The Oogenesis of Certain Invertebrata, with Special Reference to Lumbricus.

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With Plate 24.

Introduction.

The subject of the oogenesis of animals assumed a new interest with the improvements in technique recently introduced, and the understanding of the fact that two distinct types of cytoplasmic inclusions exist in all animal (and probably plant) cells, and of course in egg-cells, enabled cytologists to study the granules in eggs from a new angle.

In recent years in this country, Ludford, Woodger, Brambell, Miss King, D. R. Bhattacharya, and the present authors have undertaken work in this special field. Consequently our knowledge has grown rapidly, and our ideas have crystallized out more or less perfectly. All the above authors have worked within the last five years, and subsequently to the résumé of the whole subject made in 1920 by one of us (J. B. G.) in the 'Journal of the Royal Microscopical Society'.

Brambell has recently endeavoured, at the suggestion of one of us, to define the word 'yolk', and the nomenclature of the subject has been more clearly defined.

In the last part of the series of papers on the 'Cytoplasmic Inclusions', that on the 'Gametogenesis of Saccocirrus', the senior author has given the latest résumé of our knowledge of the oogenesis in relationship to vitellogenesis, and has pointed out many of our present difficulties.

A few months ago the junior author began collecting preparations of the ovaries of Lumbricus, because some sections of this material already made by one of us (fixed by Flemming with, and without, acetic, and by Cajal and Da Fano methods), showed that interesting results might be obtained by a study of this simple oogenesis, especially in view of the work of Gatenby on Saccocirrus.

While this work was in progress, Harvey published a paper on the 'Oogenesis of Lumbricus'. Harvey has been led into a series of criticisms of the work of Gatenby and his associates, on the oogenesis of various animals. He does not consider the only other paper on annelid oogenesis, from the point of view of the inclusions, i. e. that on Saccocirrus by Gatenby, nor does he consider Brambell's discussion on the inclusions in oogenesis, published in the 'British Journal of Experimental Biology', but merely mentions as representative of the present state of our knowledge Gatenby's first résumé of the vitellogenesis of animals, written before Ludford, Miss King, Woodger, Nath, Gatenby, Bhattacharya, Brambell, and several other workers had undertaken and published special studies on oogenesis. The more recent summary on oogenesis in Gatenby's paper on Saccocirrus is not mentioned.

Harvey has attacked Gatenby's position from the point of view of his own experiences with Lumbricus 'vitellogenesis', but, as will be seen, there is no vitellogenesis in Lumbricus in the sense of the formation of yolk-spheres, nor do Harvey's results differ radically from those of Gatenby on Saccocirrus.

The only papers published previous to the year 1923 on investigations on the oogenesis of animals, carried out by the modern technique, are those of Hirschler, Gatenby, and Ludford. There are many published studies of oogenesis dealing either with mitochondria or Golgi apparatus, but not with both. As has been pointed out repeatedly by the senior author, such studies are not of much value, because not only does the investigator invariably confuse the two systems of granulations, but it is wellnigh impossible even for the better-informed reader of these papers to discover exactly what does happen. Even the experienced worker, confronted with excellent preparations of ripe eggs made by the most suitable methods, is unable to make much headway, except after considerably prolonged study of the earlier stages of the oogenesis.

For this reason it is not proposed to review any studies not carried out by all the available methods.

There are, of course, the classic and much-quoted investigations of Schaxel on the 'chromatin-emission' in Aricia, a polychaete, and in other organisms. Harvey, though an investigator of annelid oogenesis, does not review or mention Schaxel's interesting interpretations. One of us has already commented on Schaxel's work, purely from the point of view of the oogenesis of Saccocirrus, in which there is a great deal of nucleolar activity leading to nucleolar emission, and the formation of extranuclear, nucleolar yolk. In 1922 the senior author assumed tentatively that Schaxel was dealing with nucleolar emissions or mitochondria, but neither Harvey nor the present writers have found satisfactory evidence of nucleolar emission in Lumbricus, and the inference remains that Schaxel must either have produced artefacts in his preparations, or was merely misinterpreting the mitochondrial constituents of the egg. At all events a re-examination of some of Schaxel's forms is desirable, in view of the fact that the behaviour of the cytoplasmic inclusions is so variable.

In the previous work on the archiannelid Saccocirrus, which is pertinent from the point of view of Harvey's result, but which was not considered by him, one of us obtained the following results :

Oogonium.	*Full-grown Oocyte.*
1. Nucleolus	a. Definitive nucleolus. b. Nucleolar deutoplasm, or main yolk-spheres.
2. Golgi elements	a. Definitive Golgi elements. b. Yolk-spheres (fatty) ?
3. Mitochondria	Mitochondria.

Harvey's results, for Lumbricus, put out in the same manner would be:

Oogonium.	*Full-grown Oocyte.*
Nucleolus	Definitive nucleolus.
Golgi elements . . .	Definitive Golgi elements.
Mitochondria of older oogonia	Mitochondria.
Cytoplasm	Yolk.

Harvey, however, assumes that new Golgi elements may arise from the cytoplasm, a view which we cannot uphold and for which he produces no evidence of any kind.

In the Saccocirrus work, one of us found two kinds of yolk, nucleolar and fatty yolk, and it was stated that doubt was felt as to the metamorphosis of Golgi elements into yolk of a fatty nature. The junior author, however, has insisted, elsewhere, that in Lithobius the Golgi elements do produce fatty yolk. We do not propose to consider this point at the present moment.

Returning to a comparison of the two annelids—Lumbricus and Saccocirrus, it will be noted that in both cases the mitochondria behave in the same manner, while in both cases doubt is expressed as to the metamorphosis of Golgi elements into fatty yolk. Harvey claims that in Lumbricus no such metamorphosis is demonstrable, and our previous results were in accordance. The Golgi elements of Lumbricus are less 'fatty' and more inactive than those of Saccocirrus. The chief difference between Lumbricus and Saccocirrus is the wonderful emission of nucleolar material which takes place in the latter, and which appears to be unparalleled in any other animal. Silvestri, Hegner, Jenkinson, Hogben, Ludford, Jorgensen, Gatenby, and many other authors have carefully described nucleolar emission in many forms, but in no case does this occur to the extent found in Saccocirrus, where the peculiar and marked perinuclear halo of nucleolar fragments has long been known, even in material preserved in Carnoy, Bouin, and the older Flemmings.

The view taken by the present authors is that a proper vitellogenesis is absent in Lumbricus. By a proper vitellogenesis we mean the formation of yolky spheres at the expense of some part of the cytoplasm, or its contents.

Harvey, and later Cannon, have insisted that there is yolk in the egg of Lumbricus terrestris. The literature on this subject shows the following references to yolk in Lumbricus.

CALKINS (1895).

1. 'The earliest egg-cells (oogonia) have no indication of a yolk-nucleus.'

2. 'The yolk-nucleus is not only of nuclear origin, but is derived from the chromatin, the nucleolus of the germinal vesicle taking no part in its formation.'

3. 'In older eggs the yolk-nucleus moves away from the nucleus, the disintegration continues, and the egg then contains a great number of smaller granular masses each with several fibre-like prolongations. . . . Next the disintegrated masses lose their granular structure and become large and homogeneous, forming the yolk-plates of the egg.'

WILSON (1901), however, in his book describes the 'yolk-plates' of Calkins as 'disintegration of yolk-nucleus'.

FOOT and STROBELL (1901), working on Allolobophora, deny the existence of 'yolk-plates' in that worm. They, however, describe certain osmiophilic granules whose origin they were unable to determine. They regarded these granules as products of the degeneration of the egg. Thus they say:

'In studying eggs in varying stages of degeneration, the amount of osmiophile substance in relation to the cytoplasm increases as degeneration progresses and finally we have a stage where the entire cytoplasm of the egg has become osmiophile. One of the first indications of an abnormal egg appears to be an increase in the number of osmiophile granules, and a further stage of degeneration is evidenced by aggregations of the granules or their fusing into homogeneous masses,[1] the number of these masses increasing with the progress of degeneration.'

[1] Cf. 'Yolk-Plates' of Harvey.

In a normal egg, however, the 'osmiophile granules are quite evenly distributed throughout the cytoplasm'.

'What the substance is we are unable to determine; but we do not believe that the entire yolk-nucleus is sacrificed to the formation of these granules. . . .

'In normal eggs there is nothing that can be interpreted as the "yolk-plates" which Calkins describes for Lumbricus.'

According to Harvey these yolk-plates were found in practically all preparations. Harvey himself does not regard them as true yolk (see p. 297). Nor does he know where they come from.

Harvey, however, describes yolk-droplets even in the youngest eggs. Neither Calkins nor Foot and Strobell have described any yolk-droplets. There are no yolk-droplets in our preparations either.

To sum up:

1. The yolk-plates of Calkins in Lumbricus have been described as 'disintegration of yolk-nucleus' by Wilson.
2. The osmiophile homogeneous masses in Allolobophora have been described as products of degeneration by Foot and Strobell.
3. Harvey, however, finds these yolk-plates in practically all preparations, although he admits they are not yolk.
4. The yolk-droplets described by Harvey were not discovered by either Calkins, or Foot and Strobell.

The work of Foot and Strobell is the most valuable of these previous investigations, because they used fresh cover-slip preparations, and their observations were really based on material not much inferior to what we can make to-day.

Harvey claims that in the youngest oogonia there can be detected no certain signs of the mitochondria, but a slight granular appearance in the cytoplasm may represent them. At a very early stage a distinct mitochondrial cap may be seen fitting closely over the nuclear membrane. This cap consists of a faintly staining, nebulous cloud with more definite, darkerstaining patches in it. The cap gradually grows in size and becomes distinctly fibrillar in structure. Whether the growth is due to division of the original mitochondrial elements, or to the origin of fresh ones from the cytoplasm according to Harvey, it is impossible to say. Gradually the cap moves towards the peripheral region of the cell and breaks up into smaller masses of threads, and these then spread throughout the whole of the cytoplasm of the cell.

In addition to the mitochondria Harvey describes 'yolk-plates' and 'yolk-droplets'. The 'yolk-plates' may be seen in the nearly ripe oocytes as large patches, somewhat indefinite in outline in many cases, of some homogeneous material scattered through the cytoplasm irregularly. In the living cell they occur as transparent bodies of a slightly higher refractive index than the cytoplasm. 'Undoubtedly these plates are not yolk, for their staining reactions . . . are not those of yolk.' As to how they arise nothing can be said.

In addition to the 'yolk-plates' there are present true 'yolk-droplets' in the form of spheres. These droplets do not disappear after Carnoy fixation, &c. They arise independently in the cytoplasm, and are not associated with either mitochondria or Golgi elements, or nucleolar extrusions. There are no such extrusions in the egg.

Lastly according to Harvey the Golgi apparatus consists of numbers of Golgi elements lying separate in the cytoplasm. There is never any attempt at concentration of these elements round one central mass. The Golgi elements are not rods, but probably little platelets or spheroids. They may probably arise from the cytoplasm.

The oogenesis of Lumbricus is, to the present authors, not so much a vitellogenesis as merely the growth of the ground cytoplasm with the growth and dispersion of the unchanged cytoplasmic inclusions throughout the egg. Yolk-formation in the sense understood by us, in, for instance, Saccocirrus, Lithobius, Patella, or Rana, does not occur.

Harvey mentions that he did not make successful intra vitam investigations, because 'the egg of Lumbricus is full of highly refractive granules and globules of yolk, fat, &c., which obscure any signs of the fine thread-like mitochondria, and make the study of the inclusions in the living cell impossible'.

We are compelled to disagree entirely. Not only have we succeeded in seeing both types of inclusions in all stages of oogenesis of Lumbricus terrestris, but we have been able to stain the inclusions intra vitam, and carefully to go over our results obtained by the sectioning method. Nor are any globules of fat or yolk present. Harvey has mistaken the Golgi elements, so clearly seen in this animal, for fat or yolk.

Personal Observations.

The senior author has found that the entire oogenesis of Lumbricus is best worked out with unstained whole ovaries mounted in a little salt solution, to which some 2 per cent. OsO_4 has been added. Not only do the mitochondria show clearly, but the Golgi element may be followed from its juxtanuclear position until it has divided into the numerous large granules found in the adult oocyte ready to drop off.

The Golgi apparatus is at first a single juxtanuclear, excentric, somewhat irregularly spherical bead. This divides first into two and then four parts, and so on, spreading out into the cytoplasm. In no other animal cell which we have studied is the Golgi apparatus so clearly seen in plain unstained osmic preparations. There can be no ground for disagreement about this oogenesis, if only the observer will use such simple preparations. The dictyosome, or thickened edge of the Golgi bead, may occasionally be seen as a highly refractive peripheral area.

The adult oocyte, lying in the ovarian tag, ready to drop off, contains many Golgi spherules and mitochondria, but no true yolk-granules. The Golgi spherules might be mistaken for structureless yolk-granules. This interpretation is disposed of immediately one examines Kopsch, Cajal, or Da Fano preparations, in which the normal arrangement of dictyosome and sphere is found in each Golgi element.

Examination of fixed and stained preparations confirms the results obtained by examining freshly osmicated whole ovaries. In figs. 1–5, Pl. 24, are oocytes of various ages showing the juxtanuclear Golgi element (fig. 1) growing and dividing (figs. 2, 3) and ultimately spreading out in the cytoplasm (fig. 5) to form the beads seen so plainly in the fresh osmicated ovaries.

Lumbricus provides one of the easiest forms to study, because the eggs, as is well known, are at their smallest near the septal insertion of the ovary, and at their largest in the tag, lying at the free end of the ovary. Intermediate stages, often very clearly seriated, lie in between. It is such an arrangement which undoubtedly leads on to the more highly organized insect ovary, where the seriation of growing oocytes is more perfect.

In Lumbricus, therefore, one has very favourable material for following out the changes in the cytoplasmic inclusions, and we are at a loss to understand Harvey's difficulties in giving a clear account of the behaviour of the Golgi apparatus from oogonium to ripe oocyte. In animals like molluscs, ascidians, vertebrates, &c., where no seriation of oocytes occurs, clear accounts have been given of the behaviour of the Golgi elements, and one who has only worked on such forms will be surprised at the ease with which Lumbricus oogenesis may be studied.

In fresh preparations, made by the osmic, Dahlia, Janus green, or Janus-iodine methods, the mitochondria can be seen as small delicate spheres [1] in the youngest germ-cells (fig. 6, Pl. 24).

Besides the fresh methods, we have used all the methods set out for this type of work in the ' Microtomist's Vade Mecum '.

Discussion.

Harvey states that it is very probable that the Golgi element in general is a plate, sphere, or spheroid, and not a rod. In some animals the Golgi elements may be platelets, but in others,

[1] Thread-like mitochondria figured by Harvey are artefacts due to too long exposure of the fresh ovaries to water of unsuitable tonicity, or to unsuitable fixation.

e.g. the snail (spermatocytes, &c.), the rod and crescent formation is very clear, and has been figured by many authors such as J. A. Murray,[1] Boveri, Gatenby, and by Gatenby in various animals. In vertebrate ganglion cells the classic tree-like formation is generally found: Harvey appears to view the question from a narrow aspect, and there can be no doubt that his opinions will alter after he has examined a wider field.

Regarding Harvey's objections to the descriptions of Ludford and Gatenby of yolk-formation in molluscs, from and in direct connexion with Golgi elements, the same may be said. Harvey should himself study some mollusc, or ascidian, in which the phenomenon has been described. We consider that Lumbricus provides no basis for a statement of opinion on this subject, simply because yolk-formation from Golgi elements does not occur in Lumbricus terrestris. Harvey has brought forward a criticism of Gatenby's tables of tests for the cytoplasmic inclusions, given in Bolles Lee's 'Microtomist's Vade Mecum', 1921. He says that 'a glance at either of these sources of reference will show that the two elements, fat and yolk, can easily be confused; for most of the reactions of either body have an alternative, which renders it difficult to distinguish between these two bodies'. Harvey's criticism may be answered by another quotation from the beginning of Gatenby's same article on the subject (ibid., p. 334): 'In this section, we have provided a series of tables intended to act as a tentative guide to the interpretation of the various images got by representative cytological techniques. These tables are based on work carried out on animals of most orders, but it would be injudicious for the researcher to depend upon them implicitly, because many exceptions are met with, and the personal factor is to be taken into consideration.'

[1] Dr. J. A. Murray, F.R.S., twenty-seven years ago, in his paper in the Zool. Jahrb.', 1898, appears to have been the first person to have described the intra vitam appearance of what we now call the Golgi apparatus. He writes: 'The nucleus (of the Helix spermatocyte) is clear and transparent, and at one side of it a group of highly refractive curved rodlets is seen.' These rodlets we now call Golgi bodies or dictyosomes.

It is obvious that Harvey's objections are met by the last warning paragraph. When Gatenby drew up these tables, enough work on oogenesis had been done to make one realize the difficulties ; hence the warning put at the beginning of the paragraphs containing the tables. Neither the words ' yolk ', nor ' fat ', really mean anything chemically definite : the best one can do is to define a yolk-granule as a proteid substratum impregnated by fatty and other substances.

There is an article on this subject, written before Harvey published his own paper, which was unfortunately not quoted by him. We refer to Brambell's attempt to define the word ' yolk '. Until biochemical methods are more helpful, we cannot go much farther than Brambell has done. As a matter of fact, however, we have yet to meet an example where true yolk and neutral fat cannot satisfactorily be separated, either microchemically or by centrifuge methods, the latter, by the way, which Harvey does not appear to have tried.

Harvey also writes, ' A perusal of Gatenby's paper on yolk-formation (**31**) will show that the origin of yolk is still a very much disputed question, and that various workers have claimed its origin from the ground cytoplasm, from mitochondria, the Golgi apparatus, and from the nucleus. In Lumbricus no evidence has been discovered in support of the hypothesis that the mitochondria and Golgi platelets metamorphoses [sic] into yolk.'

No modern author, equipped with, and able to use properly, the recently developed cytologic methods, has special difficulty in concluding exactly how true yolk forms in the organisms he examines. The confusion is past, and was due to the use of confusing methods such as those of Bouin's fluid and corrosive acetic, which give a totally inadequate picture of the egg. The acetic in Flemming's fluid also was a potent cause of confusion.

The view expressed by Harvey, that the Golgi elements may probably arise from the cytoplasm, is one with which we cannot agree. On the contrary, we consider that because of the complete absence of yolky material the egg of Lumbricus offers almost unique opportunities for demonstrating the fact that the Golgi elements of the adult oocyte are derived, by a process of multiplication and spreading out, from the small discrete juxtanuclear Golgi apparatus of the youngest oogonium. Both of the present writers, after careful examination, have come to the conclusion that there is no evidence for Harvey's statement, in this form or in any other we have been able to investigate thoroughly. Of course the phylogenetic origin of the Golgi apparatus (whether from the cytoplasm or nucleus) is quite a different problem, but is not under discussion here.

All the past work on the Golgi apparatus in spermatogenesis and oogenesis in development, and the work of the histologists of the Cajal and Golgi schools, goes to show that the Golgi elements are endowed with the power of division and multiplication.

One of the stumbling-blocks to the complete acceptance of the view that the inclusions pass through all stages of the germ-cell cycle, has been the work on the hen's egg and chick blastoderm. Yet the recent work of Brambell on oogenesis and development, and that of Bhattacharya on reptilian eggs, shows that the Golgi apparatus of the ovum is in the form of an extremely fine, almost ultra-microscopic powder, which ultimately runs together in early stages of embryonic development, to form the typical juxtanuclear apparatus. This seems also to be the view of Subba Rau and Ludford, who have paid some attention to this problem and with whom we have discussed this matter. In the Mollusca both Hirschler and one of the present writers have followed the Golgi apparatus through development, and in the adult oocytes of all animals, excepting the large yolked eggs referred to above, coarse scattered Golgi elements are demonstrable and are undoubtedly distributed between the blastomeres during the early stages of development. It is not proposed to labour this matter further.

Harvey has complained that workers on oogenesis are not agreed as to the method in which yolk is formed. This does not apply to modern workers. There is no single method of yolk-formation applicable to all organisms. The variability of the behaviour of these cell granules, which have been studied so much in recent years, is their most constant feature. Witness the work on sperm-formation : even Retzius, working with obsolescent methods, brought out this fact nearly seventeen years ago, during his studies on sperms.

One of us (V. N.) has recently studied comparatively oogenesis in a number of invertebrates. Such comparative work is of special value, because the same author uses the same technique on a number of different forms, and personal idiosyncrasies of technique do not introduce any possible grounds for confusion, leading to fruitless controversy. The following is the scheme of oogenesis as worked out by Nath in certain Arthropods.

LITHOBIUS.[1]

Oogonium.	*Full-grown Oocyte.*
Nucleolus . . .	*a.* Definite nucleolus (plasmosome). *b.* Nucleolar extrusions forming ordinary proteid yolk.
Mitochondria . . .	Mitochondria.
Golgi apparatus . .	*a.* Fatty yolk. *b.* Definite Golgi apparatus.

The evidence in support of the Golgi origin of the fatty yolk is very strong in this case. Firstly the fatty yolk appears only after the juxtanuclear Golgi apparatus begins to fragment and spread out throughout the cytoplasm ; secondly in Mann-Kopsch preparations all gradations exist between the Golgi elements and the fatty yolk ; and thirdly experiments with the centrifuge most clearly show an increase in the fatty yolk and a corresponding decrease in the definite Golgi elements.

[1] See also Miss S. D. King (Roy. Dub. Soc. 1924).

Julus terrestris.

Oogonium.	*Full-grown Oocyte.*
Nucleolus	Nucleolus.
Mitochondria	Mitochondria.
Golgi elements	Enormously swollen yolky Golgi elements. (Cf. Buthus and Euscorpius.)
Ground cytoplasm	Ordinary proteid yolk.

In this Diplopod the Golgi elements in the oogonium are granular and juxtanuclear. With the growth of the oocyte the Golgi grains swell up enormously, and it is extremely easy to photograph them.

Buthus and Euscorpius.

Oogonium.	*Full-grown Oocyte.*
Nucleolus	Definite nucleolus. Nucleolar extrusions. Ordinary proteid yolk.
Mitochondria	Mitochondria.
Golgi elements	Swollen Golgi elements.

That the ordinary proteid yolk is associated, either directly or indirectly, with nucleolar extrusions in these two genera of scorpions is strongly suggested by the fact that in Palamnaeus fulvipes madraspatensis in which there is no proteid yolk, the nucleolus remains inactive. The Golgi elements in Buthus and Euscorpius become swollen without in any way altering their chemical constitution.

Palamnaeus.

Oogonium.	*Full-grown Oocyte.*
Nucleolus	Nucleolus.
Mitochondria	Mitochondria.
Golgi elements	Fatty yolk (cf. Lithobius). Definite Golgi elements.

For evidence in support of the Golgi origin of the fatty yolk in this scorpion reference may be made to the original paper.

In no two examples of oogenesis do the cytoplasmic elements behave similarly. In molluscs, for instance, Brambell has shown that the activity of the mitochondria varies considerably. Bhattacharya has found similar instances in a series of reptiles observed by him.

Miss S. D. King has recently examined the oogenesis of Oniscus, and has had no difficulty in showing that there are two different types of yolk-granules, fatty yolk, and proteid or mitochondrial yolk. The scheme is as follows :

ONISCUS.

Oogonium.	*Full-grown Oocyte.*
Mitochondria . . .	Mitochondria and yolk (proteid).
Golgi bodies . . .	Golgi bodies and fatty yolk.
Nucleolus . . .	Nucleolus.

In Lithobius, Julus, Euscorpius, and Buthus, Nath has described the growth, division, and dispersion of the Golgi elements in oogenesis. No evidence for a cytoplasmic origin of these bodies was discovered. Miss King confirms this result for Lithobius and Oniscus. Similar results have been obtained by Ludford in a variety of animals, by Brambell and Bhattacharya in a variety of Vertebrata, by Hirschler in Ascidians, and by Gatenby in molluscs, vertebrates, annelids, mammals, and in fact in all types of which preparations have been made. Division of the Golgi elements has also been repeatedly observed in spermatogenesis, where these bodies often tend to be quite large.

Bhattacharya has shown that in many reptiles the Golgi apparatus of the egg forms chain-like figures produced by repeated divisions of one granule, to form a series of beads resembling bacterial formations. Such investigations provide what, after actual observation of the living egg, may be assumed to be the nearest we can get to actual proof that the Golgi elements divide in the egg.

Dr. P. Weiner, of the Histology Laboratory of the University of Leningrad, informs us (in literis) that in the eggs of various myriapods and arachnids he finds yolk-formation taking place in contact with particles of the Golgi apparatus.

There can be little doubt that one of the most remarkable of these is Julus, a form also studied recently by the junior author. The Golgi apparatus, as is usual, spreads out into the egg cytoplasm, but in this case eventually forms the major portion of the granulations of the ripe oocyte. Each Golgi element, however, swells up to form a large granule, surrounding the outer surface of which is the crenulated dictyosome. In neither Lithobius, Saccocirrus, Lumbricus, or molluscs generally, does the Golgi apparatus display such remarkable activity, even though, as Ludford, Brambell, and Gatenby have shown, the molluscan yolk is largely derived from Golgi elements.

Finally, to bring this discussion to a close, we may mention the following conclusions arrived at by Harvey:

1. The yolk-nucleus of Lumbricus is merely a mass of mitochondria.
2. The mitochondria are not clearly defined in the very young oogonia.
3. There is never any attempt at concentration of the Golgi elements round one central mass.
4. The Golgi elements are probably little platelets or spheroids somewhat resembling blood corpuscles in shape.
5. The Golgi elements may probably arise from the cytoplasm.
6. Yolk is present, and probably arises from the cytoplasm. Fat is present.
7. No direct metamorphosis of either mitochondria, Golgi apparatus, or nucleolus into yolk was observed.

There are one or two other conclusions also, not quoted here. We agree with the statements expressed in paragraphs 1 and 7, but disagree entirely with everything else.

We find :

1. Granular mitochondria clearly defined in oogonia. The degree of clearness depends on the technique.

2. Concentration of the Golgi elements is constant in the youngest oogonia.

3. The Golgi elements are not shaped like red-blood corpuscles. They resemble those found in other invertebrate animals.

4. The Golgi elements can be traced from the earliest oogonium to the oldest oocyte, and no evidence was produced for believing them to originate *de novo* from the cytoplasm.

5. The mitochondria are not filamentous but granular. The filamentous forms depicted and described by Harvey are familiar to oogenesis workers as artefacts, and will not be seen in the freshly extirpated ovary.

6. Harvey's disbelief in the previously published descriptions of the metamorphosis of Golgi bodies into yolk-spheres is based merely on his study of *Lumbricus*, where this phenomenon does not occur.

Conclusions.

1. Neither yolk nor (neutral) fat is present in the egg of *Lumbricus terrestris*.

2. The Golgi apparatus is always at first condensed, single and juxta-nuclear in the young oogonium, and spreads out little by little throughout the cytoplasm.

3. There is no evidence that the Golgi elements arise *de novo*, as suggested by Harvey. The elements may be found in process of division, the seriation of the oocytes assisting one to come to the conclusion that the numerous elements of the oocyte are derived simply from the single element in the oogonium.

4. No *intra vitam*, or wet method, shows the presence of either neutral fat or yolk. The Golgi elements, however, are slightly osmiophile but do not go black, nor do they go red in scarlet red or brown in Sudan III.

5. Occasionally (as pointed out by Foot and Strobell twenty-five years ago) the osmiophility of the egg granules is increased in degenerating oocytes. Such degenerate granules are Golgi elements and not yolk, and are not found usually in the breeding season, but after it.

6. The morphology of the Golgi elements is much like that in other animals described. There is a sphere, with a thickened edge or dictyosome, which varies in shape from a perfect banana-shaped rod to an irregular curved plate.

7. No direct metamorphosis of Golgi elements or mitochondria into yolk-spheres takes place.

8. The so-called yolk-nucleus of the older observers is merely the mitochondrial mass.

9. The osmiophile granules of Foot and Strobell are Golgi elements, which are clearly seen even in untreated *intra vitam* preparations.

10. Harvey's 'yolk-drops' are probably Golgi elements, which have degenerated (as mentioned by Foot and Strobell).

11. The oogenesis of *Lumbricus* is probably the simplest at present known, and can be worked out entirely with wet preparations. This ovary is ideal material for observing the unchanged mitochondria and Golgi elements *intra vitam*, and can be recommended for classes of medical students. The extirpated ovary may also be treated with a little weak osmic solution and examined immediately.

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DESCRIPTION OF PLATE 24.

LETTERING.

M, mitochondria ; G, Golgi apparatus ; N, nucleus.

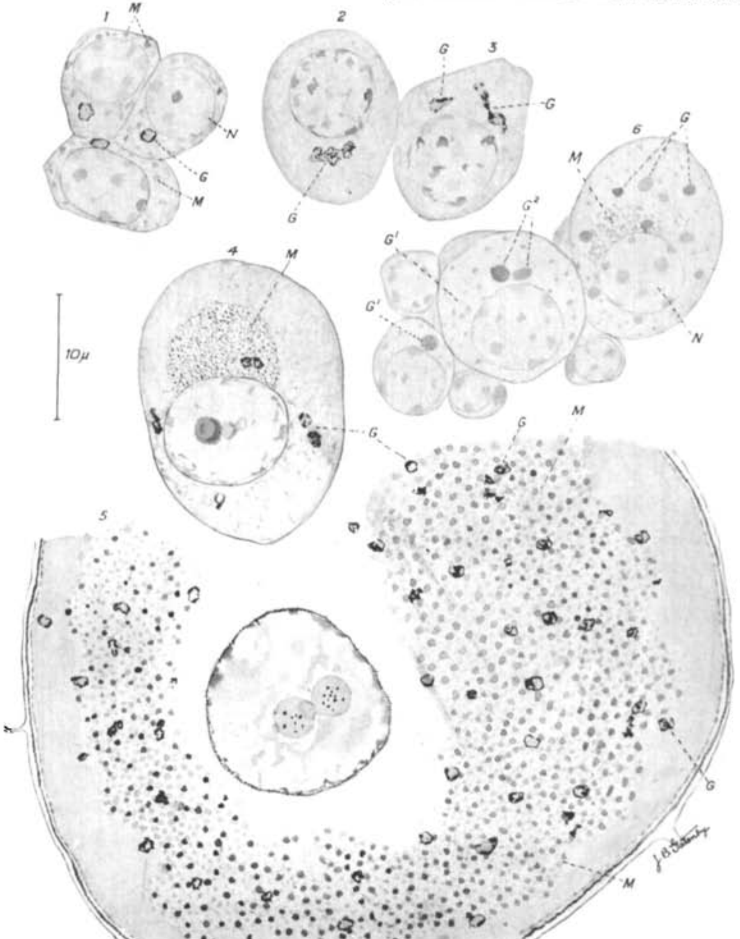
Fig. 1.—Three oogonia by modified Da Fano method, showing juxtanuclear discrete Golgi apparatus.

Figs. 2 and 3.—Division stages of Golgi apparatus. Same method.

Fig. 4.—Oocyte showing ' yolk nucleus ', i. e. aggregated mitochondria at M, dispersing Golgi elements, G. Champy, I. H.

Fig. 5.—Later oocyte, modified Da Fano.

Fig. 6.—Six oogonia in various stages showing Golgi elements juxtanuclear and single (G^1), diploid (G^2), and dispersing (G). Fresh preparation.


1
M
N
G
M
2
G
3
G
G
6
G
M
G²
G'
G'
N
4
M
10μ
G
G
M
5
G
M
J.B.Gatenby