EXPERIMENTAL STUDIES ON GERMINAL LOCALIZATION.

BY
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I. THE GERM-REGIONS IN THE EGG OF DENTALIUM[1].

WITH 100 FIGURES.

CONTENTS.

INTRODUCTION.

The following experimental studies are offered as a contribution to the theory of "Organbildende Keimbezirke" or germinal prelocalization, especially as applied to the cytoplasmic regions of the unsegmented egg. Following the enunciation of the principle of "precocious segregation" by Ray Lankester, in 1877, the im-

[1]This work was carried out at the Naples Zoological Station between February and August, 1903, on a grant from the Carnegie Institution of Washington, in which was included the use of one of the tables subscribed for by the Institution. My best thanks are due to the administration of the station for the unfailing efficiency and courtesy with which my work was aided in every possible way.

portance of the cytoplasmic factors of localization and differentiation was early recognized by Whitman in his remarkable paper on *Clepsine* ('78) and emphasized by him in later papers. Similar views were more or less clearly expressed by Van Beneden, Flemming, Platner and others prior to the definite formulation of the mosaic-theory of development by Roux in 1888.[1] Roux himself recognized from the first, as a prominent factor in his theory, the importance of a definite topographical grouping of specific cytoplasmic materials in the unsegmented egg; though unfortunately this was complicated, then and in later discussions, by the hypothesis of qualitative nuclear division, which has since been shown to be untenable and has now been relinquished by its author (Roux, 1903). Since that time the evidence, both cytological and experimental, has steadily increased that a prelocalization of the morphogenic factors in the cytoplasmic regions is a leading factor in the early development; and it has become evident that this is true not only in such "mosaic eggs" as those of mollusks or ctenophores, but even in those of echinoderms or nemertines, where an isolated blastomere or an egg-fragment may produce a perfect dwarf embryo. It has become of high importance to determine experimentally in what degree such prelocalization or cytoplasmic "organization" may exist in the unsegmented egg, and to what extent it may vary in different forms. It is even more important for our general conception of development to determine by the same method whether the prelocalization of the morphogenic factors, in whatever degree it may occur, exists from the beginning, or whether, as the cytological evidence seems to show, it is established by a progressive process; for in the latter case, as is hardly necessary to point out, prelocalization, even in the unsegmented egg, may be brought under the category of epigenetic phenomena ("epigenetic qualities" as distinguished from "preformed qualities"[2]), and falls into harmony with hypotheses that assume the nucleus to be the primary determining factor.

The present studies, which are a continuation of the preceding

[1] *Cf.* my work on The Cell.

[2] Boveri ('03), p. 356.

ones on the nemertine egg (Wilson, '03) bear upon both these questions. In that paper I approached especially the second question in an experimental study of the egg of *Cerebratulus,* which has since been extended by the work of Yatsu ('04). My results clearly showed that in this egg the cleavage-factors are not definitely localized until after the completion of the maturation of the egg, but they gave no definite evidence regarding the localization of the morphogenic factors (as distinguished from those of cleavage) at this period; it was, however, shown that in the comparatively young blastula, before the formation of the mesoblast, morphogenic localization, as shown in the predetermination of the gut and apical organ, has become much more definite than in the unsegmented egg. Yatsu subsequently obtained evidence, in the same species, that the localization of the morphogenic factors is a progressive process even in the stages preceding cleavage, since the percentage of normal larvae obtained from egg-fragments at successive periods steadily diminishes from the first discharge of the eggs (when maturation begins) up to the period immediately preceding the first cleavage; and the nature of the defective larvae, correlated with the plane of section, pointed to a increasingly definite localization, in the later stages preceding cleavage, of the bases of several important organs, such as the apical organ, gut, and ciliated lobes of the pilidium. I am now able to offer an experimental analysis along the same lines— perhaps I should say the beginning of such an analysis — of the molluscan egg, in which pure observation of the cell-lineage has produced such convincing evidence of mosaic development, sustained by Crampton's initial experimental examination of the gasteropod egg ('96), and by the interesting cytological work of Lillie ('01) and Conklin ('02) on the cytoplasmic regions of the unsegmented and segmenting egg. The cytological and experimental results coincide in demonstrating in this egg (specifically in *Dentalium*) the existence of a very definite prelocalization of some of the most important factors both of cleavage and morphogenesis, which here closely coincide. They show conclusively also, contrary to what the nemertine experiments had led me to expect, that in its main features this

prelocalization exists in the egg at the time it leaves the ovary, and probably much earlier, and long before even the initial stages of maturation and fertilization. Nevertheless, progressive changes take place during and subsequent to maturation, which, when compared with those occurring in other forms, show this egg, as I believe, to be only the extreme of a series that connects it with such forms as the nemertine or echinoderm, and brings them under one point of view.

The present paper deals mainly with the development of fragments of the unfertilized egg of *Dentalium,* the eggs being cut singly with the scalpel under the microscope and subsequently fertilized, following the method of Delage ('99). I shall here consider the development of isolated blastomeres only incidentally for the sake of comparison, reserving a fuller account for a second paper. It may be stated here, however, that the experiments on this part of the subject demonstrate, even more conclusively than do those of Fischel for the ctenophore-egg, that the cleavage of the ovum, in both *Dentalium* and *Patella,* is in fact what the normal cell-lineage so clearly indicates, essentially a mosaic-work, in accordance with Crampton's earlier experiments on *Ilyanassa.* Blastomeres isolated at any stage from the 2-cell onward continue to segment as if still forming part of a complete embryo; and apart from the changes due to shifting of the cells, which, as in the ctenophore, often lead to the displacements of the larval structures and to the closing of the partial embryos, undergo essentially the same differentiation as if united to their fellows. Thus, the first two blastomeres, upon separation, give rise to two dissimilar larvae, each of which is defective and represents essentially the same structures as would have been produced had the two cells remained united; in like manner, of the isolated cells of the 4-cell stage, the larva from the D-quadrant possesses certain structures that are lacking in the other three; and the differences among the larvae from cells of the 8- or 16-cell stages are still greater. Cells procured by successive isolations up to the 64-cell stage, or later, differentiate singly, according to their nature, into actively swimming trochoblasts of three kinds; into ordinary ectoblast- or entoblast-cells, into sensory cells bearing

the characteristic sensory hairs of the apical organ; and even into what I believe to be muscle-cells and mesenchyme-cells, though, unlike the foregoing cases, the precise origin of these was not traced. These eggs thus represent the opposite extreme to such forms as those of *Amphioxus,* the echinoderm, or the nemertine, and give a result which, apart from the hypothesis of qualitative nuclear division, agrees essentially with Roux's original conception of mosaic-development, with the conclusions of many students of cell-lineage, with the experimental results of Crampton on the gasteropod-egg, and with those of Fischel regarding the ctenophore-egg.[1]

II.

PRELIMINARY OBSERVATIONS ON THE UNSEGMENTED EGG AND THE NORMAL DEVELOPMENT.

The egg of *Dentalium,* like that of *Cerebratulus,* possesses certain features by means of which the axis may be determined in the living egg from the moment of its release from the ovary. The egg is more or less deeply pigmented, perfectly opaque, and of a color that varies in different individuals from light olivaceous to reddish brown or almost brick red. When first set free the egg is somewhat irregular, but quickly becomes more rounded. It is then seen to be very considerably flattened, so as often to be almost biscuit-shaped, one side being always more flattened than the other, and often more or less irregular in contour. Viewed by reflected light the central region of each of the flattened sides is seen to be occupied by a very distinct, though vaguely bounded, white area, nearly or quite free from pigment (Fig. 1); these areas, as shown by the subsequent development, correspond with the two poles of the egg, and the more flattened side, which

[1] The eggs of *Patella,* which were employed mainly for a study of the isolated blastomeres, were available from the middle of March until the latter part of May. Those of *Dentalium,* which were used especially for the development of egg-fragments, first became mature at the beginning of June, when less than two months remained for their study. The shortness of this period accounts for some of the obvious gaps in my work. The complexity of the subject, and the practical difficulties presented by the material are such that more extended work, with additional material, will be required for its completion.

is the side of attachment in the ovary, is found to represent the lower or vegetative hemisphere.

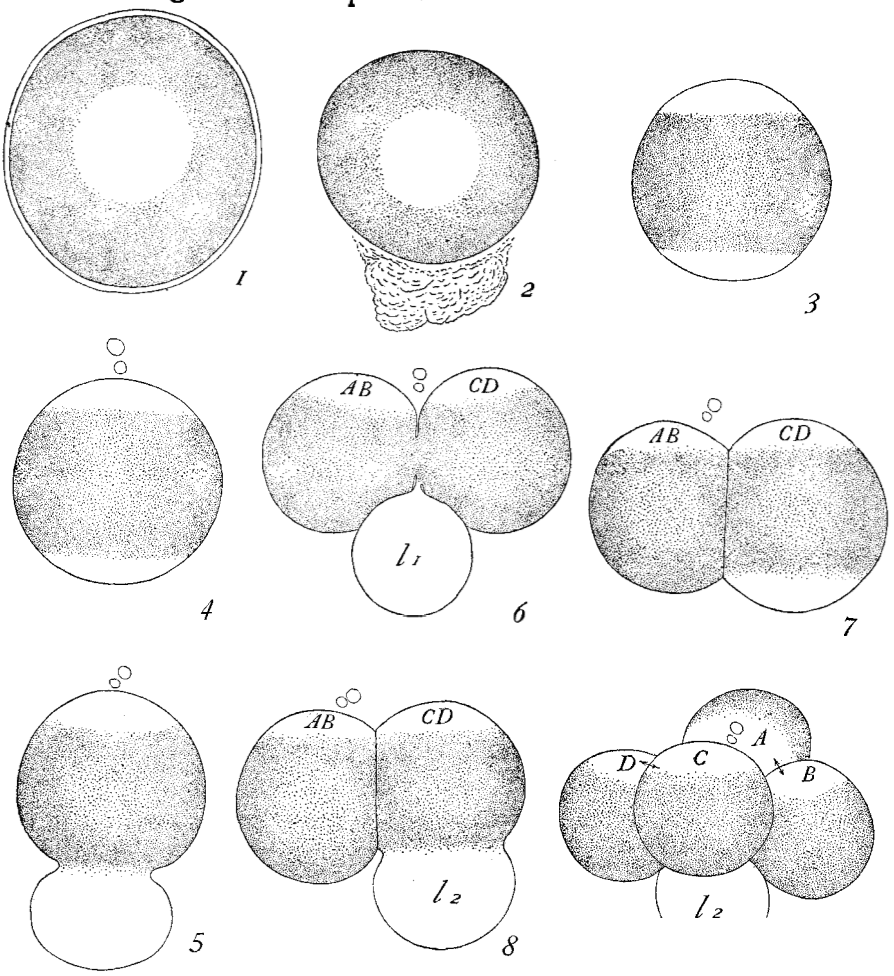


FIG. 1.

Cleavage, from living Eggs.

1, Outline of egg soon after release, in polar view, showing white polar area; 2, the same egg, 20 minutes later, after throwing off the membrane; 3, similar egg, from the side; 4, egg one hour after fertilization, with fertilization-membrane[1] and polar bodies; 5, beginning of the first cleavage, formation of the polar lobe; 6, trefoil, 1¼ hours after fertilization; 7, resulting 2-cell stage; 8, beginning of second cleavage from the side, second polar lobe forming; 9, second cleavage at its height.

[1] Accidentally omitted by engraver.

During the 20-30 minutes following its release the ripe, unfertilized egg becomes nearly spherical (and hence appears considerably smaller in polar view), the membrane by which it is at first surrounded separates more widely from the egg, finally ruptures suddenly, and then quickly draws together at one side, where it is thrown off as a mass of *débris* attached to the egg (Fig. 2).[1] Following this, a substance which at first surrounds the egg as a thin, transparent layer swells up to form a jelly, which raises the egg slightly from the bottom. The wall of the germinal vesicle breaks down at about this period (20-30 m.), leaving a clearer space in which the first maturation-figure appears. The white polar areas are still clearly visible, and the egg, still unfertilized, now gives the appearance of being surrounded by a very broad, horizontal pigment-ring, which, though often faint and with vague boundary, is always distinctly visible (Figs. 3, 4). The ring recalls that described by Boveri ('01) in the egg of *Strongylocentrotus,* though relatively broader. The egg of *Dentalium* thus shows a visible stratification of material analogous to the zones seen in *Strongylocentrotus;* but, unlike the latter, the zones of *Dentalium* clearly pre-exist before even the preparatory changes of maturation take place.

Sections and total preparations of the flattened egg, fixed shortly after its discharge or removal from the ovary, show that a distinct structural modification exists in each of the white areas, at this period much more marked in case of the lower or vegetative area. Surrounding the lower pole (Fig. 10) is a very distinct mass of dense almost homogeneous protoplasm, of approximately the same

[1] All the figures were outlined as accurately as possible with the camera, and with the exception of Figs. 10-13 and 33, 38-41, are enlarged to the same scale (150 diameters). They are only schematized in that the pigment is represented by stippling, whereas the color does not actually appear in the form of distinct granules, but as a nearly uniform hue. The stippling somewhat exaggerates the distinctness of the pigment as seen in most individuals; though in the most deeply pigmented ones, viewed under strong direct light, the color appears with great distinctness and its limits may be clearly seen. The operation of cutting usually leads to disturbances in the arrangement of the pigment, so that frequently no definite color-pattern can be clearly made out in the dwarf embryos. I have only represented the pigment in cases where its boundaries could actually be seen.

extent as the white area seen in the living egg; this contains no yolk-spheres, and stains with great intensity with a strong plasma-stain like Congo red. This mass, sharply marked off from the surrounding yolk, bulges slightly outward at the surface and at the margin is continuous with a very thin ectoplasmic zone that entirely surrounds the egg, but is only clearly visible in sections. Internally this mass is confluent with a somewhat narrow zone of similar finely granular protoplasm that extends upwards partly around the germinal vesicle. It is probably to the presence of this remarkable protoplasmic mass that the appearance of the lower white area is due, though the latter may have a different cause. In a general way, the lower protoplasmic area is undoubtedly comparable with the lower zone, composed of green material, seen in the egg of *Myzostoma* (Beard, Wheeler, and Driesch), as is proved by its later history. Comparison of my Fig. 10 with Wheeler's Fig. 2 ('97), will show how closely similar the relations of the lower protoplasmic area in the two eggs are.[1]

The upper white area cannot be distinguished as such in the fixed eggs, and is apparently produced by a different cause from the lower one. Exactly at the upper pole is a very small, superficial disc of clear, dense, intensely staining protoplasm, which, like the lower protoplasmic mass, is continuous at its margin with the general ectoplasmic layer (Fig. 10). This upper disc is so small as readily to escape observation; but sufficiently careful examination invariably reveals its presence, which is furthermore frequently indicated by a slight indentation of the egg-periphery at this point. It varies considerably in thickness and extent in different specimens, but is always very small at the beginning.[2] Evidently, the upper protoplasmic disc is not large enough to account for the appearance of the upper white area in the living egg, which must be due to some other cause,

[1] Compare also Driesch, '96, Fig. 12.

[2] Sections of the ovary show that both the upper disc and the lower protoplasmic area are present while the egg is still attached to the ovarian wall. The eggs are greatly distorted in shape, but in a general way are pyriform, and attached by the narrow end. The lower protoplasmic area occupies the narrower end, by which the eggs are attached; the upper disc is at the opposite point.

perhaps to a lighter tint in the deutoplasm in this region. In the following account, accordingly, it will be necessary always to distinguish clearly between the upper white area, or polar area, and the upper protoplasmic disc or area.

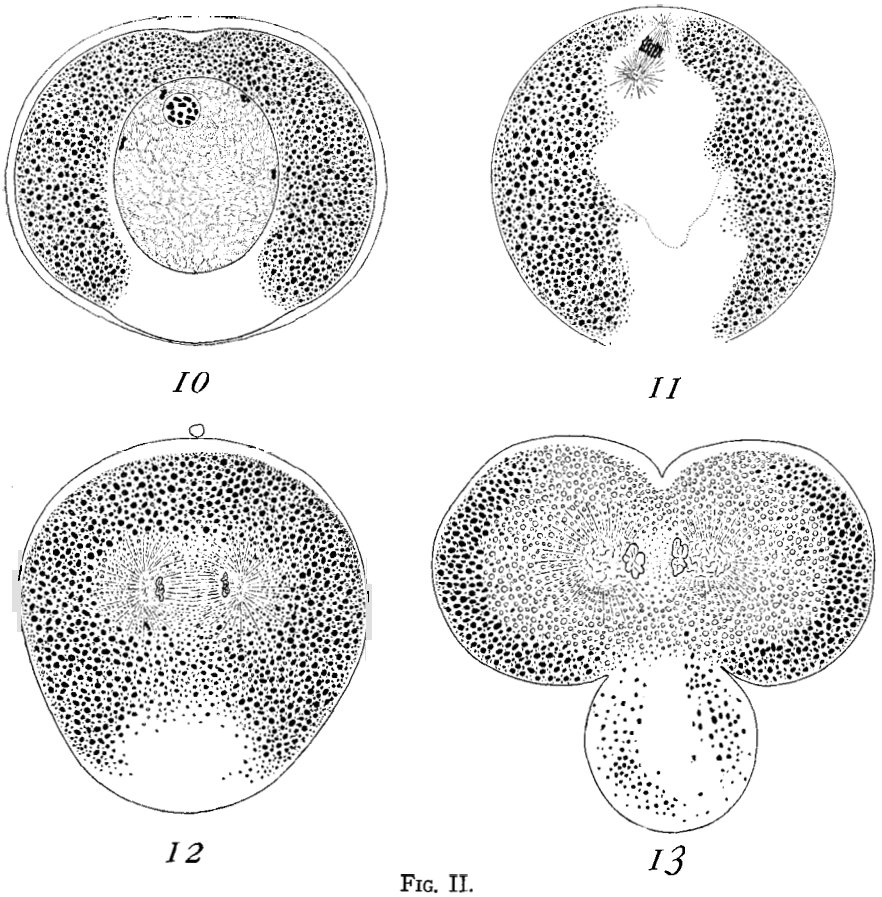


FIG. II.

Vertical Sections of the Normal Egg.

Fig. 13 directly from section (picro-acetic); outlines of Figs. 10-12 (sublimate-acetic) from optical section of total preparations, details from actual sections. The peripheral zone of deeply staining yolk shown in Fig. 13 occurs in all these stages after picro-acetic fixation, but not after sublimate-acetic.

10, Unfertilized egg, five minutes after release, showing both protoplasmic areas; chromosome-like bodies in the nucleolus; 11, fertilized egg, 30 minutes after fertilization, first polar spindle; 12, fertilized egg, 60 minutes after fertilization, initial stage in formation of polar lobe; 13, first cleavage, 68 minutes after fertilization, just before the complete trefoil stage.

I shall here give only a very general account of the later history of the two protoplasmic areas, which will require a thorough cytological study for its full elucidation. As the egg, still unfertilized, lies in sea-water, the ectoplasm in the region of the upper disc slowly increases in amount, and in some cases this region shows a faintly radiating appearance around its periphery as if clear hyaloplasm were flowing into it from the surrounding region. I am uncertain whether in this process the original disc itself enlarges or is only surrounded by an accumulation of hyaloplasm — a point of importance for the comparison with the upper polar ring of the annelid egg that is drawn further on. I shall continue to speak of the ectoplasmic thickening at the top of the egg as the "upper protoplasmic area," but would call attention especially to the fact that the original disc is composed of very dense homogeneous protoplasm that differs markedly in character from the alveolar protoplasm of the ectoplasmic thickening that afterwards extends over the whole upper surface of the egg.[1]

When the germinal vesicle breaks down, the maturation-spindle, which is relatively small, is formed just below this protoplasmic area, rotating into a radial position and moving towards the periphery so that its outer end lies in or just below it (Fig. 11). In this position it remains, in metaphase, until the egg is fertilized, when the divisions proceed, the polar bodies being successively extruded exactly at the upper pole, at the centre of the upper protoplasmic area (which is now rapidly extending and shows no definite boundary), and hence at the centre of the upper white area (Fig. 4). At this period the protoplasmic area comes into connection by a rather narrow neck of hyaloplasm, in which the spindle lies, with the central mass left after the germinal vesicle breaks down. After the polar bodies are formed this connection is severed, and the upper protoplasimc area spreads out still more

1 The general ectoplasmic layer can in the earlier stages hardly be seen in total preparations, but appears clearly in sections either after staining with haematoxylin and a strong plasma-stain such as Congo red (when it appears clear red) or after borax carmine. It is at first much thinner and less definitely bounded than, for instance, in *Rhynchelmis* as figured by Vejdovsky,'88 (in the recent paper of Vejdovsky and Mrazek, '03, it is represented as much thinner than in the earlier paper), but later becomes very conspicuous.

widely so as to appear as a general thickening of the ectoplasmic layer over the whole upper hemisphere (Figs. 12, 13). This thickening is most marked near the animal pole, where it is very conspicuous at the time of cleavage, extending thence approximately to the equator of the egg, or slightly below it, but without any very definite margin. It stains deep red in Congo red and shows a finely alveolar structure quite unlike that of the original disc.

During the foregoing stages marked changes occur also in the lower protoplasmic area, and it is evident that active movements of its material take place. These are perhaps due in part to the entrance of the spermatozoon at the lower pole, but in part also to the fact that upon the breaking down of the germinal vesicle the finely granular material derived from it becomes more or less definitely confluent with the lower area (as Wheeler describes in *Myzostoma*), so that an irregular pillar of protoplasm, surrounded on all sides by yolk, now extends from the lower pole nearly to the upper protoplasmic area (Fig. 11) and ultimately becomes connected with the latter as the first maturation spindle moves upwards.[1] In vertical section it may very clearly be seen that the material of the upper part of this pillar differs markedly from the lower, both in texture and in staining capacity (the two regions show a rather distinct boundary, indicated by the dotted line in Fig. 11), the lower region being very dense and staining in the double stain clear red, the upper one much looser (alveolar?) in structure and staining purple or blue. During the polar body formation the lower area changes its form, often becoming irregular and sometimes elongate or sickle-shaped. It is a noteworthy fact that at the time each polar body is extruded the egg becomes irregular in contour or almost amoeboid, at the center of the *lower* polar area, afterwards resuming its even outline.[2] After formation of the polar bodies the upper part of the protoplasmic pillar retreats from the periphery, while the yolk again extends across the upper region above the egg-nucleus. In the upper part of the internal protoplasmic region conjugation of the

[1] *Cf.* Wheeler's Fig. 10 or 16.

[2] This was figured by Lacaze Duthiers ('57) nearly fifty years ago.

germ-nuclei takes place. At the period shortly preceding the first cleavage, when the upper disc has been replaced by the very broad ectoplasmic thickening described above, the lower protoplasmic area, as seen in surface views of total preparations, varies a good deal in appearance in different individuals, being sometimes rounded and fairly well circumscribed, sometimes irregular, or even broken up so as to present a mottled appearance.

The first cleavage, which occurs about thirty minutes after the extrusion of the second polar body, is characterized by a trefoil stage, like that occuring in many gasteropods, lamellibranchs and annelids (Figs. 5, 6). Exactly surrounding the lower pole is formed, by a horizontal constriction, a large lobe, into which passes the whole of the lower white polar area, and which, like the area itself, appears pure white in the living object. Since the surface of the lobe is much larger than that of the original lower polar area from which it arises, it is evident that material from the interior of the egg must flow into the lobe as it forms. Vertical sections of the egg as the polar lobe begins to form show somewhat varying appearances, due in part to differences in the plane of section, but also in part to varying conditions in the protoplasmic area itself. The rather small cleavage-figure, at this period entirely surrounded by deutoplasm, lies in late anaphase or early telophase slightly above the centre of the egg. At the lower pole the dense protoplasm of the lower area is now spread out, more or less irregularly, to form a thick peripheral layer that fades away insensibly into the yolk-bearing region. Frequently, as in Fig. 12 (*cf.* Wheeler's Fig. 46) this thickening appears fairly regular and symmetrical and suggests the ectoplasmic thickening that precedes the formation of a pseudopod in *Amœba;* sometimes it is less regular than this, and occasionally gives the appearance of an asymmetrical wedge-shaped mass extending into the yolk. As the lobe forms it receives this clear protoplasm, accompanied by an inflow of yolk that seems to invade the clear substance more or less; so that in section scattered yolk-granules are found in the lobe and frequently no definite boundary of the clear substance can be distinguished (Fig. 13). In any case it is certain that the whole of the lower protoplasmic

area passes into the lobe (like the green material of the *Myzostoma* egg) to constitute its main bulk, precisely as Wheeler shows in *Myzostoma* (*cf.* his Fig 47). The term "yolk-lobe" employed by a number of earlier observers is therefore as misleading as it is inappropriate and may be replaced by the term "polar lobe." For reasons given in the discussion at the end, I believe it very probable that at least the lower protoplasmic area, and probably also the upper disc, are in a general way comparable to, if not identical with, the polar rings observed in the eggs of certain leeches and oligochaetes.

Immediately after the polar lobe is formed a vertical furrow cuts into the egg from the upper pole, dividing the upper white area into equal parts and forming with the polar lobe a trefoil, of which the two upper lobes are of exactly equal size and contain all of the pigment, while the unpigmented polar lobe is considerably less than half the bulk of each of the others (measurements give a ratio of 1 to 0.32-0.46, Fig. 6). At the height of its formation the trefoil appears at first sight to consist of three separate spheres. Close examination invariably shows however that the polar lobe is united to one of the upper lobes by a very narrow pedicle which is never severed; and as the cleavage proceeds these two lobes completely fuse while the remaining upper lobe is cut off as a separate blastomere. Thus is formed a characteristic unequal 2-cell stage (Fig. 7), consisting of a smaller anterior cell, AB, and a larger posterior one, CD, which differ in volume by exactly the bulk of the polar lobe. Each of these cells has at the upper pole a white area, representing half the original upper polar area. The lower polar area, on the other hand, is confined to the larger cell, and obviously represents that part of the substance of the fused polar lobe that appears at the surface, a part having again moved into the interior of the egg.[1] Upon the 2-cell stage thus formed is moulded the entire subsequent development, which in its general outline is of essentially the same type as in such forms as *Unio* or *Nereis*.

The experiments recorded in this paper relate mainly to the significance of the material of the lower polar area, and of the polar lobe, and form a continuation of those begun by Crampton

[1] *Cf.* Wheeler's Fig. 48.

in his interesting experimental paper on *Ilyanassa,* published in 1896. In order to understand the significance of the experiments to be described it will be necessary to trace briefly the subsequent development. The second cleavage is ushered in by the reappearance of the polar lobe at the vegetative pole of the larger cell, CD, of the same size and form as before, and again consisting entirely of white material (Figs. 8, 9). The cleavage in this cell, whether separated from its fellow or remaining united

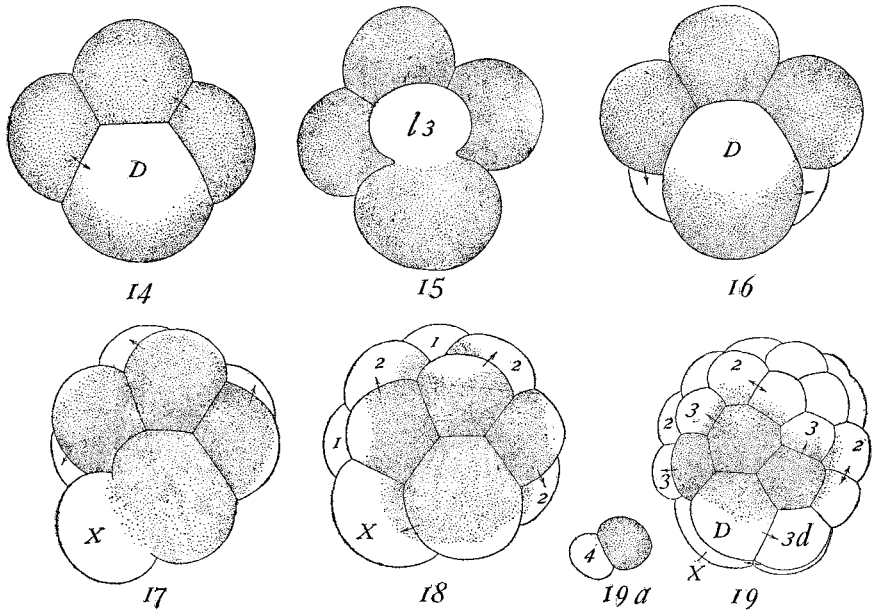


FIG. III.

Cleavage, from living Eggs.

14, Four-cell stage, from lower pole; 15, beginning of third cleavage, from lower pole, third polar lobe; 16, eight-cell stage, from lower pole; 17, beginning of fourth cleavage, first somatoblast in formation; 18, sixteen-cell stage, from lower pole; 19, view from lower pole, after the formation of the third quartet; 19a, D (pigmented) and 4d, immediately after division; surface view.

with it, follows the same general course as in the first cleavage of the entire egg, the polar lobe finally fusing with one of the cells, namely, D, the left posterior quadrant, where it again forms a very definite lower polar white area.[1] The anterior cell, AB,

[1] *Cf.* Wheeler's Fig. 49, Driesch's ('96) Fig. 12, of *Myzostoma.*

in the meantime divides equally, without the formation of a polar lobe. In the 4-cell stage, accordingly, the large posterior cell, D, exceeds A, B or C, by exactly the volume of the lobe, and the lower white area appears only in D (Fig. 14). On the other hand, the substance of the original upper white area is equally distributed among the four; but it is evident that the amount of white material visible at the surface has somewhat increased. The 4-cell stage shows the characteristic relations of the blastomeres observed in so many other eggs of this type. The two lateral cells, A and C, lie at a higher plane, and are in contact along the upper side by an upper "cross-furrow." B and D, on the other hand, are in contact along a longer transverse lower cross-furrow; and these characters, together with the large size of the posterior cell, D, thus give an immediate means of orientation from this time forwards.

As the egg prepares for the third cleavage the upper white material shifts slightly towards the left upper angle in each quadrant, anticipating the formation of the first quartet of ectomeres by the usual dexiotropic cleavage. These cells, which are of equal size and in the A, B and C quadrants are not much smaller than the basals, are formed entirely from the white material of the upper polar areas; and it is here again evident that an extensive flow of this material must take place from the interior of the egg. Their formation does not, however, exhaust the white substance of the upper areas, which still remain in the upper regions of the four basals. During this division the polar lobe forms for the third and last time, from the white material of the lower area, in the D-quadrant; but it is now noticeably smaller than before, and does not constrict so deeply (Fig. 15). After the completion of the cleavage the lobe again fuses with D, in which, as the egg enters into the "resting stage," the lower white area still appears; though this soon undergoes a great change (Fig. 16).

The fourth cleavage is of especial interest, since *a large part of the substance of the lower white area now passes into the first somatoblast, 2d,* or X, and is thus for the first time actually cut off from the pigmented region. This cleavage is preceded and

accompanied by an extensive shifting of the cytoplasmic materials in all of the cells. In the three basals, A, B and C, the white material towards the animal pole moves over towards the upper right angle of the cell and increases in amount, extending so far down the egg that in some individuals it may be seen, when the egg is viewed from the vegetative pole, as a narrow white crescentic area (Fig. 16). A similar process takes place in D, but in addition to this a great change takes place in the white material of the lower polar area, which leaves its position at the lower pole, moves over towards the same side as the upper white area, and finally fuses with it, while the pigmented part becomes lighter in color, often irregular or mottled in appearance, and extends into the area formerly occupied by the lower white substance. In the ensuing cleavage, D is usually the first to divide, giving rise by a leiotropic cleavage to the large first somatoblast, 2d or X (Figs. 17, 18). This cell consists almost entirely of white material which is certainly derived in large part from the original lower white area, but undoubtedly also in part from the upper white area, which, as stated above, fuses with the lower area in the period preceding this cleavage. In some cases X receives also a small amount of the pigment (Fig. 18), in others it seems to be composed entirely of white material. The other members of the second quartet, 2a, 2b, and 2c, are much smaller than X, and each is formed mainly from the white material of the upper polar area, but as a rule, perhaps always, each receives also a variable amount of pigment. During the foregoing changes the upper quartet divide leiotropically in the usual fashion, to form the four primary trochoblasts, which are slightly smaller than the upper cells. Owing to the foregoing changes the pigment, which in the unsegmented egg extended far up towards the animal pole, has been moved downwards so as to lie below the equator of the egg, most of it being contained in A, B and C, some in D, a little in 2a, 2b and 2c, and sometimes also a little in 2d. The pigment becomes still more restricted during the fifth cleavage, since the micromeres of the third quartet are again mainly composed of white substance.

The fifth cleavage, dexiotropic in all the cells, produces the third quartet, each cell of which is considerably smaller than the corresponding basal (Fig. 19). Of these cells 3d is much the largest, and is usually composed entirely of white material, while 3a, 3b and 3c usually, perhaps always, receive a certain amount of pigment. At the end of the cleavage the macromeres rapidly diminish in apparent size, evidently owing to their passing more deeply into the egg, and the color-pattern becomes more or less confused, though A, B and C still show the greatest amount of pigment, while D distinctly shows a white area on the side turned towards X, where 4d is subsequently formed. I have not been able to observe the formation of the entire fourth quartet satisfactorily, either in the opaque living object or in preparations. I can however state positively that as seen in surface-view of the living egg, 4d is very small (smaller than 3d and very much smaller than 2d) and appears pure white (Fig 19,a). I have been unable to determine whether the white material of this cell is derived from that of the original lower white area; though, as will appear hereafter, the experimental evidence indicates that such is the case. At this period the four basals appear much smaller, having evidently retreated into the interior.

Beyond this point it is not necessary at this time to trace the cleavage. The foregoing observations clearly show that, *in Dentalium the freshly discharged egg, prior to maturation or fertilization, shows a definite segregation of visibly different materials which accurately foreshadows a corresponding distribution of these materials among the blastomeres during cleavage.* Of the three zones of material superficially visible in the living egg, the upper one (upper white area) is allotted to the first three quartets of ectomeres, apparently in equal amount in each quadrant; the middle pigmented zone is mainly allotted to the four basal entomeres, though a portion also passes into ectomeres of the second and third quartets; while the lower zone (lower white area) certainly passes mainly into the first somatoblast, 2d, or X, probably in part into the second somatoblast, 4d, or M, and possibly in part into the left posterior micromere, 3d, of the third quartet. This agrees in general with the history of the

zones visible in the living egg of *Myzostoma,* as observed by Driesch ('96), where the lower polar area is represented by a green substance, the upper one by a reddish material, and the pigment zone of *Dentalium* by a zone of clear protoplasm. It is important not to confuse the above-described distribution of white and pigmented material with that of protoplasm and deutoplasm. As shown on a preceding page the upper white area is not, like the lower one, free from yolk; and in point of fact all the cells contain a large amount of yolk. The pigment-pattern is only a visible expression in the living object of a distribution of specific materials that can only in part be distinguished in sections.

We may now briefly consider the main outlines of the larval development. In warm weather the embryos become ciliated at about the ninth or tenth hour, and at the end of twenty-four hours are well developed trochophores that swim very actively at the surface, progressing in a spiral curve and rotating from right to left as seen from the side. At this period (Fig. 29) the body is of a blunt spindle-shape, encircled at the equator by a very broad prototroch composed of three principal rows of large trochoblasts which bear three corresponding rows of powerful cilia completely encircling the body and leaving no dorsal gap (as is also the case in *Patella*). The pre-trochal and post-trochal regions, while somewhat variable, are at this period nearly similar in form and size, being roughly conical and rounded at the tip. The pre-trochal region is wholly covered with very short vibratile cilia and bears at its apex a very long and well-defined tuft of flexible, but not vibratile, flagelliform sensory hairs. In total preparations, or in longitudinal sections, it may be seen with great clearness that the apical tuft is borne upon a large and definitely circumscribed apical thickening or plate, sharply marked off from the surrounding cells. The post-trochal region is not ciliated, but bears at its posterior extremity a small bunch of sensory hairs, which differ from those of the apical tuft in being quite stiff, and radiating from the common point of attachment. The alimentary canal at this period forms a closed sac divided into two chambers, into one of which at a slightly later period opens the mouth, formed immediately below the prototroch, but

the anus does not yet exist. The post-trochal region already shows the mantle fold and the beginning of the shell-gland. On either side the gut may be seen an irregular mass of small cells which I believe to represent the cœlomesoblast, though I have not yet traced them to the pole-cells. These masses are not to be confounded with two masses lying further forward that are proliferated off from the ectoblast in two symmetrically placed lateral areas in the pre-trochal region and perhaps represent a part of the pædomesoblast (ectomesoblast) or perhaps the foundations of the cerebral ganglia. These areas, which are figured by Kowelevsky ('83, Figs. 32, 37, 55) are shown in the lobeless embryos (Figs. 33, 40).

The ensuing changes take place very much more rapidly in the Naples species (D. *entalis*) than in the northern form studied by Lacaze Duthiers ('57), which is probably due in a measure to the higher temperature. By the 30th hour the post-trochal region has considerably elongated and the pre-trochal region is somewhat diminished (Fig. 30). In the course of the ensuing twelve hours the pre-trochal region wholly disappears from view, being withdrawn into the interior, while the post-trochal region becomes still more elongated and the larva sinks to the bottom, where it swims only sluggishly. About this time the body becomes surrounded by an extremely delicate hyaline shell into which the greatly diminished prototroch can be withdrawn; and by the end of the second day the foot appears on the median ventral side. By the end of the third day the foot has become a large protrusible organ, trilobed towards the free end, and the protrotroch is still smaller (Fig. 31, which closely agrees with Lacaze's Fig. 1, Plate VIII). In many cases the metamorphosis is complete by the end of the fifth day, the prototroch having disappeared, the otocysts and pedal ganglia being clearly visible, and the young *Dentalium* assumes the condition figured by Lacaze on Plate 8, Figs. 2, 3—a larva of 20-25 days (!).

Many details have been omitted from the above account that have already been described in the well-known memoirs of Lacaze Duthiers ('57) and Kowalevsky ('83). Many others will require for their full elucidation much more extended study than

I have thus far been able to devote to the subject. The greatest gap in my work thus far is the failure to trace the connected history of the mesoblast, which can only be done by a complete study of the cell-lineage. This presents considerable obstacles owing to the difficulty of obtaining good total preparations at every stage (the eggs and embryos stain diffusely in most dyes, and the great abundance of deeply staining yolk in all the cells renders it difficult to get clear pictures), and my time was so taken up with the study of the living material that I had not opportunity to work out a really satisfactory method. For sectioning the best results were given by sublimate-acetic, the sections being stained with thionin, which gives a sharp nuclear stain without coloring the yolk. The best total preparations were obtained by mounting in balsam without staining. Apart from the technical difficulties, the object is itself difficult, in the earlier larval stages on account of the difficulty of distinguishing between mesoblastic and entoblastic elements in the crowded mesentoblast-mass, in the later ones by reason of the complication introduced by the folding of the mantle and the shell-gland.

EXPERIMENTAL PART.

The ease with which the eggs of *Dentalium* may be operated recalls the remark of Lacaze that "L'embryon du Dentale est un de ces exemples faits pour l'étude du développement" ('57, p.196). For experimental purposes however it presents certain difficulties that should carefully be borne in mind in considering the results of the operations. First, there is a certain amount of variation, not wide but still noticeable, in the size of the eggs and the resulting larvae, and in the relative size of the polar lobe and of the blastomeres during the cleavage-stages. Second, a certain proportion of the entire eggs sooner or later develop abnormally, which results in an increasing mortality from day to day. Third, and most important, the percentage of monstrous forms, and the mortality, is always very large in the development of egg-fragments and of isolated blastomeres. This is undoubtedly due in part to the abnormal conditions under which the larvae are placed in the aquarium, in part to the shock of the operation, and in part

to the changed condition of surface-tension in the dwarf embryos and larvae, as is shown by the readiness with which they disintegrate. (I have several times seen an actively swimming dwarf larva suddenly fly to pieces on coming in contact with an obstacle or even with the surface of the water.) For these reasons, despite the great ease with which the eggs may be operated, it is difficult to base trustworthy conclusions regarding the more special features of the egg-localization on the defects observed in the individual partial larvae. I have therefore in the following work restricted my account in the main to the results that appear with unmistakable clearness, and appear in so large a proportion of the larvae as to remove all reasonable doubt. Beyond this, owing to the importance of following the development of the living larvae as far as possible, the number preserved for sectioning was not very large, and the technical difficulties indicated above, in case of the normal larvae, here appear in aggravated form. This explanation is necessary to account for certain obvious gaps in the work, which I hope to fill out by further investigation, especially those relating to the mesoblast, regarding which I can at present offer only somewhat provisional conclusions.

III.

EFFECT OF REMOVING THE POLAR LOBE.

(a) *General History of the lobeless Larvae.*—During the trefoil stage of the first cleavage the polar lobe may easily be removed, wholly in part, by means of a fine scalpel. Complete removal of the lobe produces a highly characteristic and constant, though in one respect very unexpected, result. Exactly as Crampton earlier found in *Ilyanassa,* the egg continues to segment after this operation quite symmetrically, in a manner similar to the normal cleavage of such forms as *Patella* or *Lymnaea,* giving rise by typically alternating spiral cleavages to successive symmetrical quartets of micromeres (Figs. 20-26). These cleavages differ constantly in two respects from the normal, namely, that (1) *no trace of a polar lobe is formed at either the second or the third cleavage,* and (2) *the members of the D-quadrant are no*

larger than the others. Correlated with this is the fact that these *embryos show no lower white area,* all the basal quadrants being uniformly pigmented over the lower pole (Figs. 23, 24), which sometimes shows a large opening into the cleavage-cavity (Fig.

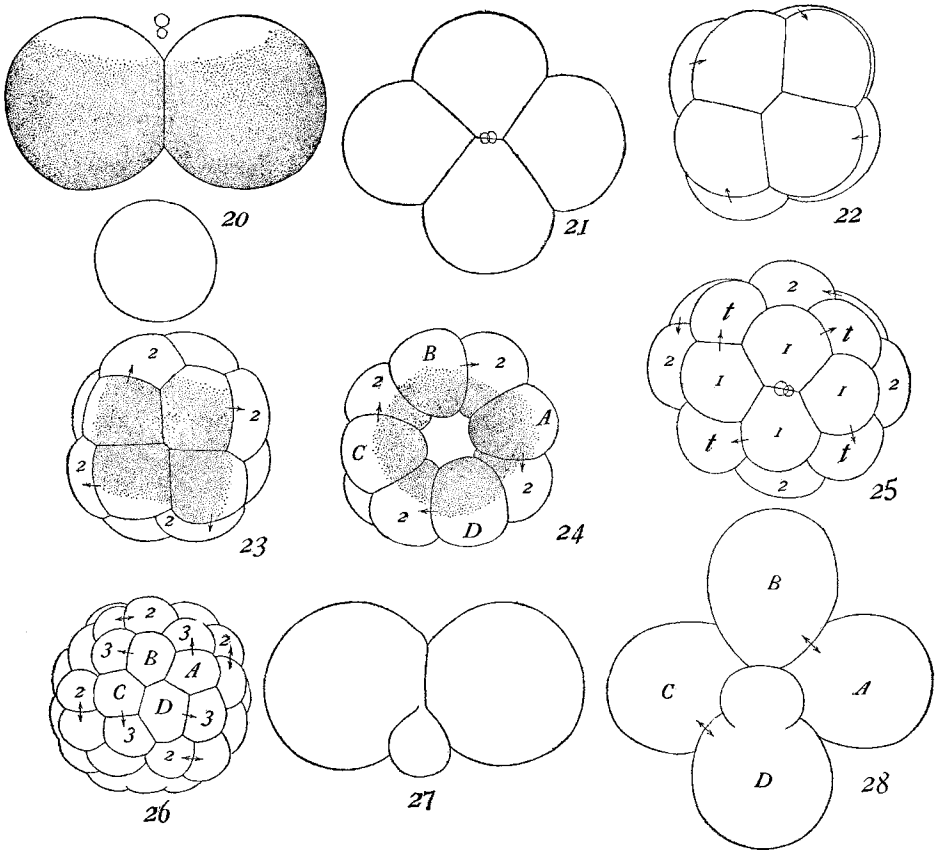


FIG. IV.

Cleavage after Removal of the Polar Lobe.

20, Two-cell stage and polar lobe after removal of the latter; 21, four-cell stage of same, from upper pole; 22, eight-cell stage of same, from upper pole; 23, sixteen-cell stage of lobeless embryo from lower pole, symmetrical second quartet; 24, similar view of the same stage, open type; 25, sixteen-cell stage, from upper pole; 26, lobeless embryo from lower pole, after formation of the third quartet; 27, second cleavage, from the side, of egg from which about three-fourths of the first polar lobe had been removed; 28, a similar form, viewed from the lower pole, after removal of about one-half of the first lobe.

24). The embryos gastrulate and develop with great regularity into larvae that swim in the same characteristic progressive spiral course as that of the normal ones. These larvae (Fig. 32) differ from the normal ones in two obvious respects, namely, (1) *the post-trochal region is absent, or represented only by a smoothly rounded surface from which no outgrowth takes place,* and (2) *they show no trace of an apical organ.*. The first of these results fully accords with expectation; for studies in cell-lineage have shown, both in annelids and in mollusks, that in forms possessing a typical trochophore larva the ectoblast and mesoblast of the post-trochal region are mainly derived from the two somatoblasts, and I have shown that the first of these cells is certainly and the second probably, derived mainly from the polar lobe (or lower white area). The second result, on the other hand, is astonishing, since the region that has been removed is diametrically opposite to that from which the apical organ develops; but a large number of operations have not shown one exception in this respect and the most convincing corroborative evidence is afforded by other experiments presently to be described.

The structure and subsequent history of these larvae is very widely different from that of the normal forms. As the cleavage advances the symmetrical cells of the second and third quartets close in around the lower pole, frequently followed in greater or less degree by the cells of the prototroch; and after the gastrulation this region (the posterior region of the larva) becomes somewhat expanded, so that the larva assumes a pyriform shape, actvely swimming with the narrower end in front, and rotating from right to left like a normal larva. The narrower anterior region is uniformly covered with fine vibratile cilia which are slightly longer near the anterior pole (as in a normal larva—Fig. 32); but an examination of more than fifty such larvae failed to show a single case in which a true apical tuft was present. Sections and total preparations reveal the remarkable additional fact that in such larvae, at least in many cases, no apical plate is formed, though the lateral areas of proliferation, referred to above, are present, as shown in Fig. 40, a, a. In a few cases I have found a somewhat vague thickening at the apical pole,

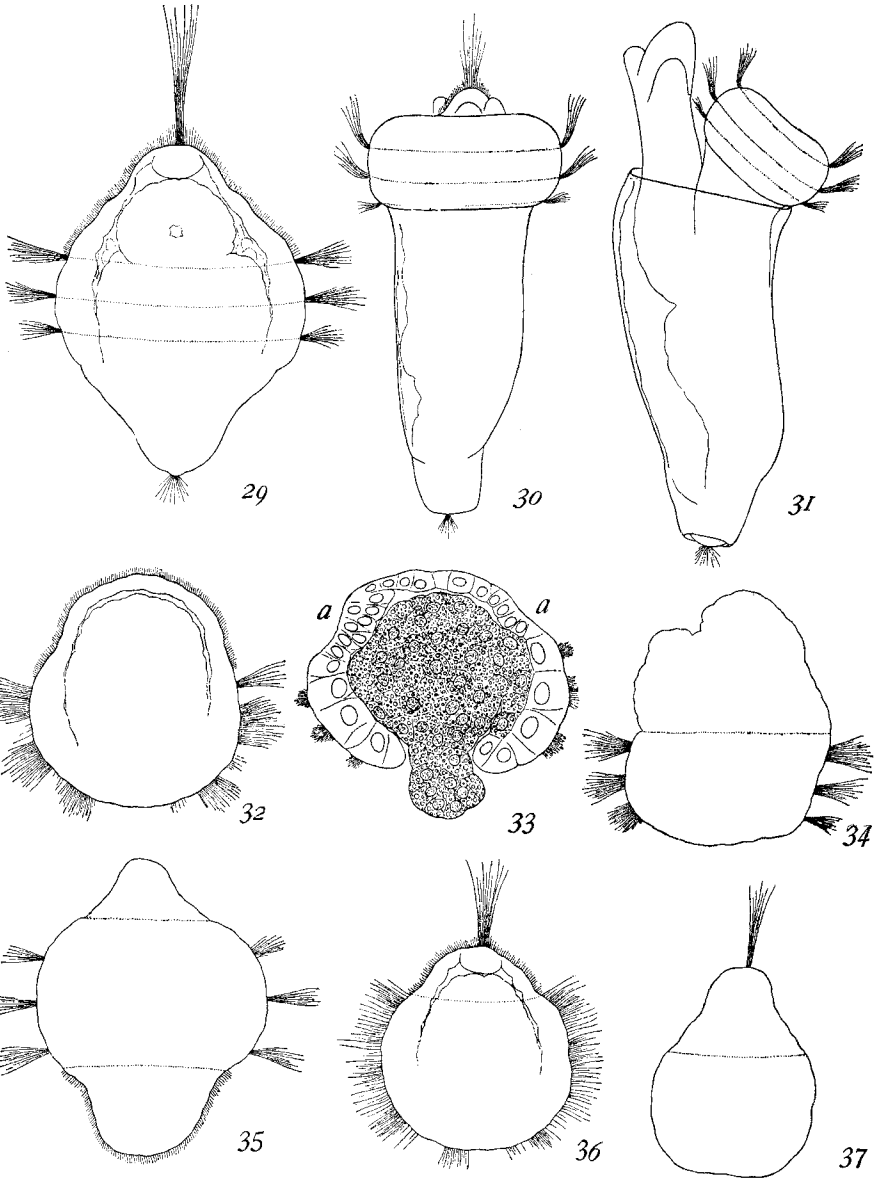

29
30
31
a
a
32
33
34
35
36
37

FIG. V.

Normal Metamorphis and lobeless Larvae.

(Excepting Fig. 33 these figures were drawn from living larva, the cilia being added from formol preparations and the inner outlines from specimens mounted in balsam.)

29, Normal trochophore of 24 hours (a rather large specimen); 30, normal trochophore of 32 hours; 31, normal larva of 72 hours, showing foot and shell; 32, larva of 24 hours, after removal of first polar lobe; 33, vertical section of lobeless larva of 24 hours, showing entoblast-plug protruding through the blastopore; 34, larva of 72 hours, after removal of first polar lobe; 35[1] larva of 24 hours, produced from a form like Fig. 28, after removal of about half the polar lobe; 36, larva of 24 hours, after removal of second polar lobe; 37, CD half-larva, after removal of second polar lobe, 24 hours.

[1] This figure has been turned upside down by the engraver.

but never one that could be mistaken for a typical apical plate. In others, however, the apical ectoderm does not differ from that by which the whole pre-trochal region is surrounded. I feel justified therefore in the statement that the lobeless larvae typically fail to develop the apical organ at any period, individuals having been reared up to the fourth day, when the metamorphosis of the normal larvae was well advanced. (*Cf.* Figs. 31 and 34.) During the development, probably owing to the deficiency of material present in the D-quadrant, the trochoblasts often become more or less displaced towards the posterior pole, and in greater or less degree lose their regular arrangement. In many specimens nevertheless the typical prototrochal belt of three rows of cilia is formed (Fig. 34), though even in these the rounded posterior region often also bears patches of cilia. In others no definite belt can be made out, and such individuals often give the appearance, when alive, and even after being killed with formol, of being ciliated over the whole posterior region. In preparations, however, the cilia of such forms may almost always be seen to be arranged in patches, leaving non-ciliated regions between them, which are doubtless occupied by cells derived from the second and third quartets. It is probable, therefore, that the appearance of uniform ciliation is misleading, and is caused by the confusion of separate tufts lying at different levels. In cases where no displacement of the trochoblasts occurs, the posterior region is covered by cells derived from the second and third quartets.

As the development proceeds there is no attempt to regenerate the missing post-trochal region or apical organ, and the later history of these larvae differs totally from that of the normal ones. The pre-trochal region shows an increase, instead of a decrease, in size, and is not withdrawn into the interior, but gives rise to a more or less irregular vesicular structure directed forwards as the embryo swims. Such larvae were reared until the beginning of the fourth day (Fig. 34), after which they invariably became more and more irregular and finally disintegrated. At this period they present a most remarkable contrast to the normal control larvae of the same age. There is still no trace of a post-trochal region, no shell, no foot, and no apical

organ. Sections show that these larvae have formed no shell-gland, no mantle-fold, and apparently also no mouth.

The foregoing account applies to the great majority of the lobeless larvae; but occasionally an apparent exception occurs, the careful examination of which only serves to confirm the rule. In these exceptional cases a more or less reduced post-trochal region appears to be present, and one individual was obtained that in life seemed to possess this region in a fully developed condition. Sections of these embryos show, however, that what appears to be a post-trochal region is in reality a plug of entoblast cells, projecting through the blastopore-region, that arises through defective gastrulation (Fig. 33). Such embryos sometimes show towards the upper pole a much larger cleavage-cavity than in the normal form,— obviously a result of the failure of the entoblast-cells to invaginate completely. This is conspicuously shown in the larva, referred to above, which appeared to have a fully developed post-trochal region. This larva, cut into longitudinal serial sections, shows very clearly the failure of the entoblast-cells to invaginate properly, a large space being left in the upper hemisphere above the archenteron. For this very reason this larva showed very clearly, both as a total preparation and after sectioning, the entire lack of an apical organ.

The foregoing observations fully establish the conclusion, I believe, *that the material of the polar lobe is indispensable for the formation of the post-trochal region and the apical organ, and as shown beyond they give considerable reason for extending this conclusion also to the cœlomesoblast.*. That the failure to produce a normal larva *is not due to the lack of sufficient material,* is conclusively shown by several additional facts. First, in *Patella* the D-quadrant is no larger than the others, yet a post-trochal region is formed that is relatively as large as in *Dentalium.* Second, as will be described in Part V, much smaller larvae, possessing all of the typical parts, may be produced from fertilized egg-fragments. Third, the same conclusion is afforded by the history of isolated blastomeres, which also fully corroborates the results obtained by removing the polar lobe from an entire egg. If in the 2-cell stage the two blastomeres, AB and CD, be sep-

arated, both continue to segment for a time as if still forming part of an entire embryo, the second and third polar lobes forming in normal fashion in the CD half; but in the end both completely close, gastrulate, and form actively swimming larvae. The two larvae agree in possessing a closed, though often somewhat asymmetrical or confused prototroch, but otherwise show the following characteristic and constant differences. The AB (smaller) larva, closely resembles, except in size, that derived from an entire egg from which the polar lobe has been removed, invariably lacking a post-trochal region and apical organ (Fig. 46). The CD (larger) larva, on the other hand, possesses both these structures, both of which may be as large as in a whole embryo (Figs. 42-45). These larvae vary greatly in form, but in general are asymmetrical and, as may be seen by a comparison of Figs. 45 and 29, possess a post-trochal region that is almost invariably relatively too large, and a pre-trochal region relatively too small as compared with a normal larva. As in the AB half, the prototrochal cilia frequently show a confused arrangement, the regular rings of the normal larva being more or less broken up. In like manner, if the four blastomeres of the 4-cell stage be isolated, only the larva from the D (largest) quadrant develops these two structures (Fig. 47), while those from A, B or C are nearly like those derived from the AB half, though only half as large (Figs. 48, 51). Like the CD ½-larvae the D ¼-forms are variable in form; but whenever they complete what may be considered their normal development they show the post-trochal region very much too large, and the pre-trochal region much too small (Fig. 47).

All these larvae show a very high mortality, but I have kept the ¼-larvae as late as the beginning of the fourth day (Fig. 51), and the ½-larvae nearly as long. The smaller larvae (the AB half, or the small quarters) show a greater tenacity of life, swim more actively, and become less irregular than the larger ones. In the end, however, all the forms become irregular and finally wholly disintegrate, without producing normally formed trochophores or regenerating the missing structures. The CD ½-larvae of 24 hours sometimes approach the form of normal larvae of the same age, though always showing the false proportions of the pre-tro-

chal and post-trochal regions described above. Like the AB halves and the ¼-larvae, they often swim actively at the surface, rotating in the same way as an entire larva; though the progressive movement is almost always slower and less regular than that of the smaller halves. These forms, however, seem to live no longer than the less regular ones, and in spite of every precaution they become more and more irregular and finally disintegrate in the same aquaria containing the normally developing whole larvae. Those that lived to the end of the second day invariably became monstrous in form and showed no resemblance to a normal larva. The history of the AB halves or the smaller quarters in general very closely resembles that of the lobeless larvae, the pre-trochal region enlarging, becoming irregular, and finally disintegrating, often while the embryo is still actively swimming by means of the trochoblasts, which, as Fischel has observed in case of the swimming cells of ctenophores, are most tenacious of life of all the cells.

The relative volumes of protoplasmic substance contained by these various forms of larvae, may be determined either by measuring the volumes of the blastomeres after isolation by means of calcium-free sea-water, or by measuring the polar lobe and estimating the other volumes, the two methods giving fairly consistent results. It should be remembered, however, that both the whole eggs and the relative size of the polar lobe (and hence of the blastomeres) vary somewhat, both in the eggs produced by a single female, and to some extent in those produced by different females. I observed one lot of eggs, for instance, the greater number of which produced lobes considerably smaller than usual. Measurements of the lobe in typical average trefoils give a value ranging from one-fifth to one-sixth that of an entire egg. A typical case gave a volume of 0.18 for the lobe, from which the other volumes are as follows:

Entire embryo	1.00
Embryo without polar lobe	0.82
CD ½ embryo	0.59
AB ½ embryo	0.41
D ¼ embryo	0.385
A, B or C	0.205

Since the CD ½ larva is less than ¾ and the D ¼ larva less than ½ the volume of the lobeless embryo, yet both produce apical organ and post-trochal region, the conclusion is unavoidable that *the failure to form these structures after removal of the polar lobe must be due to a qualitative and not a quantitative difference;* in other words, the material of the lobe must be specifically different from the remaining material, and as such is the determining cause of the development of the structures in question.

The above conclusion is fully sustained by the effect of cutting off only a part of the polar lobe. In such embryos during the second and third cleavages the polar lobe is correspondingly diminished in size (Figs. 27, 28), and the D-quadrant is too small by the same amount. Such eggs produce larvae with a corresponding reduction in the post-trochal region (Fig. 35) and these larvae sometimes possess, sometimes lack, the apical organ. It is not improbable therefore that further experiments of this kind may show a localization, within the polar lobe itself, of the determining materials of the apical organ and of the post-trochal region. This experiment adds to the foregoing the important result that after the polar lobe has formed there is a direct quantitative relation between the amount of specific material it contains and the size of the post-trochal region, there being apparently no regulative process in the later stages (though I have not yet sufficiently examined this latter point). As will appear in Part V, *this conclusion does not apply to the material of the lower polar area before the formation of the lobe.*

(b) *The mesoblast question.*—We may now consider what is in some respects the most interesting, as it is certainly the most difficult, of the questions relating to the lobeless larvae, namely, that of the mesoblast. The fact that certainly the first and probably the second somatoblast is derived mainly from the substance of the polar lobe, and that after the removal of this substance the post-trochal region fails to develop, suggests that the material of the cœlomesoblast as well as of the ectoblastic structures, is localized in the polar lobe and hence in the original polar area. In point of fact

Crampton ('96) in his interesting paper on *Ilyanasssa,* found that after removal of the polar lobe the second somatoblast (4d) differs from the normal not only in being no larger than the other members of the quartet, but also in texture, being filled with yolk-spheres instead of being mainly composed of clear protoplasm as in the normal, and it also lies at first at the surface, exactly like 4a, 4b and 4c. This observation I can confirm from a reëxamination of the original preparations, kindly placed at my disposition for this purpose by Dr. Crampton. He found further, that the larvae produced from such eggs lacked the mesoblast-bands present in the normal larva, 4d apparently entering, like its fellow-members of the same quartet, into the formation of the archenteron.

This highly interesting result, which has atracted considerable attention, was based on the examination of total preparations only; and the desirability of a more adequate study of the matter by means of sections has long been obvious. I have accordingly given especial attention to this point as far as my material would allow; but must admit that neither in point of abundance nor of fixation is this material quite adequate for the full investigation of the question, which indeed would demand a complete study of the cell-lineage, both in the normal and in the lobeless forms. Nevertheless such evidence as I have obtained is distinctly in favor of the correctness of Crampton's result.

The mesoblast may be most clearly seen in the normal larvae in cross sections through the region of the prototroch, where the gut shows two chambers and the complication produced further back by the shell-gland and mantle-folds are not present. In such a section (Fig. 38) the gut appears in the form of two distinct chambers, the wall of the ventral one being a little further back intimately connected with the stomodæal invagination (Fig. 39) though its cavity does not yet appear to communicate with the outside. The walls of both chambers are composed of large cells, more or less columnar and radially disposed, completely filled with yolk-spheres (as are all the cells at this time) and with large nuclei. On either side is a loose group of much smaller cells with small nuclei, that appear irregular or often spindle-

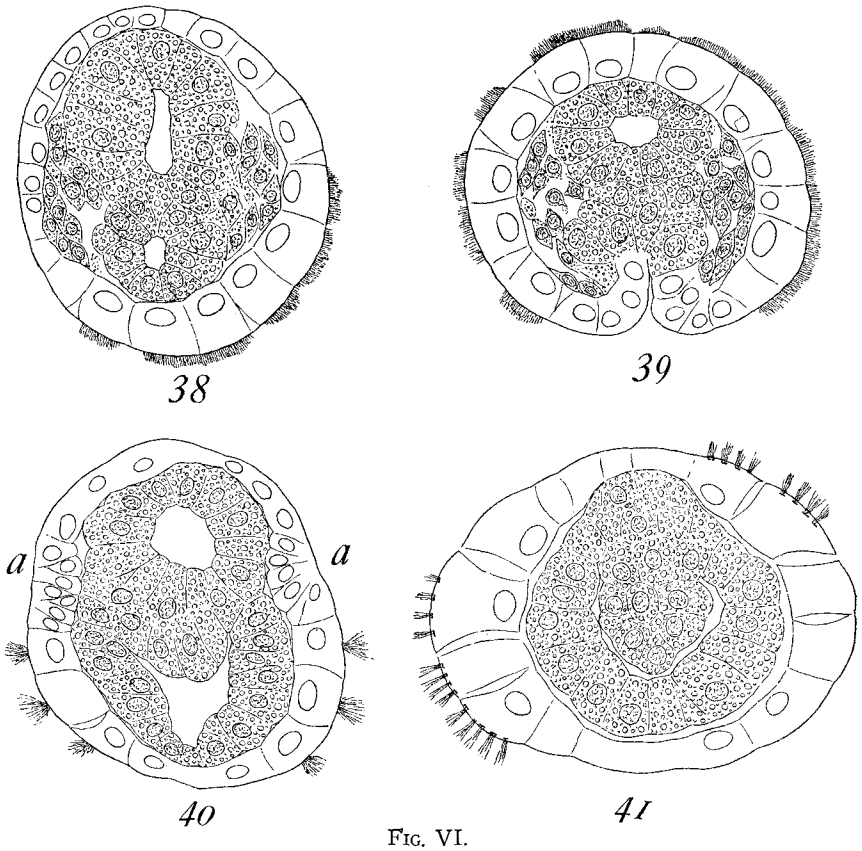


FIG. VI.

Sections of normal and lobeless Larvae.

(Each of these is drawn from a single section, supplemented by a few details from the two adjacent sections of the series. The deutoplasm is only shown in the entoblast and mesoblast.)

38, Slightly oblique cross-section of normal larva, 24 hours, just anterior to the mouth; 39, cross-section through the mouth; 40, vertical section of lobeless larva, 30 hours; 41, cross-section through prototroch-region of lobeless larva, 48 hours.

shaped. There can, I think, be no doubt that these are mesoblast cells,[1] though I have not determined whether they are the products of the second somatoblast, 4d, or arise from another source. A possibility of error on this point is given by the fact, already referred to, that just anterior to the prototroch on either side are two lateral ectoblastic areas of proliferation (of unknown significance) that may contribute to the small cells in question. In any case *these lateral masses of mesoblast fail to appear in the lobeless embryos of corresponding age or older.*. In the earlier stages, of which Fig. 33 is an example, it is impossible to determine this point with any degree of certainty, owing to the crowding together of the entoblast cells in a compact mass in which frequently no cavity can be seen. In later stages, however, both longitudinal and transverse sections give pretty clear evidence that the small mesoblast-cells are either wholly absent or very few in number. Fig. 40 is from a complete series of longitudinal sections of a lobeless embryo of 30 hours. This shows the gut as a two-chambered sac directly applied to the ectoblast with no sign of smaller cells between them, though both the anterior ectoblastic areas of proliferation are shown (*a, a*). It might well be supposed that the small cells are present in a different plane, as would be the case in Fig. 38 if cut in the sagittal plane; but their absence appears no less clearly in cross-section, as shown in Fig. 41 (from a complete transverse series). This embryo of 48 hours swam actively and normally. Though not so well fixed as the preceding one, it clearly shows the gut as a simple sac, enclosing a single cavity that opens at the posterior pole and anteriorly is nearly filled with a thickening bulging inward from the wall at one side. I am quite sure that no mesoblast-cells are present in this embryo unless at the extreme anterior end, where the layers are cut tangentially and cannot be clearly analyzed. The sections of this embryo clearly show further

[1] The relations as figured by Kowalewsky ('83, Fig. 48) in the Marseilles species are essentially similar to those here shown, except that the mesoblast-cells are shown very much larger and fewer. This is stated to be from a larva of 24 hours, but probably represents a relatively earlier stage of development than mine. Compare the mesoblast-cells in Kowalewsky's Fig. 66, from a larva of 38 hours.

the absence of any structure comparable with the foot, mantle-folds, shell-gland, or mouth (unless the posterior opening can be so considered) though all these structures are present in the normal control embryos. The absence of an apical organ is shown as in other series, by the two from which Figs. 33 and 40 are taken.

I would not speak too positively before examining additional material, for in some of the other series a few small cells appear that may be of the same nature as those seen in the normal embryos, though they are far less numerous; yet the foregoing evidence is sufficient to create a strong presumption that Crampton's result was correct. Crampton showed due caution in guarding against the conclusion, from his observations, that the polar lobe "contains prelocalized mesoblast material," being probably influenced by the fact that in *Ilyanassa* the lobe appears to be composed mainly of deutoplasm. He only concluded "that the presence of the yolk mass in the cell D may be the stimulus which causes that cell to act differently from the other macromeres, A, B and C" ('96, p. 14). I believe, however, the facts brought forward in this paper render it probable that the polar lobe (and hence the cell D) does in fact contain a specific kind of cytoplasm which, if not actually "prelocalized mesoblast-material" is the direct and necessary antecedent of that material.

IV.

LOCALIZATION OF THE APICAL ORGAN AND ITS CORRELATION WITH THE POST-TROCHAL REGION.

The failure of the AB half-larva to produce an apical organ, though wholly consistent with the history of the lobeless embryos, was to me a surprising fact; for the development of this organ in other forms indicates that all of the four quadrants contribute to its formation; and in point of fact I had found in *Patella* that not only do both the AB and CD halves produce an apical organ, but also any of the ¼-embryos, and even any isolated micromere of the first quartet. I therefore turned with much interest to a more detailed examination of the localization of this organ in *Dentalium;* and this involved the inquiry whether the correla-

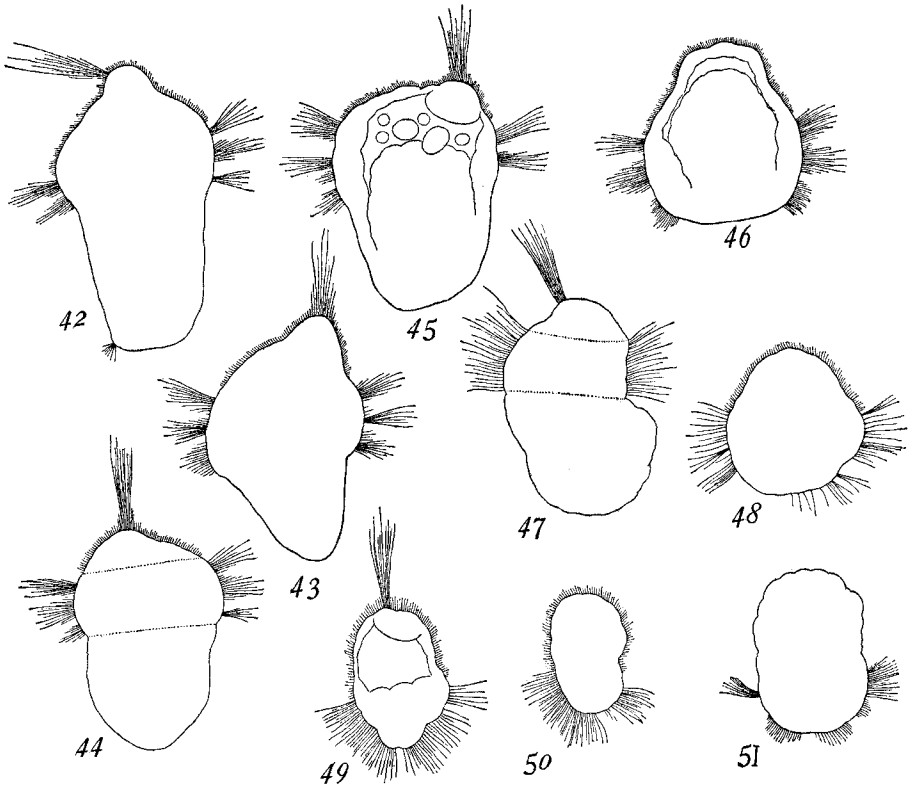


FIG. VII.

Larvae from isolated Blastomeres.

42, 43, 44, Various forms of larvae from isolated CD halves, 24 hours; 45, 46, twin larvae from the isolated CD and AB halves of the same egg, 24 hours; 47, larva from isolated D-quadrant, 24 hours; 48, larva from isolated C-quadrant of the same egg, 24 hours; 49, larva from isolated posterior micromere, 1d, of 8-cell stage, 24 hours; 50, larva from isolated micromere, 1c, of the same egg, 24 hours; 51, one-fourth larva from one of the small quadrants (A, B or C), 72 hours.

tion between apical organ and post-trochal region is direct or indirect—*i. e.*, whether the development of the one depends on that of the other, or whether the development of the two is only connected through their common relation to the polar lobe. Further experiments conclusively show that the latter is the case; for in several ways larvae may be produced that possess the apical organ but lack the post-trochal region. My first experiment to test this consisted in the isolation, separately, of the four micromeres of the first quartet (1a, 1b, 1c, 1d), which may easily be effected by means of Herbst's calcium-free sea-water. The result of this experiment, several times repeated, is that while all four of these micromeres may develop into actively swimming ectoblastic embryos, *the one derived from the D quadrant* (1d), *and this alone, develops an apical organ* (Figs. 49, 50). All of these four small embryos are of approximately the same size, ovoidal or somewhat pear-shaped in form, with a group of active trochoblasts at the larger (posterior) end. The anterior region is covered with fine cilia (as in the AB ½-larva or the A, B or C ¼-larva); but only the 1d larva bears in addition the characteristic apical tuft, which is nearly or quite as large as in a whole embryo, and is borne upon the usual ectoblastic thickening or apical plate. None of these larvae gastrulate or develop a post-trochal region; from which it follows that *after the completion of the third cleavage not only is the development of the apical organ independent of that of the post-trochal region, but at this time the posterior micromere of the first quartet,* 1d, *is already definitely specified for the formation of that organ,* independently of its relation to the remainder of the embryo. The result of isolating the cells of the 4-cell stage is entirely in harmony with this, as already mentioned. The A, B or C ¼ develops into a closed pyriform larva swimming normally with the smaller and turned forwards, but entirely devoid of apical organ or post-trochal region (Fig. 48). The D ¼, on the other hand, though often distorted, shows typically the apical organ, and an exaggerated and usually irregular post-trochal region. (Fig. 47.) This result is in striking contrast to the fact, mentioned above, that in *Patella*, each of the quadrants, whether of the 4-cell stage or of the first quartet, may develop an

apical organ. The only conclusion that can be drawn from this contrast is that the definitive basis of the apical organ is more closely localized in *Dentalium* than in *Patella*, being concentrated in a single cell.

The above results prove that the determination of the development of the apical organ takes place at some period between the first and the third cleavages. Further experiments fix the period of determination still more nearly. If the egg be allowed to advance as far as the second cleavage and the polar lobe formed at that time be removed, the egg continues to segment in a manner indistinguishable from that of an egg from which the lobe has been removed at the time of the first cleavage. *From such eggs arise larva agreeing exactly with those arising after removal of the first polar lobe in every respect save one, namely, that the apical organ is typically present, though this is not invariably the case.* (Fig. 36.) Sections clearly show that the apical tuft is borne upon a very definite apical plate, in striking contrast to the larvae arising after removal of the first polar lobe. It is thus possible to produce at will larvae which lack the post-trochal region and either possess or lack the apical organ; and *the determination of the apical organ is thus proved to be effected during the short period between the first and second cleavages.* Complete corroboration is given by removal of the second polar lobe from the isolated CD ½ during its first division. The resulting larva resembles that arising from the AB half in having no post-trochal region, but possesses an apical organ as well developed as though the polar lobe had not been removed. (Fig. 37.)

The experiments just described prove, first, that the correlation between post-trochal region and apical organ is due to their common determination by the first polar lobe. The second polar lobe, though apparently precisely similar to the first, has no longer any influence on the apical organ, though it still determines the development of the post-trochal region. It seems impossible to explain these facts, save under the assumption that the first polar lobe contains specific stuffs that are in some manner essential to the formation of both structures, and that during the period

between the first and second cleavages the "apical stuff" (if such a term be allowed) exerts once for all its specific effect. The most natural explanation of this is given by the hypothesis that this stuff moves upward to the apical pole, to be isolated in the large posterior quadrant, D, during the second cleavage, and subsequently in the corresponding micromere, 1d, during the third cleavage. The basis of correlation between post-trochal region and apical organ may thus be sought in the physical association of the corresponding specific stuffs in the first polar lobe, while the specification of the posterior micromere, 1d, is due to the final isolation within it of the "apical stuff."

V.

LOCALIZATION IN THE UNSEGMENTED EGG.

The preceding sections are in a measure only preliminary to the present one which includes the most important part of the present paper, namely, the results of experiments on the localization of the polar lobe, and of the structures that it involves, in the unsegmented egg. As has already been stated, the clear substance forming the polar lobe is already visible in the egg prior not only to cleavage, but even to fertilization and maturation. *Experiments on the unsegmented egg show with great clearness that this area possesses in a general way the same promorphological value as the polar lobe itself,* though at this early period the egg possesses a greater regulative capacity than at later stages. The unfertilized living eggs of *Dentalium* may readily be cut in two with the scalpel under the microscope, and the plane of section determined with considerable accuracy not only during the operation but by a subsequent examination of the fragments in which the polar areas are often still clearly visible. As Yves Delage first showed, such fragments when fertilized may segment and give rise to ciliated embryos and in certain cases even to dwarf trochophores. In a considerable proportion of such experiments, both fragments develop. For convenience of description I shall divide them into two classes, including (*a*) those obtained by horizontal or oblique section, and (*b*) those obtained

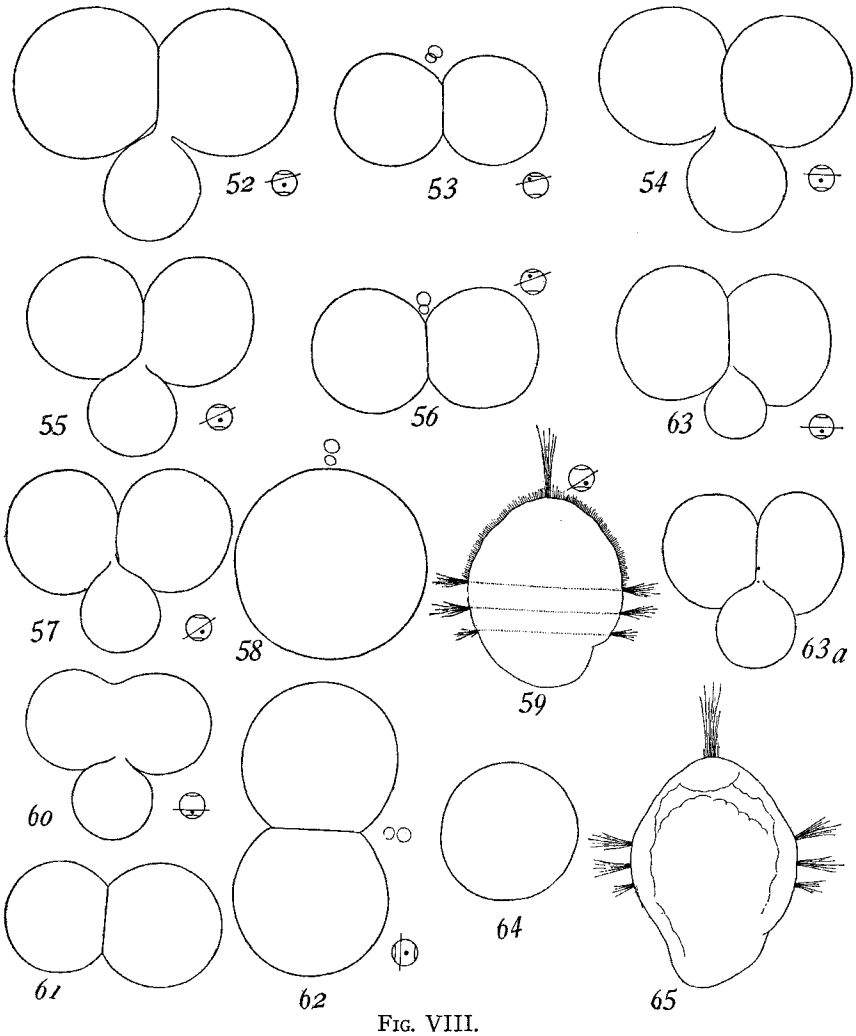


FIG. VIII.

Development of Egg-fragments after horizontal or oblique Section.[1]

52, 53, Twins, after oblique or horizontal section near upper pole; 54, trefoil, lower half, horizontal section above equator; 55, 56, twins, after oblique section, larger lower, smaller upper fragment; 57, 58, 59, twins, after slightly unequal oblique section; 57, trefoil from lower fragment, 58, upper fragment (failed to segment), 59, trochophore of 24 hours developed from 57; 60, 61, 62, twins, horizontal section below equator; 60, trefoil, lower fragment, 61, 2-cell stage of same, 62, upper fragment, 2-cell stage; 63, trefoil, lower fragment, horizontal section, polar lobe too small; 63a, trefoil lower fragment, with polar lobe slightly too large; 64, 65, twins, plane uncertain, 64 undeveloped fragment, 65 trochophore, 24 hours.

[1] In these and the following figures the plane of section is indicated by the small accompanying diagram, the fragment studied being marked with a black dot.

by exactly vertical section passing through the axis and bisecting the polar areas.

(*a*) *Fragments obtained by horizontal or oblique section.*— Under this heading may be grouped all fragments obtained by sections passing in such a plane as to separate the polar areas, so that one fragment contains only the upper, the other only the lower, of these areas. These may be designated respectively as the upper and the lower fragments. Before maturation I have not found it possible to distinguish the upper from the lower fragment; but as soon as the polar bodies form, the upper fragment may be at once identified with certainty, since it alone produces these bodies. I have not thus far observed any difference between the results of horizontal and of oblique sections.

The upper and lower fragments differ in a characteristic way, both in the form of cleavage and in the structure of the resulting larvae; though it should be added that this appears most clearly in the cleavage-process, since many of the embryos die before reaching the trochophore stage, and many of the remainder become wholly monstrous in form. Nevertheless the main result is given with great consistency by a comparison of the larvae. This contrast is especially striking when two fragments from the same egg are compared; and within rather wide limits it is independent of the plane of section and the size of the piece, certainly as far as the form of cleavage is concerned, and apparently also as regards the larval type. Whether large or small the upper fragment forms the polar bodies in normal fashion, and in many cases *segments in essentially the same way as an egg from which the polar lobe has been removed.* The first cleavage takes place without the formation of a polar lobe and is invariably equal (Figs. 53, 56, 62, etc.), and the same applies to the second cleavage. Frequently the two pairs of cells shift during or after the second cleavage, so as to produce a "cross-form," the succeeding divisions of which are difficult to analyze. In many cases, however, the four cells remain in nearly the same plane; and in such cases the succeeding divisions conform to the regular rule of spiral cleavage, quartets of micromeres being found by alternating dexiotropic and leiotropic divisions. (Fig. 69.)

A considerable proportion of these embryos fail to develop into larvae, breaking up sooner or later into loose groups of cells that perish. Many, however, develop into actively swimming larvae, but these, whether large or small, are never normal trochophores. While showing many variations, and often being more or less irregular in form, these larvae tend in general towards, and sometimes agree precisely with those derived from whole eggs minus the polar lobe, from the AB half, or the A, B or C quarters (Figs. 70, 86). They are in general more or less distinctly pyriform, swimming actively by the long cilia that are more or less irregularly disposed about the posterior enlarged region. A typical case is shown in Fig. 70 (from a preparation, the cilia from the living larva) produced from the upper two-thirds of an egg after exactly horizontal section. The cleavage of this fragment was similar to that shown in Fig. 69. This larva is in every respect closely similar to the lobeless larva, though the pre-trochal region is more expanded than usual, forming a large hollow vesicle enclosing a few loose cells, and with a slight thickening at the anterior pole, but without anything like a true apical organ. The posterior region is filled with a crowded mass of rounded cells. Transverse sections of this larva show that this mass incloses a very small central cavity; but it is impossible to determine whether mesoblast cells are present or not. In a very few cases an apical organ is present in such larvae; but this is so rare that I attribute its occasional presence to the fact that the plane of section was not quite correctly determined, a portion of the lower polar area having been in fact included in the piece. Another possibility is that the specific material of the polar lobe extends so far up into the interior as to be removed by a section that externally passes quite outside the polar area. This interpretation is supported by the fact that in a very few cases, when the upper fragment is considerably larger than the lower one, I have seen the upper fragment form a very small polar lobe.

The development of the lower fragment—*i. e.,* one that includes the lower polar area—differs in a remarkable way from that of the upper one, both in the form of cleavage and in the end-result. Whether obtained by horizontal or oblique sections,

and (within rather wide limits) whatever its size, *this fragment may segment in every detail like an entire egg of diminished size, forming the polar lobe in normal fashion, and may give rise to a dwarf larva nearly or quite normal in form and possessing an apical organ.* The study of a large number of these fragments shows that while there is considerable variation in the size of the polar lobe *it is as a rule of approximately and often exactly, of the correct proportional volume;* and this is true even after a horizontal section that passes quite outside the limits of the polar area. By varying the plane of section it is thus possble to obtain a graduated series of forms leading down from a full-sized embryo to one not more than one-fourth this size, the fragments from the other halves forming a similar series grading in the opposite direction. That the form of cleavage is within wide limits, independent of the size of the piece, is thus strikingly demonstrated. Such a graded series of trefoils and the corresponding equal 2-cell stages, is shown in Figs. 52-60, the last of these showing the smallest one observed. Regulation of the size of the polar lobe sometimes fails however, examples being shown in Fig. 63, where, even after horizontal section, the lobe is too small (this egg produced a larvae possessing an apical organ, but with the post-trochal region greatly reduced), Fig. 63*a*, where it is slightly too large, and Fig. 66, where it is much too large; but these are exceptional. It is hardly possible that this apparent regulation is owing to the fact that the specific polar material extends so far up into the interior of the egg that a section in almost any plane includes the right amount of material to form a normally proportional lobe. Such an explanation is rendered very improbable by the usual failure of the upper fragment to form a lobe even after horizontal section far down in the vegetative hemisphere or after oblique section; and still more improbable by the fact that so many of the fragments form a normally proportioned lobe, whatever be the plane of section. The conclusion therefore appears unavoidable that the size of the polar lobe, and hence of the structures dependent upon it, is subject to a regulative process, from which it follows that the *predetermination of the region of the polar lobe is qualitative, not quantitative, or if*

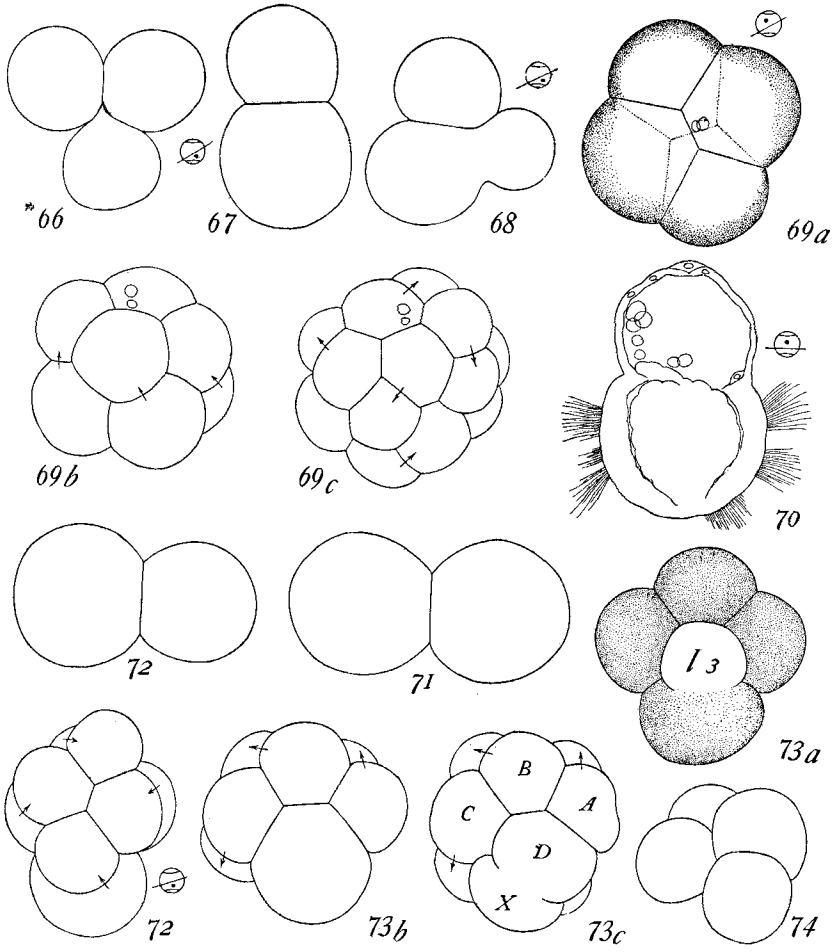


FIG. IX.

Development of Egg-fragments

66, 67, Lower fragment, oblique section; 66, trefoil, polar lobe much too large, 67 resulting 2-cell stage, CD half too large; 68, 69a,-69c, cleavage of twin fragments, oblique section, 68 trefoil, from smaller lower fragment, 69a-69c, symmetrical cleavage of larger upper fragment, 4-cell to 16-cell stages; 70, larva of 24 hours, from upper fragment of a case exactly similar to 69; 71, 72, twins, oblique section, nearly equal fragments, 71, 2-cell stage of upper fragment. 72 and 72, 2-cell and 8-cell stages of lower fragment; 73, 74, twins, probably oblique section near upper pole, 74, upper fragment, 4-cell stage, 73a, normal third cleavage of lower fragment from lower pole, 73b, resulting 8-cell stage, 73c, beginning of fourth cleavage, formation of first somatoblast.

quantitative, it is still subject to the operation of a regulative factor that lies behind the topographical distribution of the egg-materials. This appears to me one of the most significant results that my experiments have yielded.

The embryos may in succeeding stages cleave in every detail like whole eggs. Typical 4-cell stages are shown in Figs. 73a, 78b, 83, 8-cell stages in Figs. 72, 73b, 84, and the fourth cleavage, with the formation of the first somatoblast, in Fig. 73c. Fig. 73a shows the third cleavage with the formation of the third polar lobe. Many individuals were observed showing the formation of the second polar lobe in normal fashion, though none are figured.

The larvae arising from fragments of this type differ as markedly from those derived from the upper fragments as does the cleavage. Although many of the embryos perish, and of those that live many are abnormal, they frequently possess both the apical organ and a post-trochal region; and occasionally a dwarf larva is produced that is essentially similar, except in size, to an entire trochophore. One of the best of these is shown in Fig. 59, which arose from a lower fragment obtained by oblique section, slightly smaller than half the volume of the egg, and including the whole of the polar area. The typical trefoil stage of this larva is shown in Fig. 57; it has exactly the normal proportions, and segmented normally in later stages. This larva is somewhat less pointed posteriorly than the normal, but the whole larvae vary considerably in this regard. It swam in quite normal fashion. Another larger larva from a lower fragment in shown in Fig. 65. The total preparation of this larva shows with great clearness a typical apical plate at the upper pole. Out of a very large number of operations I have obtained altogether not more than five or six such perfect larvae, at least half the embryos dying during the cleavage, and a large proportion becoming abnormal during the later development.

That so large a proportion of the embryos die or develop abnormally is to be expected when we consider the very different mechanical conditions of surface-tension and the like in these small embryos. The fact remains that abnormal larvae may be pro-

duced from lower fragments less than half the size of the egg; and that such larva may possess a typical apical organ when the section passes far away from the apical pole; while in no case does the upper fragment produce a larva that ever approaches the normal form. It may therefore safely be concluded that the dwarf trochophores obtained by Yves Delage ('99) arose from fragments including at least a part of the lower polar area.

The abnormalities observed in larvae from the lower fragments range from only slight defects to wholly irregular and monstrous forms, and thus far do not permit any more detailed conclusions regarding the prelocalization than those stated above. A common defect, illustrated by the pair of twins shown in Figs. 85, 86, is a more or less imperfect development of the post-trochal region, even when the whole lower area is included in the fragment, and sometimes this region appears to be wholly lacking. Much more rarely the apical organ is lacking while the post-trochal region is in greater or less degree developed. Such a case is shown in Fig. 87 (from a preparation), the absence of the apical tuft having been certainly determined in the living larva.

As in the case of the lobeless larvae, the experiments demonstrate that the failure of the upper fragment to produce the missing structures is not due to an insufficient mass of protoplasm; for I have obtained larvae showing the characteristic defects from upper fragments fully two-thirds the bulk of the egg (Fig. 70), and perfect dwarfs from much smaller fragments (Fig. 59). The conclusion is therefore unavoidable that, like the polar lobe to which it gives rise, *the lower polar area contains specific materials that are essential for the formation of the apical organ, and of a post-trochal region;* and that it is these materials that enter into the formation of the polar lobe, as simple observation of the normal development indicates.

(*b*) *Fragments obtained by vertical section through the axis.* —In view of the foregoing results we should expect to find that when the egg is cut exactly vertically, so as to bisect the lower polar area, both fragments should form the polar lobe; and such is in fact the case. The experiments of this type were not very numerous, and only a few cases were obtained in which both frag-

ments developed. I have only one pair of camera sketches to show the polar lobes in such a case (Fig. 75, 76). In both these the lobe is relatively too small, as if produced from insufficient material; but this not always the case (as shown beyond), and it should be remembered that the polar lobe is sometimes too small even in a lower fragment containing the whole of the lower polar area (Fig. 63). Figs. 77a, 78a show a pair, one of which has a lobe of normal proportions; the other is a very nearly normally formed 2-cell stage, though the larger cell is perhaps a trifle too small. Both these produced nearly normally proportioned 4-cell stages (Figs. 77b, 78b). Several other cases, in which only one fragment developed, showed a normal trefoil. These data are somewhat meagre, yet they justify the conclusion, I believe, that after vertical section bisecting the lower polar area both fragments may segment like whole eggs of half size.

The above conclusion renders it probable that by such vertical section two perfect dwarf trochophores may be produced from a single egg, which is apparently impossible when one fragment alone contains the lower polar area. In point of fact, I have never obtained even a single wholly normal larva after such section; but in view of the comparatively small number of successful operations and the very small number of such larvae obtained by section in other planes this is not surprising. A number of larvae from more or less nearly vertical sections is shown in the following figures. Fig. 88 is a nearly normally formed larva with two apical organs, from an oblique section passing outside the lower white area. Fig. 89 is a nearly normal larva from a section that removed a part of the lower area. Fig. 93 is from an exactly vertical section bisecting both areas. In section this larva is closely similar to a normal one, and seems to show that the trochoblasts are as large as in a whole embryo. Fig. 90 is from the smaller fragment after a slightly oblique section bisecting the lower area; a very distinct apical organ is present and also an abnormally formed post-trochal region. Figs. 91, 92 are twins from a slightly unequal vertical section (developed from the respective twin fragments 81, 82), the post-trochal region is lacking in both, while one lacks an apical organ.

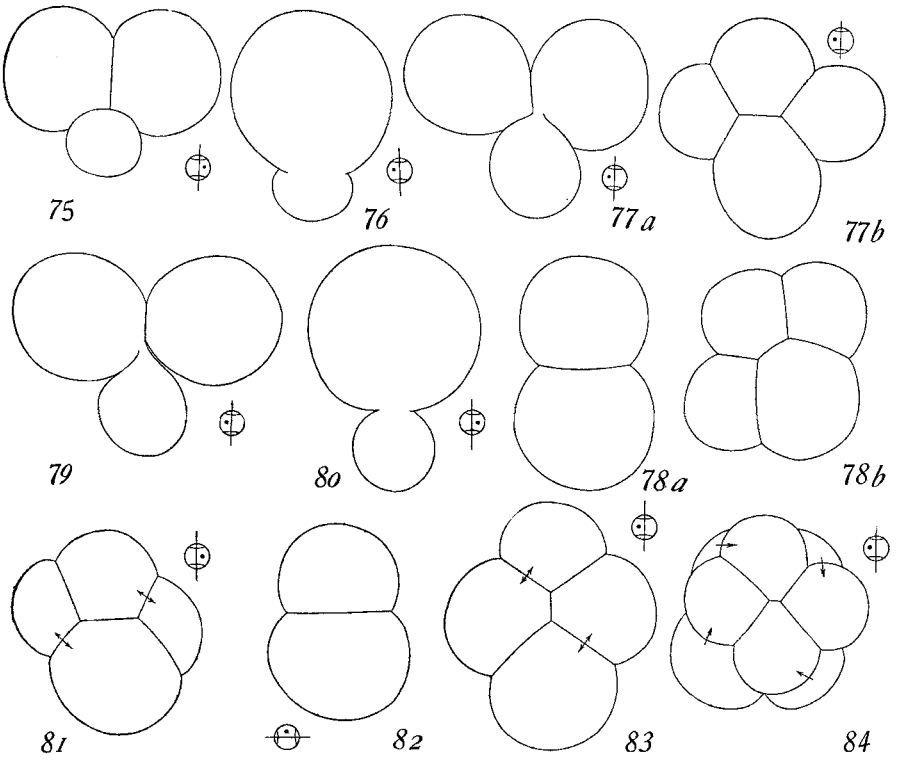


FIG. X.

Development of Egg-fragments after vertical Section.

75, 76, Equal twins, respectively in trefoil and polar lobe-formation; lobes too small; 77, 78, equal twins, nearly correct proportions; 77a, 77b, typical trefoil and 4-cell stages of one fragment; 78a, 78b ,typical 2-cell, slightly abnormal 4-cell stages of the twin fragment; 79, 80, twins, from a fertilized egg, 79, nearly normal trefoil, 80, the twin, with reduced polar lobe; 81, 82, nearly equal twins, 81, typical 4-cell stage, 82, its twin, nearly typical 2-cell stage; 83, 84, typical 4-cell and 8-cell stages, from upper pole, of the same fragment.

It may be pointed out that not one of these larvae shows a fully developed post-trochal region, though 91 and 92 arose respectively from 2- and 4-cell stages that show nearly the normal proportions and must have been produced from nearly normal trefoils. This may seem to contradict the conclusion, drawn above, that the predetermination of the lower polar area is not quantitative; but a similar reduction sometimes exists in this region when the whole polar area is present (as in Fig. 85), and I do not think a trustworthy conclusion can be drawn without additional data.

I may add that after a large number of unsuccessful attempts I obtained two nearly normal dwarf trochophores from fragments of the unsegmented egg of *Patella*. One of these, which is about half the volume of a normal larva, clearly shows the cells of the prototroch. In the full-sized normal trochophore of *Patella* the prototroch, as may be seen with the greatest clearness in total preparations, consists of a closed principal ring of cells that vary in number (as seen in optical section) from 19 to 21. In the dwarf the cells are more variable in size and less regularly arranged, but on the average as large as in the normal individual; equatorial optical section of this larva shows 13 cells in the principal ring.

V I.

OBSERVATIONS ON FRAGMENTS OF THE FERTILIZED EGGS AND ON THE ISOLATED LOBE.

Extremely interesting and curious results are obtained by a comparison of the behavior of fragments of *fertilized* eggs, and of the isolated polar lobe, with that of fragments of the unfertilized eggs described above.

(*a*) *The behavior of fragments of fertilized eggs obtained before cleavage.*—In order to make sure that the eggs were fertilized the operation was delayed until one or both polar bodies had been formed, and the egg was then cut as nearly as possible horizontally, so as to separate the lower polar area from the nucleated part. As already described, if this operation be performed on the unfertilized egg, and the two fragments be fertilized, both may, and frequently do, develop. When, however,

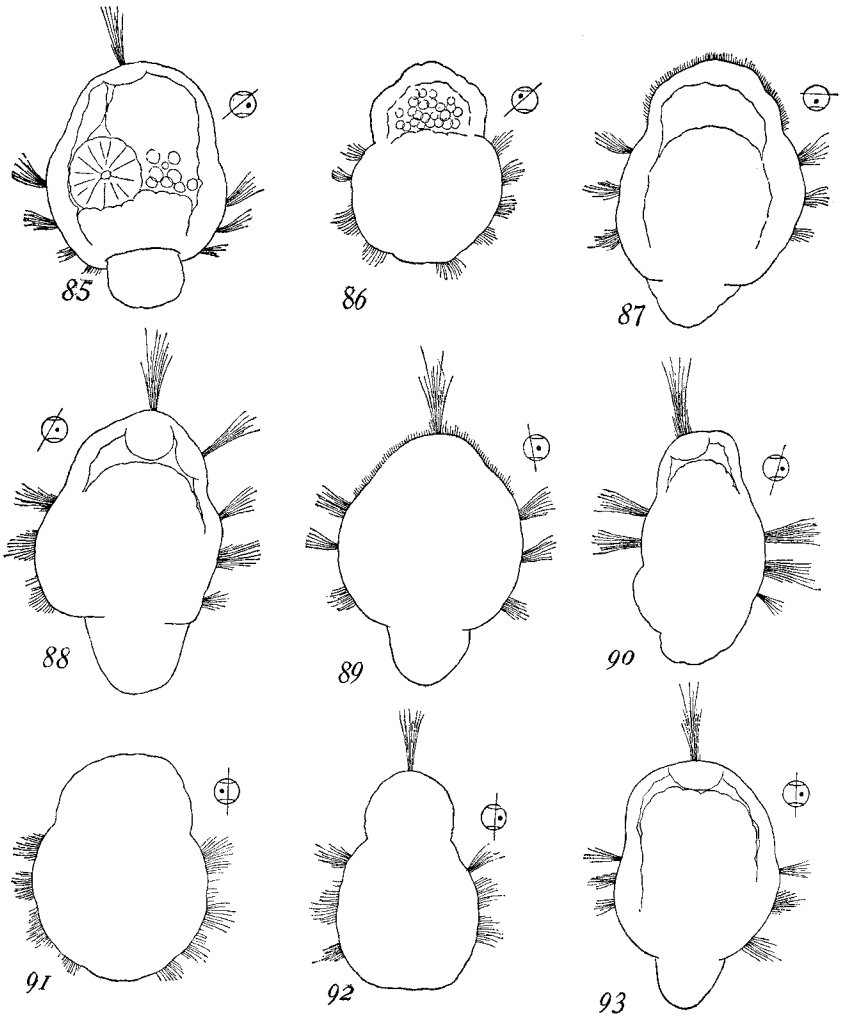
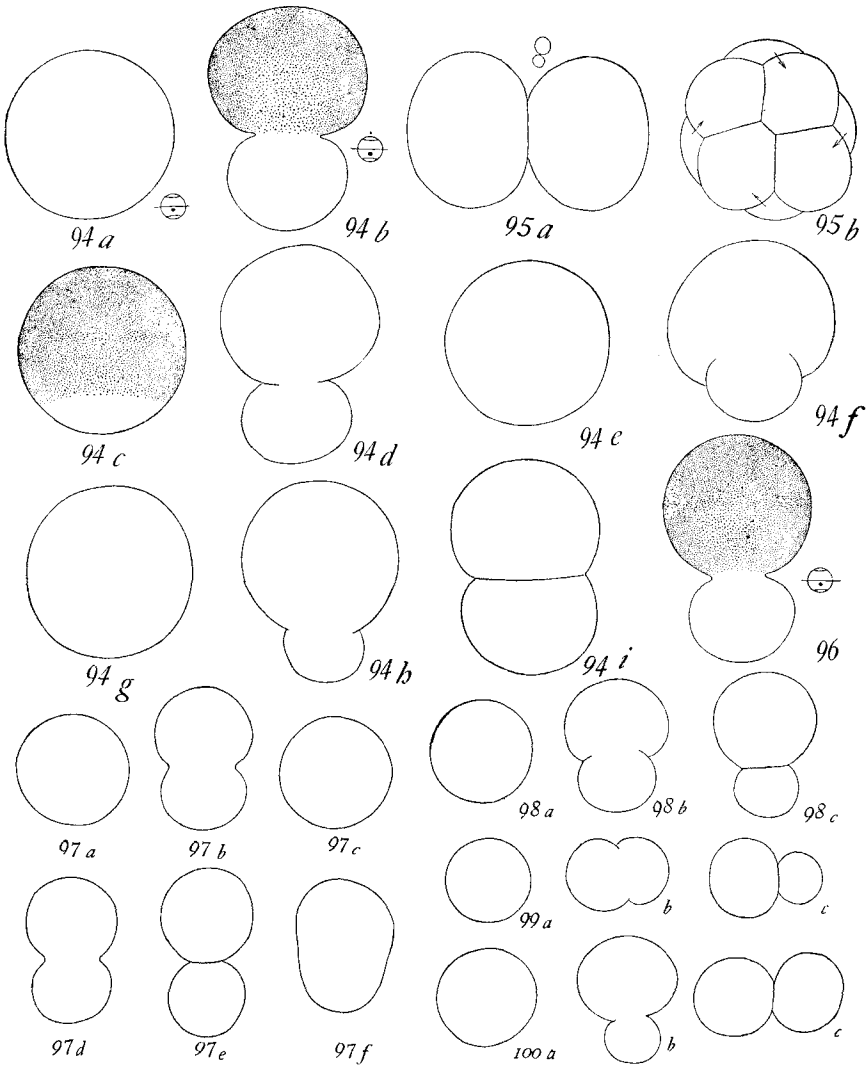


FIG. XI.

Larvae of 24 Hours, from Egg-fragments.

85, 86, Twin larva of 24 hours, oblique section passing outside lower polar area, 85, the lower, 86, the upper larva; 87, larva from lower two-thirds, horizontal section, without apical organ; 88, larva from lower two-thirds, oblique section, two apical organs; 89, larva from nearly vertical section; 90, larva from smaller fragment, slightly oblique section bisecting lower area; 91, 92, twin larvae, produced from 81 and 82 respectively, vertical section; 93, larva from exactly vertical section.


94 a
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97 f
99 a
b
c
100 a
b
c

FIG. XII.

Fragments of fertilized Eggs, horizontal Section; isolated Polar Lobes and Fragments of Lobes.

94, 95, Equal twins from the same egg; 95a, 95b, upper half, 2- and 8-cell stages; 94a-94i, successive changes in the lower enucleated fragment; 94a, soon after operation; 94b, first polar lobe (drawn immediately before 95a); 94c, first resting stage, upper fragment in 2-cell stage (23 m. after b); 94d, second polar lobe (21 m. after c, the upper fragment just divided into 4); 94e, second rest (16 m. after d); 94f, third polar lobe (16m. after last, at nearly the same time with 95b); 94g, third rest (7 m. after last); 94h, fourth lobe (46 m after last, fourth cleavage in progress in upper fragment); after 16 minutes the fragment appeared to be divided into two and so remained; 94i, the same four hours later; 96, lower fragment, like last, but showing correct proportions of first polar lobe; 97, successive changes in isolated polar lobe from the individual shown in Fig. 21; 97a, soon after removal; 97b, first active period (16 m., the egg just divided into 4); 97c, ensuing first resting period (14m. after b); in the second period of activity (not sketched), 15 m. later, as the eggs divided into 8, the lobe constricted as in b, but not so deeply, and again became spherical in a second resting period; 97d, e, third period of activity, 74 and 78 m. after the first period; 97f, final result, 36 m. later; 98, another isolated lobe, 98a, third resting period; 98b, second lobe; 98c, final result, in which condition it remained without further change; 99, 100, two fragments obtained by cutting a polar lobe in two (the original lobe was slightly larger than usual), showing active changes shortly after division of the egg into 4; 99a, 100a, are shown 42 m. after 99 and 100; 99b, 100b, 8 m. later; 99 remained in this condition, while 100 again became spherical by fusion of the two halves and underwent no further change.

a fertilized egg is thus sectioned only the nucleated (*i. e.*, the upper) fragment develops—a result that agrees with my observations on the nemertine egg and that of *Renilla* ('03), and with the earlier ones of Delage ('01) on those of echinoderms. This fragment has essentially the same mode of development as a corresponding fragment of an unfertilized egg, segmenting equally into two and four without the formation of a polar lobe, forming successive symmetrical quartets of micromeres by alternating spiral cleavages (Fig. 95), and producing a larva that is either an irregular monster or a pyriform larva closely similar to those arising from the lobeless egg or the AB half. This is what would be expected in view of the preceding results; but the behavior of the non-nucleated lower half is most remarkable in that *it forms three times in succession a polar lobe from the white area at the same time that the nucleated half is dividing, becoming spherical after each period of activity without dividing.* When this was first observed, I believed that I must in some way have confused the fragments with those of unfertilized eggs; but repetitions of the experiment under conditions that precluded all error, gave the same result. A typical case is shown in Fig. 94, from consecutive camera drawings of the same fragment. The first lobe is shown (Fig. 94b) about 15 minutes after the operation, while the nucleated half (Fig. 95) has just divided into equal halves. Twenty-three minutes later the fragment was again perfectly spherical (94c), while the upper fragment was in a resting 2-cell stage. The second lobe (94d) was formed 44 minutes after the first, while the upper fragment was dividing into 4 equal cells, after which the lower fragment again became spherical (94e, 16 minutes later than 94d). The third lobe (94f) was formed 32 minutes after the second, and was considerably smaller than either the first or the second, as in a whole egg; the upper fragment meanwhile divided into eight cells (Fig. 95b). A third period of rest followed (Fig. 94g). Following the fourth cleavage of the upper fragment the lower one passed through a change no less remarkable than the preceding (it is at this period in the normal development that a large part of the lower polar area passes into the first somatoblast). This

change begins with the formation of a fourth lobe, composed of white material, which is at first much smaller than any of the preceding (94h, 46 minutes after 94g). Unlike the preceding lobes this one was not resorbed into the fragment, but was permanent, slowly increasing in size until after two or three hours it was nearly as large as the remaining portions, the fragment now appearing as if divided into two (94i).

This case is fairly typical of several that were followed through the entire cycle of changes, and one or more of the stages were seen in many individuals. The lobes are not always so distinctly formed as in the one figured, and the final stage, though usually like that described, varies considerably in appearance.

(*b*) *Behavior of the isolated polar lobe.*—Previous to making the observations just described, I had several times observed changes of form in tne isolated polar lobes after their removal from the trefoil stage. On reëxamining the matter I found that these changes are also periodic, *taking place approximately at the same time as the cleavage in the lobeless nucleated portion.* The activities of the isolated lobe at these periods vary considerably in different individuals. Sometimes the activity is no more than a slight change of form, the spherical lobe becoming slightly pyriform or even almost amoeboid. Frequently, however, *the isolated lobe actually forms a smaller lobe by a process that closely simulates the formation of a polar lobe by a whole egg or an egg-fragment.* In any case, each period of activity is followed by a spherical resting-stage that coincides approximately in time with the resting stages of the segmenting lobeless portion. I regret that I had not time to study this remarkable phenomenon with sufficient care, but give series of sketches illustrating two particular cases. Fig. 97a shows a lobe soon after its removal; 97b, the same, 16 minutes later just after the egg had divided into four; 97c, the ensuing resting stage, 14 minutes after 97b; a second period of activity followed, in which the lobe again constricted, but not so deeply as at 97b, followed by a second spherical stage; 97d and 97e show the third active period, and 97f the final result, after which no further change occurred. In 98 is shown the final active period of a lobe, which resulted in the permanent apparent

division of the lobe into two. Even if the lobe be cut in two after its removal, the fragments likewise pass through alternating periods of activity and rest closely similar to those of the whole lobe, as is shown in Figs. 99, 100 (the original lobe was somewhat larger than in the other cases shown). This proves that the power of a rhythmic change of form involving the temporary formation of lobe-like structures, is not a property of the lobe as a whole, or of the lower polar area, but is inherent in the substance of which it is composed. It would be interesting to compare in this respect the behavior of the isolated lobe, or fragment of a lobe, with fragments from other regions of the fertilized egg. Such fragments would probably also exhibit rhythmic changes, but I hazard the conjecture that their activity would be found to differ in some definite way from that of the lobe-fragments.

The phenomena above described, which deserve further careful study, are of interest both cytologically and embryologically. First, since both the nuclei and the centrosomes are absent, it follows with great probability that even in the cleavage of a whole egg the constriction of the cell that leads to the formation of the polar lobe takes place wholly independently of either these structures or the astral rays, which suggests the possibility that the same may be true of the constrictions that lead to complete cell-division. Second, since the rhythm in the formation of the polar lobes in the enucleated fragment coincides with that shown in the division of the nucleated fragment, it is clear that as far as the lobe-formation is concerned the cytoplasmic division rhythm is quite independent of that of either the centrosome or the chromosomes. This fact may be placed behind the one earlier determined by Boveri ('97), Ziegler ('98) and myself ('01), that the rhythmic activities of the chromosomes and of the centrosomes are likewise independent, or at least separable. But beyond this it is remarkable that the periodic activity in the non-nucleated fragment is not merely of a rhythmic character, *but changes its character at the time of the fourth cleavage* when in the normal development the material of the polar lobe no longer forms a merely temporary structure, but is permanently cut off by a cell-division. We here catch a glimpse, as it were, of a

definite order of events predetermined in a particular cytoplasmic area and wholly independent of the immediate action of nucleus or centrosome. An additional point of great embryological interest is the fact, shown by a comparison of Fig. 6 with Fig. 94, that in these fragments the polar lobe is, at least in some cases, nearly or quite as large absolutely as in one entire egg; whereas in the lower fragment of an unfertilized egg it is typically reduced to the correct proportional volume of the lobe in a whole egg. This is however not invariable, for in some cases, an example of which is shown in Fig. 96, the lobe is reduced to its proper proportional size. I have not accurately studied this matter in a sufficient number of cases to speak very positively; yet I feel confident that the contrast in this respect between the lower fragments from unfertilized and fertilized eggs is a general, though not an invariable rule. The interest of this fact is pointed out in the sequel.

VII.

COMMENT.

Without undertaking at this time a complete discussion of the foregoing observations, I may briefly indicate their bearing on the general questions referred to at the beginning.[1] My observations demonstrate conclusively, I think, both the mosaic character of cleavage in these eggs, and the definite prelocalization of some of the most important morphogenic factors in the unsegmented egg. The *Dentalium* egg shows, even before it breaks loose from its attachment in the ovary,and long before even the initial changes of maturation, a visible definite topographical grouping of the cytoplasmic materials. This is proved by the experiments to stand in definite causal relation to the subsequent differentiation of the embryo in such wise that the removal of a particular cytoplasmic area of the unsegmented egg results in definite defects in the resulting embryo that are not restored by regenerative or other regulative processes within the time-limits of the experiment. Since both the egg-fragments and the isolated blastomeres

[1] A more general discussion of the mosaic-theory of development, with a fuller review of the literature, will be given in a following paper.

become perfectly spherical before development proceeds, the resulting defects cannot be due to a failure of regulation traceable to the shape of the fragment, as was formerly assumed by several writers. Neither are they due to insufficient mass; for perfect dwarfs may arise from fragments much smaller than those that show the characteristic defects. Further, these facts, like those earlier determined by Crampton ('96) in the gasteropod egg, and by Driesch and Morgan ('95) and more recently by Fischel ('98) in the ctenophore egg, are fatal to the view that embryonic differentiation is brought about through qualitative nuclear division during the cleavage. The conclusion is therefore unavoidable that the specification of the blastomeres in these eggs is due to their reception, not of a particular kind of chromatin, but of a particular kind of cytoplasm; and that the unsegmented egg contains such different kinds of cytoplasm in a definite topographical arrangement. How many such specific stuffs exist in the unsegmented egg of *Dentalium* and what is their arrangement it is impossible at present to say; for the pigment-band and the two polar areas can only be considered as an outward sign of an organization that for the most part doubtless escapes the eye. My experiments have only positively determined the cytoplasmic prelocalization in the lower polar area of material essential for the development of that complex of structures that I have included in the term "post-trochal region," and of one other structure, the apical organ. The first of these includes material that is essential to the development of the typical larval form, including the foot, to certain characteristic ectoblastic structures of the post-trochal region, such as the shell-gland, mantle-fold, and probably also the pedal ganglia; it also appears probable that it includes material essential for the formation of the cœlomesoblast. I do not doubt that further experiments on this egg will show a still more definite and detailed prelocalization; though, as already stated, it is not easy to determine this, owing to the difficulty of distinguishing between defects in the partial larvae that result directly from the plane of section and those that are due to other causes.

Two additional facts clearly appear from the experiments, on which I would lay stress. First, the amount of material removed

with the polar lobe or lower polar area is wholly disproportionate to the effect produced. The polar lobe includes less than one-fifth the volume of the egg; yet its removal does not merely cause a structural defect of like extent, but inhibits the whole process of growth and differentiation in the post-trochal region and the concomitant withdrawal of the pre-trochal region. The cleavage of the lobeless embryos shows that both the second and the third quartets are formed; and it is fair to conclude that certainly in the AB half of the embryo, and probably also in the CD half, these cells contain ectoblastic material, which in a normal embryo would contribute to the formation of the post-trochal region. These cells, as stated above, close in around the posterior region, and perhaps are partially turned in with the invaginating entoblast-cells. In any case, however, the power of active growth in the post-trochal region, so conspicuous in the normal larva, is wholly lost with the removal of the excess of material in the D quadrant. It does not seem possible that this loss in power of growth is due to mechanical obstacles, since the same defects exist in fragments of the unsegmented egg from which the lower polar area has been removed and which are free to segment as best they can. The conclusion therefore appears unavoidable that the material of the lobe is not only specifically necessary for the formation of the bases of the post-trochal structures, but also for the whole growth-process that is here brought to a focus. Apart from its more general bearings, this conclusion is important from the light that it may throw on the teloblastic growth of annelids and other segmented forms, and it seems altogether probable that if the polar lobe could be removed from such an egg as that of *Sabellaria* or *Myzostoma* the resulting larva would fail to develop a metameric trunk-region.

A second point of interest that clearly appears from the experiments is that the topographical grouping of specific materials in the unsegmented egg may be in its *ensemble* widely different from that of the definitive bases of the organs which they determine; for the experiments demonstrate that the apical organ, lying at the upper pole, is determined by material originally lying far down in the vegetative hemisphere in the lower polar area.

On this point an analogous result has recently been obtained by Yatsu, who has shown with great probability that in the unsegmented nemertine egg the basis of the apical organ does not lie at the upper pole, where we should expect to find it, but in, or slightly above, the equatorial region.

These facts have an important bearing on our interpretation of development in general. In my previous paper on the nemertine egg I have developed an hypothesis of differentiation agreeing broadly with Sach's well-known theory of formative stuffs, and with the general conclusions regarding mosaic development independently published by Fischel ('03) nearly at the same time, the essential assumptions being that the prospective value of a cell is determined by its cystoplasmic content, that this content is determined by the form of cleavage in connection with an antecedent formation and segregation of specifically different materials (which may itself determine the form of cleavage), and that the morphogenic function of cleavage, so to say, is to isolate the materials thus segregated.' This conception, it is hardly necessary to point out, receives very definite support by the observations now brought forward; but I wish to bring them more closely into relation with those made on the nemertine and echinoderm eggs, especially with regard to the general question of progressive (*i. e.,* epigenetic) localization in the egg. In the nemertine (*Cerebratulus*) I found that either an isolated blastomere or a fragment from any region of the unsegmented egg may produce a perfect dwarf larva; but the two differ in the form of cleavage, the blastomere segmenting as if still forming part of a whole embryo and producing an open blastula (as in the echinoderm), while the egg-fragment segments like a whole egg and produces a closed blastula—that is, it develops as a whole from the beginning. I explained the contrast in development between the two as the result of a regrouping of the egg-materials, occurring during and subsequent to the process of maturation and fertilization, which initiates the morphogenic process and determines also the form of the earlier cleavages. I pointed out that such regrouping of materials is known to occur at the maturation-period of many eggs—for instance, in the sea-urchin—and suggested

that the contrast between the development of an egg-fragment in the nemertine and in a sea-urchin (where it segments like a whole egg only after section in certain planes) is owing to the fact that in the latter, egg-fragments have only been obtained in the period subsequent to maturation when the regrouping has been effected. Localization of the cleavage-factors was thus conceived, essentially in agreement with Roux's early conclusions regarding the frog's egg, as a progressive (*i. e.*, epigenetic) process, and the same conception was applied to the general morphogenic process which, as is shown with especial clearness by the facts here brought forward, may be so closely connected with the cleavage-process.

As far as the progressive character of localization is concerned, the result obtained in *Dentalium* may seem at first sight to be in disagreement with the conclusions just reviewed, for the germ-regions are here defined by a definite segregation of materials that exists even in the attached ovarian egg long before either maturation or fertilization, and the isolated blastomere is not capable of producing a complete embryo. But the contradiction disappears upon comparison with certain other forms, which are intermediate in character between the extremes represented by *Dentalium* and the nemertine or echinoderm egg; and this comparison demonstrates, as I believe, the validity of the theory of "precocious segregation," formulated as a pure speculation by Ray Lankester in 1877. I have already expressed the opinion that the horizontal stratification of the egg expressed by the three zones of material visible in *Dentalium* or *Myzostoma* is comparable, or at least analogous, to that which finds an expression in the formation of the well-known polar rings of leeches and oligochaetes. This comparison is based both on the position and mode of formation of these rings and on their fate. Vejdovsky ('88) very clearly shows that in *Rhynchelmis* both the polar rings arise as local thickenings of a general ectoplasmic layer, and both assume at one period the form of protoplasmic discs lying at either pole of the egg (as Whitman also observed in *Clepsine*). Except for the fact that the upper and lower protoplasmic areas have not at any period been seen to appear in the form of actual

rings, the resemblance to these relations of those observed in *Dentalium* is unmistakably obvious. It is entirely possible that the correspondence is not complete; but that in a general way the resemblance indicates a similar form of stratification in the molluscan and annelidan egg, seems hardly open to question; and the comparison is sustained by the fact that in *Clepsine* both rings were traced by Whitman into the AB half, and the upper one into the D quadrant, while in *Rhynchelmis* Vejdovsky traced both rings into the D quadrant, where the material of the two fuses into one mass in the 4-cell stage and later *passes into the mesomeres,* which are undoubtedly to be identified with the somatoblasts.[1]

If this comparison be admitted a further comparison of these and some other forms is highly significant. In *Dentalium* three structural zones are present from the beginning, the lower one coinciding in extent with the lower white area, the upper one lying at the centre of the upper white area, at first very small, but rapidly increasing in extent during and after the maturation period. A condition similar to this exists in *Sternaspis,* where Vejdovsky ('81) showed that a distinct protoplasmic area, *which he compares to a polar ring* ('88, p. 122) lies at each pole of the ovarian egg, the upper one being much smaller than the lower one, though larger than in *Dentalium*.. In *Clepsine* and *Rhynchelmis* three structural zones are likewise present, *but these first appear during the maturation period* with the development of the polar rings, like the three zones described by Boveri ('01) in the *Strongylocentrotus egg.* The egg of *Myzostoma* occupies, at least in some respects, an intermediate position. No upper protoplasmic disc has here been observed as yet, but the lower protoplasmic area is obviously represented by the green mass, which, as Driesch ('96) has shown passes into the polar lobe, and subsequently certainly in part into the first somatoblast, and probably in part into the second somatoblast, precisely as in *Dentalium.* The interest of this case, compared with the foregoing, lies in the fact observed by Driesch (which I can confirm) that before ma-

[1] *Cf.* Vejdovsky and Mrazek ('03, p. 454); see also the highly interesting statement (p. 534) that the dense protoplasm of the polar rings ("Polplasmen") can be recognized as such "in den Zellen des Mesoblasts inbesondere in den grossen Mesomeren."

turation the egg shows at first but two colored zones, of which the lower green one exactly represents the lower white area of *Dentalium,* while the upper one first segregates during maturation into an upper red zone and an equatorial colorless one. Like the lower zone the two upper ones correspond very closely in fate to those in *Dentalium;* for the upper (red) area passes into the ectomeres, like the upper white area of *Dentalium,* while the middle (colorless) zone passes into the entomeres, as is the case with the greater part of the middle (pigmented) zone in *Dentalium.* It is possible that sufficiently careful search may reveal the presence in *Myzostoma* of an upper protoplasmic disc, comparable with a polar ring; and as far as the visible colored zones are concerned, it is evident that the *Myzostoma* egg stands midway between those of *Dentalium* and *Strongylocentrotus,* and it is probably intermediate also between *Dentalium* or *Sternaspis* and *Clepsine* or *Rhynchelmis.*

It seems a legitimate interpretation of the foregoing series that these eggs present an essentially similar form of stratification *which is attained at different periods in the ontogeny,* and that as compared with the leech or oligochaete, *Myzostoma* and *Dentalium* or *Sternaspis* represent two earlier stages in the precocious segregation of specific cytoplasmic materials that have a like prospective value in the development.[1] But if this be admitted, it follows that in none of these cases can the segregation in question be considered as a primary character or "preformed quality" of the egg. Upon this secondary localization of material, as my experiments prove, depend many of the most important features of the later morphogenic localization; and I think a presumption is thus established that cytoplasmic prelocalization is in general of like secondary or epigenetic origin, though to what extent this holds true can only be determined by further experiment.

Although the characteristic segregation is in its main outlines effected very early in the egg of *Dentalium,* it may be pointed out that, like so many other eggs, there is the clearest evidence of

[1] *Cf.* Vejdovsky "Während aber bei Sternaspis die Concentration des Bildungsplasma an beiden Polen bereits im Laufe der Eibildung stattfindet, sammelt sich dasselbe bei Rhynchelmis erst nach der Polzellenbildung und dem Eindringen des Spermatozoön in das Ei an" ('88, p. 122.)

later movements and progressive segregation of the cytoplasmic materials. I will only call attention, among these, first, to the determination of the apical organ by material originally lying in the lower polar area, which, if my interpretation of the experiments is valid, moves upwards to the apical pole in the period between the first and second cleavages. That such a movement occurs is only a matter of inference; but this interpretation appears to me far simpler and more intelligible than to assume a brief "Fernwirkung," or the like emanating from the first but not the second polar lobe. It is however not a matter of inference but of fact that the remaining material of the lower white area moves upwards and towards one side in the 8-cell stage preceding the fourth cleavage, when it apparently fuses with the material of the upper white area in the D-quadrant. It is interesting to compare this with the facts described by Vejdovsky in *Rhynchelmis,* where the remains of the upper and lower polar rings fuse in the D-quadrant at the 4-cell stage.

I have endeavored to show that cytoplasmic prelocalization in *Dentalium* differs only in degree from the conditions existing in such eggs as those of the nemertine or sea-urchin. The same may be said, I think, of the development of isolated blastomeres, despite the fact that in *Dentalium* such blastomeres are incapable of producing complete dwarf embryos. As in the nemertine or sea-urchin, although the isolated blastomere segments as a part and not as a whole, the embryo finally closes, in the course of which process structures like the prototroch, the post-trochal and pre-trochal regions, and the gut, close to form whole structures. That this process, which in the case of the nemertine I compared to Morgan's "morphallaxis" in regenerating planarians or hydroids, falls short of producing a complete embryo in *Dentalium,* may be due to different causes in different cells. In the AB half or one of the smaller quarters this is obviously due in the main to lack of the specific material of the lower polar area. The failure of the CD half or the D quarter may in part be due to a like cause; but since these embryos contain the materials (those contained in the lower polar area) that are missing in the other cases, their failure may be due to a different cause. The CD half

larvae are sometimes nearly normally formed except for the false proportions of the post-trochal and pre-trochal regions. Their invariable subsequent degeneration into irregular and monstrous forms is not improbably due to the abnormal mechanical conditions created by their mode of development. It seems possible, however, that if these larvae could sustain themselves sufficiently long they might in some cases succeed in attaining a normal condition. They die before attaining this end; and hence succeed no better than the AB halves in the "attempt" to produce a perfect embryo.

One cause of the difference between the isolated blastomeres of the nemertine or sea-urchin and the mollusk thus doubtless lies in a difference in the segregation-pattern such that in the former the specific materials are symmetrically divided between the first two blastomeres, while in *Dentalium* such is not the case. In the former, accordingly, the earlier cleavages are purely quantitative, but in the latter are qualitative as far as the cytoplasm is concerned, and to this extent produce from the first cleavage onward a mosaic-work in entire accordance with Roux's general conception, as I long since indicated in the case of *Nereis* ('94). But beyond this the results especially of Driesch's later studies on the isolated blastomeres of sea-urchins indicate that here, although a definite polarized segregation of material has taken place at the time of the earlier cleavages (directly proved by Boveri's ('01) observations on *Strongylocentrotus,* indirectly by Driecsh's ('00) comparison of the development of the upper and lower quartets of the 8-cell stage) this segregation is not only symmetrical with respect to the axis but is also less definite or less complete than in the molluscan egg,— again a difference which finds its natural explanation in the theory of precocious segregation (or differentiation). I should therefore interpret the differences between the isolated blastomeres of the mollusk and those of the sea-urchin or nemertine as due to a difference, on the one hand, in the pattern, on the other hand in the degree, of segregation.

It is hardly necessary to point out that the foregoing conclusions will in large measure reconcile the apparent conflict between the fact of cytoplasmic prelocalization and the continually increasing

evidence that the primary determining factors of development are to be sought in the nuclear organization. The well-known hybridization experiments of Boveri ('92, p. 469) and Driesch ('98) on sea-urchins have shown that the earlier cleavage-factors conform to the maternal type and hence must be predetermined in the egg-cytoplasm; and up to the blastula-stage, at least, the embryos remain of the pure maternal type. But the same experiments demonstrate no less clearly that the nucleus begins to affect the cytoplasmic phenomena at least as early as the late (prismatic) gastrula, and according to Boveri's latest work ('03) as early as the mesenchyme-formation, though the latter point is disputed by Driesch ('03). It therefore appears possible, not to say probable, that every cytoplasmic differentiation, whether manifested earlier or later, has been determined by a process in which the nucleus is directly concerned, and that the regional specifications of the egg-substance are all essentially of secondary origin.

Another question, which has been often discussed, is raised by these observations, namely, as to the relation in the regenerative process between the moulding of the mass as a whole (which falls under the general conception of Roux's "Umordung der Zellen" or Morgan "morphallaxis) and the specification of the individual cells. Like the facts determined by Fischel ('98) in the ctenophore egg (following the earlier work of Driesch and Morgan) those observed in *Dentalium* bring out with great clearness the independence, in this case, of the two groups of factors by which these are determined. It is a very noteworthy fact that all the partial larvae that lack the lower polar area, whatever their size or mode of origin, tend to assume the same form, and all are alike devoid of further regenerative capacity. The larvae arising from entire eggs after removal of the polar lobe only, the CD half from which the second polar lobe has been removed, the AB half, the A, B or C quarter, or an upper fragment, of any size, of the unsegmented egg—all these typically assume the characteristic pyriform shape with the trochoblasts surrounding the larger posterior end. This form, which results after closure of the embryos and gastrulation, is essentially a prolate

spheroid modified by the presence at one end of the large trochoblasts which have not like the other cells the power of continued multiplication, and it evidently represents a state of equilibrium towards which any segmented mass of the egg tends that is devoid of the lower polar area. Whether the closure of the embryos (which in the case of isolated blastomeres are at first strictly partial structures) to produce this form should be considered as a regulation or regenerative process is largely a question of definition.[1] In any case the facts very clearly show that the process is not perceptibly influenced by the nature of the cells individually considered; nor does it, on the other hand, appear to exert any appreciable effect on the nature of the individual cells ("Umdifferenzierung" of Roux), as will be more clearly shown in my second paper.[2] Certainly the closing of the embryos does not lead to the least perceptible tendency towards the restoration of the missing structures that are dependent on the material of the lower polar area.[3] I am in agreement with the opinion of Fischel ('98) that, whether a regulative process or not, the closing in to form a closed structure is probably explicable as a result of relatively simple physical factors, though I doubt whether the explanation is as simple as Fischel assumes in the case of the ctenophore.[4] It is difficult to avoid the conclusion that these same factors are operative in the establishment of the normal form in a whole embryo; but to them are add in the material of the lower polar area a far more complex group of factors, at present not analyzable, that involve the whole process of growth and metamorphosis. That a mass of cytoplasm so small should

[1] Roux ('93, p. 837) interpreted the closure of the open blastula as part of the regenerative process, in opposition to Driesch ('92, p. 585), who asserted that this had nothing to do with the regenerative process proper; though he afterward took the ground that it should be considered as an initial regulative process ('96, p. 88). Morgan ('01, p. 13, etc.) classes morphallaxis under the head of regeneration, though not the closing in of a cut surface, which is considered as a preliminary process. Cf. Child, on "Mechanical Regulation" ('02).

[2] *Cf.* Crampton, '97, p. 55.

[3] *Cf.* the remark of Driesch, based especially on Crampton's experiments on *Ilyanassa;* "Ist, wie bei Gastropoden und Anneliden, echte Lokalisation der Bildungsfaktoren im Ei anzunehmen, so schliesst das eine Regulation zum Ganzen wirklich aus." ('96, p. 89.)

[4] *Cf.* Rhumbler, '02, Zur Strassen, '03.

exert so great an effect on the morphogenic process is a most convincing piece of evidence in favor of the theory of specific formative stuffs in development. The only intelligible view of the polar lobe seems to me to be that it is, so to say, a reservoir of such stuffs destined for allotment to particular cells which thereby become definitely specified, irrespective of their subsequent relation to the embryo as a whole. This is a very different result from the oft-quoted one of O. Hertwig that the lineage of particular structures from particular blastomeres is nothing more than an incidental result of the continuity of development. It is equally opposed to the conclusions of other writers who have too hastily rejected the principle of mosaic development for which Roux and others have contended.

Lastly I may point out that in so far as these observations show the course of differentiation, and the correlation of parts, to be determined by a preëxisting topographical grouping of specific egg-materials they sustain an essentially mechanistic (as opposed to a vitalistic) interpretation of development. To conclude however that these eggs are devoid of regulative capacity would be to overlook some of the most striking of the phenomena I have described. The experiments give clear evidence that a power of regulation exists in the unsegmented egg that is no less striking in form, if more limited in degree, than in the nemertine or echinoderm. As in the case of the nemertine, the typical spiral cleavage, alternately dexiotropic and leiotropic, is not affected by section in any plane. Far more striking is the fact that *in the cleavage of an egg-fragment the size of the polar lobe, on which the proportions of the trochophore largely depend, is proportional to the size of the piece.* Since this is true even after horizontal section, when the whole of the lower polar area is included in the piece,it follows that *the predetermination of this area is qualitative, but not quantitative,* or only quantitative in so far as it is subject to regulative control by other factors. This conclusion receives further support from the one reached above that the material of the lower polar area is as such specifically concerned not merely with the formation of the structures that arise from it but with the form of growth that results in the metamorphosis. But if this par-

ticular area shows such a qualitative, as distinguished from a quantitative, pre-determination, one is led to suspect that a like conclusion may apply to other egg-regions, such as those that form the gut, the prototroch, and the like; and to conclude that however detailed a prelocalization may exist in the form of regional segregations of material, a regulative factor may always be present that controls their normal combination. In this respect the unsegmented egg, may, I believe, be directly compared with such an adult animal as a planarian or hydroid, which, while possessing more or less definitely specified tissues, in a typical grouping, nevertheless may possess a high regulative capacity shown in the process of regeneration after injury.

The facts observed give as little clue to the nature of the regulative factors by which the quantitative relations are determined in the egg-fragment as in the fragment of a planarian or hydroid; but one or two considerations deserve brief mention. It is noteworthy that although the polar lobe regularly forms in the non-nucleated vegetative half of a fertilized egg it is as a rule, though not always, *not reduced,* but nearly or quite as large as in a whole egg, whereas in a fertilized fragment, representing the same region of an unfertilized egg, the lobe is as a rule reduced to its proper proportional volume. While I would not lay too much stress on this without further study, it seems to indicate that the power of regulation, on which the size of the lobe depends, is more complete in a nucleated fragment than in an enucleated one. Second, when once the polar lobe has formed, the power of regulation seems to be lost, at least temporarily; for if a part of it be cut away the second lobe is of correspondingly reduced size, as is also the post-trochal region of the resulting larva. This result is supported by the fact that, like the post-trochal region to which it gives rise, the polar lobe in the first (virtual second) division of the isolated CD half, though sometimes slightly reduced, is in general nearly or quite as large as in a whole embryo. These facts prove that the size of the lobe is not determined merely by the size of the piece, but by more complex conditions existing apparently for only a brief period, and apparently also more effective in a nucleated than in a non-nucleated protoplasmic

mass. This sufficiently indicates the complexity of the problem with which we are dealing, and the importance of further more precise studies of the facts. At the same time, it seems clear that the problem of proportionate development in a fragment of an organism here appears in a much simpler form than in a blastula-fragment, or a piece of an adult organism such as a planarian or a hydra; and I think we should not abandon the hope of finding for it a relatively simple solution. While I am not able to offer such a solution, it seems to me that it would be rash to deny its possibility, not merely in the present instance, but in all analogous processes, even when they take place under the more complex conditions existing in multicellular masses.

VIII.

SUMMARY.

1. The *Dentalium* egg shows from the beginning three horizontal zones, an equatorial pigment-zone and two white polar areas. Each of the polar areas includes a specially modified protoplasmic area probably comparable to a polar ring.

2. During cleavage the pigmented zone is allotted mainly to the entomeres, the upper white area to the ectomeres, the lower white area to the first and probably also the second somatoblast. At the first, second and third cleavages the lower white area temporarily passes into the "yolk-lobe" or polar lobe.

3. Removal of the first polar lobe leads to a symmetrical cleavage without the subsequent formation of polar lobes, and to the formation of a larva devoid of post-trochal region and apical organ. Removal of a portion of the first lobe produces a larva with reduced post-trochal region, and with or without apical organ. Removal of the second polar lobe produces a larva without post-trochal region but with an apical organ.

4. The lobeless larvae undergo no metamorphosis, form no foot, shell-gland or shell, no mantle-folds, no pedal ganglia, apparently no mouth, and probably no cœlomesoblast-bands.

5. The isolated AB half or A, B, or C quarter, produces a closed larva closely similar except in size, to the lobeless ones. The isolated CD half or D quarter produces a larva possessing a

post-trochal region as large as in a normal larva, and an apical organ, which dies without undergoing metamorphosis. The CD half from which the second polar lobe is removed produces a larva like that from an AB half, but possesses an apical organ.

6. The isolated micromere 1d produces a mass of ectoblast-cells bearing an apical organ, while 1a, 1b, 1c produce no apical organ.

7. Fertilized fragments of the unsegmented unfertilized egg, obtained by horizontal or oblique section, differ in development according as they do or do not contain the lower white area. The upper fragment segments symmetrically without the formation of polar lobes and produces a larva similar to the lobeless ones. The lower one segments like a whole egg of diminished size, and may produce a normally formed dwarf trochophore.

8. Fragments obtained by vertical section through the lower white area may segment like whole eggs and may produce nearly normally formed dwarf trochophores.

9. Enucleated fragments, containing the lower white area, of fertilized eggs, pass through alternating periods of activity and quiescence corresponding with the division-rhythm of the nucleated half, and form the polar lobes as if still forming part of a complete embryo. The same is true of the isolated polar lobe.

10. The foregoing observations demonstrate the prelocalization of specific cytoplasmic stuffs in the unsegmented egg and their isolation in the early blastomeres. The lower white area contains such stuffs that are essential to the formation of the apical organ and the complex of structures forming the post-trochal region, including the shell-gland and shell, the foot, the mantle-folds and probably the cœlomesoblast. These stuffs are contained in the first polar lobe, but the second lobe no longer contains those necessary for the basis of the apical organ. Progressive changes therefore occur in the original distribution of the specific cytoplasmic materials.

11. Comparison indicates that the conditions observed in the molluscan egg differ only in degree from those in the nemertine or echinoderm. These differences reduce themselves to differ-

ences in the period of segregation (or differentiation) and in its pattern, and are explicable under the general theory of precocious segregation.

12. The early development of egg-fragments indicates that the specification of the cytoplasmic regions is primarily qualitative, but not quantitative, or if quantitative is still subject to a regulative process that lies behind the original topographical grouping of the egg-materials.

13. The development of the molluscan egg is in its essential features a mosaic-work and sustains the theory of "Organbildende Keimbezirke."

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