Zoology. — "*The Gastrulation of Rana esculenta and of Rana fusca*". By Dr. H. C. DELSMAN. (Communicated by Prof. J. BOEKE).

(Communicated at the meeting of November 25, 1916).

In my note of May 27, 1916, I was able to mention that similar pricking experiments to those described at that time for *Rana fusca*, were performed by me on the eggs of *Rana esculenta* also, an object, which in investigations on the earliest development of the frog egg we encounter much less frequently than the eggs of *Rana fusca*, which are to be obtained so much more easily. In some respects for pricking experiments like the present ones the eggs of *Rana esculenta* appeared to me to offer advantages over the eggs of the other species, but on the other hand certain disadvantages are to be noticed, which in the latter are at least less conspicuous. Among the advantages it may be noted that in pricking, which in this case too was performed with the point of a hedgehog's quill, one did not need to operate with nearly so much caution, to prevent the production of a voluminous extraovate, which has a disturbing influence on the further development. The egg content namely is in *Rana esculenta* far less liquid than in *Rana fusca*; indeed it is much more solid and tough, so that every prick not too clumsily made produces a little wound which in the last mentioned species can only be attained with the greatest caution and after several failures. Accordingly it was not difficult to apply to one egg several marks, e.g. one at the animal pole (a), and one or more at the crossing points of the third, equatorial cleavage furrow with the other, meridional ones, which, as in Fig. 1, we can indicate here again as b (dorsal), c (ventral) and d (the two lateral ones). Also the lighter colour of the egg has a great advantage, as it renders the surface images more distinct. On the reverse, the marks, so much more easily applied, also come off more easily, the wounds healing too soon. In not one of the eggs marked by me — all from one spawning — did it prove possible to rear them until the appearance of the medullary plate, without all the marks coming off beforehand. Next year therefore I hope to try and renew the marks in time during the development and thus to attain what this year was not reached. Yet the results reached until now seem to me sufficiently interesting to communicate them, and in completeness they are only a little behind those for *Rana fusca*.

To my surprise, I found that the external features of the gastrulation process and the behaviour of the dorsal and the ventral blastoporic rim in *Rana fusca* and *esculenta* differ from one another pretty considerably, so that a comparison of the two cases becomes especially interesting. Let us first consider the facts and afterwards look for an explanation.

The eight-celled stage of *Rana esculenta* agrees in the main with that of *Rana fusca*, as a comparison of Fig. 1 with the figure for

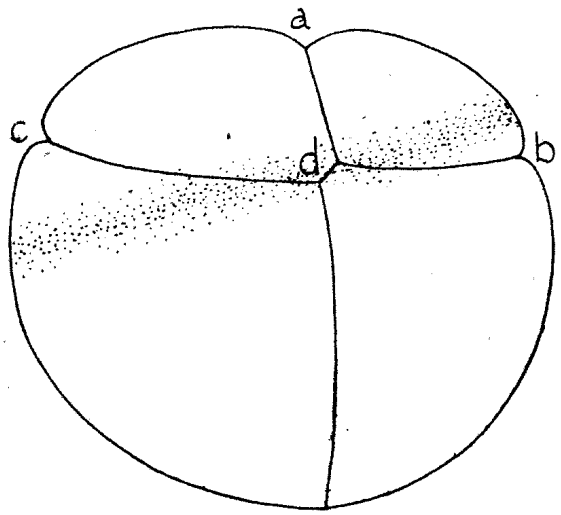


Fig. 1. Egg of *Rana esculenta*, 8 cells, from the side. The zone of demarcation between the darker and lighter area is defined by spots.

R. fusca of my former communication shows at once. The proportion of the size of the blastomeres in both cases is nearly the same. Nevertheless the distribution of the pigment points to a difference in the internal structure: the line of demarcation of darker and lighter hemisphere, in both figures indicated by a dotted band, not only lies much nearer to the animal pole in *Rana esculenta*, but it has also a much more horizontal situation. Now this boundary-line does not coincide in the least with the boundary of the future ecto- and entoderm, but it is apparently of importance in so far as in both frog species, as we will see, the border of the blastopore shortly after its appearance nearly runs parallel to it. We will revert to this in due course.

Turning to the figures 2—6, all drawn with a drawing-prism from the same egg, which was marked at the animal pole and at the point *b*, we see in fig. 3, how the first indication of the blastopore appears as a short, transverse slit, a little beneath the equator of

the egg, at a place therefore which wholly corresponds to what we found in *Rana fusca*. It may be observed, that the boundary between the darker and lighter hemispheres has wandered downward

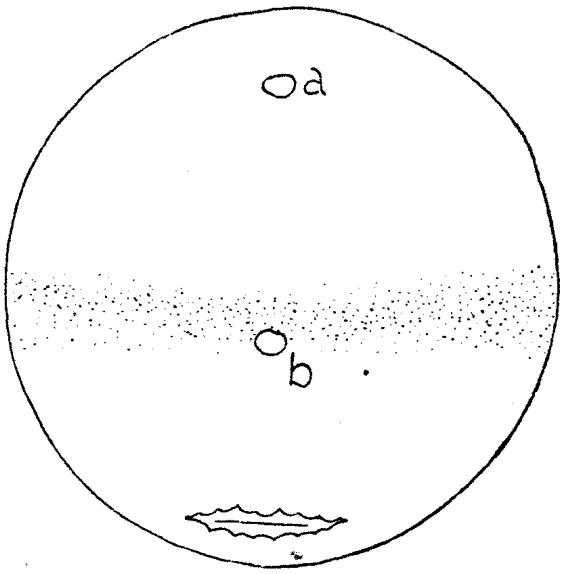


Fig. 2. Egg of *Rana esculenta*, marked on May 6 in the eight-celled stage at the points *a* and *b*. First appearance of the blastopore (*bl.*) From the dorsal side, May 7, 2.30 p.m.

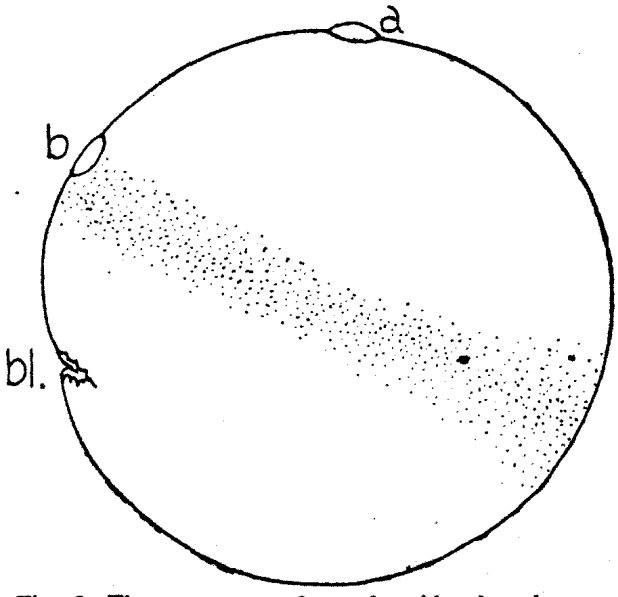


Fig. 3. The same egg, from the side, dotted zone as in fig. 1.

a considerable distance parallel to itself, away from the animal pole, as appears from a comparison with fig. 1, which as a matter of

fact does not represent the same egg, a circumstance, which having regard to the great uniformity of the eggs in this respect, does not imply any difficulty. From this however one must in no way conclude, that the cells containing the pigment perform such a wandering downward themselves. The behaviour of the marks at *b*, *c*, and *d* in the different eggs tells us otherwise: their distance from the animal pole just as in *Rana fusca* increases only very slightly. Besides, former investigators have already pointed to the fact that during development the formation of new pigment goes on, especially at places of great cell-activity.

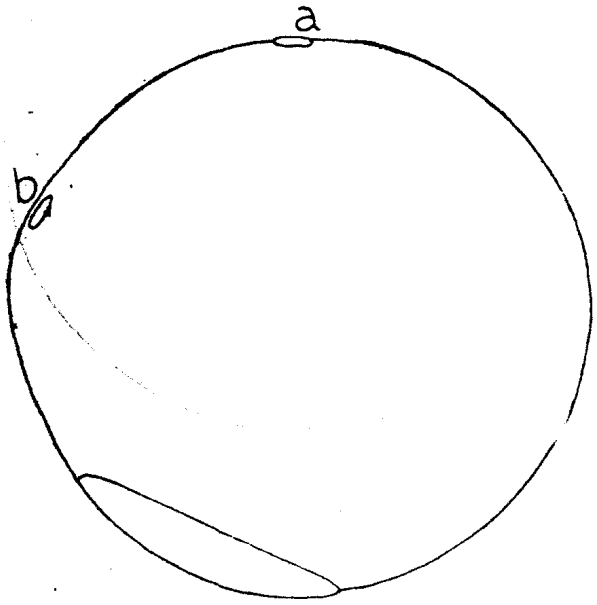


Fig. 4. The same egg, from the side, May 8, 6.30 a.m.

'Unfortunately the next figure of the egg, was drawn much later (fig. 4), when the blastopore had already been contracting for some time. Other eggs however teach us, that, when the border of the blastopore has just closed at the rear side to a ring, this ring is much wider than in *Rana fusca*. While in the latter species the longitudinal diameter of the blastopore is about 60°, in the former it amounts to no less than 120°, about twice as much. So the exact situation of the anterior and posterior border in this stage in regard to the points *a* and *b* could not be made out and in the fig. 8, which is a composition of the other figures, I have accordingly indicated the border of the blastopore with a dotted line, as it will probably run. I have indicated the anterior border as lying a little in front of the place where the first trace of the invagination became visible, which accordingly would point to a primary backward

movement of the dorsal rim. Such a primary wandering backward may be noticed in fact in such eggs, which to this end have been provided with marks at a shorter or longer distance in front of and behind the blastopore border. Evidently it is the result of the forming of an invagination border at this place, where cells, lying originally

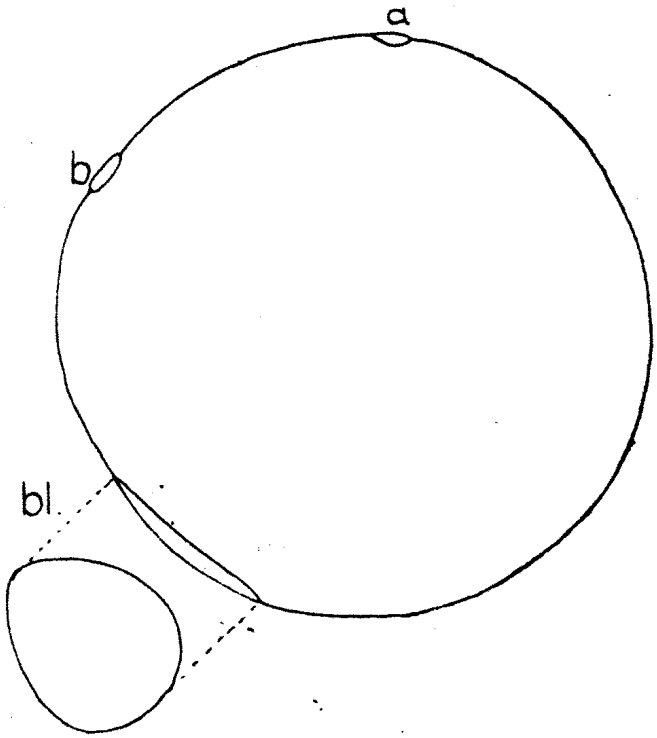


Fig. 5. The same egg, from the side, 8 May, 1.50 p m.

in front of the primary transverse rim of fig. 2 and 3, are carried inward. This however does not mean, that epiblast cells wander into the interior to participate in the construction of the archenteron roof. To me the view of MAC BRIDE[1]) seems to be preferable, according to which the first transverse slit does not appear at the border of the ecto- and entoderm area, but within the entoderm area, a little under the demarcation line. Thus the slit does not represent so much the first beginning of the blastoporic rim, as that of the archenteric invagination beneath it, and the cells in front of it, which disappear under the just forming blastoporic rim, are to be counted to the entoderm. Hence it is no wonder, that in a somewhat further advanced stage we find the blastoporic border a little in front of the rim of fig. 2 and 3, which is rendered the

[1]) E. W. MAC BRIDE, 1909, The Formation of the Layers in Amphioxus etc. Quart. Journ. Vol. 54.

more intelligible when we see that during gastrulation the whole entoderm area performs a wandering forward. The assumption that the situation of the blastoporic rim at this stage as dotted in fig. 8, is right, is also favoured by the fact, that the line 1—1 thus runs parallel to the boundary between the darker and the lighter area of the egg, as indicated in fig. 3. This becomes evident, if we hold the figures 3 and 8 up to the light one upon the other, in such a way that the points *a* coincide. Just as in *Rana fusca* we also find in *Rana esculenta* that this line of demarcation in the different stages always runs parallel to the blastoporic border, approaching it gradually, until at last it reaches it.

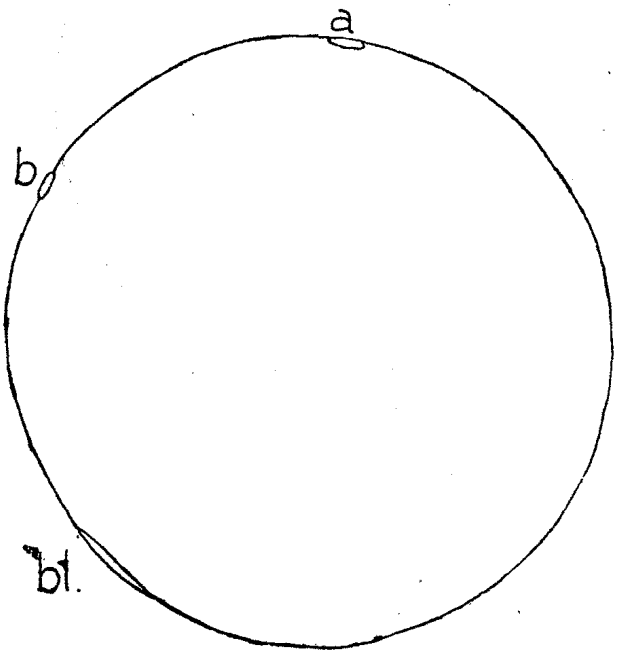


Fig. 6. The same egg, from the side, May 8, 9.15 p.m.

Now in holding up to the light one upon the other the figs. 4, 5 and 6, in such a way that the marks *a* each time coincide, it may be stated further that the distance of the points *a* and *b*, just as in *Rana fusca*, increases only very slightly, and moreover the way in which the blastopore contracts may be studied in detail. This same method was adopted again in composing the summary figure 8. While in *Rana fusca* the ventral blastopore border, which there appears approximately diametrically opposite the animal pole, does not make any forward movement, in *Rana temporaria* it not only does so, but the ventral border even progresses still more rapidly than the dorsal one!

Although some time after drawing fig. 6, I found the marks

detaching themselves, yet it may be stated already that the closing of the blastopore here does not occur, as in *Rana fusca*, diametrically opposite the animal pole, but more to the dorsal side. So the appearance of the medullary plate in this egg was not observed anymore before the detachment of the marks, just as little as in the other eggs. Now, however, it does not rarely occur, that in eggs, where

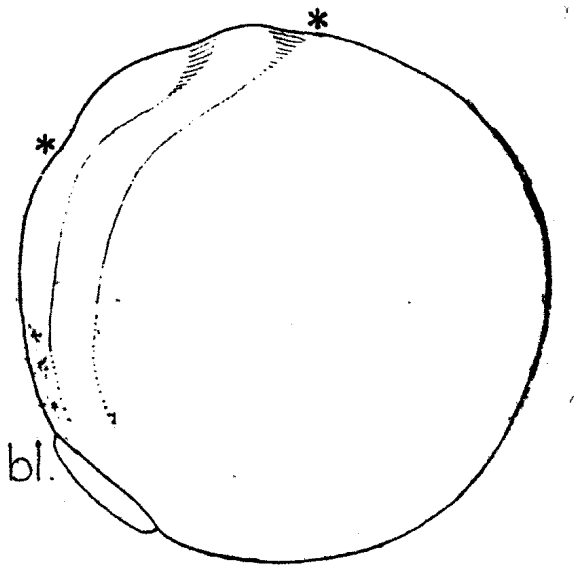


Fig. 7. Another egg, with foundation of medullary plate. * *a* and *b* as transferred from fig. 6.

the blastopore has not yet quite closed, the first rudiment of the medullary plate becomes visible already. Such an egg is represented e.g. in fig. 7, where we see that the foundation of the embryo does not, as in *Rana fusca*, encircle nearly 180° of the egg, but is somewhat shorter. If now we hold up to the light this drawing together with that of fig. 7 and we transfer to fig. 7 the position of the marks *a* and *b* from fig. 6, it appears that they find themselves at exactly the same place as we stated in *Rana fusca*, i.e. respectively just in front of the transverse head-fold and at the transition of cerebral and medullary plate (fig. 7*). The objection might be raised that the possibility is not excluded, that during or before the appearance of the medullary plate there might still occur cell wanderings, which would raise doubts as to the correctness of the above conclusion. As we have seen, however, that in *Rana fusca* there is no question of anything of the kind, we may safely assume the same in this case. So this result for *Rana esculenta* again confirms the conclusions drawn from the theory which has engendered the present investigations.

For the sake of completeness in fig. 8 the position of the marks *c* and *d*, from another series, has also been indicated. As already

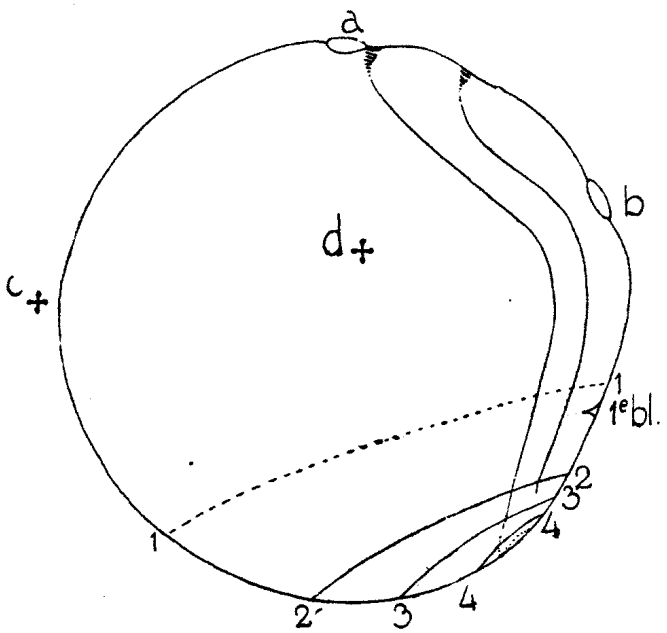


Fig. 8. Combination of figs. 3—7 and others, from which the situation of *c* and *d* and the extension of the blastopore in phase 1 are borrowed. 1e *bl* = first indication of the blastopore (fig. 2 and 3).

observed before, the mutual distance of the marks *a*, *b*, *c*, and *d*, just as in *Rana fusca*, changes but little during cleavage and gastrulation. Yet it could be stated that the distance *a*—*c* increases somewhat.

Very conflicting views have up to the present day been held as to the gastrulation of vertebrates. To many an adherent of one of these views the result of the recorded pricking experiments will be somewhat surprising. Who, after studying fig. 8, could maintain any longer that the foundation of the dorsal parts of the embryo originally lies as a ring round the border of the blastopore and is formed from it by concrescence? By far the greater part of the embryo is formed in front of the place, where the dorsal blastoporic rim first appears, and the contraction of the blastopore proceeds nearly concentrically. An explanation of the facts mentioned seems to me to be afforded by the views concerning the gastrulation, which follow from my theory on the derivation of vertebrates from annelids.

To this end let us first consider once more the movement of the ventral blastoporic border. Have we to deal here with a similar overgrowth of the yolk as at the dorsal lip? In that case we ought to

find in sections under the ventral lip, just as under the dorsal one, an archenteric slit or cavity. Not, that this archenteric cavity under the dorsal lip owes its existence solely to the overgrowth of the yolk by the dorsal lip. In this case the cavity would not reach further forward than the place where this dorsal lip appeared first. As a matter of fact, however, it soon reaches considerably further forward, so that doubtless also an active enlargement of the archenteric cavity by dehiscence of the entoderm cells occurs, though it seems to me less suitable to assume a sharp demarcation of the parts of the archenteron formed in these two manners, and to distinguish these as archenteron and metenteron, as ASSHETON [1]) did. Only by the overgrowth of the ventral blastoporic lip however, should there be formed already an archenteric cavity or slit under it, reaching to the place of its first

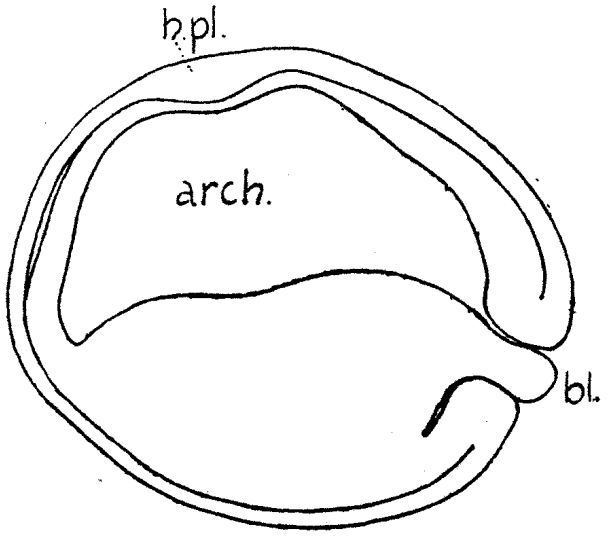


Fig. 9. Sagittal section of a gastrula of *Rana esculenta*. *h. pl.* cerebral plate, *arch.* archenteron, *bl.* blastopore with yolk plug.

appearance. This now proves not to be the case, as shown in fig. 9; only a short slit is present under the ventral lip, not nearly reaching up to where this lip first appeared. So the conclusion must be drawn that not only the ventral blastopore lip, but also the whole entoderm area in front of it performs a wandering to the dorsal side, and that accordingly the entoderm is not only overgrown by the dorsal blastoporic lip in a backward direction, but also actively wanders forward to disappear under it. This reminds us of the controversy between ROUX and SCHULTZE, mentioned in my former

[1]) R. ASSHETON, 1909, Professor HUBRECHT's Paper on the Early Ontogenetic Phenomena in Mammals. Quart. Journ. Vol. 54.

communication, on the wandering of the dorsal blastopore border. ROUX's opinion was, that only the dorsal rim wanders over the yolk, which for *Rana fusca* proves to be right, though not 180°. SCHULTZE on the contrary declared all movement of the dorsal border to be illusory and to be explained by a rotation of the egg. Actively, according to him, the entoderm wanders forward under the dorsal border, and it appears by our present results that SCHULTZE's view, at least as far as *Rana esculenta* is concerned, is not quite erroneous either.

Now apparently we have in this wandering of the entoderm area during gastrulation in *Rana esculenta* the same dorsally directed movement before us, which in *Rana fusca* is performed immediately after fertilization, and which there causes in the eight-celled stage the demarcation line between the darker and lighter area of the egg surface to make a so much greater angle to the egg equator than in *Rana esculenta*, while for the blastopore border, just after it has closed to a ring, the same holds. All this is shown at once by a comparison of fig. 1 and 8 of the present paper with fig. 1 and 2 of the former.

Before looking now for the explanation of the phenomenon, a short discussion must precede of the views, to which my theory of the origin of vertebrates leads concerning the gastrulation of vertebrates, in the first place of anamnia. In studying this theory many a one will have wondered how from two in Protaxonia (HATSCHEK) diametrically opposite areas as the apical plate (round the animal pole) and the stomodaeum (round the blastopore) in craniote vertebrates an organ could arise, which so much gives the impression of a unity, as the cerebral and the medullary plate. A considerable displacement at any rate must have occurred, to bring together these two parts.

This approach we now see performed before our eyes in the ontogeny of annelids. While the entoderm, which remains after the production of the three quartets of ectomeres, originally lies diametrically opposite to the animal pole, we find the mouth, which is directly to be traced back to the blastopore, in the trochophora lying just under the prototroch, which forms the border of the apical plate. As discussed in my article on the development of *Scoloplos armiger*, the displacement is to be ascribed to three factors.

In the first place we observe a wandering of the whole entoderm area to the ventral side (Fig. 10*a*), a result of the active multiplication and extension of the ectoderm cells at the rear side, i.e. mainly the *d*-quadrant of the egg, whereas the cells of the anterior side,

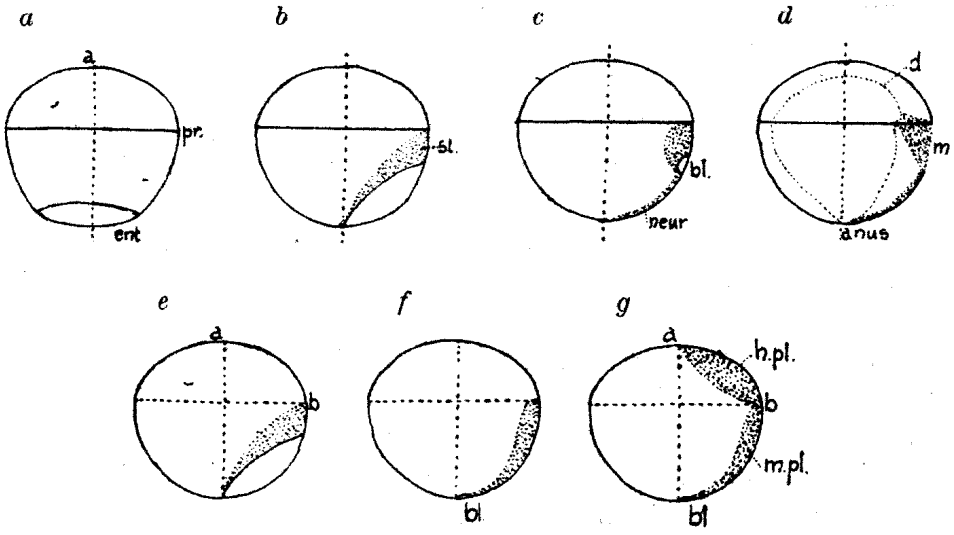


Fig. 10. Diagrammatic representation of the behaviour of the blastopore, see text. *a*, *b*, *c*, *d* in polychaete annelids, *e*, *f*, *g* in chordates. *bl.* blastopore, *d.* gut, *ent.* entoderm, *h. pl.* cerebral plate, *m.* mouth, *m. pl.* medullary plate, *neur.* neurotroch, *pr.* prototroch.

the *b*-quadrant, are backward in development. This causes the entoderm area to wander to the ventral side to such an extent, that no longer its centre but its hind border is found opposite the animal pole. In this region afterwards the anus is formed.

Secondly the blastopore does not close concentrically, but excentrically in a forward direction (Fig. 10*b*), be it with or without concrescence of the lateral borders. This depends on the relative speed with which either the lateral borders or the hind border move forward over the entoderm, and this again depends on the way in which the descendents of 2*d*, the so-called somatic plate, spread over the left and right side and over the posterior end of the embryo. Evidently concrescence here seems to be the rule and at the suture, where left and right blastopore borders have met, the neurotroch arises.

In the third place the foundation of the stomodaeum here does not any longer surround the blastopore as a ring of uniform breadth, as in Protaxonia, but lies more in the way of a crescent round the anterior border. For of the third quartet it is only the cells of the anterior two quadrants, 3*a* and 3*b*, of the second quartet only 2*a*—2*c*, which participate in the formation of the stomodaeum. After the sinking in of this crescentic rudiment to the formation of the stomodaeum-tube, which arises outside the final, narrowed blastopore, the mouth comes to lie just underneath the prototroch (Fig. 10*d*).

We shall see now what we find of these phenomena in the frog

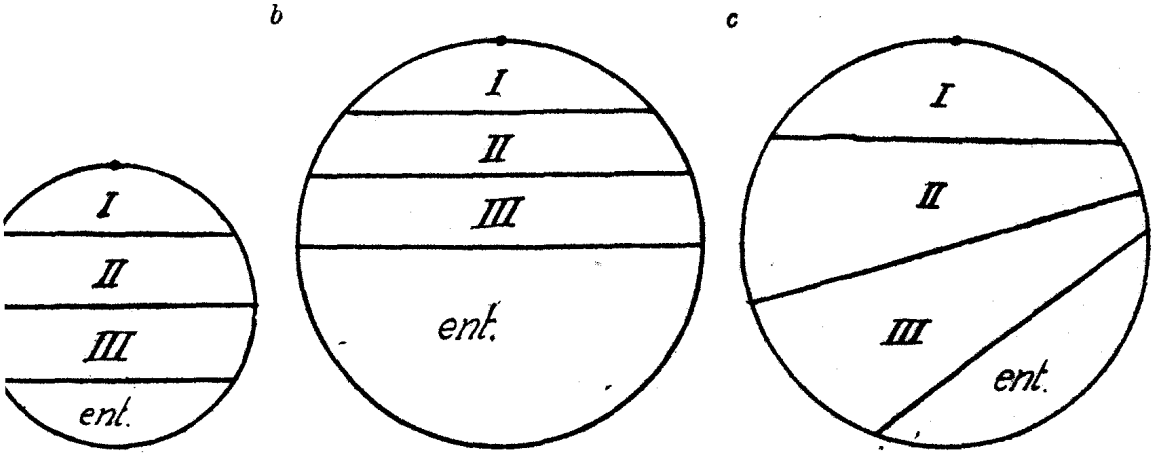
egg and to this end begin with the egg of *Rana fusca*. At once it appears that the first of the three above-mentioned processes, the wandering of the entoderm area to the ventral, c. q. to the dorsal side, is here performed very precociously, immediately after fertilization, and consequently is already finished in the unsegmented, fertilized egg. At least we find, as is shown by fig. 1 of my former communication, that the white area here does not lie at all diametrically opposite the animal pole, but much more to the future dorsal side. The boundary between the ecto- and entoderm areas probably runs parallel to the demarcation of the darker and lighter areas of the egg, as may be also concluded from the place, where afterwards the dorsal and ventral borders of the blastopore appear (fig. 2, ibid.). Evidently we have to deal here with a case of precocious segregation, though it concerns here more a wandering than a segregation.

The second process mentioned above, the rostrad-excentrical closure of the blastopore, we do not find in *Rana fusca*; on the contrary, the closure proceeds caudad-excentrically. As mentioned already in my former communication and elsewhere, I see in this caudad-excentrical closure a result of the interference of the contraction of the blastoporic border with a backward movement of the blastopore, following directly from my theory on the homology of stomodaeum and epichordal neural tube in annelids and vertebrates. As a result of the strong elongation which we must assume that the stomodaeum of annelids undergoes to be transformed into the medullary tube of vertebrates (cf. the scheme in my article in Anat. Anz. Bd. 44, p. 493), the entrance to the stomach (Schlundpforte, HATSCHEK), into which the blastopore passes, must perform a wandering over nearly the whole length of the body to become the neurenteric canal (also resulting from the blastopore). This backward wandering now in chordates is performed in anticipation of the formation of a tube, already during the contraction of the blastopore border. By this process the final, narrowed blastopore is carried back to the place where it was originally found in Protaxonia, viz. diametrically opposite the animal pole. Whether this caudad-excentrical closure of the blastopore is performed by concrescence or not, is here of no importance; as stated earlier, I do not believe that concrescence, at least in amphibians, occurs to any considerable extent. The medullary plate, of which the foundation in stage 10*e*, just as the foundation of the stomodaeum in fig. 10*b*, surrounds as a crescent the anterior border of the blastopore — a conclusion reached for Amphioxus also, e. g. by KORSCHELT and

HEIDER in the last edition of their "Lehrbuch" — during the contraction undergoes a change in shape as indicated in fig. 10c and discussed already in my former paper. We see in this the backward growing out of the stomodaeum of annelids into the epichordal neural tube of chordates, projected as it were on a plane. In fig. 10*g* it has been indicated, how in craniotes, in addition to the epichordal neural plate, the praechordal cerebral plate is now added, while in acrania the condition of fig. 10*f* continues (Anat. Anz. T. 44.)

How are now our statements for *Rana esculenta* to be brought into accordance with those for *Rana fusca*, how are they themselves to be interpreted and what are the points of difference from the latter species? Simply in this way, that 1 in *Rana esculenta* the egg contains more yolk or at least is less isolecithal in structure, and 2 that the wandering of the entoderm area, shown in fig. 10*a* and *b*, here occurs later.

Let us revert firstly once more to the annelids. In my article on the development of the annelid *Scoloplos*, published this year (1916), I have tried to show that among the eggs of polychaete annelids three types are to be distinguished. In the first place we have the small, poorly yolked eggs of *Polygordius*, *Hydroides* etc., in which the cleavage results in a very equal coeloblastula (Fig. 11*a*). Now in the larger eggs of other species two types of polarity may very early be recognized, which exert their influence on the here very determinate cleavage. In the first place the polar or radially symmetrical polarity, expressing itself in accumulation of yolk at the vegetative pole, which again causes the entoderm cells to be much larger than the cells of the three quartets of ectomeres. In the second place the bilateral



. 11 *a*, *b*, *c*. Diagrammatic representation of the 3 types of polychaete eggs. *I*, *II*, *III* = 1st, 2nd and 3d quartet of ectomeres, ent. = entomeres.
minute, yolkless egg. *b*. egg with pronounced polar polarity. *c*. egg with pronounced bilateral polarity.

polarity, which expresses itself in that the cells of the rear side (*d*-side) from the beginning are much larger than the corresponding cells at the anterior side (*b*-side), so that the entoderm area from the beginning does not lie diametrically opposite the animal pole. The scheme of fig. 11 may serve to illustrate this. As a rule we see at the same time both kinds of polarity in the larger eggs exerting their influence on the cleavage, but in one case the first predominates, in the other the second prevails. As an example of the prevalence of polar polarity, I mentioned *Nereis* where the macromeres (entoderm) are especially large in regard to the ectomeres, which lie over them as a little cap, while on the other side the bilateral polarity is only slightly expressed, the cells of the rear side not being much larger than those of the anterior side. In the reverse this last condition prevails very strongly in *Scoloplos*, which accordingly can serve as an example of the predominance of the bilateral polarity (fig. 10*c*). Especially 2*d* is of extraordinary size, while the entoderm cells are not at all remarkable for special bulk. So the entoderm area is displaced here from the beginning to the ventral side.

Hence the eggs of *Rana fusca* and *esculenta* evidently are in the same relation to each other as *Scoloplos* and *Nereis*. In the first species a precocious displacement of the entoderm area and less yolk, as appears e. g. from the extension of the blastopore. In *Rana esculenta* a later wandering of the entoderm area and a greater amount of yolk, at least a less isolecithal structure of the egg, as appears from the large blastopore together with the fact, that the foundation of the embryo encircles considerably less than 180° of the egg circumference; the belly accordingly is relatively more swollen than in *Rana fusca*. Originally the entoderm area in *Rana esculenta*, though not perfectly, yet lies much more diametrically opposite the animal pole than in *Rana fusca*, as appears from the fact, that the demarcation line of the lighter and darker hemispheres of the egg and later the border of the blastopore make a much smaller angle with the egg equator than in the last mentioned species. So in *Rana esculenta* the polar or radial symmetry is originally more strongly pronounced, in *Rana fusca* the bilateral symmetry.

In conclusion, attention may be drawn to the fact of how little this difference in the internal constitution of the egg influences the cleavage. Were things as in annelids, with their determinate cleavage, we might expect, that in the eight-celled stage in *Rana esculenta* the four upper cells would be relatively smaller than the four lower ones as compared to *Rana fusca*, and, reciprocally that in the latter

species the four ventral cells would be larger than the four dorsal ones. Nothing of the kind proves to be true: the eight-celled stages in *Rana fusca* and *esculenta* are nearly uniform. Besides, we saw in the foregoing communication, how relatively independent the direction of the first cleavages is of the internal constitution of the egg.

Chemistry. — "*Röntgen-investigation of allotropic forms*". (Preliminary communication). By Dr. J. OLIE JR. and Dr. A. J. BIJL. (Communicated by Dr. ERNST COHEN).

(Communicated in the meeting of January 27, 1917.)

DEBYE and SCHERRER have in the "Nachrichten der Königlichen Geschellschaft der Wissenschaften zu Göttingen" [1]) published their investigations about "Interferenzen an regellos orientierten Teilchen in Röntgenlicht". Led by theoretic considerations DEBYE [2]) had come to the conclusion that secondary Röntgenlight, emitted by a body shone upon by Röntgenlight is not equally strong in every direction. The arrangement of the electrons in the atoms must necessarily give to that light a maximum of intensity in certain definite directions. Even if the atoms should not be arranged regularly, the resultant of all secondary light-emission will be a definite division of the light in space into maxima and minima of intensity.

DEBYE expected and actually obtained in his investigations with SCHERRER results, which clearly proved the existence of such a division of lightrays (interference). But by the side of the phenomenon he expected he noticed in several cases, whenever crystalline material had been used for the investigation, a much more striking phenomenon. Besides the diffuse maxima, which were visible in the photos as so many spots with vague outlines, there were to be seen some rather distinct lines, which made one think of a spectrum. DEBYE and SCHERRER pointed out that this should not be explained from the interference of the Röntgen rays in the electron-complex of the atom, but in an analogous way from the interference of those rays falling upon the crystalline structure that are to be formed in the macroscopically unarranged mass. [3]) This is contrary to the generally accepted opinion that Röntgen-interference images can only be obtained with large and properly-shaped crystals. From the theoretical considerations as well as from experiments it becomes

[1]) Mathem-physikal. Klasse 1916 Heft 1. See also Phys. Zeitschr. 17, 277 (1916).
[2]) Nachr. der K. Ges. der Wissensch. in Göttingen math.-physikal Klasse 1915.
[3]) Fine crystalpowder or quasi amorphous material.