THE NATURE AND ORIGIN OF YOLK.*

Experimental Studies of the Oöcytes of *Helix aspersa* and *Patella vulgata*.

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The advance of modern cytology has necessitated the division of the cytoplasmic inclusions into two groups, the protoplasmic inclusions or living organellæ capable of spontaneous growth, division and movement within the cell, and the deutoplasmic inclusions or non-living reserve materials of the cell built up by the activity of the living protoplasm, representing a stage in the anabolic processes of the cell. The Golgi apparatus and the mitochondria both belong to the former group and appear, in the present state of our knowledge, to be specially associated with cell anabolism and often, if not always, in oögenesis with the special case of yolk formation. Sometimes the yolk is produced indirectly by the action of these organellæ, at other times either the Golgi elements or the mitochondria swell up and themselves transform into yolk spheres. In the latter case it is difficult to decide whether an element at any given stage of the transformation process should be considered a deutoplasmic or a protoplasmic body.

Although our knowledge of the chemistry of the bird's egg is the most complete, in them vitellogenesis is so complex morphologically that we are at present unable to synthesise these two lines of study and produce a reasonably clear account of the different processes concerned, with the parts played by the various cytoplasmic inclusions. On the whole, however, vertebrate yolk seems to contain three chief substances—protein, fat, and lipin. This form of yolk may be present often in invertebrate eggs, but in many cases other deutoplasmic constituents are also present. It seems probable

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that these various kinds of yolk may be formed in many different ways, directly or indirectly, from the Golgi apparatus, mitochondria, or nucleolar material, or independently in the ground cytoplasm. The term should therefore be qualified whenever possible by a description of its apparent composition and of its origin, and might be expressed as Golgi-yolk, nucleolar-yolk, etc. Dr Gatenby suggests that these terms might be conveniently shortened to M-yolk, G-yolk, N-yolk, and C-yolk, to designate mitochondrial, Golgi, nucleolar, and ground-cytoplasmic-yolk respectively. It is well to bear in mind that several of these species of yolk may be present in the one cell.

Among zoologists there seems to be much mental confusion concerning the processes of vitellogenesis in different Mollusca. This has arisen partly owing to the lack of a clear definition of the term "yolk" and the various categories of cytoplasmic inclusions to which it is applied, and partly because the different cytoplasmic elements vary in the relative importance of the parts they play in its formation even among closely related forms. The part played by the yolk and other cytoplasmic inclusions in segmentation and early embryogeny is of much interest, especially in view of the experimental work of Conklin and others on the effects of centrifuging the eggs of Mollusca. Professor J. Brontë Gatenby suggested this work in the hope that further light might be thrown on the origin of yolk in molluscan oögenesis and its significance and importance in segmentation. It is a great pleasure to me to be able to take this opportunity of thanking him not only for suggesting the work, but for his advice and criticism in carrying it out.

1. Summary of Previous Work.

Conklin[2]* in an important paper dealing with the effects of centrifuging molluscan eggs, especially those of Physa, Lymnæa, and Planorbis, describes the eggs at time of laying. They then contain a large spherical transparent germinal

* I would like to take this opportunity of expressing my indebtedness to my friend, Mr A. Subba Rau, of University College, London, for preparing a "résumé" of this paper for me.

vesicle which is slightly excentric. The remainder of the egg is more opaque from the presence of yolk granules and a diffuse yellow pigment uniformly distributed. At no stage do the egg-contents orient themselves with respect to gravity. The germinal vesicle dissolves and the first maturation spindle appears, the former giving rise to a deep clear "well" of protoplasm leading from the surface of the egg at the animal pole into the centre. A finely granular yellow substance surrounds the clear protoplasm. The clear protoplasm forms a cap at the animal pole during the extrusion of the polar bodies and then spreads over the entire upper hemisphere of the egg. Cleavage results in the micromeres (or ectomeres) containing little, if any, of the yellow substance of the egg, all of which is left in the macromeres, in the subsequent division of which, to form the endoderm and mesoderm, it is distributed to all the cells. Thus the quantity of clear substance relative to the yellow substance increases greatly from maturation to gastrulation. This change Conklin considers to be due in large part to the transformation of yellow substance into clear substance.

Conklin showed that three distinct layers were formed in the egg by centrifuging the eggs at a speed calculated to produce a pressure of approximately 600 times that of gravity. These were an upper grey layer of light substance, a middle clear layer, and a lower yellow layer of heavy substance. Before the first maturation division the yellow substance composes at least one-half of the entire egg, but before the first cleavage it only composes one-eighth. In strongly centrifuged eggs the yellow substance consists almost entirely of yolk spherules. The grey substance is small in quantity and finely granular before maturation but later becomes more abundant and coarsely granular or alveolar. The ease with which the layers can be sharply separated decreases from maturation to the first cleavage. Conklin, by measuring the time taken by centrifuged eggs at various stages to orient themselves, deduced that a change in relative weight of the oöplasmic substances also took place between maturation and cleavage. Further, Conklin was able to conclude that the chief axis of the egg is fixed at all stages and may not be

altered by the shifting of the egg substances or maturation spindles or the point of extrusion of the polar bodies. It is also evident that the injurious effects of centrifuging increase rapidly from the time of the first maturation to that of the first cleavage, and Conklin suggests that this is due to (1) Increasing differentiation of the egg, and (2) decreasing opportunities for readjustment of the displaced substances. Conklin says :—

"Finally, these experiments show that the differently coloured substances of these eggs are not 'organ-forming' in the sense that each can give rise to only one organ or set of organs. In the normal development the clear and grey substances are largely contained in the micromeres or ectomeres, the yellow substance in the mesomeres and entomeres. But in the centrifuge eggs the stratification of these substances may take place in any axis and yet the form of development may be perfectly normal in every case, and young snails may hatch from eggs and live indefinitely. Indeed the grey material may be thrown entirely out of the egg without interfering in the least with its normal development."

"In the study of these eggs one cannot avoid the impression that both grey and yellow substances are mere inclusions in the protoplasm, and that neither are essential to development. The clear substance on the other hand seems to be the real protoplasm of the egg in which the heavier and lighter inclusions are contained: and when these inclusions are separated from the protoplasm by centrifugal force, the latter is still capable of typical development."

Morgan[7] concludes from centrifuge experiments on the eggs of Cumingia that "the visible substances of the egg that can be centrifuged are not organ-forming." He considers that normal development may follow centrifuging, and that abnormal development results from the mechanical difficulties set up and not directly by the segregation of the visible substances.

Gatenby[4] working on *Limnæa stagnalis*, describes the cytoplasm of the ripe egg as frothy, owing to the presence of a very large number of vacuoles. The substance of these vacuoles is almost entirely absent in the finished sections, but leaves behind a slight coagulum. He thinks that these vacuoles are probably watery, but might be oily, and that the coagulum may be a protein substance. The vacuoles appear late in oögenesis but are always present in the ripe ovarian egg. They are speedily used up in development.

He also describes "yolk spheres," mitochondria, and Golgi grains, lying in the ground cytoplasm between the vacuoles. His diagram of the structure of the cytoplasm is reproduced in fig. 1. The mitochondria contain a yellow pigment (a lipochrome?) and in the full-grown egg become enlarged by the addition of some lecithin or other fatty matter. Gatenby separated the inclusions into three layers in the egg by

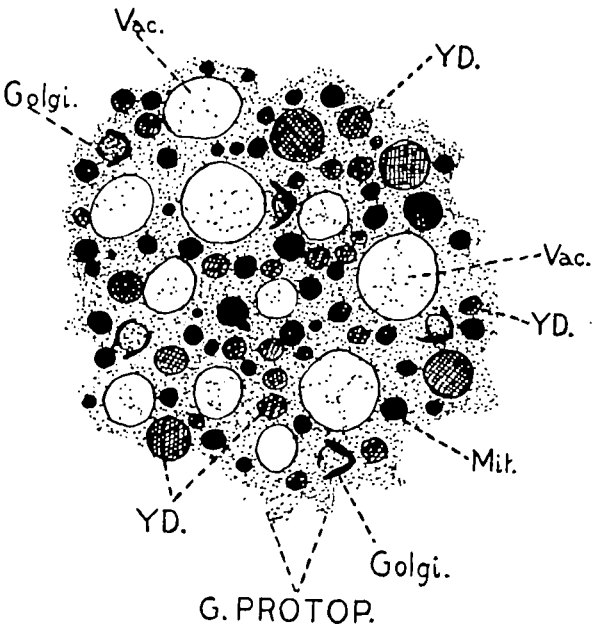

FIG. 1.—Diagrammatic high-power drawing of a part of the cytoplasm (*Limnæa stagnalis* (Linn.)) to show its structure. The cytoplasm is vacuolated, and the vacuoles (Vac.) contain a coagulum. In the regions between the vacuoles is protoplasm (G. Protop.) containing yolk-granules (Y.D.), mitochondria (Mit.), and Golgi elements (Golgi). (After Gatenby.)

centrifuging (fig. 2). He showed that: (1) The upper grey substance consists of yolk-discs and a very little protoplasm; (2) the middle "clear substance" is pure protoplasm without any inclusions; (3) the lower "yellow substance" consists of the bright yellow mitochondria, and of the Golgi apparatus suspended in protoplasm. He pointed out that the diffusion of the inclusions segregated by the centrifuging is very rapid. In reference to the previous author's work he says:—

"Conklin found the grey zone (which I consider yolk) could sometimes be completely disrupted from the egg without affecting subsequent development.

The fact that this grey substance is yolk explains Conklin's experience. Conklin is wrong, I believe, in considering the yellow zone contains 'yolk.' It consists entirely of yellow mitochondria and of the Golgi apparatus, together with protoplasm."

Gatenby and Woodger[5] in a paper "On the Relationship between the Formation of Yolk and the Mitochondria and

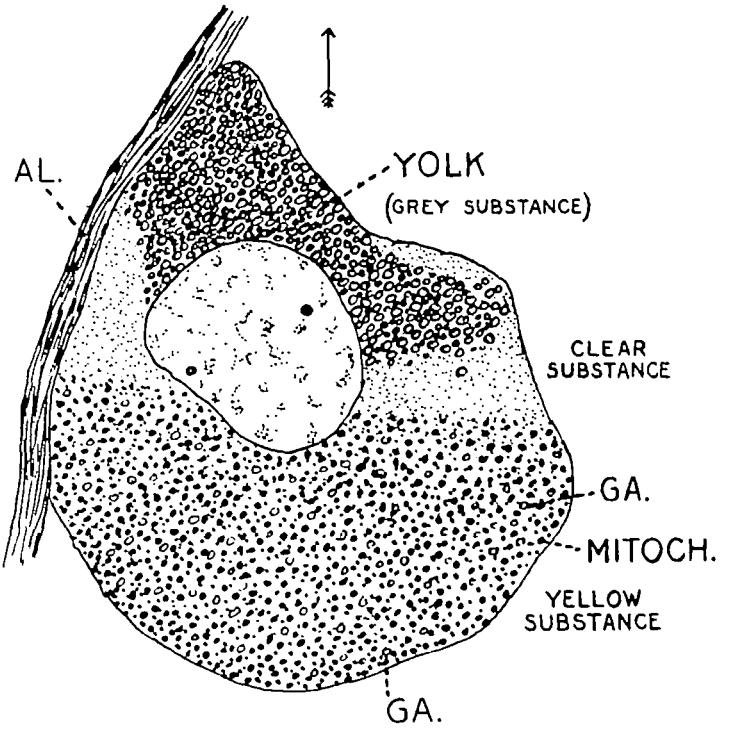

FIG. 2.—Camera lucida drawing of a centrifuged ovarian oöcyte (*Limnæa stagnalis* (Linn.)). G.A., Golgi apparatus ; A.L., Ancel's layer of ovotestis wall. (After Gatenby.)

Golgi Apparatus during Oögenesis" give an interesting summary of their work on Mollusca. They state:—

"It has been shown that in such a mollusc as Helix or Limnæa the mitochondria and Golgi elements gradually spread out throughout the oöcyte, and the grains forming these systems increase in number. It seems quite probable that the diffuse Golgi elements actively take part in the formation of yolk bodies; we cannot say so much in the case of the mitochondria. From a study of a number of Pulmonate Mollusca we have concluded that much of the evidence in these forms is against the view that part of the mitochondrial constituents of the cytoplasm metamorphose into yoke. The latter seems to form either from Golgi elements, or *per se* in the ground cytoplasm."

These authors also state that the Golgi apparatus takes a more important part in deutoplasmagenesis in Patella than in Helix or Limnæa, and that whether the yolk spheres are formed from mitochondria, or the centrosphere, or in the ground cytoplasm, the Golgi elements become stuck upon their surfaces. They state :—

"We believe that in the case of Patella the Golgi apparatus provides most of the yolk spheres of the full-grown egg, but in Limnæa either the mitochondria or the ground cytoplasm are most active in this respect."

Ludford[6] confirms Gatenby and Woodger's results on Patella. In the young oöcyte the Golgi apparatus consists of the usual batonettes lying on the archoplasmic sphere. As the cell grows some of the rods spread out in the cytoplasm, while the sphere and the pieces of archoplasm probably attached to each of the separate rods become loaded with fat and represent the initial stages of yolk formation. The individual elements divide rapidly as they spread out in the cytoplasm and lead to the formation of yolk spheres in the following manner: In many places several Golgi rods collect together and the cytoplasm between them becomes more stainable under their influence. Gradually this stainable cytoplasm swells up into a yolk sphere with the Golgi rods applied to its surface and almost completely surrounding it. This process of yolk formation continues throughout oögenesis. As each yolk sphere is completed the majority of the surrounding batonettes break away from it and fragmenting pass, either to the periphery, where they form a layer under the vitelline membrane, or towards the nucleus, around which they form another layer. Some, however, of the batonettes remain attached to the yolk spheres. The mitochondria do not become active in the growing oöcyte until the process of yolk-formation is well advanced. They do not fuse with the Golgi elements or become swollen to form yolk bodies; the latter staining differently and always being considerably larger than the largest mitochondria.

2. Material and Technique.

This present research was carried out with a view to clearing up the questions connected with vitellogenesis in the Mollusca, and more especially to decide whether the mitochondria take any part in the process. Another aim was to decide the composition of the upper grey layer of Conklin's centrifuged eggs, the removal of which he claims does not effect development.

The material studied was the hermaphrodite gland of *Helix aspersa* and the ovary of *Patella vulgata*. The research was carried out during the winter months. In the case of Helix the head was cut off and the hermaphrodite gland quickly excised and placed in its own lymph. In Patella a drop of ether was placed in the gill cavity, the shell broken with a hammer, and the ovary quickly excised and placed in salt solution. The gonads were then centrifuged for half an hour at approximately 3000 to 4000 revolutions per minute, the radius of revolution being $5\frac{1}{2}$ in., thus producing an acceleration of 2000 times gravity. When the centrifuge was stopped the gonads were speedily transferred to the various fixatives and slightly teased to ensure rapid penetration. The Mann-Kopsch, Flemming-without-acetic-acid, Champy, and Da Fano techniques were employed and produced excellent results. Some of the Mann-Kopsch preparations were treated with turpentine to remove the fat; some of these and also some untreated preparations were stained with crystal violet. The chrome-osmium material stained well with iron-hæmatoxylin followed by orange G., or with the Champy-Kull method.

3. The Centrifuged Oöcytes of Helix.

On examination of the material it was seen that there were three distinct layers formed in the mature oöcytes which were clear cut and sharp, showing little tendency to merge into each other (fig. 3). The upper of these did not constitute more than 10 per cent. of the whole egg. The middle layer contained the nucleus, the lower layer constituted

about 50 per cent. of the egg. The nucleolus was seen to be flattened out on the lower side of the nucleus, sometimes pressing so much on the nuclear membrane as to cause it to bulge out towards the lower or heavy end of the cell. The lower layer was found to consist almost entirely of

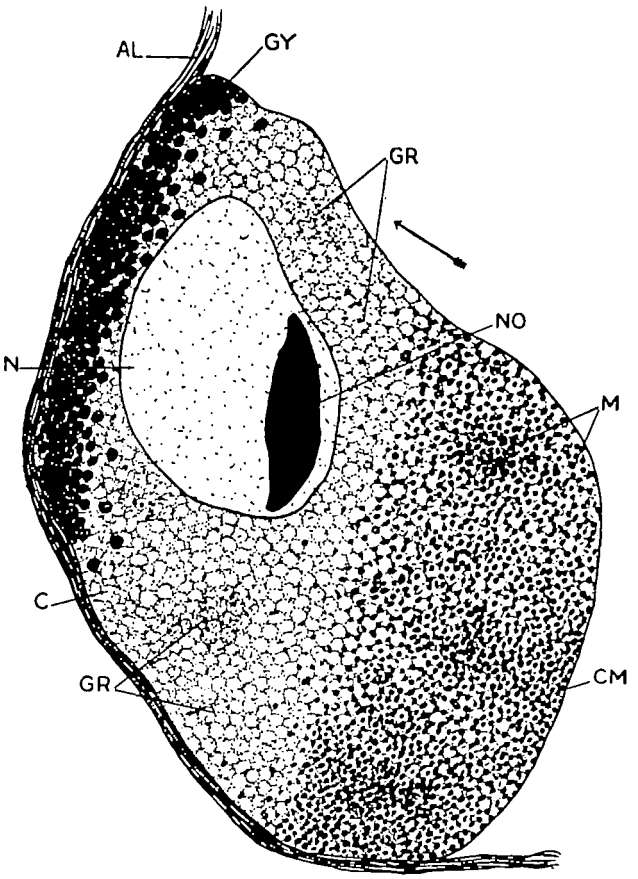

FIG. 3.—Diagrammatic drawing of a centrifuged ovarian oöcyte of *Helix aspersa*. (Original.) The arrow points to the top of the cell. G.R., Golgi rods; N., Nucleus; C., Ground cytoplasm; C.M., Cell membrane; G.Y., Golgi-yolk; N.O., Nucleolus; M., Swollen Mitochondria; A.L., Ancel's layer of ovotestis wall.

mitochondria which were absent from the other layers. They appeared as a grey-brown layer in the unstained Mann-Kopsch preparations. In the stained preparations they took the crystal violet strongly. In Da Fano preparations this layer was brown and partially vacuolated. In Champy-Kull preparations it took the fuchsin strongly, and went black in

Champy-iron-hæmatoxylin preparations. The Golgi apparatus, showing as black granules, in the Da Fano and Mann-Kopsch preparations remained scattered throughout the cell and did not become separated into a definite layer. Except for its share of Golgi elements the middle layer was free of cytoplasmic inclusions, consisting of the ground cytoplasm alone. The upper layer consisted of spheres which go black or dark grey in Champy's fluid, but only appeared as vacuoles in Da Fano preparations. These did not show up in the large oöcytes in my Mann-Kopsch preparations.

The Golgi apparatus in the growing oöcytes of Helix consists of scattered rod-shaped granules which in places form groups or clouds in the cytoplasm. In my Mann-Kopsch preparations they are beautifully shown, and in the younger oöcytes small spheres are also present stained with the osmic in different shades of grey to black. Every gradation can be found between these and the definitive Golgi elements, which they also resemble in distribution. I have no doubt that these are formed by a direct metamorphosis of the individual Golgi rods, by a process of swelling up of the elements owing to the formation or deposition of some substance in them. That these small spheres in the young oöcyte ultimately produce the spheres in the mature oöcyte, which constitute the upper or light layer of centrifuged cells, is clearly shown by the Champy, and to some extent by the Mann-Kopsch preparations, every grade between the two being demonstrable.

4. The Centrifuged Oöcytes of Patella.

In the centrifuged mature oöcytes of Patella, as in those of Helix, three distinct layers were formed (fig. 4). Of these the upper was much the largest, constituting about 75 per cent. of the whole egg and containing the nucleus, while the lower or heavy layer only occupied about 10 per cent. This heavy layer was found to consist almost entirely of mitochondria as in Helix. The upper layer consisted chiefly of spheres which blackened sometimes but not always in Mann-Kopsch, and did not blacken in any of my Flemming-without-acetic-acid preparations, but appeared in these and in the Da Fano preparations as vacuoles.

Mitochondria were also present in considerable numbers in this layer, apparently being entangled and retained in the interstices between the deutoplasmic spheres. The mitochondria do not blacken in the Mann-Kopsch technique, but they stain intensely with crystal violet afterwards. They do not blacken in Flemming's fluid without acetic acid, but if this method

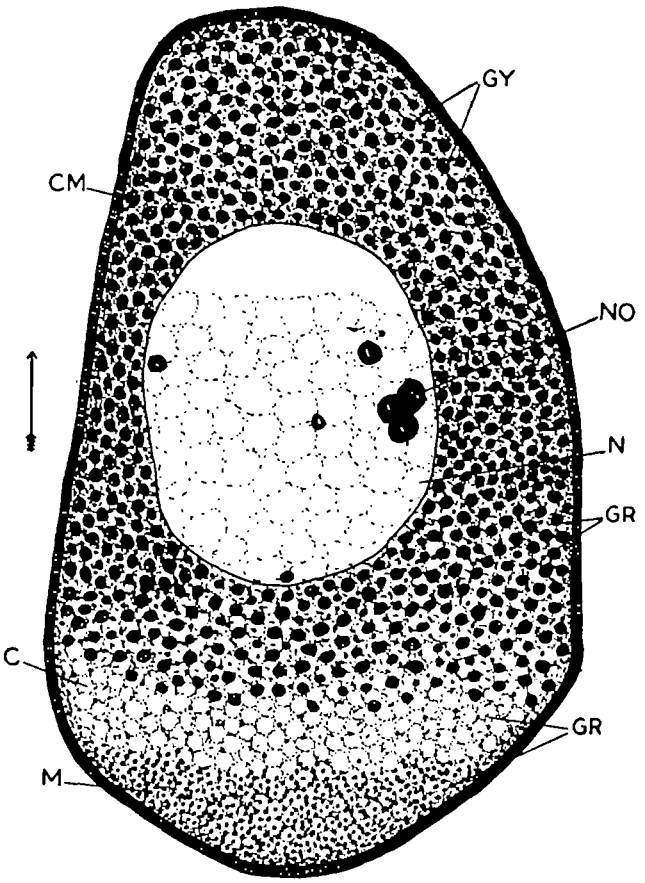

FIG. 4.—Diagrammatic drawing of a centrifuged ovarian oöcyte of *Patella vulgata*. (Original.) The arrow points to the top of the cell. Lettering the same as fig. 3.

is followed by Champy-Kull staining they take the fuchsin strongly, and they also stain well in iron-hæmatoxylin. In untoned Da Fano preparations the mitochondrial layer appears dark brown, badly fixed, and partially vacuolated. On toning with gold-chloride the colour changes to blue-black, easily distinguishable from the clear black of the Golgi.

The middle layer consisted of ground cytoplasm which stained with orange G. in the F.W.A. iron-hæmatoxylin technique, and with toluidine blue when the same fixative was followed by Champy-Kull staining. This layer was free from cytoplasmic inclusions, except for occasional mitochondria which had apparently recently escaped from entanglement in the upper layer and were in process of sinking to the lower layer, and scattered Golgi elements. It may be remarked that these mitochondria straggling in the upper and middle layers were identical in size and staining affinity with those in the lower layer, and do not appear to be in any way intermediate between them and the deutoplasmic spheres.

The Golgi apparatus consists of numerous scattered rod-shaped elements stained black in the Mann-Kopsch section whether extracted with turpentine or not, and in the Da Fano toned and untoned sections. They do not appear in chrome-osmium preparations. These elements are scattered throughout the entire cell, irrespective of the different layers, and do not appear to be effected by the centrifuging. They are often plastered on to the deutoplasmic spheres in the manner described by Ludford and are clearly concerned in their formation. I have observed the various stages in this process figured by Ludford and am entirely in agreement with his interpretation. In the larger oöcytes I have also observed the peripheral layer of Golgi elements described by him. The cell membrane is very thick and is violently fuchsinophil.

5. Discussion.

I am convinced that in both the molluscs I have studied the process of vitellogenesis is strictly comparable in nature, and results in the production of two distinct kinds of nutritive substance in each egg, although the relative proportions of these substances are very different in the two species.

In Helix and in Patella the mitochondria in the oöcytes become swollen and rapidly increase in number during the growth period. At the end of this period they constitute roughly 50 per cent. of the entire egg in Helix and 10 per cent. in Patella. That they then form an important part

of the reserve food material of the mature ovum and are subsequently more or less used up in development can hardly be doubted. Gatenby's [4] observations on the early embryogeny of Limnæa support this expectation, and show that although the mitochondria give up substances to the cytoplasm during embryogeny they do not disappear. He says :—

"Careful examination of the mitochondria in the unsegmented egg and in the advanced differentiating organ or germ-layer seems to establish the fact that the mitochondria shrink gradually in size *pari passu* with the differentiating somatic or germ-cells during any stages I have examined."

The swelling of the individual mitochondria during the growth stage must result from the accumulation of substances in them either built up within themselves or absorbed from the surrounding cytoplasm. I can see no evidence which throws any light on the question of which of these two processes is actually taking place. It is obviously a very difficult matter to decide whether the resulting "yolk" spheres should be considered as protoplasmic inclusions still retaining their identity as living mitochondria, or as deutoplasmic inclusions formed from the mitochondria. In view of the fact that Gatenby's observations on Limnæa seem to show that although they shrink they still retain their individuality throughout embryogeny and eventually constitute the normal mitochondria of the somatic cells, I am inclined to classify them under the former category. In view of their microchemical reactions it seems probable that they consist of a protein substance and a lipoid, possibly in loose combination, as has been suggested for normal mitochondria. In this case they would bear a certain chemical similarity to the bird's egg. It is well known that in vertebrates the mitochondria play an important part in vitellogenesis.

In Helix and Patella the upper or light layer consists chiefly of deutoplasmic spheres and constitutes about 10 per cent. of the ripe egg in the former type and 75 per cent. in the latter. Although their microchemical reactions to the various methods employed are slightly different in the two species, I consider that they may be regarded as strictly comparable. I believe from my own observations that they

are formed in Helix by a direct metamorphosis of some of the Golgi elements, each individual element concerned gradually swelling up, losing its rod-like shape, and altering in its staining affinity until it forms one of these deutoplasmic spheres. I did not find any appearance of the Golgi rods being plastered on the surface of these spheres as in Patella, and am convinced that they are not formed by the rods but from them. As in the case of the mitochondria there is no evidence to show whether the swelling of the rods is due to the accumulation of substances formed by the rods (or the part of the centrosphere attached) or absorbed from the ground cytoplasm surrounding them. Many of the Golgi elements do not take part in this process but remain unchanged throughout oögenesis, and probably constitute the definitive elements of the resulting embryo. In the absence of any evidence to the contrary, I am inclined to think that the greatly altered elements forming the upper layer of the centrifuged oöcyte of Helix are completely used up in development and do not persist as definitive Golgi rods in the embryonic cells.

In the case of Patella I believe that the deutoplasmic spheres forming the upper layer of the centrifuged oöcyte are formed by the Golgi rods in the manner described and figured by Ludford. I agree with him further in stating that they are formed by the accumulation of a fatty substance, probably in the archoplasm, under the influence of the Golgi rods, and not by a direct metamorphosis of the rods themselves. It is thus probable that all the rods of the mature ovum of Patella remain and retain their identity throughout embryogeny. I think from their reactions that these Golgi-yolk spheres of Helix and Patella are fatty in nature, and that they probably do not contain protein substances.

So far as the mitochondria are concerned my results agree with those of Gatenby on Limnæa. I would suggest, however, that the vacuoles he describes as "probably watery" may be fatty and almost certainly represent the Golgi-yolk spheres of Helix and Patella; with Ludford's work on Patella I am entirely in agreement, except in his view that the mitochondria take no part in yolk-formation. The behaviour of the various

cytoplasmic inclusions during oögenesis and embryogeny in the Mollusca might be represented graphically thus :—

Oöcyte before growth stage.	Mature ovum.	Somatic cells of embryo.
Mitochondria	Swollen Mitochondria	Mitochondria.
Golgi Rods	Golgi Rods	Golgi Rods.
	Golgi Yolk	?

From the fact that the Golgi rods in the oöcytes are not affected by centrifuging, it is obvious that their specific gravity must approximate to that of the ground cytoplasm. Gatenby and I [1] found that this was the case in the neurones of Helix.

6. Discussion of Conklin's Centrifuge Experiments.

It is extremely instructive to consider Conklin's [2] and Morgan's [7] results of centrifuging eggs in the light of our knowledge of the cytoplasmic inclusions. In three species of Gasteropods Conklin found that centrifuging the eggs resulted in the production of three layers, an upper grey layer of light substance, a middle clear layer, and a lower yellow layer of heavy substance. My observations are therefore in agreement with his, and enable me to identify his "grey" layer with my "Golgi-yolk," and his "yellow" layer with my "swollen mitochondria." Conklin states that the yellow substance (mitochondrial) composes at least one-half of the entire egg before the first maturation, which is exactly what I found in Helix and Gatenby found in Limnæa. He then shows that by the time the first cleavage is ready to take place it only composes one-eighth of the egg. It would therefore appear that the substance loading the swollen mitochondria is used up chiefly during the period between the first maturation and the first cleavage division. He further shows that the grey substance (Golgi-yolk) only constitutes one-eighth of the egg at the time of laying, but increases greatly afterwards until, at the time of the first cleavage, it may constitute as much as seven-eighths. This is especially interesting as my experiments do not extend to this period, and also because it is similar to the state of affairs found in the mature ovarian oöcytes of Patella. It seems possible

that this reciprocal change in the bulk of the swollen mitochondria and the Golgi-yolk, without any marked change in gross bulk of the egg, may merely indicate a passage of substances from the former to the latter. Such an hypothesis admirably accounts for the change in the relative weights of the various oöplasmic substances during the period between the first maturation and first cleavage divisions which Conklin claims to have demonstrated.

Conklin also states that the grey substance may be thrown out of the egg entirely without affecting its subsequent development. In view of the fact that much of the Golgi apparatus remains unchanged and scattered throughout the centrifuged egg, we find no difficulty in accepting this.

He shows further that the grey substance normally goes to the micromeres during cleavage and the yellow substance to the macromeres and ultimately to endoderm and mesoderm cells. Both Conklin and Morgan agree, however, that the normal distribution of these visible substances of the egg may be disturbed by centrifuging without affecting the subsequent development. They therefore conclude that these substances are not organ-forming; in other words, that the distribution of the mitochondria and Golgi-yolk in molluscan eggs does not determine the development of the blastomeres into the various embryonic layers.

7. Summary.

1. A convenient form of nomenclature for the various forms of yolk is suggested.

2. In the centrifuged oöcytes of Helix three layers are produced; an upper layer of G-yolk (Golgi-yolk) (10 per cent.) formed by a direct metamorphosis of some of the Golgi rods, a middle layer of ground cytoplasm free from yolk and containing the nucleus, and a lower layer of swollen mitochondria (50 per cent.). The unaltered Golgi rods remain scattered throughout the cell.

3. In the centrifuged oöcytes of Patella three layers are also produced; an upper layer of G-yolk (75 per cent.) formed by, not from, the Golgi rods, and containing the nucleus together with a number of mitochondria caught between the

G-yolk spheres, a middle layer of ground cytoplasm free from yolk, and a lower layer of swollen mitochondria (10 per cent.).

4. The bearing of these results on early development is discussed, especially in the light of Conklin's work.

5. A generalised account of yolk-formation in the Mollusca is given and graphically represented.

8. References.

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[5] Gatenby and Woodger, J. H. (1920), *Journ. Roy. Micr. Soc.*

[6] Ludford, R. J. (1921), *Journ. Roy. Micr. Soc.*

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