question of stopping the backward movement of the blastopore viz. neurenteric pore in front of the anus (comp. fig. 4), and the difference between Anuran and Urodelan consequently does not lie in the fact that in the former the neurenteric pore stops a little before the anus is reached, in the latter only after

[1]) While in Anurans both processes stop nearly at the same time, in fishes, as stated above, we fairly frequently find that somatogenesis continues after gastrulation has been completed, so that the anus eventually lies somewhere about halfway between the yolk-cell-mass and the tip of the tail.

this has occurred, but in that in Anurans the tube-formation, i. e. the closure of the medullary folds, occurs a little before the anus is reached, in Urodelans, Dipnoans and Cyclostomes only after this has occurred. And this, only graduated difference evidently again depends on the circumstance that in Urodelans the activity of the periporal growing zone is stronger than in Anurans, the activity of the perianal on the contrary weaker than in the latter. This manifests itself, as stated above, in the medullary plate in Urodelans being developed very strongly, the ventral side very little in comparison with the Anurans. The same holds for Dipnoans and Cyclostomes. Now, as we have seen, the perianal growing zone acts mainly ventrally and on both sides of the (future) anus, for the simple reason, that, as long as the medullary plate is open, the future trunk ectoderm also lies only ventrally and on both sides of the egg. But in front of the (future) anus too, there seems to be some feeble activity, directed against the ventral blastopore lip, which accordingly is developed more strongly where the perianal growing zone is most active (Anurans, fig. 1, plate), less so, where the perianal growing zone is less active (Urodelans etc., fig. 5).

Now the action of this dorsal part of the perianal growing zone is opposed by the periporal growing zone, which pushes the blastopore backwards. And it is no doubt due to the relative strength of the two growing zones that in Urodelans the blastopore is pushed back to the anus before the tube-formation [1]), in Anurans on the contrary it does not reach it till after the tube-formation. I hope that the brevity with which I am obliged to express myself will not militate too strongly against the clarity of this exposition. A more explicit review will doubtless be published later.

While I feel that the application of my theory has thus thrown light on a number of obscure problems, the facts and results recorded above afford yet further support to my theory of no inconsiderable value.

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[1]) The beginning of which is determined again by the end of gastrulation, just as in Protostomia the stomodaeal tube is formed directly after gastrulation. In Selachians, where the accomplishment of the gastrulation is so much retarded by the great yolk-richness, urogenesis actually sets in before the tube formation, the neurenteric canal thus originally being an open groove (sulcus neurentericus).

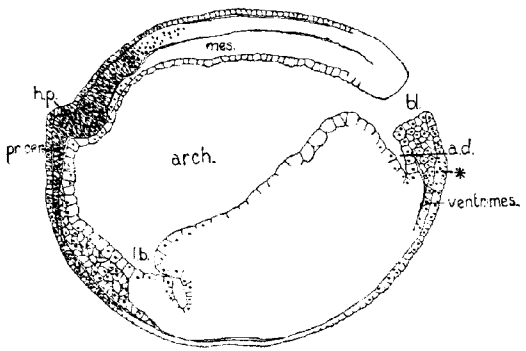

Fig. 1. *Rana esculenta.*
Median section through the egg of text fig. 1*a*.

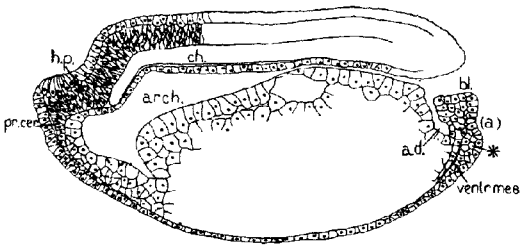

Fig. 2. *Rana esculenta.*
Median section through an egg as in text fig. 1*b*.

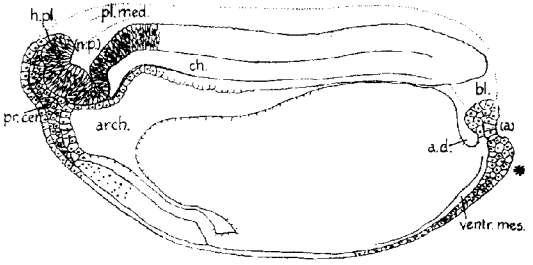

Fig. 3. *Rana esculenta.*
Median section through the egg of text fig. 1*c*.

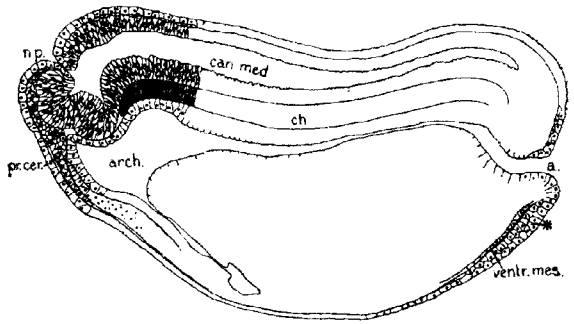

Fig. 4. *Rana esculenta.*
Median section through an egg with closed medullary folds.

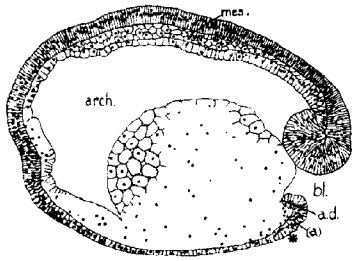

Fig 5. *Amblystoma tigrinum.*
Median section through the egg of text fig. 2*a*.

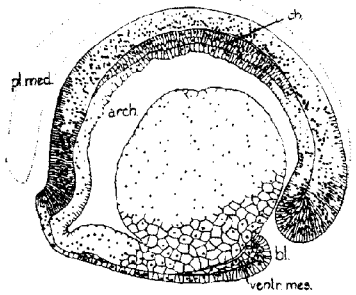

Fig. 6. *Amblystoma tigrinum.*
Median section through the egg of text fig. 2*b* and *c*.

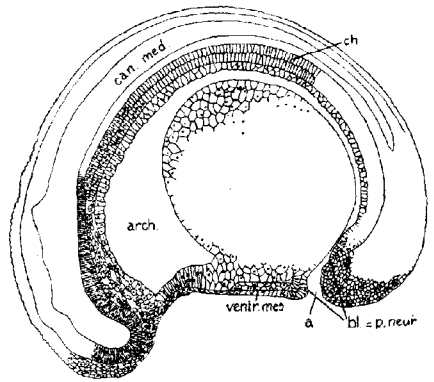

Fig. 7. *Amblystoma tigrinum.*
Median section through the egg of text fig. 2*d*.

ABBREVIATIONS.

a. anus, *(a)* anal pit, *a.d.* anal diverticulum of gut, *arch.* archenteron, *bl.* blastopore, *can. med.* medullary canal, *h.p.* cerebral plate, *l.b.* liver cove, *mes.* mesoderm, *n.p.* neuropore, *(n.p.)* place of the future neuropore, *p. neur.* neurenteric pore, *pl. med.* medullary fold, *pr. cer.* praecerebral thickening of the ectoderm, *ventr. mes.* ventral mesoderm.

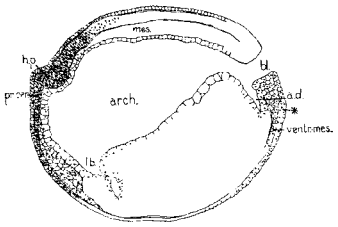

Fig. 1. *Rana esculenta.*
Median section through the egg of text fig. 1*a*.

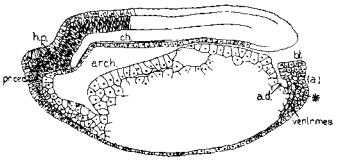

Fig. 2. *Rana esculenta.*
Median section through an egg as in text fig. 1*b*.

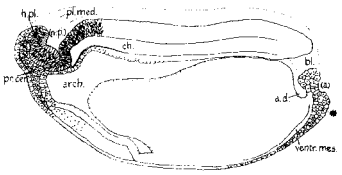

Fig. 3. *Rana esculenta.*
Median section through the egg of text fig. 1*c*.

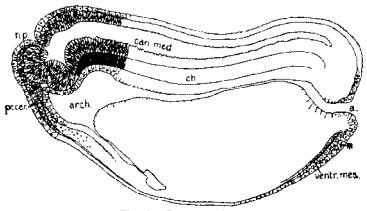

Fig. 4. *Rana esculenta.*
Median section through an egg with closed medullary folds.

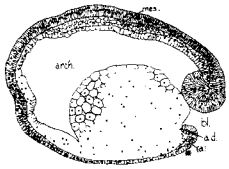

Fig. 5. *Amblystoma tigrinum.*
Median section through the egg of text fig. 2*a*.

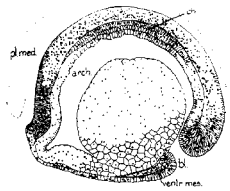

Fig. 6. *Amblystoma tigrinum.*
Median section through the egg of text fig. 2*b* and *c*.

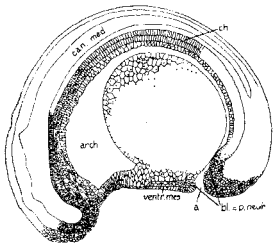

Fig. 7. *Amblystoma tigrinum.*
Median section through the egg of text fig. 2*d*.

ABBREVIATIONS.

a. anus, *(a)* anal pit, *a.d.* anal diverticulum of gut, *arch.* archenteron, *bl.* blastopore, *can. med.* medullary canal, *h.p.* cerebral plate, *l.b.* liver cove, *mes.* mesoderm, *n.p.* neuropore, *(n.p.)* place of the future neuropore, *p. neur.* neurenteric pore, *pl. med.* medullary fold, *pr. cer.* praecerebral thickening of the ectoderm, *ventr. mes.* ventral mesoderm.

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Microbiology. — *"The Enzyme Theory of Heredity."* By Prof. M. W. BEIJERINCK.

(Communicated in the meeting of March 31, 1917).

"Nothing is perfect at birth."

Combining the results of the enzymological researches of recent years with those obtained by the experiments on heredity, an insight is obtained into the nature of the thereby concerned substances which deserves attention.

The most acceptable theory of heredity is the conception that the living part of the protoplasm of the cell is built up from a great number of factors or bearers, different from one another, which determine the hereditary characters of the organism; at the cell division these bearers double or multiply, in consequence of which the characters, latent or unfolded, are transferred to the daughter-cells. They are called: *differirende Zellelemente* (MENDEL), *gemmules* (DARWIN), biophores, pangens, gens, character units, heredity units, MENDELIAN factors, or factors.[1])

[1]) G. J. MENDEL, Versuche über Pflanzen-Hybriden. Verhandl. d. naturforschenden Vereines in Brünn, Bd. 4, Abh. Pag 42, 8 Februar u. 8 März 1865. — C. DARWIN, Provisional hypothesis of Pangenesis. Domestication, 1st Ed. T. 2, Pag. 357, 1868. 2nd Ed. T. 2, 349, 1875. — HUGO DE VRIES, Intracellulare Pangenesis, Jena 1889, and the American edition, Intracellular Pangenesis, Chicago 1910. — V. HAECKER,

How they appear in the cell, how they behave to nucleus, chromidia, chromosomes, and other cell-organs, and many questions more, form the subject of the heredity researches of to-day, which however start from the supposition that the said theory is in the main right. Nor does the observation that heredity units or factors may occur in latent condition and must then be activated by special kinds of food, by alcalies or acids, or other stimuli, touch the fact of their existence.

By the side of this view stands another, only apparently quite different, namely that the living part of the protoplasm is built up of a large number of various enzymes. A nearer consideration of these two views shows that "heredity units" and "enzymes" means the same. [1])

Hence the fundamental conception here to be proposed, that every hereditary character of an organism corresponds to one or more enzymes, which exert a reaction on specific substrates.

Long ago already I came to the conviction that the ontogenetic evolution of the higher plants and animals can be best explained by admitting that it is caused hy a series of enzymes, for the greater part endoenzymes, which, becoming active in a fixed succession, determine the morphological and physiological properties gradually manifest in the development. These enzymes in the formation of plant-galls are likewise concerned, and in a study on the galls of the saw-fly *Nematus capreae* on the leaves of *Salix amygdalina*, I gave them the name of "growth enzymes".[2]) It is still my

Allgemeine Vererbungslehre. Pag. 265, 1911. — M. W. BEIJERINCK, Mutation bei Mikroben. Folia microbiologica. Bd. 1, Pag 24, 1912. — W. JOHANNSEN, Elemente der exakten Erblichkeitslehre. 2nd Ed. Pag. 143, 1913. etc.

[1]) Younger physiologists (as E. ABDERHALDEN, Physiologische Chemie, 3te Aufl. Theil 2 Pag 997, 1915) wrongly use anew the old and equivocal word "ferment", instead of the practical and clear word "enzyme". The history of the introduction of the word enzyme is as follows. In "Verhandlungen des Naturhistor. und Medicin. Vereins zu Heidelberg", Sitzung am 4 Februar 1876, Bd. 1, N. F., the account of a lecture of KÜHNE begins thus: "Herr W. KÜHNE berichtet über das Verhalten verschiedener organisirter und sogenannter ungeformter Fermente. Um Missverständnissen vorzubeugen und lästige Umschreibungen zu vermeiden, schlägt Vortragender vor die ungeformten oder nichtorganisirten Fermente, deren Wirkung ohne Anwesenheit von Organismen und ausserhalb derselben erfolgen kann als Enzyme zu bezeichnen". This proposal is still acceptable. That KÜHNE only thought of exoenzymes was in accordance with the times. The term "endoenzyme" was introduced in 1900 by M. HAHN (Zeitschr. f. Biologie Bd. 40 Pag. 172, 1900). But the conception existed already long before. Enzyme comes from the Greek "en" in, and "zymè" leaven, and is related to "zeo" I boil.

[2]) Das Cecidium von *Nematus capreae* auf *Salix amygdalina*. Botan. Zeitung, 1888, Pag 1.

opinion that this view is in the main correct, but while I formerly thought that the growth enzymes partly derived from the gall-insect, I now recognize that they belong to the plant only and that the animal does not introduce enzymes into it.

Research material.

In the free living unicellular organisms morphological differentiation, joined with cell division, is quite or almost quite absent, which much simplifies the ontogenetic development. That in this case the properties must be represented just in the same way by specific factors, that is by heredity units or MENDELIAN factors, as in the cell protoplasm of the higher organisms, is beyond question. Although it would be erroneous to admit that the number of characters, and so of the heredity units or factors of the unicellular organisms must be small, we certainly have to deal here with a simpler case than in the multicellular. Hence it seemed probable that heredity experiments with the former would give some chance better to understand the nature of the heredity units in general.

But not all properties are equally well adapted to such a research. To show that some character of a cell corresponds to one or more units or MENDELIAN factors, that character must be able to change by mutability in such a way that the mutants prove to be hereditary constant races, distinctly different from the original form, for the conception of heredity units must also for the unicellulars start from the possibility of race formation.

The character to be studied must further be observable with ease and accuracy and it must be possible to cultivate the concerned organism in a simple way, so that in few days thousands of individuals can be examined and that no doubt is left as to their distinction from foreign infections. These requirements are very well answered by some pigment- and by the luminous bacteria as I repeatedly stated before. [1]) Especially the phosphorescence of the latter I have minutely examined, no character being better qualified to show the process of mutability and to enable us more quickly and precisely to judge of the vital energy of the culture material. Errors in the nutrition are in this way prevented, which so easily occur in microbiological experiments, in particular by too strong concentration and too alcaline reaction. Besides, the function of phosphorescence is not only found in certain luminous bacteria, but it is widely spread throughout the natural system and a remarkable

[1]) These Proceedings, 21 November 1900 and 9 February 1910.

similarity exists everywhere, [1]) notwithstanding the enormous differences in the respective phosphorescent organs.

Another consideration which induced me to study with particular care the production of light by living microbes was the following.

I saw the great difficulty of explaining by the enzyme theory a function so obviously the attribute of the living protoplasm. Yet I had the conviction that if it were possible to account for this exceptional character by that theory, the same would be the case for any other character, physiological or morphological. Presently we shall see that the facts are in accordance with the expectation.

Not all luminous bacteria are equally well qualified for this investigation. *Photobacter splendidum*, common in the North Sea at the end of summer, [2]) and *Ph. phosphoreum* COHN, always present on sea-fish, whose properties are very different and in many respects complementary, are recommendable. *Ph. splendidum* produces trypsin, urease, diastase and invertase, and assimilates mannite with light production. *Ph. phosphoreum* has none of these enzymes and does not attack mannite. [3])

The chief result of this study is that the function of phosphorescence may be ascribed as well to living protoplasm as to one or more enzymes.

I chose this function to elucidate the theory with regard to a physiological character; the production of the cell-wall shall be treated to test it from a morphological point of view, and also in the latter case it can be shown that the protoplasm as well as one or more enzymes may be regarded with the same right as the cause of its formation.

The subsequent considerations must be given in a short and somewhat aphoristic but I think not unclear form.

Enzymes considered as the bearers of phosphorescence. Irritability.

Already in 1898 RAFAËL DUBOIS endeavoured to demonstrate that phosphorescence should be considered as caused by an enzyme-action. [4])

[1]) Perhaps with exception of the higher Fungi, where the luminosity seems to be in correlation with a state of collabescence.

[2]) Die Leuchtbakterien der Nordsee im August und September. Folia microbiologica, Bd. 4, Pag. 1, 1915.

[3]) Aliment photogène et aliment plastique des bactéries lumineuses. Archives Néerlandaises T. 24, P. 369, 1891 (Feeding of *Ph. phosphoreum* COHN.)

[4]) R. DUBOIS, Leçons de Physiologie générale, Pag 450 and 524. Paris 1898, Drawings of the phosphorescing organ of *Pholas* by ULRIC DAHLGREN: The production of light by animals. Franklin Institute, February 1916, Pag 38.

He experimented particularly with the luminous sipho-slime of *Pholas dactylus* and calls the enzyme, he thinks he has found "luciferase" and the unknown matter it acts upon "luciferine". The latter substance corresponds to what is called an "enzyme-substrate", but which might better be denominated "enzymoteel", [1]) the word "enzyme-substrate" being evidently equivocal. To prepare a luciferase solution, free from luciferine, he leaves the luminous mucus till it becomes dark. He makes a solution of luciferine, free from luciferase, by slightly heating the mucus whereby the luciferase is destroyed. By mixing the two dark solutions light is evolved, from which he concludes that the luciferase acts as a catalysator similarly as other enzymes. The luminous slime consists of the cell-content of peculiar glands of the epiderm and flows from the cell through a fine canal; it seems not impossible that it contains protoplasm.

Various other sea animals as some Annelides, Cephalopodes and Coelenterates likewise secrete a luminous slime, which spreading in the sea-water illumines the surroundings of the animal.

E. Newton Harvey has examined the phosphorescence of insects and comes to the same results as Dubois, but he calls the related substances "photogenine" and "photopheleine". [2]) It is also easy to show that the phosphorescent cells of our glow-worms, after mechanical destruction do not loose their luminosity. But these facts cannot be considered as proving incontestably the accuracy of the enzyme theory, it not being impossible that in all these cases not yet destroyed protoplasm is still active.

A better evidence for the view that the bearer of the phosphorescence consists of one or more endoenzymes is to be derived from the luminous bacteria. Here the production of light is inseparably bound to the bacterial body and secretion of a luminous slime never occurs. [3]) If thus there is question here of an enzyme as cause of the phosphorescence it can only be an endoenzyme, and that this supposition is in accordance with the facts may be shown by exposing the luminous bacteria to the influence of ultra-violet light. It is namely possible by means of the light of a quartzlamp, to bring them into the necrobiotic state, wherein they have lost their power of reproduction, but preserved their phosphorescence. [4]) If the time of the radiation is well chosen, the necrobiotic condition may last for

[1]) Of "telos", aim.

[2]) Science N. S. T. 44, Pag. 208, 440, 652, 1916.

[3]) The slimy matter produced by some kinds of luminous bacteria is non-phosphorescent cell-wall substance.

[4]) For the particulars of this experiment see Folia microbiologica, Bd. 4, Pag. 10, 1915.

hours and it may be shown that the luminosity of *Ph. phosphoreum* during this period is greatly intensified by glucose. Hence the very same argument which leads us to consider the alcohol function of the necrobiotic yeast-cell as an enzyme action, caused by one or more enzymes, called zymase, holds likewise with regard to the connection between phosphorescence and its factor or factors the luciferase. The still unknown "luciferine" which, as said, can result in the case of *Ph. phosphoreum* from glucose, is the natural analogon of the "glucose-phosphoric-acid ester", i. e. the substrate or enzymoteel of the zymase.

The necrobiotic yeast-cells have lost their semi-permeability, as shown by the ease wherewith they are dyed by methylene-blue, their power of reproduction and certainly the motility of their protoplasm, whence they are considered as dead by several investigators. The same is probably the case with the necrobiotic luminous bacteria; but change of permeability could not be stated, since also in the condition of normal life they have a great affinity for pigments. I venture to think that the loss of the above properties when based, as is supposed, on the becoming inactive or on the destruction of the more sensitive heredity units or enzymes, can quite well go side by side with the continued activity of another part of the protoplasm, so that then it cannot be said that the cell is "dead" in the same sense as when all its functions are destroyed. The importance of this view is obvious if we bear in mind that the theory of the units of heredity consists in the very supposition that from their combination energies and activities may arise strange to the units separately. The demonstration of the properties to be ascribed to special factors and of those due to the co-operation of two or more factors is the chief subject of the heredity researches of to-day and the difficulties met with are well known. That the enzyme theory will here be useful is obvious.

About irritability I need not be long here, as for the lower immotile microbes this conception is only then based on observable facts if we think it coinciding with the power of metabolism and of reproduction.

In this connection I call to mind that the peculiarity of actions caused by stimuli, consists in their showing an optimum for certain intensities of these stimuli, which is also the chief character of enzyme action. So the influence of temperature and of different concentrations of poisons on the process of cell division and on that of amylolysis by diastase is analogous, and this is of course one of the best evidences for the correctness of the enzyme theory.

Phosphorescence considered as bound to protoplasm. Combination of the two views.

That the function of phosphorescence of the luminous bacteria is bound to the living protoplasm is supported by the following facts.

Anaesthetics, such as chloroform and aether, stop the light production almost completely, while after vaporisation of these substances it sets in anew, only slightly diminished. A short heating of temperatures near 40 ° to 45 ° C. of *Ph. splendidum* and of 30° to 35 ° C. of *Ph. phosphoreum*, with subsequent cooling, has the same effect. By the action of acids and alcalies the phosphorescence disappears and returns after neutralisation. A strong salt concentration darkens, after dilution the light is completely restored. Diminution of luminosity in these cases is caused by the dying of part of the germs. The phosphorescence of very active broth cultures, kept at rest for some time, undergoes a sudden and remarkable enhancement in its intensity by mechanical stimuli, such as shaking. The thus produced light reminds of the behaviour of higher luminous animals, possessing a nervous system, which by contact, or other mechanical stimuli, suddenly react with light production.

All these facts induced me already long ago [1]) to call the bearer of the phosphorescence "photoplasm" and its elementary units "photophores". Also for the Flagellate *Noctiluca miliaris* DE QUATREFAGES has demonstrated that the light issues from the protoplasmic threads that run from the nucleus to the cell-wall which, when seen under the microscope, presents a large number of minute light centres, corresponding to the ends of the threads, closely grouped near the flagellum, but farther on the surface at greater relative distances. [2]) The sudden radiance of *Noctiluca* by shaking the sea-water wherein it is suspended is well-known. When "fatigued" the cells become entirely luminous and DE QUATREFAGES called the so produced light "pathological light", but he does not say whether it originates from the cell-wall or the cavity.

A principal argument for the view that the photoplasm of the luminous bacteria possesses the properties of the protoplasm lies in the relation between food and luminosity. For if peptones are present in sufficient quantity the phosphorescence is considerably

[1]) De Ingenieur, 15e Jaarg. Pag. 53, 27 Januari 1900.

[2]) Mémoire sur la phosphorescence de quelques invertebrés marins. Ann. d. sc. nat. Zoologie, 3me Sér. T. 14. Pag. 326, 1850. Vide also R. DUBOIS. Leçons de Physiologie générale, Pag. 498, Paris 1898.

increased by several carbon compounds either free from or containing nitrogen, as glucose, levulose, glycerin, malates, asparagin, and many others that do not act as stimuli, but as in the normal respiratory process are oxidised to carbonic acid and water. Peptones alone can also be broken off by the photoplasm, likewise under production of ammonium carbonate, carbonic acid, and water. Phosphorescence thus proves to be bound to the photoplasm in the same way as the respiratory process in general is bound to the protoplasm, so that it may be said that the photoplasm of the luminous bacteria forms part of their respiration protoplasm.

As now the chief criterion of enzyme action consists in the fact that enzymes act only on a specific substrate, in the case of phosphorescence this criterion at first sight seems to fail, and the process more reminds of a catabolism bound to the protoplasm as a whole and which is rather unanalysable.

But considering what should be understood by a catabolism we find in many cases that it is based on the co-operation of various factors of the nature of enzymes. The respiratory process itself supports this view, for recent enzymological investigations have shown that the respiration protoplasm is composed of different factors, in general called oxidases, with the specific distinction of peroxidases, oxigenases and oxidones.

These units possessing the character of enzymes, and only oxidising special substances, or but few nearly related ones, we must accept that in this case, too, a preformation of enzyme-substrates or enzymoteels takes place on which they exert their function. The composition of the photoplasm of several of such factors or oxidases is thereby rendered probable, and the ease wherewith by means of mutation experiments with the luminous microbes hereditary constant races arise of very unequal phosphorescence (but as it seems always of the same colour), is evidently connected with these facts.

That the factors of the photoplasm of the various species of luminous bacteria are not always the same follows from the before described experiments about the relation between nutrition and phosphorescence. [1])

So, in the photoplasm of *Bacterium phosphoreum* an oxidase must exist associated with a substrate resulting from peptones only, and another oxidase whose substrate is an unknown matter, produced by peptone and sugars and perhaps by peptone and glycerin too. In the photoplasm of *Bacterium splendidum* another factor occurs

[1]) For *Ph. phosphoreum*, Aliment photogène, Archives Néerl. 1851. For *Ph splendidum*, Folia microb. 1915.

adapted to a still unknown substrate deriving from peptone and mannite. Really these still hypothetical substrates are but different "luciferines" in the sense of DUBOIS. It should be borne in mind here that DUBOIS knows nothing at all of his luciferine of the pholades, whereas regarding the photobacteria at least the substances are known from which they result.

By multiplying the nutrition experiments it will be possible to come to a complete "factor analysis" of the photoplasm. For other bacteria the difficulties will be greater, but for *B. prodigiosum*, where race formation easily occurs, a corresponding factor analysis of the "chromoplasm" will be possible, since, according to former demonstrations, it must quite like the photoplasm be regarded as a complex of heredity units possessing the character of oxidases.

So we arrive also here at a result analogous to that already obtained for the alcohol function, which may be ascribed as well to "alcohol protoplasm" as to some enzymes, the zymase of BÜCHNER.

In consequence of the foregoing it is clear that conceptions such as "chromoplasm", "photoplasm", "alcoholprotoplasm" etc., are not in contradiction with the wider view that considers the protoplasm in general as composed of enzymes, as they themselves are built up of these.

There being nothing to object to the further generalisation of the view here forwarded, it is allowed to consider the heredity units as enzymes and these as heredity units, clearly two different names for the molecules or micells of the living part of the protoplasm.[1])

Cell-wallfactors are enzymes.

For the higher plants and animals factor analysis is based on crossing experiments between forms of which we wish to state by what and by how many heredity units they differ. For the bacteria and the other microbes, where for want of sexuality crossing is impossible, factor analysis is then possible when the factors of special properties can be recognised by race formation through mutation, which I already put forward before. The recognition of the heredity units as enzymes may likewise lead to factor analysis by applying the property of enzymes only to act on special substances.

We saw how this principle may be applied to a physiological

[1]) This theory I first advanced, though with some doubt, in: Mutation bei Mikroben, Folia microbiologica, Bd. 1, Pag. 2, 1912, but now the difficulties are overcome.

function; that it can likewise lead to the factor analysis of a morphological character I will now endeavour to show with regard to the cell-wall.

The formation of the cell-wall is commonly considered as a function of the parietal protoplasm and must necessarily repose on the action of factors or heredity units. For some microbes this process is clearly caused by one or more enzymes and this is distinctly the case when the wall substance consists of levulan. This matter results from cane-sugar (and slower and less profusely from raffinose), but from no other substances. It forms the cell-wall of many species of sporulating bacteria, such as *B. megatherium* and also the common hay bacterium *B. mesentericus*, but only if fed with cane-sugar. The levulan arises in two ways: it either remains in contact and entirely united with the bacterial body as a slimy cell-wall, in which case on cane-sugar-agar plates strongly swelling colonies develop, or the levulan is deposited outside the bacterial body at some distance from the colony. If the latter takes place the remarkable reaction occurs which I have called the "emulsion reaction".[1]) Its explanation was given by the discovery of a specific exoenzyme, viscosaccharase, which acts on cane-sugar and converts it into levulan slime, which is incapable of diffusion but attracts water, so that droplets are formed causing a strong swelling of the agar. This enzyme, acting synthetically and evidently polymerising the cane-sugar, might as well be called saccharo-levulanase and is obviously one factor of the factor-complex that governs the cell-wall formation. That it is not the only one follows from the fact that some levulan bacteria, for instance the hay bacterium itself, when fed with other sugars, produce another not slimy wall-substance, probably cellulose, which likewise derives from cane-sugar beside levulan, but only in slight quantity. If the production of cellulose is brought about by one or more factors is not yet known. As to the viscosaccharase, however, there is not the least doubt but that it consists of one single enzyme or factor.

Hence it may be concluded that it is quite well possible to become acquainted with the separate factors of a process at first sight so complicated as the formation of the cell-wall, and it may safely be predicted that further experiments will show whether the cellulose production also depends on one single or on more than one enzyme.

On the other hand, at the factor analysis by crossing experiments with higher plants and animals, without the guidance of the enzyme conception, we are continually in doubt whether a factor, thought to

[1]) These proceedings 9 February and 2 Mei 1910. Folia microbiologica Bd. 1 Pag. 382, 1912.

be elementary, will not, on continued examination, prove to be composed of other still unknown factors.

As to dextran I have stated elsewhere [1]) that it is a wall substance comparable to levulan, likewise only resulting from cane-sugar, but produced by some lactic acid ferments, belonging to the physiological genus *Lactococcus*. Dextran, however, never originates independently from the cell, as may occur with levulan, but exclusively at the surface of the outer layer of the protoplasm and in direct contact with it. But the knowledge of the relation between levulan and its producing enzyme, viscosaccharase, indicates clearly that dextran, whose properties are so analogous to those of levulan, must have a similar origin. It is therefore most probable that dextran also arises under the influence of one single factor or specific enzyme, which might be called saccharo-dextranase, but which, being an endoenzyme, cannot leave the cell.

The formation of the slime wall by *B. prodigiosum viscosum* [2]) must be brought about by at least two factors, differing from levulanase and dextranase since the slime produced by this bacterium, belongs to the celluloses or cellulan-slimes. That beside the slime factor, which might be called cellulanase and which produces cellulan from carbohydrates, still quite another factor operates here is proved by the following observations. By feeding this bacterium with glucose, cane-sugar, maltose or lactose, wall slime is readily yielded. In several other species, for instance *Aerobacter viscosus* and *Bacillus polymyxa* we find the same. But *B. prodigiosum* can besides produce slime from albuminous substances such as gelatin and peptone, which *B. polymyxa* and *A. viscosus* cannot. As now it is quite unacceptable that one and the same factor could be able to produce cellulose slime as well from proteids as from carbohydrates, *B. prodigiosum* must possess a specific factor able to split off from the albuminous matter an enzyme-substrate, converted into cellulose slime by the wall-forming factor. But this proteid-splitting factor does not exist in *B. polymyxa* and *A. viscosum*. *B. prodigiosum viscosum* is thus a mutant, distinct by at least two factors from *B. prodigiosum* itself, which produces no slime at all, neither from carbohydrates nor from proteids. It must thus be possible to detect another still unknown mutant lacking the factor to produce from proteids a substrate that

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[1]) Die durch Bakterien aus Rohrzucker erzeugten Wandstoffe. Folia microbiologica. Bd. 1. Page 392, 1912.

[2]) *B. prodigiosum viscosum* is no natural form but a mutant or race, easily obtained from *B. prodigiosum*. Folia microbiologica, Bd. 1, Pag. 35, 1912.

can be converted into slime, that is a mutant capable to produce slime from carbohydrates only and not from proteids.

A great number of other examples might be added demonstrating that the speculations about the heredity units or factors have relation to enzymes.

Limitation of the enzyme conception.

In my opinion the preceding may lead to a better enzyme conception than the existing. I will try to elucidate this by a few instances taken from the cecidia or galls and the substances called ferments in immunology.

Elsewhere I pointed out that the change of the plant at gall-formation is not hereditary. From the galls of *Nematus viminalis*, kept on moist sand, quite normal roots of the gall-bearer *Salix purpurea*, and from those of the gall-fly *Neuroterus lenticularis* on oak-leaves, quite normal oak roots may arise.[1])

From the axil-buds of the willow-rose, caused by *Cecidomya rosaria* on *Salix alba*, I have cultivated quite normal willow trees; likewise I grew normal plants of *Poa nemoralis* from the bud in the remarkable gall of *Cecidomya poae*, whose strange metamorphic roots readily develop into normal roots, when the whole gall is planted in earth.[2]) By strongly pruning the twigs of *Rosa canina* whereon Bedeguars developed, caused by the gall-fly *Rhodites rosae*, the wonderful appendices of this gall changed into long-petiolated, simple, green leaflets, whose anatomic structure and external appearance were quite identic with those of the leaf on which the gall originates.

These instances, to which I could easily add others, show that in the formation of galls two groups of substances are concerned: the protoplasm of the plant, consisting of the unchanged heredity units, and substances deriving from the egg of the gall-animal, or from the larva of *Cecidomyia*, which evidently have the character of enzymesubstrates. It is however clear that the heredity units concerned in the morphologically higher galls, multiply more intensely, in any case become more numerous under the influence of the gall-animal than under normal circumstances. Hence we come to the conclusion that either the enzyme-substrates may serve as food for the heredity units or enzymes to which they belong and may give rise to their multiplication, or that the gall-animal, beside the enzyme

[1]) Only very few *Lenticularis*galls possess this disposition, which is probably connected with the spot where the gall grows on the leaf.

[2]) Botanische Zeitung 1886.

substrate, also supplies "enzymosites",[1]) that is to say a special "enzyme food". The latter supposition will probably be the right one, for the real enzymes are in their origin in no way dependent on their substrates, as we learn from almost every experiment with microbes.[2])

The enzymosites apparently correspond to ABDERHALDEN's "Bausteine" of the specific living proteids, that is of the protoplasm. That, in case these enzymosites differ, different heredity units or protoplasm micells will develop from the mixture of units from which the latter is built up, is to be expected. For if we remember in how remarkable a way in elective culture experiments with microbes, the thereby obtained floras depend on nutrition, we may safely conclude that the same will be the case in the subtle world of protoplasm molecules.

That from the gall-animal no enzymes pass into the plant, is in accordance with the fact that foreign exoenzymes commonly do not enter living cells. The diastase, which in the distilleries occurs in great quantity in the food of yeast, which consists for a great part of malt, does not penetrate into the yeast-cell. Experiments purposely carried out with other exoenzymes and various kinds of other microbes have invariably given the same result. The possibility of endoenzymes passing by diffusion from one living cell into another is of course wholly excluded.[3])

On the other hand, in the range of immunology, facts are known which prove that living cells sometimes take up enzymes from their surroundings.

In those cases namely when acquired immunity is hereditary the thereby concerned substances must needs belong to the heredity units, hence to the enzymes.

They give evidence that DARWIN's view, according to which the "gemmules" of his pangenesis hypothesis freely move within the

[1]) Sitos, food.

[2]) Many diastatic bacteria for example produce diastase without the presence of amylum in their food. This must be ascertained by a special experiment, amylum being the only known reactive on diastase; the literature proves that this has sometimes been forgotten by the investigators.

[3]) It is not impossible that endoenzymes such as zymase are to some degree capable of ordinary diffusion (which is quite another thing than penetrating into living protoplasm). Gelatin can slightly penetrate into agar, likewise starch and even the carbon of Indian ink. Gold seems able to penetrate into lead. In the protoplasm of luminous bacteria no disposition for diffusion is to be observed. However the pathological light of *Noctiluca miliaris*, described by DE QUATREFAGES, seems to repose on the entering of the photoplasm or luciferase into the cell-sap in which the luciferine must then be dissolved.

organism, is true in certain cases, at least for the higher animals. Non-hereditary immunity might be caused by freely moving enzymes, unable to enter the cells.

VAN CALCAR's opinion that the anti-bodies of the serologists are ferments, that is enzymes, is thus undoubtedly right. He says: [1]) Whichever immunity reaction is examined, it is constantly found that the whole course of these reactions depends on the action of two substances, one of which having in all respects the character of a ferment, the other that of an enzyme-substrate to be decomposed by that ferment. The ferment-like substances are called "anti-bodies", the various substrates they act upon, "antigens".

In my opinion there is however no sufficient ground also to call the antigens and the complement "enzymes", as is done by several investigators.

If these substances are considered as enzymes only because of their action after injection into the blood of higher animals, it will be necessary, in order to be consistent, likewise to bring to the enzymes toxins and even some common coagulable proteids, which would make this word lose its real significance. Whereas in the descriptive sciences the necessity is felt to designate by special names even but slightly differing objects, it would be an error to attribute to the words enzyme and ferment a continually varying and wider meaning no more in accordance with the original conception. On the other hand it is clear that further knowledge about the enzymes or factors may necessitate the creation of new names to mark the vast differences between them, as now we are already compelled to use the words exo- and endoenzymes.

There is still another group of bodies worth being considered from the new point of view, namely the viri in general and in particular those of plant diseases, such as the mosaic disease of the tobacco. They clearly belong to the enzymes or factors, although commonly not hereditarily transported. But the further discussion of this point must be deferred to later.

The only place in literature, hitherto come to my knowledge, where an hypothesis is indicated somewhat corresponding to my view, is to be found in BATESON. He says [2]): "Ueber die physikalische Natur der Erbeinheiten können wir noch nichts aussagen; die Folgeerscheinungen ihrer Gegenwart sind aber in so vielen Fällen

[1]) R. P. VAN CALCAR, Voordrachten over algemeene biologie, Pag 182 and 188, Leiden 1915.

[2]) W. BATESON, MENDEL's Vererbungstheorien, Pag. 269, 1914 (Translation of the English edition of 1909).

mit den durch Fermente hervorgerufenen Wirkungen vergleichbar, dass wir mit einiger Bestimmtheit annehmen, dass die Fähigkeiten einiger Erbeinheiten im wesentlichen in der Bildung bestimmter Substanzen bestehen, welche in der Art von Fermenten wirken".

Although the observations on which this statement is based are in accordance with the enzyme theory, it is clear that BATESON's view is quite different from mine.

Physics. — "*Contributions to the kinetic theory of solids.* I. *The thermal pressure of isotropic solids.* By Prof. L. S. ORNSTEIN and Dr. F. ZERNIKE. (Communicated by Prof. H. A. LORENTZ).

(Communicated in the meeting of February 26, 1916).

P. DEBIJE [1]) has in his Wolfskehl-lecture developed a theory of the equation of state of solid matter which has been elaborated by Dr. M. I. M. VAN EVERDINGEN [2]). DEBIJE assumes as a physical principle that the forces between the molecules in solid matter are not quasi-elastic, but depend also on higher powers of the deformations. He points out that only this principle enables us to understand the expansion of solid matter which gains energy under constant pressure. This assumption enables him to give a deduction of the GRÜNEISEN-theorem about the connection between the coefficient of expansion and the specific heat.

DEBIJE calculates the free energy of a solid body with the help of a canonical ensemble, using the method of normal vibrations, and introducing from the beginning the hypothesis of energy-quanta.

We shall indicate in this paper another way to find the equation of state with the aid of the physical principles of DEBIJE. The quantum-theory will be applied to our final result if we wish to use it for low temperatures. DEBIJE has taught us to replace in the calculations the space-lattice of molecules by a continuum, BORN [3]) has shown this artifice to be right. Therefore, in considering the isotropic body, we shall use a continuum as a limiting case. For explanation we shall treat the case of a row of points and for this case we shall perform the transition to a continuous bar. Our method consists in determining the thermal pressure, i.e. the pressure that

[1]) Vorträge über die kinetische Theorie der Materie, Leipzig 1914. "Zustandsgleichung und Quantenhypothese u. s. w.".

[2]) De toestandsvergelijkingen van het isotrope vaste lichaam. Diss. Utrecht 1914.

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