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

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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 (1931 *a*) 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., H_2O 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 Rita. 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 Ophiocephalus, 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