Microchemical Tests for Fats, Lipoids, and Vacuoles with special reference to Oogenesis.

By

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Introduction

If a substance goes black in osmic acid in a short time, for example, in twenty-four hours' immersion in Champy's fluid, and can be subsequently decolorized in turpentine, the cytologist is generally content to label it as fat. If, on the other hand, a substance goes black only after a prolonged period of osmication, for example, in four days' incubation in 2 per cent. osmic acid at 40° C., and cannot be subsequently decolorized by turpentine, he complacently considers it as lipoidal in nature, and assigns it to the category of Golgi apparatus. Observations which are recorded in this paper prove that the above test is by itself very unsatisfactory. Its exclusive use by the majority of cytologists has resulted in much confusion.

The time taken by a particular sample of fat or lipoid to blacken in osmic acid depends entirely on the degree of unsaturation, and the amount of oxidation it has already undergone. Recently a sample of completely hydrogenized fat, whose iodine value was nil, and which had been prepared from whale fat, was supplied to me through the courtesy of Dr. J. N. Ray of the Punjab University Chemical Laboratory. Films of this completely saturated fat did not blacken in the slightest degree, even in seven days' incubation in 2 per cent. osmic acid at 40° C. It is, however, very uncertain whether completely saturated fats and lipoids exist in such a substance as protoplasm which is in a state of continual chemical flux. Similarly Walker and Allen (1927) failed to blacken in osmic acid methyl myristate, which is another example of a completely saturated fat. Thus it is clear that samples of fat can be found which will actually take longer time to blacken in osmic acid than many samples
of lipoids, simply because the latter are more unsaturated than the former. It follows, therefore, that osmic acid is an unsuitable test for distinguishing fats from lipoids.

There are of course other tests which have been discussed by Gatenby and Cramer in Lee's 'Vade Mecum', to which reference may be made. One of these seems trustworthy, and at the same time is highly convenient to employ. This is the Sudan III or Scharlach R test. True fats are stained deeply with these dyes, whereas the majority of lipoids are not. Lipoidal substances such as cholesterin-esters and cholesterin fatty acid mixtures also stain, but much less intensely. This test again seems to be uncertain; but for the purposes of the thesis developed in this paper it is enough that true fats, according to Gatenby and Cramer, always stain deeply. In other words, if a granule in the cell is not stained with Sudan III and Scharlach R, it is not fat.

The classical lipoidal Golgi apparatus has been blackened after a short treatment with osmic acid. Bowen (1919 and 1928) succeeded in blackening the Golgi apparatus in the testis of Hemiptera and other insects after twenty-four hours' immersion in Mann's corrosive osmic mixture. Weigl (1910, as quoted by Bowen) found that after five to ten minutes' exposure to 2 per cent. osmic acid at 25° C. some traces of the Golgi apparatus (presumably in somatic cells) were just barely visible. After one hour the blackening is more obvious. Similarly Nath has demonstrated the Golgi apparatus in several eggs after short periods of osmication. This has led Harvey (1931) to state that the material which Nath and his co-workers have described as Golgi in several eggs is in reality fat. He thinks he has proved in the case of the earthworm that the Golgi spherules of Gatenby and Nath (1926, Lumbricus) and of Nath (1930, Pheretima) are droplets of fat. I welcomed this statement because it has induced me to use fat tests which I had not so far used, except in one or two cases.

Staining with Sudan III and Scharlach R has proved that not only in the earthworm egg but in all other eggs worked out by Nath and his co-workers, the substance described as Golgi apparatus material is anything but fat, and it is only in later
stages of oogenesis in some eggs that it is converted into fat. Harvey (1931a) has admitted that in *Antedon* the Golgi elements go dark after ten to twenty minutes' immersion in 1 per cent. osmic acid.

At the end of this paper a chart is published recording the reactions of fats, lipoids, and vacuoles to various microchemical tests employed.

**Material and Methods**

Ovaries are kept overnight in saturated alcoholic (90 per cent. alcohol) solutions of Sudan III and Scharlach R either directly or after fixation in formalin (formol 10 c.c., H₂O 50 c.c.). The results with both the dyes are absolutely identical. The material is brought down through lower grades of alcohol to water or glycerine in which they are studied. There is no danger of washing out these stains in this process because they are not at all soluble in grades of alcohol lower than 90 per cent.

On account of the presence of a large amount of accessory tissue covering the oocytes this technique could not be employed in the case of the rabbit. In the absence of a freezing microtome the following technique was employed: Ovaries are fixed in formalin. After a wash in water they are first transferred to 90 per cent. alcoholic solutions of the dyes and then to their solutions in absolute alcohol. They are cleared in cedar-wood oil and embedded in paraffin in the usual way. Sections are mounted in Canada balsam after removing the paraffin by xylol.

These experiments have been carried out on eggs of animals representing the following groups: Fishes, Amphibia, Reptiles, Birds, Mammals, Insects, Crustaceans, Spiders, and Annelids. The scolopendrid *Otostigmus*, on the oogenesis of which Nath and Husain published a paper in 1928, could not be collected from Lahore, and no work, therefore, could be carried out on this material.

**Observations.**

Fishes (*Rita rita* and *Ophiocephalus punctatus*).

Nath and Nangia (1931) have already reported that the Golgi elements of young oocytes of *Rita* (oocytes measuring more...
than 0·5 mm. were not available for study) are not fatty. They do not go black in Champy's fluid and they required thirty-two days to be impregnated in Mann-Kopsch. Repeated trials with Kolatschev were negative, although the eggs were incubated in 2 per cent. osmic acid for ten days at 38° C. They went black in Da Fano.

Recently it has been possible to obtain older oocytes of Rīta. Staining with Sudan III and Scharlach R shows that there is fatty yolk in this egg, that it appears when the egg measures about 1 mm., and that in younger eggs the Golgi elements are not at all coloured.

In Ophioccephalus, according to Nath and Nangia (1931), the Golgi elements of early oocytes are non-fatty, and become black, not only in Kolatschev and Da Fano, but also after forty-eight hours' osmication at room temperature. Gradually they grow in size, become fatty and form the fatty yolk which in eggs measuring 1 mm. goes brilliantly red in Sudan III.

Younger oocytes measuring up to 0·375 mm. have been recently stained with Sudan III and Scharlach R and it has not been found that red granules appear in the cytoplasm. It has not been possible to obtain oocytes measuring anything between 0·375 mm. and 1 mm., so that it is unknown at what stage the Golgi elements become fatty.

Amphibia (Rana tigrina and Rana cyanophlyctis).

Nath (1931) has already reported that in Rana tigrina the Golgi elements remain non-fatty throughout oogenesis inasmuch as they cannot be stained with Sudan III. They are blackened not only in Da Fano and Kolatschev but also in Champy. It must be noted that oocytes measuring more than 1·08 were not studied.

Recently it has been possible to stain oocytes of all stages both with Scharlach R and Sudan III. It is found that the Golgi elements stain with these dyes only when the oocyte reaches 1·2 mm. in size. The biggest egg of this species studied by me measures 1·5 mm.

In Rana cyanophlyctis, on the other hand, the Golgi
elements begin to stain with these dyes at a much earlier stage when the egg measures a little more than 0.5 mm.

**Reptiles (Emyda granosa).**

In the oocytes of this tortoise the Golgi elements begin to stain with Sudan III and Scharlach R only when the egg has grown to about 0.6 mm. Work on the oogenesis of this species has been completed in this laboratory by Aziz Ahmad, and will be published in due course. It will suffice to mention here that the Golgi elements of this species go black not only in Da Fano and Kolatschev but in Champy also, even in the earliest oocytes.

**Birds (Gallus bankiva).**

In the earliest oocytes in which the Golgi elements are in the juxta-nuclear position, they are blackened even in Champy, although they tend to get decolorized after mounting. They can be blackened in Kolatschev throughout oogenesis. Experiments with Sudan III and Scharlach R revealed that there is variation in the time of the appearance of fatty yolk. Oocytes measuring 1 mm. sometimes show red granules and sometimes not. However, oocytes measuring 0.2 mm. never showed any red granules. It has not been possible to obtain oocytes measuring anything between 1 mm. and 0.2 mm.

**Mammals (Rabbit).**

The oogenesis of this animal has been worked out in this laboratory by Sukh Dyal, and his results will be published shortly. It will suffice here to mention that the Golgi elements of this species go black even in Champy, although they tend to get decolorized after mounting. They are blackened in Kolatschev and Da Fano. But they do not go red in Sudan III and Scharlach R, even in the slightest degree throughout the whole oogenesis.

**Insects (Luciola gorhami, Periplaneta americana, Culex fatigans, and Dysdercus cingulatus)**

In Luciola, according to Nath and Mehta (1929), the Golgi vesicles of even the primordial germ-cells contain some quantity
of fat, inasmuch as they are blackened in short periods of osmication. In the course of oogenesis they swell up, become more fatty, and form the fatty yolk of the egg. They go jet-black in Mann-Kopsch like the typical Golgi apparatus.

As the result of experiments with Sudan III and Scharlach R, it appears that the Golgi elements become fatty only when the egg measures about 0.6 mm. At this and later stages they stain brilliantly with these dyes, but in the younger oocytes, in the undifferentiated germ-cells, and in the follicular epithelium, they are not coloured even slightly.

According to Nath and Piare Mohan (1929) the Golgi vesicles in the youngest oocytes of Periplaneta americana hardly contain any fat as they do not go black in osmic acid in twenty hours or even in forty-eight hours. Early in oogenesis, however, they become fatty and give rise to the fatty yolk. They are blackened in Mann-Kopsch, Kolatschev, and Da Fano like the typical Golgi apparatus. These conclusions and others have now been confirmed in every detail by Gresson (1931), who has worked on the allied species, Periplaneta orientalis.

Experiments with Scharlach R and Sudan III show that the Golgi vesicles do not show the slightest amount of colour in eggs measuring 1.675 mm. or less. When, however, the egg measures 3 mm. the Golgi vesicles go brilliantly red. No oocytes measuring anywhere between 1.675 mm. and 3 mm. were obtainable.

In Culex, according to Nath (1929), the Golgi vesicles of the youngest follicle, in which the oocyte is not differentiated from the nurse-cells, are not fatty. They react to Mann-Kopsch and Da Fano like the typical Golgi apparatus. The contents of the Golgi vesicles of the nurse-cells and the follicular epithelial cells remain non-fatty throughout the growth period, while those of the majority of the oocyte vesicles become fatty, inasmuch as they go black in Champy, even though they do not grow in size.

Repeated treatment of fresh as well as of formol-fixed ovaries with Scharlach R and Sudan III has completely failed to stain the Golgi vesicles of the egg during all stages of oogenesis. The same is true of the vesicles of the nurse-cells and the follicular epithelial cells.
In *Dysdercus*, according to Bhandari and Nath (1930), the Golgi vesicles of even the earliest oogonia contain some fat inside them, as they go black in osmic acid in short periods. They react to Kolatschev like the typical Golgi material. In the course of oogenesis many of them swell up, become more fatty, and give rise to the fatty yolk.

Repeated treatment of fresh and formol-fixed ovaries with Scharlach R and Sudan III produced interesting results. Even in eggs measuring about 1 mm. the Golgi elements are not at all coloured. The size of the 'ripe' egg of this species is not known, but the writer has never seen eggs which are bigger than about 1 mm. It may be that there are bigger eggs, and in these the swollen Golgi elements (fatty yolk) are stainable with these fat dyes.

**Crustaceans (Palaemon lamarrei and Paratelphusa spinigera).**

In *Palaemon*, according to Bhatia and Nath (1931), the non-fatty Golgi vesicles of the youngest oocyte grow in size and become fatty in the course of oogenesis, forming the fatty yolk of the egg.

Experiments with Scharlach R and Sudan III show that the Golgi vesicles are coloured with these dyes only in oocytes measuring more than 0.15 mm. Similarly in the fresh-water crab, *Paratelphusa spinigera*, the Golgi vesicles cannot be stained with these dyes in oocytes measuring less than 0.6 mm., although they go dark in osmic acid in four hours.

**Spiders (Crossophriza lyoni and Plexippus paykulli).**

In *Crossophriza*, according to Nath (1928), the Golgi vesicles are non-fatty in the youngest oocytes, but in later stages they give rise to the fatty yolk. They go black in Mann-Kopsch.

The laid eggs of this species measure between 0.7 and 0.8 mm., but the ovarian oocytes are not known to measure more than 0.6 mm. Scharlach R and Sudan III have failed to stain the Golgi elements of ovarian eggs, even though they can be
<table>
<thead>
<tr>
<th>Species</th>
<th>a, b, c; for explanation see under remarks on right.</th>
<th>Kolatschev or Mann-Kopsch prolonged osmication</th>
<th>Chaupcy or F Pwning without acetic for 24 hours</th>
<th>Immersion in 2 per cent. osmic acid (whole mounts or contents studied after rupturing)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ophiocephalus punctatus</td>
<td>(a) (1 mm. possibly earlier; absent up to 0.375 mm. stage)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark in 48 hours in youngest oocyte but earlier in older. (b) Black. (c) Slightly yellowish.</td>
</tr>
<tr>
<td>Rana tigrina</td>
<td>(a) (1 mm.)</td>
<td>(a) Black.</td>
<td>(a) Not blackened.</td>
<td>(a) Not blackened. (b) Black. (c) Slightly yellowish.</td>
</tr>
<tr>
<td>Rana cyano-phlyctis</td>
<td>(a) (1-2 mm.)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark within 10 minutes. (b) Black. (c) Slightly yellowish.</td>
</tr>
<tr>
<td>Emyda granosa</td>
<td>(a) (0-3 mm.)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark within 10 minutes. (b) Black. (c) Slightly yellowish.</td>
</tr>
<tr>
<td>Gairus bankiva</td>
<td>(a) (1 mm. possibly earlier; absent up to 0-2 mm. stage)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark. (b) Black.</td>
</tr>
<tr>
<td>Lepus cuniculus</td>
<td>(a) (absent)</td>
<td>(a) Black.</td>
<td>(a) Not blackened.</td>
<td>(a) Dark within 10 minutes.</td>
</tr>
<tr>
<td>Dysdercus cingulatus</td>
<td>(a) (absent)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark within 10 minutes.</td>
</tr>
<tr>
<td>Culex fatigans</td>
<td>(a) (absent)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark within 2 hours.</td>
</tr>
<tr>
<td>Lociola gorhami</td>
<td>(a) (0-6 mm.)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark within 10 minutes.</td>
</tr>
<tr>
<td>Periplaneta americana</td>
<td>(a) (3 mm. possibly earlier; absent up to 0-75 mm. stage)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark in 48 hours. (b) Black.</td>
</tr>
<tr>
<td>Palaeon lamarrei</td>
<td>(a) (0-15 mm.)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Dark in 22 hours. (b) Black.</td>
</tr>
<tr>
<td>Paratelphusa spinigera</td>
<td>(a) (0-6 mm.)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
</tr>
<tr>
<td>Grossopriza lyoni</td>
<td>(a) (absent)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
</tr>
<tr>
<td>Plectrurus paykulli</td>
<td>(a) (absent)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
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<tr>
<td>Phorotima posthuma</td>
<td>(a) (absent)</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
<td>(a) Black.</td>
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<tr>
<td>(a) Black.</td>
<td>(a) Not coloured.</td>
<td>(a) Not coloured.</td>
<td>(a) From 0.2 mm. stage as dark-greyish refractile bodies.</td>
<td>I.</td>
</tr>
<tr>
<td>(b) *, but should appear as empty spaces.</td>
<td>(b) Deep red.</td>
<td>(b) Not coloured.</td>
<td>(b) Refractile spheres.</td>
<td>(b) = Golgi elements, (c) = Vacuoles.</td>
</tr>
<tr>
<td>(c) White empty spaces.</td>
<td>(c) Not coloured.</td>
<td>(c) Red.</td>
<td>(c) White droplets.</td>
<td></td>
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<tr>
<td>(d) *</td>
<td>(d) Very refractile granules with definite bluish rings.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(e) White empty spaces.</td>
<td>(e) Very refractile spheres.</td>
<td></td>
<td>(e) Whitish.</td>
<td></td>
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<tr>
<td>(f) White empty spaces.</td>
<td>(f) Whitish.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(g) Black.</td>
<td>(g) Not coloured.</td>
<td>(g) Not coloured.</td>
<td>(g) Very refractile granules dark-greyish.</td>
<td>II.</td>
</tr>
<tr>
<td>(h) Empty spaces.</td>
<td>(h) Deep red.</td>
<td>(h) Not coloured.</td>
<td>(h) Very refractile spheres.</td>
<td>* indicates the observation with the particular technique is lacking.</td>
</tr>
<tr>
<td>(i) Empty spaces.</td>
<td>(i) Not coloured.</td>
<td>(i) Red.</td>
<td>(i) Whitish.</td>
<td></td>
</tr>
<tr>
<td>(m) Black.</td>
<td>(m) Not coloured.</td>
<td>(m) *</td>
<td>(m) Very refractile spheres.</td>
<td></td>
</tr>
<tr>
<td>(n) Black.</td>
<td>(n) Not coloured.</td>
<td>(n) Very refractile greyish spheres.</td>
<td></td>
<td></td>
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<tr>
<td>(o) *</td>
<td>(o) Very refractile greyish granules.</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(p) Deep red.</td>
<td>(p) Not coloured.</td>
<td>(p) Dark-greyish granules.</td>
<td></td>
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<tr>
<td>(q) Empty spaces.</td>
<td>(q) Centre portion stains very slightly but the cortex is dark-greyish.</td>
<td>(q) Dark-greyish.</td>
<td>(q) Dark-greyish.</td>
<td></td>
</tr>
<tr>
<td>(r) Black.</td>
<td>(r) Deep red.</td>
<td>(r) Not coloured.</td>
<td>(r) Refractile spheres.</td>
<td></td>
</tr>
<tr>
<td>(s) Black.</td>
<td>(s) Not coloured.</td>
<td>(s) Not coloured.</td>
<td>(s) Cannot be seen.</td>
<td>(s) Refractile spheres.</td>
</tr>
<tr>
<td>(t) Empty spaces.</td>
<td>(t) Deep red.</td>
<td>(t) Not coloured.</td>
<td>(t) *</td>
<td></td>
</tr>
<tr>
<td>(u) Black.</td>
<td>(u) Not coloured.</td>
<td>(u) Not coloured.</td>
<td>(u) *</td>
<td></td>
</tr>
<tr>
<td>(v) Black.</td>
<td>(v) Not coloured.</td>
<td>(v) Dark-greyish.</td>
<td></td>
<td></td>
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<tr>
<td>(w) Black.</td>
<td>(w) Not coloured.</td>
<td>(w) Dark-greyish.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(x) Black.</td>
<td>(x) Not coloured.</td>
<td>(x) Dark-greyish.</td>
<td></td>
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</tr>
</tbody>
</table>

**Remarks:**

I.  
(b) = Fat. 
(c) = Vacuoles.

II.  
Measurements within brackets against (b) indicate the size of the egg when fat appears for the first time.

III.  
* indicates the observation with the particular technique is lacking.

IV.  
A glance at this chart will at once show that neither the Golgi material nor the fats stain with neutral red. The fatty yolk of the Scoplenurid Oto-stigmus has been reported by Nath (1923) to stain with this dye. This was based on a single highly advanced oocyte and should be taken with reserve. It seems likely that fats in oogenesis watery vacuoles appear in the egg which might have stained with neutral red.
blackened in Champy's fluid in twenty-four hours. Identical results have been obtained in *Plexippus*.

**Annelids (Pheretima).**

Fresh ovaries are transferred directly into alcoholic solutions of Sudan III and Scharlach R and kept there for varying periods. Nothing whatsoever in the oogonia or in the oocytes of any stage is tinged even slightly by these dyes. The Golgi spherules are slightly corroded, but they still appear as dark greyish vesicles as in the fresh material. After fixation in formalin and treatment with Sudan III and Scharlach R for varying periods exactly the same result is obtained, except that the Golgi spherules are not distorted. The period of immersion in Scharlach R was extended to a fortnight, but the Golgi spherules remained absolutely unaffected by the stain.

In *Lumbricus*, Harvey reports that fat is present in the egg, that it gives a negative reaction to one fat test (Nile blue sulphate), a positive one to another (Scharlach R), and a weak one to another (Sudan III). This is difficult to believe, and I regard his recent interpretations of the *Lumbricus* oogenesis as very doubtful.

**Discussion.**

The most important conclusion of the present investigation is that if a granule goes black in short periods of osmication it must not be labelled as fat unless it also goes red in Sudan III and Scharlach R. These two dyes stain brilliantly all true fats, whereas osmic acid will quickly blacken lipoids also if they are highly unsaturated or are very little oxidized.

In *Pheretima*, *Culex*, *Dysdercus*, *Crossopriza*, *Plexippus*, and the rabbit the substance that I have described as Golgi material goes black in Da Fano and Kolatschev or Mann-Kopsch. But it also goes black, not only in Champy in twenty-four hours, but in much shorter periods of immersion in 2 per cent. osmic acid. In the youngest, as well as in the most advanced oocytes studied, it remains absolutely uncoloured after treatment with Sudan III and Scharlach R. It must, therefore, be interpreted as a material which consists of highly unsaturated or very little oxidized lipid.
In Ophiocephalus, Rana, Emyda, Gallus, Luciola, Periplaneta, Palaemon, and Paratelphusa the substance described as Golgi material reacts to Da Fano, Kolatschev, or Mann-Kopsch like the typical Golgi apparatus, and also goes dark in short periods of osmication. In early stages of oogenesis, it is not stained in the slightest degree with Scharlach R and Sudan III and must again be interpreted as highly unsaturated or very little oxidized lipoid. But at some stage in oogenesis (see observations for exact measurements) these lipoids are converted into fats when they stain brilliantly with Scharlach R and Sudan III. They may now be truly described as the fatty yolk of the egg.

Lastly, in Rita the Golgi material of early oocytes consists of very nearly fully saturated lipoids, as it does not go black at all in Champy in twenty-four hours or in shorter periods of osmication, and takes thirty-two days to impregnate in Mann-Kopsch. It does not stain with Sudan III and Scharlach R, but by the time the egg measures 1 mm. it is converted into fat and begins to stain brilliantly with these dyes.

Harvey (1931a) suspects that Nath may not be dealing with a Golgi apparatus at all, but with developing fatty droplets. In a way Harvey's suspicion is justified because biochemists tell us that lipins form fat in the cell (Hammersten, 1904; Maclean and Williams, 1909; and Heffter, 1891. Quoted by Walker and Allen, 1927), and the lipoidal Golgi elements of many eggs (Rita, Ophiocephalus, Rana, Emyda, Gallus, Luciola, Periplaneta, Palaemon, and Paratelphusa) may aptly be described as 'developing fatty droplets'. Harvey (1931a) has himself reported that in the accessory cells of the ovary of Asterias, fat arises under the influence of Golgi bodies. Among the recent supporters of the view that the lipoidal Golgi apparatus forms fat in the cell may be cited Gresson (1929, 1929a, and 1931) and Bell (1929). Gresson has confirmed in every detail Nath and Piare Mohan's account of the origin of fatty yolk from the Golgi apparatus in the egg of the cockroach, and Bell has shown that even in the male germ-cells fat may be derived from the same source.

Apart from the tests described in the introductory pages of
this paper, there are other ways of distinguishing fat globules
from the Golgi material. The juxta-nuclear or the circum-
nuclear arrangement of the Golgi elements in the earliest
oocytes and their subsequent orderly dispersal are of great
diagnostic value. The presence of exactly similar material in
the tiny undifferentiated germ-cells and in the follicular epithelia
is still another point against regarding them as inert globules
of fat.

Recent research from this laboratory has clearly demon-
strated that in the eggs of Rita rita, Ophiocephalus
punctatus, and Rana tigrina (see Nath, 1931; and Nath
and Nangia, 1931) watery neutral red-staining vacuoles (Parat’s
vacuome) exist independently of the classical lipoidal Golgi
apparatus, and that in Ophiocephalus these vacuoles con-
dense albuminous yolk in their interior as claimed for Perca
and Pygostèus by Hibbard and Parat (1928), and for
Discoglossus by Hibbard, 1928. Similarly in the egg of the
tortoise Emyda granose (Aziz Ahmad, unpublished) the
vacuoles and the Golgi apparatus are independent cytoplasmic
components. A series of papers (for references see Nath, 1931) on
the germ-cells of both sexes, on the somatic cells, and on plant-
cells also have now conclusively proved that the vacuome of
Parat is not the Golgi apparatus. So that the claim of Parat
that the Golgi apparatus (his vacuome) gives rise to albuminous
yolk in eggs and to aleurone grains in plants is shown to be
incorrect.

So far as I am aware Harvey (1931a, in Carcinus, Ante-
don, and Asteries) and Weiner (1925 in Tegenaria and
Lithobius) are the only workers who claim that proteid yolk
is formed by the lipoidal Golgi apparatus. No evidence has
been discovered for this view by Bhatia and Nath (1931), in
such crustaceans as Palaemon and Paratelphusa, by
King (1926), in the isopod Oniscus, by Hilton (1931), in
the copepod Calanus, by Nath (1928), in the spider Croso-
priza, by Bambèke (1898), in the spider Pholcus, and by
Sukh Dyal and Nath (in press), in the spider Plexippus
paykulli, whose so-called ‘yolk-nucleus’ is exactly like that
of Tegenaria as described by Weiner.
With regard to Lithobius, I have recently had an opportunity of studying Weiner’s brief account through the courtesy of Professor Bhattacharya who kindly sent me the original paper. I was interested to discover that Weiner does not make any mention of nucleolar extrusions which are so big and prominent in this egg. The fact is that he never made the necessary control preparations (e.g. Bouin and iron-haematoxylin) which very clearly demonstrate the remarkable process of nucleolar budding in this material. Weiner describes groups of Golgi elements scattered in the cytoplasm, each group being embedded in a grey field (his ‘Champs gris’). This grey field later becomes rounded and vacuolated with the Golgi elements lying on its surface. This is described as the yolk-nucleus. As I will show in detail in a separate publication, the ‘Champs gris’ of Weiner is nothing but the slightly darkened cytoplasm in which the Golgi elements are embedded and his ‘yolk-nucleus’ is nothing but a big vacuolated nucleolar extrusion, the presence of Golgi elements on the surface of which is merely fortuitous. Reference may be made to Nath’s figures 6 and 12 of nucleolar extrusions which have been figured as vacuolated round bodies exactly like the ‘yolk-nucleus’ of Weiner (see Nath, 1924).

Recent research has very clearly shown that the Golgi apparatus, like the mitochondria, is polymorphic. It may exist in the form of a granule, a ring or a vesicle, a crescent, a dictyosome, or a platelet, and finally in the form of network. Very often it has a duplex structure, showing an osmiophilic and an osmiophobic part. The former is generally understood to be lipoidal in nature, although direct evidence to support this view is still lacking. The latter has been interpreted by some as a specialized piece of cytoplasm and by others (including myself) as a vacuole. I am now inclined to accept neither of these views. A more rational view is that the osmiophilic part of the Golgi element consists of unsaturated lipoids and the osmiophobic part of very nearly fully saturated lipoids. This view is not only in accord with the well-known osmic acid reaction, but also easily explains the absence of the chromophobic part in the granular and the network type of Golgi apparatus. It also explains
those cases in which attempt after attempt has failed to im-
pregnate the Golgi apparatus. Here we have simply to suppose
that the Golgi material is so nearly fully saturated, or is so nearly
fully oxidized, that it does not take up any oxygen from osmic
acid.

Summary.

1. It is generally believed by cytologists that fats blacken
quickly in osmic acid, whereas lipoids take a much longer time.
This view seems to be erroneous, as the time required by osmic
acid to blacken a particular sample of fat or lipid depends
entirely on its degree of unsaturation and its previous state of
oxidation.

2. Films of a sample of completely hydrogenized fat, with
iodine value nil, failed to blacken in osmic acid, even in seven
days at 40°C. Samples of fat, therefore, must exist which
actually take longer time to blacken in osmic acid than some
highly unsaturated lipoids.

3. It follows, therefore, that a granule in the cell, which goes
black in osmic acid in a short period, cannot be identified as
true fat unless it also stains intensely in Sudan III and Schar-
lach R.

4. These fat tests have been employed on eggs of animals
representing many groups.

5. In Pheretima, Culex, Dysdercus, Crossopriza, Plexippus, and the rabbit, the Golgi material can
be blackened in short periods of osmication, but it cannot be
stained even slightly with Sudan III and Scharlach R during
any stage in oogenesis. It must, therefore, be interpreted not
as fat but as very highly unsaturated or very little oxidized
lipoid.

6. In earlier stages of the oogenesis of Ophiocephalus
Rana, Emyda, Gallus, Luciola, Periplaneta, Palaemon, and Paratelphusa, the Golgi material does
not stain at all with Sudan III and Scharlach R, even though
it can be blackened in short periods of osmication. It must
again be interpreted, not as fat, but as highly unsaturated or
very little oxidized lipoid. At a certain stage in oogenesis these
lipoids are converted into fats when they stain brilliantly with Scharlach R and Sudan III.

7. In R. ita the Golgi material of early oocytes consists of very nearly fully saturated lipoid, as it does not go black in short periods of osmication, and takes thirty-two days to impregnate in Mann-Kopsch. It does not stain with Sudan III and Scharlach R, but by the time the egg measures 1 mm. it is converted into fat and begins to stain intensely with these dyes.

8. A chart is published recording the reactions of fats, lipoids, and vacuoles to various microchemical tests.

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