

THE OOGENESIS OF *SALMACIS BICOLOR* (AGASSIZ)
WITH A SUGGESTION AS TO THE
FUNCTION OF GOLGI BODIES.

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Received November 20, 1934.

(Communicated by Prof. R. Gopala Aiyar, M.A., M.Sc.)

1. *Introduction.*

THE discovery of fatty and albuminous yolk arising in relation with the Golgi apparatus in *Clibanarius*¹ together with the occurrence of a seasonal variation in the cytoplasmic inclusions led the author to suggest that these may explain the diversity of results obtained by various workers on the cytoplasmic phenomena during oogenesis. The cause of the disagreement as to the function of the Golgi bodies appears to be enhanced by the lack of a clear definition of the term 'fatty yolk'; for under this term seem to be included two different inclusions, namely, fat and fatty yolk. The behaviour of the Golgi grains after fertilisation in *Acentrogobius neilli* (*Gobius neilli*. Day)² suggested that fat, fatty yolk, or yolk formation is but one of the functions of the Golgi as soon after, its activities may be directed in an entirely different direction.

Cytologists like Bowen (1922, 1924, 1926*a, b, c, d*) and Nasonov (1923, 1924*a, b*) who tried to elucidate the function of the Golgi apparatus seem apparently to have paid scant consideration to its possible general function in all cells and the views of the few like Gatenby (1919, 1921), Ludford (1922, 1925) and Brambell (1923) who attempted an explanation, have not been given the attention they deserve. Oogenesis workers of all schools of thought have failed to consider the general function of Golgi bodies in all cells even though they have seen these elements in eggs where apparently no deutoplasmic bodies occur. If these bodies can have a particular function in specialised cells, it is only reasonable to

¹ "Oogenesis of *Clibanarius olivaceus* (Henderson) with special reference to a seasonal variation in the various cytoplasmic inclusions." A note was published in *Curr. Sci.*, December 1933. Paper in course of publication.

"Cytoplasmic Inclusions in *Acentrogobius neilli* (*Gobius neilli*. Day)." *Curr. Sci.*, December 1933. Paper in course of publication.

² "Effect of Fertilisation on the Golgi bodies in *Acentrogobius neilli* (*Gobius neilli*. Day)." Unpublished.

expect that they should have some function in non-specialised cells also. The author finds no justification to consider oogenesis as a special phenomenon in any way different from secretion or spermatogenesis.

An attempt has been made in this paper to evaluate the various theories and a suggestion is made as to the function of the Golgi apparatus.

2. *Material and Methods.*

Salmacis bicolor is a very common form occurring in the Madras harbour. Fully mature specimens are available throughout the year and fixations were carried out immediately the animals were brought to the Laboratory. The standard techniques like Champy-Kull, Flemming without acetic, Da Fano, Nassonov, Mann Kopsch, Bouin and Corrosive acetic were employed and in addition fresh eggs were treated with 2% Osmic acid and solutions of Neutral Red and Janus Green B. Sudan III and Scharlach R tests were also employed with or without previous fixation in formalin. The eggs of *Salmacis* were not very suitable for the study of fresh eggs intra-vitam. The stains generally used were Acid Fuchsin, Aurantia, Thionin, Toluidin Blue, Mann's Methyl Blue Eosin, Iron Alum Hæmatoxylin, Orange G. and Pyronin Methyl Green.

3. *Previous Work.*

The behaviour of the cytoplasmic inclusions during oogenesis in Echinoderms has been studied by Hibbard (1922) and Harvey (1931) and a cytological and biochemical study of the ovaries of *Echinometra lucunter* by Gardiner, Smith and Tennent (1931).

The conclusions of Hibbard are the following:—

(1) Fat drops occur in the unfertilised egg. These drops are emulsified and the fine droplets of fat thus formed gradually become used up or transformed during the early cleavage stages.

(2) Small spherical mitochondria are found scattered throughout the cytoplasm and there is evidence to show that they are the direct products of the fine fat droplets.

(3) The cytoplasm is packed with plates of nutritive material which have some fatty component in their make up. They are probably yolk. They are in some cases closely associated with the mitochondria and it is probable that they are instrumental in their synthesis. The plates become gradually fewer in number and in the blastula the cytoplasm is quite spongy and full of vacuoles once occupied by the plates.

As some of the conclusions are contrary to what has been observed by other workers it would be worth while to consider the results of

Hibbard in a critical manner. The author does not seem to have succeeded in the Golgi techniques but at the same time does not make any allowance in the conclusions about the possible errors that may creep in due to such a defect.

The continued splitting of the large fat drops into minute droplets and the dispersal of the latter through the cytoplasm is illustrated by the author in Figs. 2 and 3. As Figs. 2 and 3 are those of eggs 25 minutes and 1 hour and 40 minutes after insemination the splitting of the large drops into minute droplets continues or is taking place even after fertilisation. Hibbard further observed a decrease in the amount of the blackened material during cleavage and final disappearance in the two-celled stage. This is taken as an indication of a change occurring. She has not specified the type of change and as in the summary it is mentioned that the fine droplets of fat either gradually become used up or transformed in the early cleavage stages we have only to surmise that the change is one of transformation into mitochondria. The argument in favour of such a change is that there is a continuous variation in colour from the small fat droplets which are brown after osmic impregnation, through similar granules which are less and less brown to pink granules. The figure which is supposed to represent such a stage is that of an unfertilised egg. The description of splitting is after fertilisation but the formation of mitochondria is described in an unfertilised egg. If actually we have to accept the change postulated, the formation of mitochondria has to be taken as occurring even after fertilisation. This, taken together with the transformation of the mitochondria into yolk which is stated to take place after fertilisation, gives one the impression of a peculiar phenomenon happening in *Echinarchnius parma*. From what is already known about these inclusions (Gatenby, 1919) it is inconceivable that such a double change of fat into mitochondria and mitochondria into yolk could take place after fertilisation. Regarding the change of fat into mitochondria there is also the objection that the 'less and less brown' granules which she identifies with intermediate stages between fat and mitochondria may be the Golgi. Whether all these changes occur after fertilisation is a doubtful point and hence much reliance cannot be placed on the conclusions of the author.

Turning now to Gardiner, Smith and Tennent's paper (1931) we find certain sweeping statements unsupported by actual results. A complete criticism being out of place it would be well to point out a few discrepancies. One of the most important conclusions is that the Golgi

bodies are nothing but fatty acid crystals. They state that in a concentrated ethereal solution of mixed fatty acids, crystals of needle-like form were abundant (p. 25). A droplet of this solution on being shaken in a test tube of water resulted in an emulsion. These crystals, according to them, are also hygroscopic. Nowhere have these authors figured or described Golgi batonettes in *Echinometra oocytes* and yet they state, "These results suggest that the Golgi batonettes may be fatty acid crystals having separated out of solution of fatty acids or a mixture of neutral fats and fatty acids." Though they themselves declare in a later part of the paper that few, if any, of these substances are present in the pure form in the tissues they are ready to suggest that the Golgi batonettes may be fatty acid crystals forgetting for the moment that fatty acids do not occur in ethereal solutions in eggs! Further, even if fatty acids occur as crystals there is every probability of their forming an emulsion with the sea water in view of the free interchange between the body fluids and the surrounding medium in marine animals—a fact which these authors have overlooked. If they had suggested fatty acids as one of the probable components of Golgi material it may have been acceptable.

Harvey (1931) describes yolk droplets arising under the influence of the Golgi apparatus: "On the concave sides of the scales, and inside the cups formed by two or more scales, appear minute granules, colourless in the living, pale green or brown after Bensley-Cowdry, black after iron hæmatoxylin. They enlarge to a certain extent *still in association with the Golgi bodies*, and finally migrate away from them and form little clumps of upto 20 or 30 granules in the peripheral cytoplasm of the egg"* (p. 423). The developing yolk droplets sever their connection with the Golgi when they are a third in size of the fully formed yolk spherules. Further enlargement according to Harvey is by accretion. After all this he states, "It will be noticed that there is an extraordinary resemblance to some of the figures published of secretion production in various gland cells, the most striking difference being that in no case do the yolk spherules *ever appear in contact with the scales*, whereas the secretion droplets often arise closely applied on the strands of the Golgi network in gland cells."* A perusal of Bowen's paper (1926 *d*) reveals that not in all cases have the intimate relation between the Golgi and the secretory product been well demonstrated. There also seems no justification for the statement that there is a striking

* Italics mine.

difference between what is actually seen in secretory cells and what Harvey describes and figures. Even the severance of connection between the Golgi and yolk is not against a comparison with what is occurring in secretory cells, since in the developing spermatid the acrosome is capable of remarkable changes subsequent to its separation from the Golgi apparatus.

4. *The Ovary.*

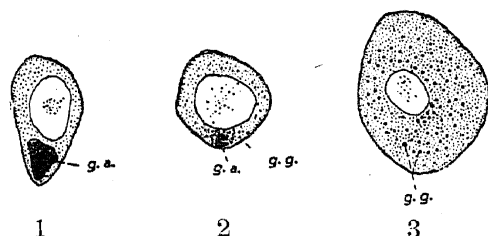
The walls of the ovarian tubules are composed of three layers (Figs. 5 and 6): (a) an outer layer of coelomic epithelium (*coel. ep.*) which gets torn up when the tubules are packed with ripe ova; (b) a middle layer of muscles (*mus. l.*) and connective tissue; and (c) an inner layer of germinal cells which gives rise to oocytes as well as albuminous and lipoidal nutritive spheres. The germinal layer cells are irregular and oocytes of different stages of growth are found side by side. Only oocytes more than 70μ in size lie loose in the cavity of the ovarian tubule.

5. *The Golgi Apparatus.*

Hibbard (1922) remarks that the silver nitrate techniques were a failure in *Echinarchnius parma* and Harvey (1931) also records a similar experience. In *Salmacis*, a modified form of Da Fano, gave very confirmatory results, these being checked by the Mann-Kopsch Altmann method. Harvey's papers on *Lumbricus* (1925 and 1931) seem to show that the Da Fano technique often distorts the Golgi and that there is a possibility of difference in appearance between fixed and living Golgi. The author's experience with this technique has been entirely different. The Golgi grains, scarcely distinguishable in the fresh oogonia of *Salmacis*, become clearly visible after osmication for six hours. In *Dasychone*, the elements have been observed to have the batonette form in fresh eggs as well as in Da Fano material.

The eggs of *Salmacis* are very small and very young oocytes were visible only after 2000 magnification. Treatment of such early oocytes with 2% osmic acid proved a failure and hence reliance had to be placed on fixed material. In the early oocytes where the Golgi apparatus has been noticed to occur as an irregular mass (Fig. 1; *g.a.*) the nucleus occupies almost two-thirds of the cell. With the growth in size of the cell the main mass (Fig. 2; *g.a.*) begins to cut off small pieces, the mass itself disintegrating into granules only after the first-formed elements (*g.g.*) have begun to migrate to other parts of the cytoplasm. No particular arrangement of these grains has been observed for they appear irregularly scattered throughout the cytoplasm (Fig. 3; *g.g.*). Concurrent with this process there is also an increase in the area of the cytoplasm (Figs. 2 and 3) and during this

period the Golgi become scattered uniformly. This stage is soon followed by a migration of all the bodies towards the nuclear membrane. When this process is progressing the Golgi begin to divide into finer grains which closely



FIGS. 1, 2 & 3.

1. Youngest oocyte showing the Golgi apparatus. Da Fano. $\times 3,000$.

2. Breaking up of the Golgi apparatus. Mann-Kopsch Altmann. $\times 3,000$.

3. Spreading of the divided Golgi elements prior to the formation of a concentration round the nucleus. Da Fano. Alum Carmine. $\times 3,000$.

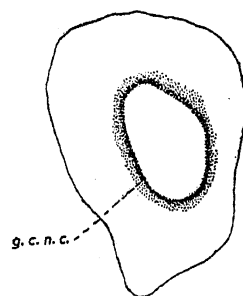


FIG. 4.

Figure showing the Golgi elements forming a circum-nuclear concentration. Da Fano. $\times 1,500$.

embrace the nuclear membrane and form a concentration (Fig. 4; *g.c.n.c.*). The complex phase does not last long and the granules once again begin to scatter throughout the cytoplasm. The division of the grains begun earlier seems to continue but a few of the bodies persist as such. These big grains migrate to the periphery and become arranged as shown in Fig. 11 (*p.g.g.*). A similar migration of Golgi has been observed by the author in *Acentrogobius neilli* where they were seen along with the grains formed by the breaking up of the rims of fatty yolk vacuoles to be the agents responsible for the transformation of the zona radiata into a mucilaginous envelope. In *Salmacis* no formation of such a structure has been noticed, and it is believed that the bigger grains may be instrumental in giving rise to the fertilisation membrane which, according to Mortensen, is lipoidal in composition. Just before the commencement of yolk formation the Golgi, though scattered, appear in well-marked patches among the mitochondria.

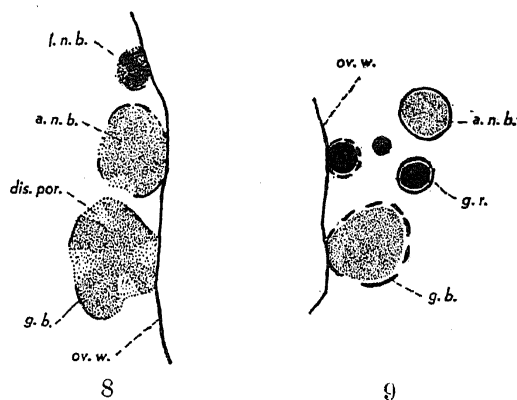
6. The Nutritive Bodies.

In a previous paper* the author has traced out the cytological changes during the transformation of a cell of the germinal layer into a nutritive body. These spheres have also been found to be of two types, lipoidal and albuminous, and they lie scattered throughout the ovarian tubule. Their attachment to the oocytes is haphazard for some of the

* "A Cytological Study of the Structure and Formation of Nutritive Bodies in *Salmacis bicolor*." (In course of publication.)

growing oocytes have only lipoidal while others have only albuminous nutritive spheres attached to them. A considerable number of the oocytes have no nutritive bodies attached throughout their development. Curiously enough some germ cells transforming into albuminous type of deutoplasmic spheres have other spheres, lipoidal as well as albuminous in close association.

The stage at which the nutritive bodies are absorbed seems also to vary. The spheres appear to persist for a considerable period in some oocytes while in others they seem to be absorbed immediately. In both the types of bodies, it is degeneration of the Golgi rim (Fig. 9; *g.r.*) that precedes the absorption of the material in the spheres. This is not uniform, for batonnettes are left sticking (Fig. 8; *g.b.*) to the sides of the bodies owing to the degeneration of the intermediate portions.



FIGS. 8 & 9.

Stages in the absorption of the nutritive bodies.

8. Mann-Kopsch Altmann. $\times 1,200$.
 9. Da Fano. Alum Carmine. $\times 1,200$.

contact with any oocyte, to that of one in close association with a growing egg. The fact that in nutritive bodies the Golgi rim exists without a nucleus leads to the question, can a Golgi body remain as such without the aid of a nucleus? Brambell (1925) notes in the case of the oviducal glands of the Fowl that the extruded Golgi retain their form even outside the cell and Bowen (1926*d*) remarks that logically it follows that if a piece of non-nucleated cytoplasm can persist for a short time the Golgi which form the living inclusion in the cytoplasm has necessarily to live even after it has been separated from the nucleus. The only difference according to him is that whereas a non-nucleated piece dies after a short time, the nucleated piece regenerates because it contains the nucleus. Judging by this standard in the nutritive bodies also the

Golgi may persist till they are absorbed into the cytoplasm of the oocyte or degenerate in the ovarian tubule. Just like a piece of non-nucleated cytoplasm the nutritive body persists only for a short time. The author's belief is that the process of solution of the nutritive body is started by the Golgi rim, for till a late stage of absorption of the nutritive bodies, batonnettes could be seen sticking to the unabsorbed portions of the nutritive spheres. But this is only a suggestion which explains the beginning of the process of solution and it is hoped that further work, which is being done in this direction, will throw light on this problem.

7. The Nucleolus.

The nucleolus occurs in the youngest oocytes (Fig. 5; *nu.*) as a lightly staining body occupying the centre of the nucleus. It is darkened by osmic fixatives and is not well stained by alum carmine after Da Fano. In corrosive acetic followed by Mann's Methyl Blue Eosin, it is light pink. In the earliest oocyte observed the nucleolus measured 0.55μ and the nucleus 1.1μ . In the very early stages the growth of the nucleolus keeps pace with that of the nucleus as the appended table will show.

Nucleus	Nucleolus	
1.65 μ	0.55 μ	
1.65	0.55	
2.2	1.1	
2.75	1.65	
3.3	1.65	} Period of Extrusion.
3.85	1.65	
4.1	1.85	
4.4	2.2	
4.95	2.2	
6.6	2.2	
7.7	2.2	
9.9	2.2	
12.8	3.2	
13.2	4.4	
16.3	5.5	} Period of Extrusion.
19.8	6.6	
23.1	6.6	
26.4	6.6	
27.5	7.7	} Final Extrusion.
42.9	13.2	
52.8	13.2	

When the nucleolus attains a size of 1.65μ extrusion may be said to begin. In many oocytes extrusion occurs at this stage and may persist till the nucleolus measures 2.2μ . Now, its growth instead of being proportional to that of the nucleus is comparatively negligible. If it is not extruded at this stage it enters another phase of slow growth and when it measures 6.6μ extrusion may be said to take place as evidenced by the presence of extruded nucleoli in many oocytes. If, however, it is not thrown out now, it grows to a maximum size of 13.2μ apparently remaining stationary until the nuclear membrane breaks down letting it out.

An attempt has been made to study the cause of the occurrence of the three periods of extrusion. One of the factors which seems to delay the process of extrusion is probably the attachment of the nutritive bodies to the oocytes. Early attachment of nutritive bodies retards the extrusion, but in a few even after such an attachment extrusion was observed. But these were in Champy and F. w. a. slides. Hence when next attention was focussed on the composition of the nutritive bodies the primary cause for the postponement of extrusion appeared to be the attachment of albuminous nutritive spheres. As has been noted in an earlier paper in material fixed in corrosive acetic, the albuminous spheres are well preserved while the lipoidal ones are distorted. An intensive study of corrosive acetic preparations revealed the fact that where extrusion takes place in early stages no albuminous nutritive bodies were found in relation with such oocytes. Thus nutritive bodies and the nucleolus seem to share among themselves the function of supplying the oocyte with raw materials for the growth of the mitochondria in the first place and formation of yolk next.

Another interesting phenomenon was observed. In nucleoli in corrosive acetic slides, measuring more than 4.4μ , less deeply staining vacuoles were found arising. The vacuoles were generally found in the centre and they never exhibited any basophil reaction. The number of such vacuoles also varied. In some nucleoli there was one large vacuole while in others there were as many as four small ones. No explanation for this phenomenon could be offered with the material at present available, but it appears possible that this may have something to do with the attachment of the nutritive bodies and a slow diffusion of material from the nucleolus into the cytoplasm.

The mode of extrusion is by the rupture of the nuclear membrane. The coelomic fluid of *Salmacis* has a pH near that of sea water. As in marine animals there is a free exchange between body fluids and the surrounding medium. Hence the coelomic fluid was employed instead of normal saline for the study of fresh oocytes. In many, extruded nucleoli were found just

outside the nuclear membrane and experiments followed by fixation in corrosive acetic such as (1) raising the pH to 9.4 by addition of Na_2CO_3 , (2) lowering the pH to 6 by the addition of dilute HCl, (3) increasing the salinity to 40, and (4) finally reducing the salinity to that of normal saline only confirmed the belief that in *Salmacis* the extrusion is a real phenomenon. Neither did these experiments suggest that the vacuoles inside the nucleolus were artefacts due to the action of the fixatives.

The nucleolus once extruded into the cytoplasm (Fig. 12; *nu.*) dissolves quickly near the nuclear membrane.

8. Mitochondria.

The mitochondria occur as a few grains scattered in the cytoplasm (Fig. 5; *m.*). The cell at this stage measures 9.9–13.2 μ . These grains

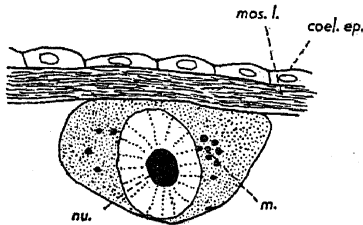


FIG. 5.

Youngest oocyte attached to the wall of the ovarian tubule. Flemming without acetic. $\times 2,000$.

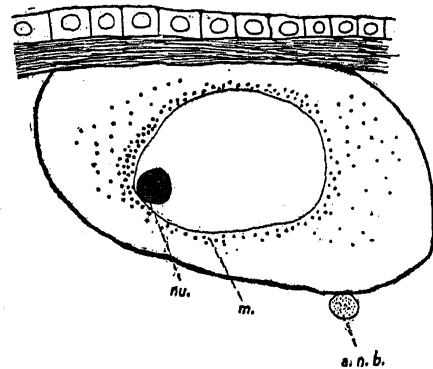


FIG. 6.

Illustrates the mitochondrial concentration round the nucleus. Flemming without acetic. Iron Hæmatoxylin. $\times 2,000$.

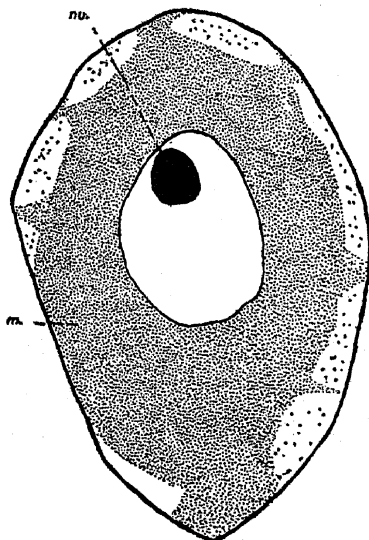


FIG. 7.

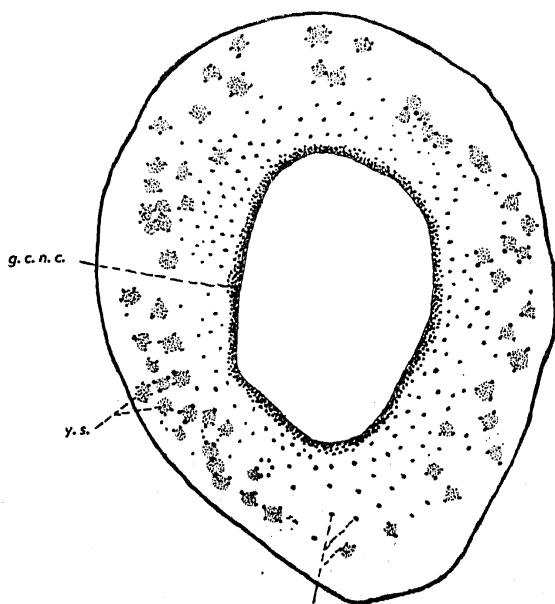
The mitochondria just before they fill up the cytoplasm of the growing oocyte. Champy. Iron Hæm. $\times 1,200$.

divide and give rise to a number of mitochondrial granules. Unlike the Golgi at the same stage, the mitochondria begin to migrate towards the nucleus (Fig. 6; *m.*) instead of being distributed throughout the cytoplasm, and form a concentration round it. Fig. 6 (*m.*) will show that even when a concentration is formed by the mitochondria granules, the middle area of the cytoplasm contains scattered mitochondria. Such a condition persists for some time and this is followed by a stage of spreading and division which occurs simultaneously with that of the Golgi (Fig. 7; *m.*). During

the process of spreading well-marked concentrations are seen proceeding from the circum-nuclear concentration to portions of cytoplasm farthest situated from the nucleus. Once the process begins, the spreading throughout the cytoplasm is completed quickly. The mitochondria from the commencement of the migration begin to grow in size. The materials brought by the nucleolus or by the absorption of the nutritive bodies are evidently utilised in this process since either absorption or extrusion is usually well marked during this period. Growth continues till the mitochondria occupy the whole area of the cytoplasm.

9. Yolk Formation.

Yolk formation begins when the oocyte measures $75.9-85.6\mu$. The mitochondria which are in the process of spreading come into relation with the Golgi bodies (Figs. 10, 11 and 12; *y.s.*) which are also in a similar stage



g.g.

FIG. 10.

Beginning of yolk formation. The enlarged peripherally lying Golgi bodies could also be seen. Da Fano. Alum Carmine. $\times 2,000$.

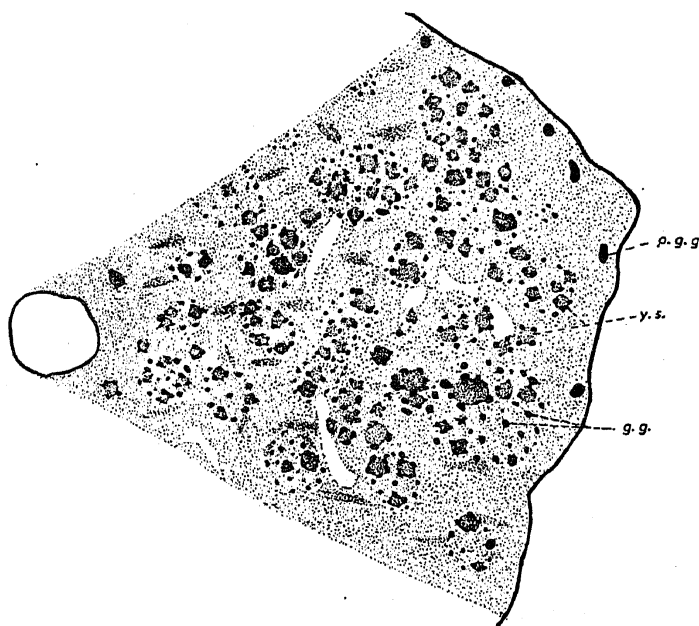


FIG. 11.

Portion of a fully grown oocyte showing the yolk spherules with their attached Golgi bodies. Da Fano. Alum Carmine. $\times 3,000$.

at this time. Each mitochondrion seems to be plastered over with 2-5 Golgi bodies (Fig. 14; *m.* and *g.g.*). The Golgi bodies are numerous and not all of them have been observed at the final stage in relation with all the mitochondria (Fig. 11; *g.g.*) for in the oogonium, in addition to yolk, free mitochondria and free Golgi have been observed (Fig. 13; *m.*). Yolk is formed by the transformation of material in the mitochondria and probable addition

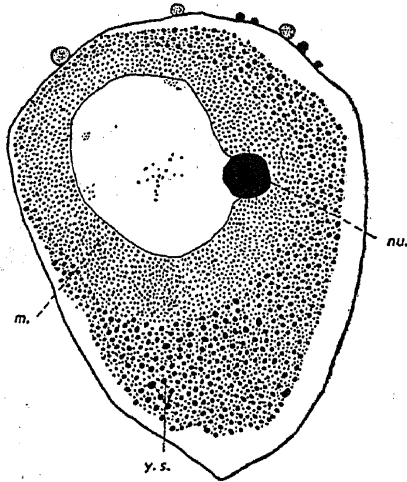


FIG. 12.

A growing oocyte showing transformation of mitochondria into yolk. The nucleolus is also seen in the process of being extruded. Nasonov. Iron Häm. $\times 800$.

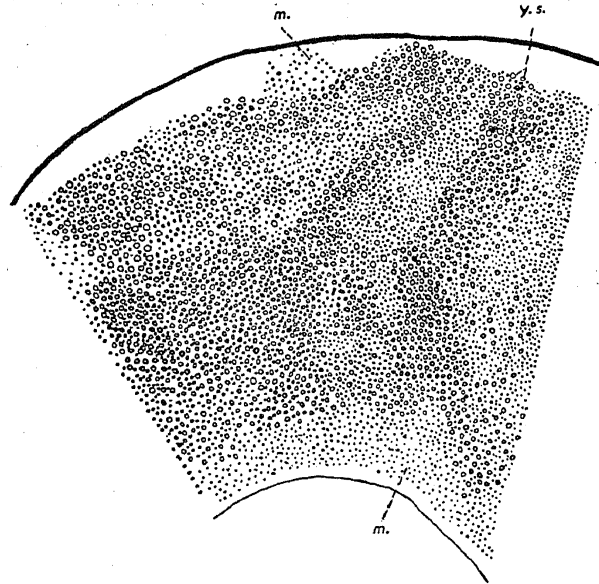


FIG. 13.

A fully grown oocyte showing the yolk spherules and the mitochondria. Champy Kull. $\times 3,000$.

of other material derived from the nucleolus and the nutritive bodies. By the time the Golgi come into relation with the mitochondria the growth of the latter seems to have come to an end. Transformation of mitochondria into yolk is not followed by an increase in size. The reduction in size due to shrinkage during transformation cannot be observed, as evidently there is a simultaneous addition of albuminous material condensed by the Golgi.

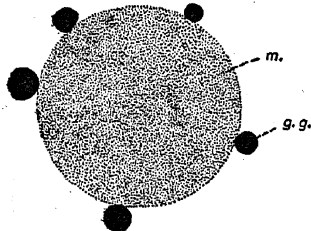


FIG. 14.

A diagrammatic representation of the attachment of the Golgi bodies to a mitochondrion prior to its transformation into a yolk spherule. Reconstructed from material fixed by the various techniques.

formation. In Mann-Kopsch and Nasonov slides yolk is but slightly fuchsinophile. In Da Fano, where the mitochondria are distorted, the yolk spherules are well preserved and stain light brown. In corrosive acetic preparations the discs were preserved and took on eosin readily, whereas the mitochondria were distorted beyond recognition.

The formation of yolk begins near the periphery and seems to proceed inwards (Figs. 10 and 12; y.s.). The mitochondria which stain blue after Champy and Flemming without acetic begin to take Orange G from the commencement of their trans-

10. Discussion.

1. *Theories regarding the function of Golgi bodies.*—Cowdry (1924) summarising the function of Golgi bodies comes to the conclusion that "Its activities may be bent in one direction in spermatogenesis and along entirely different lines in cells specialised to perform other duties." MacBride and Hewer (1931) observe "From data such as these has grown up the general theory of the function of the Golgi apparatus." Nasonov first put it forward, but Bowen has been instrumental in obtaining its general acceptance, in these terms:—The Golgi apparatus is a centre of synthetic processes. It is engaged primarily in the production of secretory granules. These may be excretory in nature. These products are of a temporary character such as mucous, serous, lipoid granules, yolk, acrosome, Nissle granules, etc. The apparatus undergoes hypertrophy during the process and is not transformed into the various products. This feature is constant in all the foregoing examples and is the fundamental postulate for the so-called "Process theory of action". The above theory does not take into consideration the general function of the apparatus in all cells. Gatenby (1919 and 1921), Brambell (1923), Ludford (1922 and 1925) and Rau and Ludford (1925) attribute to it a metabolic function with or without the production of visible substances. Brambell (1923) from his work on neurones comes to the conclusion that possibly they are concerned with the production of protein substances. But in Patella (1924) he finds substances of a fatty nature arising in relation with the apparatus. Similarly Ludford (1922) concluded from his work on *Dytiscus marginalis* that the apparatus would play by no means an unimportant part in lipoid metabolism. But his further work (1925) has brought out the fact that the apparatus has a number of other functions also. Anyway the fundamental fact remains that they are concerned in some way or other with cell metabolism. The first definite suggestion regarding its general function in all cells seems to be that of Gatenby (1919). "It may be considered that this spreading out is to enable the activities of the Golgi rods to be felt in every corner of the cytoplasm, or that the spreading out of the apparatus is only in preparation for subsequent segmentation of the egg. I think that both interpretations are true, and there seems little doubt that the two categories of cytoplasmic inclusions while spreading out are taking some part in the building up of the oocyte" (pp. 473-474). The products of metabolism may be visible or invisible. The three fundamental conditions laid down by Bowen (1926 *d*) are not strictly applicable to all these cases, especially the last, if the intracellular enzymes are not demonstrated by the usual techniques. A critical demonstration of the relation between the Golgi apparatus and the secretory

product, the final condition of Bowen, is according to him, not fully satisfactory and universal even in secretory cells. Regarding the question whether the relation between the Golgi and the secretory product should continue till maturity appears to be doubtful as evidence in spermatogenesis shows remarkable changes of the acrosome after separation from the apparatus.

Coming now to the actual method by which the Golgi apparatus performs its function, one finds the greatest difficulty. Gatenby's, Ludford's and Brambell's suggestion that the Golgi apparatus is concerned with metabolism is but a bare statement of fact. How this is done still remains to be answered. Similarly the actual process of formation of secretory products is also unexplained. Bowen (1926 *d*) rejects Nasonov's theory that the Golgi material forms a surface of separation between the granule and the surrounding plasma, which is responsible for the differentiation of the granule as the acrosome does not necessarily occupy a position within the Golgi material. He suggests that the surface membrane is really provided by the wall of the clear vesicle within which the secretory granule appears to be differentiated. "Thus materials for the secretory granules would be elaborated primarily in the Golgi apparatus, and thence transferred to the granules." Nasonov's explanation that they form a separating membrane does not clearly explain how the materials which the membrane separates are absorbed or are brought within the focus of activity of the apparatus. Neither does Bowen's explanation that the materials for the synthetic processes of the Golgi are derived from the nucleolus, mitochondria, cytoplasm, etc., demonstrate how these materials are made available to the apparatus which synthesises them into the various metabolic products. Even his observation that the materials for the secretory granules are first synthesised in the Golgi apparatus and then transferred to the granules implies a sort of transference which requires the aid of a medium. Similarly Gatenby's and Ludford's view that the scattering of the apparatus is to enable the activities of the apparatus to be felt in every corner of the cytoplasm wants also the aid of an agent by means of which the effect is made to be felt on the cytoplasm. Finally Cramer and Ludford's (1925) work on intestinal fat absorption where glycerine and fatty acids absorbed are reconverted into neutral fat is only explicable on the assumption of an agent bringing it within the focus of the activity of the apparatus. In addition, how the same apparatus synthesises products of various chemical compositions such as mucous, serous and lipoid droplets brings in the question whether the first products of synthesis are the visible products or whether the visible products are only secondary in their origin.

It was from difficulties such as these that the explanation of the production of an intra-cellular enzyme was first postulated by the author, in the case of the transformation of the zona radiata into a mucilaginous envelope and in the formation of two kinds of nutritive bodies in *Salmacis bicolor*. The formation of two kinds of nutritive bodies, *i.e.*, lipoidal and albuminous, in relation with the Golgi apparatus reminds one of the fatty and albuminous yolk arising in relation with the apparatus in the oogenesis of *Clibanarius* and hence attention may be directed to the views of workers in oogenesis.

2. *Theories regarding the function of Golgi bodies in Oogenesis.*—It would be well worth while to analyse the conclusions of Nath and his collaborators, especially in view of Nath's strong views regarding the shape and function of the apparatus. It is by no means easy to arrive at a definite conclusion regarding his views. Hence the papers are analysed under the following headings:

- (a) Golgi vesicles becoming fatty even in the early stages of oogenesis, but in which there is no fatty yolk.
- (b) Golgi bodies becoming fatty even in the early stages, but in which there is fatty yolk.
- (c) Golgi bodies remaining as a rim to the fatty yolk globules even after the completion of the oogenesis.
- (d) Golgi bodies becoming converted into fat in the final stages.

(a) *Golgi bodies becoming fatty even in the early stages of oogenesis, but in which there is no fatty yolk.*—Under this heading comes the oocyte of the earthworm (Nath, 1930) and the mosquito (Nath, 1929). He is of opinion that in these two cases even though the Golgi vesicles do not swell up, yet they are fatty (p. 502, 1930). I conclude from his descriptions that they are not strictly comparable. The fatty content of the Golgi vacuoles of *Pheretima* has not been substantiated by his recent work with the vital dyes (Nath, 1933). The reasons adduced by Nath for considering the Golgi elements as fatty are: (1) in whole ovaries mounted after fixation in Champy for 24 hours, the Golgi vacuoles appear colourless or at most as slightly grayish bodies after the lapse of one month; (2) when the ribbons containing Champy fixed material are exposed to the action of xylol to remove the paraffin, the Golgi which appear as blackened elements in the ribbons appear colourless or slightly grayish (p. 492, 1930). Naturally, one expected the facts to react to Scharlach R and Sudan III but strangely enough Nath (1933) says, "Nothing whatsoever in the oogonia or in the oocytes of any stage is tinged even slightly by these dyes" (p. 138, 1933).

He further continues that "In *Pheretima*, *Culex*, *Dysdercus*, *Crossopriza*, *Plexxippus* and the Rabbit, the substance, described by him as Golgi bodies and which blackens after short periods of immersion in 2 per cent. osmic acid has to be considered as highly unsaturated or little oxidised lipid as it remains absolutely uncoloured after treatment with Scharlach R and Sudan III."

Now let us turn to the question whether the fatty Golgi vesicles of *Culex* and *Pheretima* are strictly comparable. In *Pheretima* the Golgi bodies appear as granules in Bouin preparations which according to him answer to the description of the 'yolk droplets' of Harvey (p. 497, 1930); further the Golgi vesicles of *Pheretima* appear as solid granules in Da Fano. Even the statement about the appearance of these bodies after treatment with Bouin and Da Fano gives one the clue that what he claims to be fat inside the Golgi vacuole is after all not fat. He himself criticises King's observation thus: "Now it is well known that the Da Fano method fails to show fat in finished slides, while the Golgi apparatus is preserved" (p. 404, 1929).

In *Culex* his reasons for concluding that the vesicle contains fat are: (1) in the oocyte the microspheres appear black after about 48 hours immersion in F. w. a.; (2) they are completely decolourised if the slide is kept in either turpentine or xylol for about 20 minutes after which they could not be made out in the preparations (p. 662, 1929); (3) in centrifuged decolourised Mann-Kopsch preparations the microspheres are vacuolar and occupy the upper pole; and (4) in centrifuged eggs fixed in Da Fano they appear as distorted vacuoles (p. 664).

He is really not justified in this conclusion that the Golgi in *Pheretima* and *Culex* have a similar composition. In *Culex* what he describes is a transformation—of course into an unsaturated lipid if we have to accept his latest interpretation, whereas in *Pheretima* the elements do not contain fat and at the same time do not also transform. The question now arises how the Golgi bodies having the same composition in *Culex* and *Pheretima* give different reactions? The microspheres in *Culex* appear as vacuoles in Mann-Kopsch followed by turpentine and Da Fano, while in *Pheretima* the Golgi appear as solid black granules in Da Fano and are preserved even after Bouin.

(b) *Golgi bodies becoming fatty even in the early stages, but in which there is fatty yolk.*—This leads us to the oogenesis of *Luciola* (Nath and Mehta, 1930) which is still more difficult to understand. Hence Nath's own description is put in and analysed. He informs the reader (p. 12), "Text-Fig. 16 represents the greater portion of the sack containing the primordial

germ cells. The nuclei of these germ cells appear distinctly, but the cell boundaries cannot be made out. In spaces between the nuclei one can see distinct vacuoles with *a rim gone black due to osmication and a clear central substance*. These are the Golgi vacuoles. If, however, the ovary is kept in 2 per cent. osmic acid for more than half an hour, *these vacuoles are blackened considerably and then appear solid*. Such quick blackening in osmic acid unmistakably *shows that their contents are fatty*. The number of these vacuoles is about three or four in each cell, as can be determined more accurately by the study of fixed preparations, and they are not closely aggregated in each cell. *In Chrome-osmium and Mann-Kopsch preparations they appear solid and black, but they can be easily decolourised with xylol, or turpentine, after which they appear as clear vacuoles.*"* In this explanation of his results it is not at all clear how the rim present in Golgi vacuoles after ten minutes of immersion in 2 per cent. osmic acid could be easily decolourised in Chrome-osmium and Mann-Kopsch preparations with turpentine or xylol. Either the rim should be an artefact, as entire reliance could not be placed on treatment of tissues with 2 per cent. osmic acid, or he is dealing with inclusions which are not Golgi bodies. My justification for the latter assertion is that if the rim is fatty even in primordial germ cells and continues to be so, how is it converted into the typical lipo-protein Golgi apparatus of the somatic cells. The little work that has been done on dictyokinesis (Gatenby and Ludford, 1921, Ludford, 1922 and Hirschler, 1918) and early developmental stages at least demonstrate that the apparatus has its typical composition in dividing cells as well as in segmenting stages. As such an interpretation strikes at the root of our knowledge about the composition of the Golgi, no worker on cytoplasmic inclusions having ever attributed to it a fatty composition, it is difficult to understand the assertion of Nath. Because of the presence of fat in the Golgi vesicles Nath considers *Luciola* a very valuable material for the demonstration of the origin of fatty yolk vacuoles from the Golgi vacuoles. It would be relevant here to consider (1) how in *Luciola* these Golgi vacuoles give rise to fatty yolk vacuoles, and (2) whether the Golgi element has anything to do with its formation. In Text-Fig. 14 he represents a developing oocyte and remarks that the Golgi vacuoles which are uniformly distributed have increased in number and show a very distinct black rim and a clear interior. In the highly developed oocyte the Golgi vacuoles are blackened. But this is all in oocytes which he studied after treatment with 2 per

* Italics mine.

cent. osmic acid. In fixed preparations in Text-Fig. 5 he shows an advanced oocyte in which "many of the Golgi elements have swollen up". Their appearance seems to be solid and he further mentions that xylol decolourises some of them (pp. 15, 16 and 17; Figs. on pages 20 and 11). No mention of the decolourisation of the chromophobic part alone is mentioned and the reader is left in a dilemma whether to rely on the Mann-Kopsch and other standard fixatives or on treatment of fresh eggs with osmic acid. Anyway as Nath deduces the fatty nature from fixed preparations it need only be understood that the rim as well as the interior becomes fatty, rejecting of course his conclusions on fresh eggs treated with 2 per cent. osmic acid.

Nath himself is doubtful as to when such Golgi vacuoles may be called fatty yolk vacuoles and to avoid such a confusion uses the term Golgi vacuoles throughout. His final conclusion regarding the mode of fatty yolk formation is that 'the Golgi vacuoles simply grow in size and give rise to fatty yolk vacuoles'.

Analysing these conclusions to elucidate the function of the Golgi bodies, it will be seen that Nath has nothing new to offer. If the Golgi vacuoles are fatty and simply grow in size one would like to know what the Golgi has to do with the growth of the vacuole. It is merely like the growth of a crystal or to put it more clearly, origin of fat from the cytoplasm. It can be nothing but that, for there is no secretion and no transformation.

(c) *Golgi bodies remaining as a rim to the fatty yolk vacuoles even after the completion of oogenesis.*—In *Scolopendra* (Nath and Hussain, 1929), spider (Nath, 1929), *Dysdercus* (Bhandari and Nath, 1930) and *Periplaneta* (Nath and Mohan, 1929) the Golgi bodies contain a watery non-fatty fluid. Even in these he describes two different processes. It would be convenient to consider first the case of *Dysdercus cingulatus* where the Golgi seem to exist as such in the fully developed egg as Nath and collaborator mention that even after Bouin fixation "the Golgi vesicles appear as clear vacuoles surrounded by a corroded rim, especially in the advanced oocytes where they have grown in size (Fig. 23)". The process described by Nath is one of secretion of fatty yolk by the Golgi but he says (p. 620), "It is impossible to have stronger evidence than that furnished by the red cotton bug in favour of the theory of Nath that in the eggs of the spider, *Scolopendra* and the cockroach *the vacuolar non-fatty Golgi vesicles of the youngest oocyte directly give rise to vacuolar fatty yolk, in as much as in Dysdercus the Golgi vesicles are fatty even in the earliest oogonia.*"* As will be seen he makes no mention of secretion but on

* Italics mine.

the other hand tries to correlate these results with those he obtained in spider, *Scolopendra* and cockroach.

(d) *Golgi bodies becoming converted into fat in the final stages.*—Attention may now be directed to the process of fatty yolk formation in the spider (1929). The Golgi elements are described as vacuoles with a very sharp chromophilic rim and a central chromophobic substance and their contents are supposed to be watery and non-fatty. The fatty yolk vacuoles do not show any rim and according to him appear dull, black and solid. When the Mann-Kopsch slides were treated with turpentine the fatty yolk vacuoles appeared as clear vacuoles and he refers the reader to Fig. 8. In describing Text-Fig. 11 also he remarks that the decolourised fatty yolk appeared as clear vacuoles giving a frothy appearance to the egg. In the case of *Scolopendra* he mentions (pp. 408, 409 and 411) that acid fuchsin tends to decolourise osmicated fatty bodies and that in Mann-Kopsch slides stained with acid fuchsin the fatty yolk vacuoles “appear as colourless vacuoles without a sharp black chromophilic rim of the Golgi vacuole”. What apparently happens in the two cases is that the Golgi itself is transformed.

Yet in his paper on the cockroach (1929) he observed: “In the spider, *Scolopendra* and *Luciola* the Golgi vesicles enlarge considerably when the egg grows, with the result that the membrane of the vesicle becomes more and more attenuated. *Pari passu* with their growth, free fat, not miscible with the general cytoplasm is deposited in the interior of these vesicles.” As shown above, Nath's accounts of fatty yolk formation in spider and *Scolopendra* clearly indicate a transformation and one cannot understand how he can describe an attenuated rim in these cases. In the cockroach also (Nath and Mohan, 1929) his description of the process of fatty yolk formation is one of transformation. The circum-nuclear ring of Golgi vesicles—in his account of the oogenesis of the cockroach—break away from the nuclear membrane and the majority of these continue to grow in size becoming more and more fatty till they assume huge dimensions. In this process the rim, it appears, becomes attenuated. In Kolatschev preparations treated with turpentine he records their appearance as clear vacuoles of different sizes (pp. 267, 268 and 269). Even here, his description is one of transformation as treatment of Kolatschev material with turpentine suggests, and it is really difficult to conceive of an attenuated rim existing in these before treatment with turpentine. What Nath describes is apparently an optical illusion.

Further analysis of Nath's work seems unnecessary, for he has mixed up a number of processes to be included under his theory of fatty yolk

formation. The above analysis of his papers will show that there is no such thing as Nath's theory which could be tested at least in some cases. An evidence for such a conclusion will be found in his conception of 'fatty yolk' (p. 501, 1930). The term according to him is misleading because it is not the case of a metamorphosis of a substance A into B "but a case of a small vesicle simply enlarging and storing up fat inside it". One would like to know whether the formation of fatty yolk from Golgi bodies in the spider and *Scolopendra* is not the metamorphosis of a substance A into B.

Let us now turn our attention to another school of cytologists which believes in the possibility of albuminous yolk arising in relation with the apparatus. It would be well to consider Hogben's theory (1921) before entering into a discussion about the views of Harvey (1925, 1927, 1929, 1931*a*, 1931*b*, 1931*c*) who may be taken as a representative of the school. The reason for this course of procedure is due to the fact that Harvey has accepted Hogben's hypothesis and tries to explain away all phenomena in that light. In justice to Hogben it should be admitted that he is only concerned with nuclear and nucleolar phenomena. His conclusions regarding the mode of yolk formation are based on the work of other writers like Gatenby, Hirschler and Hilarowicz. The theory as postulated by Hogben—"a very intricate inter-action of the metabolic functions of the plasmosome, mitochondria and the Golgi apparatus, a hypothesis, which while unifying the data, is perfectly compatible with the observed transformation of deutosomes (nuclear emissions), chondriosomes and dictyosomes in individual cases"—is based on the observations of transformations and not on secretory or synthetic activity. Moreover, Hogben and later Harvey consider only one aspect of the activity of the Golgi, namely, yolk formation. Both the workers do not seem to have taken into consideration the metabolic function suggested by Gatenby even before the publication of Hogben's paper. The theory does not also include the observed transformations of mitochondria into fat, nucleoli into fat and the Golgi into fat. The theory as it stands represents only one side of the question. Harvey finds the Golgi having no apparent function in *Lumbricus* (1925 and 1931*a*) and Hogben mentions similar experience of Gatenby and Hilarowicz. If, as Harvey admits, the apparatus is concerned with the production of yolk spherules in *Carcinus* (1929) and *Antedon* (1931*b*) surely in the earthworms which he has examined it should have some function. The absence of any visible products need not in any way deter one from considering it as not active.

Reverting to Harvey's elaboration of Hogben's theory the first reference to it is made in his paper on *Carcinus moenas*. He observed yolk

being deposited in the chromophobe part of the Golgi apparatus and considered the proteid yolk formation as a condensation into droplet form by the Golgi of material synthesised by the mitochondria, the plasmosome and the ground cytoplasm. This theory has been elaborated further in his papers on *Antedon* and *Asterias* (1931b and 1931c) where he mentions that in its widest application it will be found to fit practically all cases.

Fat like yolk is of common occurrence in a large number of eggs and Golgi bodies in cells of the germinal layer which transform into deutoplasmic spheres do not have a composition different from that of the one in the oocyte. Harvey seems to have overlooked the work of Cramer and Ludford (1925) when he makes the statement "The possibility that fat might arise in some eggs in connection with the Golgi apparatus and not in others has always appeared doubtful to me on theoretical grounds"; but when he finds fat arising in relation with the Golgi in degenerating germinal cells (1931b) he considers the process a paradox and argues that there is no conversion of the latter into fat until the cell has become so degenerate as to have no cellular structure.

Bowen's theory is not far different from that of Hogben or Harvey's except that he considers the Golgi as the synthetic centre. But both these theories do not seem to have paid enough attention to the function of Golgi bodies in ordinary cells as well as in eggs in which there is no deposition of fat or yolk. Surely, the apparatus should have some definite function in all cells and from the evidence available it is perfectly reasonable to consider them as controllers of metabolism. In addition it appears justifiable to conclude that in specialised cells they will have in addition specialised functions.

Returning now to its function in oogenesis the author is fully convinced that the primary function of the apparatus is the production of intra-cellular enzymes and that yolk and fat are only secondary products resulting from the action of these enzymes. Such a hypothesis would explain the cases where intimate contact is not ensured and also cases where the products undergo further growth in other parts of the cytoplasm not necessarily in relation with the apparatus. If it is admitted that the first result of the activity of the Golgi is the production of intra-cellular enzymes it becomes all the more reasonable why the first origin of these products should be in contact with or in the immediate vicinity of the apparatus owing to the fact that initiation of condensation is easier in the fountain-head of these enzymes, whereas further growth may take place in other regions. Such a hypothesis will also explain how these bodies exert their influence in all parts of the cytoplasm.

One may enquire how this will throw light on the transformation of Golgi into fat or yolk. The author would just refer to the phenomena occurring in gland cells, especially the extrusion of a large portion of the apparatus at the end of secretion. In eggs such a process is replaced by the conversion of the superfluous elements into fat or yolk but a conception like that lays down clearly that *not all the Golgi elements are transformed*. Transformation, at least of part, and absorption have been noticed during spermatogenesis (Gatenby and Wigoder, 1929) and there is no reason why in an egg where there are numerous Golgi elements, a good number may not be transformed as a measure of cellular economy. If in gland cells secretions of various types could occur one may reasonably enquire why such a process could not take place in oogenesis.

Finally is it not probable that neutral red vacuoles (Douglas, Duthie and Gatenby, 1933) instead of being products of reaction of the cell to neutral red may really be the visible expression of enzymes secreted by the Golgi? (Vera Koehring, 1930 and 1931).

3. *Mitochondria and Yolk Formation*.—It has been mentioned that in *Clibanarius* the mitochondria do not transform into albuminous yolk but remain as such, and that transformation of mitochondria into yolk should be followed by histochemical changes. The majority of the mitochondria of *Salmacis* transform into yolk and such a phenomenon is followed by different reactions of these spherules to fixatives and stains. I would like to suggest from the evidence of the transformation of mitochondria unaccompanied by any increase in size, that the mitochondria after all are not the synthetic centres of yolk formation. The Golgi elements plastered on to the mitochondria and persisting in a similar condition in the yolk spherules seem to be the real agents responsible for such a change. It does not appear to the author quite necessary to consider the size as an important factor. The fact that the yolk spherules are not bigger than the mitochondria does not form a serious objection as the composition of the spherules are more important than their size.

11. Summary.

1. Cytoplasmic phenomena during the oogenesis of *Salmacis* have been worked out.
2. The mitochondria first become uniformly distributed throughout the cytoplasm.
3. The nucleolus exhibits three definite periods of growth when it may be extruded. Extrusion seems to be retarded by the attachment of albuminous nutritive bodies.

4. The Golgi bodies while in the process of spreading come into relation with the mitochondria. Yolk appears to be formed by a conversion of the material of the mitochondria with further secretion by the Golgi of material derived from nucleolus and nutritive bodies.

5. Theories regarding the function of the Golgi apparatus in various cells are discussed.

6. It is suggested that the primary function of the Golgi bodies is secretion of various intra-cellular enzymes visible or invisible and that the secretory products like fat, yolk, acrosome, mucous, serous and lipid granules are only secondary products resulting from the action of these enzymes.

It is a great pleasure to acknowledge my indebtedness to Professor R. Gopala Aiyar for his very active interest and help throughout this work.

BIBLIOGRAPHY.

1. Beams, H. W., and Goldsmith, J. B. "Golgi bodies, Vacuome and mitochondria in the salivary glands of *Chironomous larva*." *J. Morph.*, 1930, **50**.
2. Bhandari, K. G., and Nath, V. "Studies on the Origin of Yolk. V. Oogenesis of the red cotton bug *Dysdercus cingulatus*." *Zeit. Zellf. mikr Anat.*, 1930, Bd. **10**.
3. Bhattacharya, D. R. .. "The Golgi apparatus and the Vacuome Theory." *Allahabad Univ. Studies*, 1927-28.
4. Bowen, R. H. .. "On the idiosome, Golgi apparatus and Acrosome in male germ cells." *Anat. Rec.*, 1922, **24**.
5. Bowen, R. H. .. "On the possible relation between the Golgi apparatus and the secretory products." *Amer. Jour. Anat.*, 1924, **33**.
6. Bowen, R. H. .. "Studies on the Golgi apparatus in Gland Cells. Pt. I." *Quart. Jour. Micr. Sci.*, 1926, **70**.
7. Bowen, R. H. .. Part II, *Ibid.*, 1929b.
8. Bowen, R. H. .. Part III, *Ibid.*, 1926c.
9. Bowen, R. H. .. Part IV. "A critique of topography, structure and function of the Golgi apparatus in gland cells." *Quart. Jour. Micr. Sci.*, 1926d, **70**.
10. Bowen, R. H. .. "The cytology of Glandular Secretion." *Quart. Rev. of Biol.* (In two Parts), 1929, **4**.
11. Brambell, F. W. R. .. "The activity of the Golgi apparatus in the Neurones of *Helix aspersa*." *Jour. Phy.*, 1923, **57**.
12. Brambell, F. W. R. .. "The Nature and Origin of Yolk." *Brit. Jour. Expl. Biol.*, 1924, **1**.
13. Brambell, F. W. R. .. "Part played by the Golgi apparatus and its subsequent reformation in the Oviducal glands of the fowl." *J. Roy. Micr. Soc.*, 1925.

14. Chubb, G. C. "The growth of the oocyte in *Antedon*: A morphological study in cell metabolism." *Phil. Trans. Roy. Soc., London, B*, 1906, **198**.
15. Cowdry, E. V. .. *General Cytology*. Univ. Chicago Press, 1924.
16. Cramer, W., and Ludford, R. J. "On cellular changes in intestinal Fat absorption." *Jour. Phy.*, 1925, **60**.
17. Cramer, W., and Ludford, R. J. "On cellular activity and cellular structure as studied in the Thyroid gland." *Jour. Phy.*, 1926, **61**.
18. Douglas, Hilda, Duthie, E. S., and Gatenby, J. B. "Further investigations of the reaction of certain cells to Neutral Red solution." *Zeit. fur wiss. Zool.*, Oct. 1933, Bd. **144**.
19. Dyal, Sukh and Nath, Viswa. "On the Nature of the 'Yolk Nucleus' of Spiders." *J. Roy. Mic. Soc.*, 1933.
20. Gardiner, M. S. .. "Oogenesis of *Limulus polyphemus* with special reference to the behaviour of the nucleolus." *Jour. Morph.*, 1927, **44**.
21. Gatenby, J. B. .. "Cytoplasmic Inclusions in Germ Cells: Part V. The Gametogenesis and early development of *Limnaea stagnalis* with special reference to the Golgi apparatus and the mitochondria." *Quart. Jour. Micr. Sci.*, 1919, **63**.
22. Gatenby, J. B. .. "Cytoplasmic Inclusions in Germ Cells: Part X. Gametogenesis of *Saccocirrus*." *Quart. Jour. Micr. Sci.*, 1922, **66**.
23. Gatenby, J. B. and Ludford, R. J. "Dictyokinesis in Germ Cells or the division of Golgi apparatus during cell division." *Proc. Roy. Soc. London, B*. 1922, **92**.
24. Gatenby, J. B., and Nath, V. "Oogenesis of certain Invertebrata with special reference to *Lumbricus*." *Quart. Jour. Micr. Sci.*, 1926, **70**.
25. Gatenby, J. B., and Wigoder, S. "The effect of X-radiation on the spermatogenesis of the Guinea-pig." *Proc. Roy. Soc. London, B*, 1929, **104**.
26. Gatenby, J. B., and Woodger, J. H. "On the relationship between the Formation of Yolk and the Mitochondria and the Golgi apparatus during Oogenesis." 1920.
27. Gresson, R. A. R. .. "Yolk formation in certain Tenthredinidæ." *Quart. Jour. Micr. Sci.*, 1929, **73**.
28. Gresson, R. A. R. .. "Yolk formation in *Periplaneta orientalis*." *Ibid.*, 1931, **74**.
29. Gresson, R. A. R. .. "A study of the cytoplasmic inclusions and Nucleolar phenomena during the oogenesis of the mouse." *Ibid.*, 1933, **75**.
30. Harvey, L. A. .. "On the relationship between mitochondria and the Golgi apparatus to yolk formation in the egg of the common earthworm, *Lumbricus terrestris*." *Ibid.*, 1925, **69**.
31. Harvey, L. A. .. "The History of the Cytoplasmic Inclusions of the egg of *Ciona intestinalis*, (L.) during oogenesis and fertilisation." *Proc. Roy. Soc., London, B*, 1927, **150**.

32. Harvey, L. A. "The oogenesis of *Carcinus moenas* (Penn.) with special reference to yolk formation." *Trans. Roy. Soc. Edin.*, 1929, 150.
33. Harvey, L. A. "Oogenesis of *Lumbricus*: A restatement." *Quart. Jour. Micr. Sci.*, 1931a, 74.
34. Harvey, L. A. "Studies on Echinoderm Oogenesis: I. *Antedon bifida* (Pennant)." *Proc. Roy. Soc. London*, B, 1931, 1931b, B. 107.
35. Harvey, L. A. "II. *Asterias rubens* Linne." *Proc. Roy. Soc. London*, B, 1931c, 107.
36. Hibbard, Hope "Cytoplasmic Inclusions in the egg of *Echinarchnius parma*." *Jour. Morph.*, 1922, 36.
37. Hirschler "Uber den Golgischen Apparat embryonaler zellen." *Arch. f. mikr. Anat.*, Jan. 1918, 91.
38. Hirschler "Studien uber die Plasmakomponenten (Golgi-Apparat u. a.) an vitalgefärbten männlichen Geschlechtzellen einiger Tierarten." *Zeit. Zellf. mikr Anat.*, Jan. 1928, Bd. 7.
39. Hirschler and Monne, Ludwik. "Studien uber die Plasmakomponenten (Golgi-Apparat u. a.) an vitalgefärbten männlichen Geschlechtzellen einiger Sauger (*Cavia*, *Lepus*, *Mus*.)" *Ibid.*, 1928, Bd. 7.
40. Hendee, Esther C. .. "Formed components and Fertilisation in the egg of the sea-urchin *Lytechinus variegatus*." *Carnegie Instn. Wash. Pubn.* No. 413, 1931, 27.
41. Hogben, L. T. "Studies on Synapsis III. The Nuclear organisation of the germ cells in *Libellula depressa*." *Proc. Roy. Soc. London*, B, 1931, 92.
42. King, S. D. "A note on the oogenesis of *Peripatopsis capensis*." *Quart. Jour. Micr. Sci.*, 1926, 70.
43. King, S. D. "The Oogenesis of *Oniscus asellus*." *Proc. Roy. Soc. London*, B., 1926, 100.
44. Koehring, V. "The Neutral Red Reaction." *Jour. Morph.*, 49, 1930.
45. Koehring, V. "Thermal Relationships in Neutral Red Reaction." *Jour. Morph.*, 1931, 52.
46. Ludford, R. J. "Contributions to the study of the oogenesis of *Patella*." *Jour. Roy. Micr. Soc.*, 1921.
47. Ludford, R. J. "The behaviour of the Golgi bodies during nuclear division with special reference to amitosis in *Dytiscus marginalis*." *Quart. Jour. Micr. Sci.*, 1922, 66.
48. Ludford, R. J. "The general and experimental cytology of Cancer." *Jour. Roy. Micr. Soc.*, 1925.
49. Ludford, R. J. "Cell organs during secretion in the epididymis." *Proc. Roy. Soc. London*, B., 1925, 98.
50. Ludford, R. J. "The vital staining of Normal and Malignant cells. III. Vital staining of Acinar cells of the pancreas and its bearing on the theories of vital staining with basic dyes." *Proc. Roy. Soc. London*, B., 1931, 107.

51. Ludford, R. J. and Carmer, W. "The mechanism of secretion of the Thyroid gland." *Proc. Roy. Soc. London, B.*, 1928, **102**.
52. MacBride, E. W., and Hewer, H. R. "Recent Advances in Microscopy, *Zoology*, Edited by A. Piney J. & A. Churchill, London, 1931.
53. Miller Ruth, A., and Helen, B. "Observations on the formation of the Egg of *Echinometra lucunter*." *Carnegie Instn. Wash.*, 1931, **27**, Pubn. No. 413.
54. Nasonov, D. .. "Das Golgische Binnentz und seine Beizeihungen zu der sekretion Amphibiandruse." *Arch. f. mikr. Anat.*, 1923, Bd. **97**.
55. Nasonov, D. .. "Forsetzung Unterschungen an einiger Saugetierdrusen." *Ibid.*, 1924, **100**.
56. Nasonov, D. .. "Der Exkretionsapparat (Kontraktile Vakuole) der Protozoa al Homologon des Golgischen Apparat der Metazoa zellen." *Ibid.*, 1924b, **103**.
57. Nath, V. .. "Golgi origin of fatty yolk in the light of Parat's work." *Nature*, 27th Nov. 1926.
58. Nath, V. .. "Studies on the Origin of Yolk: I. Oogenesis of *Crosopriza Lyoni*." *Quart. Jour. Micr. Sci.*, 1929, **72**.
59. Nath, V. .. "Studies on the shape of the Golgi apparatus: I. Egg follicle of *Culex*." *Zeit. Zellf. mikr Anat.*, 1929, Bd. **8**.
60. Nath, V. .. "Studies on the shape of the Golgi apparatus: II. Observations on the fresh egg of the Indian Earthworm *Pheretima posthuma*." *Quart. Jour. Micr. Sci.*, 1930, **73**.
61. Nath, V. "Microchemical Tests for Fats, Lipoids and Vacuoles with special reference to Oogenesis." *Quart. Jour. Micr. Sci.*, June 1933.
62. Nath, V., and Hussain, M. T. "Origin of Yolk in *Scolopendra*." *Nature*, 6th Nov. 1926.
63. Nath, V., and Hussain, M. T. "Studies on the Origin of Yolk: II. Oogenesis of *Scolopendra*, *Obostigmus fecæ*." *Quart. Jour. Micr. Sci.*, 1929, **72**.
64. Nath, V., and Mehta, D. R. "Studies on the Origin of Yolk: III. Oogenesis of the fire-fly *Luciola gorhami*." *Quart. Jour. Micr. Sci.*, 1930, **73**.
65. Nath, V., and Mohan, P. "Studies on the Origin of Yolk: IV. Oogenesis of *Periplaneta americana*." *J. Morph.*, 1929, **48**.
66. Nath, V., and Nangia, M. D. "A demonstration of the Golgi apparatus and Vacuome as independent cytoplasmic components in the fresh eggs of Teleostean Fishes." *J. Morph.*, 1931, **52**.
67. Rau, A. S., and Ludford, R. J. "Variation in the form of the Golgi bodies during the development of neurones." *Quart. Jour. Micr. Sci.*, 1925, **69**.
68. Tennent, D. H., Gardner, M. S., and Smith, D. E. N. "A Cytological and Biochemical Study of the Ovaries of *Echinometra lucunter*." *Carnegie Instn. Wash.*, 1931, **27**, Pubn. No. 413.

KEY TO LETTERING OF TEXT-FIGURES.

- a.n.b.* .. Albuminous nutritive spheres.
- coel. ep.* .. Outer epithelium of the gonadic tubule.
- dis. por.* .. Dissolved portion of the nutritive spheres.
- g.a.* .. Golgi apparatus.
- g.b.* .. Golgi batonette.
- g.c.n.c.* .. Golgi circum-nuclear concentration.
- g.g.* .. Golgi grains.
- g.r.* .. Golgi rim of the nutritive bodies.
- l.n.b.* .. Lipoidal nutritive spheres.
- m.* .. Mitochondria.
- nu.* .. Nucleolus.
- ov.w.* .. Oocyte wall.
- p.g.g.* .. Enlarged peripherally situated Golgi grains.
- y.s.* .. Yolk spherules.