The Lipoid Contents of the Golgi Bodies in the Oocytes of the Indian Water Spider, *Lycosa birmanica*

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SUMMARY

1. The Golgi bodies of the oocyte of *Lycosa birmanica* do not contain triglyceride.
2. They contain phospholipine, lipo-protein, and protein.
3. They are granular and all of approximately equal size. Any variation in their size or shape is due to the irregular deposition of phospholipine on them.
4. Fine bodies resembling Golgi bodies in appearance and reacting like them with osmium tetroxide and silver nitrate consist of phospholipine.
5. The Golgi bodies in the material studied appear not to multiply by division, but to arise in the ground cytoplasm independently of one another. They may be regarded as nutritive bodies.

INTRODUCTION

IT is the intention here to study the chemical nature of the Golgi bodies in the oocytes of *Lycosa birmanica* Thor. during the growth period. The Golgi bodies in the oocytes of this animal are of fairly large size and can be seen even in the living condition. Observations were made on frozen sections by the use of modern cytological techniques.

TECHNIQUE

Silver and osmium techniques were employed to identify Golgi bodies. During the breeding season (February to April) fresh ovaries were fixed for 24 hours in the fluids of da Fano, Ramon y Cajal, and Aoyama, and silver impregnation was carried on for 48 hours in 1·5 per cent. silver nitrate solution. The impregnated silver was reduced and fixed, and the wax sections of this material were toned to remove excess of silver. Material was also treated by Ludford's modification of the Mann-Kopsch osmium technique. Post-osmication was done for 6 days and the wax sections of this material were bleached with potassium permanganate. The formulae of the fluids in both the techniques were taken from Bolles Lee's *The microtomist's vade-mecum* (1946 edition). The Golgi bodies looked black with these techniques; the mitochondrial region appeared as a light-brown patch with the osmium technique. This patch was ignored in the present study.

For the study of lipoids ovaries were fixed in calcium-formaldehyde (Baker, 1944) and cut into frozen sections. Triglycerides, phospholipines, and lipoproteins were identified by selective staining and solubility tests (Krishna,

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1950). The frozen sections were stained with nile blue sulphate for triglycerides (Cain, 1947*a*, 1948). These were then dissolved in acetone and the phospholipines were stained with sudan black B (Lison, 1936 and 1953; Baker, 1944) and acid haematein (Baker, 1946 and 1947; Cain, 1947*b*). They were also removed with ether and alcohol and the remaining lipoids, the lipoproteins, were stained with sudan black B. Sudan IV (Baker, 1944; Gatenby and Painter, 1946) was also used for triglycerides and phospholipines as a control. The lipo-proteins were stripped of their lipoids in boiling alcohol, boiling ether, and finally in frozen ether (McFarlane, 1942). The extracts of the sections were always tested for their contents by classical spot tests (Feigl, 1947).

During the course of this study certain tests for proteins were also employed. These were standard tests of ninhydrin, aldehyde tests for tryptophane, and Millon's test (Bensley and Gersh, 1933). Proteins were also removed by the enzyme action of pepsin. The frozen sections were firmly stuck to the slides over a gelatine film and the slides were incubated with pepsin solution (0·06 per cent.) in acetic acid medium (Tiselius and Erikson-Quensel, 1939) at pH 1·5 (Sahyun, 1944) at 37° C. The enzyme action was complete in 12 hours.

Observations

In young oocytes the Golgi bodies appear near the nucleus and follow the yolk nucleus throughout the growth period. In osmium and silver preparations the Golgi bodies appear as small and large granules and also as bodies of irregular shape (fig. 1, A). In living oocytes, too, the irregular bodies and large granules could be seen when the light was to some extent cut off by tilting the mirror of the microscope. These bodies in frozen sections are coloured blue or blue-black with sudan black B (fig. 1, B), and orange with sudan IV. They are not coloured red by nile blue. These reactions suggest that they do not contain triglycerides.

The intensity of the sudan stains did not appreciably change when the sections were treated with acetone. The Golgi bodies gave a positive reaction with the acid-haematein test, showing in them the presence of phospholipines. The sections were then treated with alcohol and ether in succession. This removed phospholipines from the Golgi bodies and also from other parts of the section. The small granules had all dissolved. The irregular bodies lost most of their substance and only circular bodies were left in their place. The large granules also lost some of their contents and now looked slightly reduced in size, smooth and circular (fig. 1, C). These bodies did not stain with sudan IV, but took a blue colour with sudan black B. Further treatment with alcohol and ether did not dissolve any more lipoids. Thus the phospholipines had been removed and the blue colour with sudan black B indicated the presence of lipo-proteins in the Golgi bodies. The sections were then treated with boiling alcohol and boiling ether and then with frozen ether for 12 hours. This treatment completely stripped the Golgi bodies of their lipoid contents.

They could now no longer be coloured by sudan black B, but gave positive results with all the protein tests employed here. The removal of the lipoids considerably reduced the size of the Golgi bodies, but their granular shape did not change; rather it became more distinct than before. The remnants of the Golgi bodies, consisting mainly of protein, then looked like smooth granules and took the protein stains uniformly.

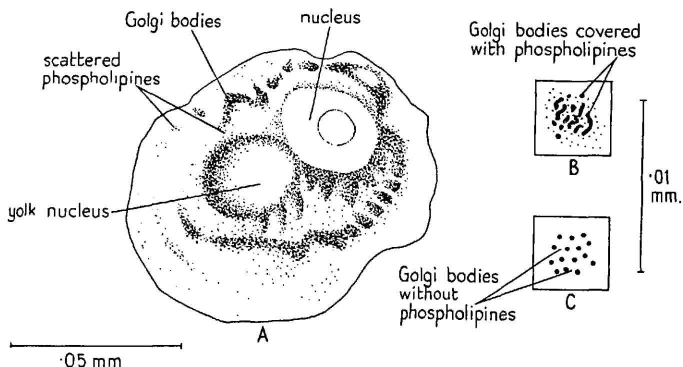

Fig. 1. A, section of an oocyte fixed in Ludford's modification of Mann-Kopsch's fluid. Bleached. B, a part of a frozen section of an oocyte fixed in calcium-formaldehyde and coloured by sudan black B. C, the same as B after treating the section with alcohol and ether in succession.

These sections were incubated with pepsin solution at 37° C. and the reaction was watched every hour for 12 hours, when the sections almost disappeared in the enzyme solution. For about 3 hours there was no appreciable action. After that the signs of disintegration became distinct. The remains of the Golgi bodies were the first to be attacked by the enzyme, irrespectively of their position in the section. First they became thin in the centre and after an hour or so looked hollow. The process of thinning proceeded towards the periphery, and in about 6 hours, when the general cytoplasm began to disintegrate, the remains of the Golgi bodies were visible only by the thin circular outline that marked their margin. This outline gave positive results with protein tests and was seen till the last remains of the cytoplasm dissolved. The pepsin solution apparently had little action on this outline, although the surrounding cytoplasm was rapidly dissolving. Disintegration of the general cytoplasm also began in the centre of the oocyte and proceeded radially towards the periphery. In the end there remained only a very thin outline marking the margin of the section. This did not take any stain and did not show any structure even when it was seen through an oil-immersion objective. The number of the Golgi bodies varied with the

size of the oocyte. They were few in young oocytes and many in the old ones. There was no evidence to suggest any relation among the granules at any stage of the oocyte growth. On the other hand, they appeared to be formed in the cytoplasm independently of one another. When the phospholipine had been removed, they all appeared to be of the same size.

Discussion

The observations lead to the conclusion that the Golgi bodies in *Lycosa birmanica* are granular, mainly consisting of proteins and lipo-proteins. Covering the granule there is a membraneous structure of a doubtful chemical nature—probably proteins, different from those in other parts of the granule. On these granules phospholipines are deposited. Sometimes the deposit covers more than one granule lying near each other, and the Golgi bodies, under a common cover of phospholipines, appear in various shapes and sizes. When the phospholipines are removed the granules become distinct and look separate from each other. What looked like fine Golgi bodies in the silver and osmium techniques were really scattered phospholipines and all of them dissolved in alcohol and ether without leaving any trace behind. Only the large granules showed the usual structure of the Golgi bodies. The lipo-protein and the protein contents of the Golgi bodies could be seen only when the phospholipines had been removed. The enzyme action by its peculiarity in first attacking the centre of the Golgi granule showed that the proteins in the centre were different from those on its periphery. It was interesting to observe that after the removal of the phospholipines all the Golgi granules were of the same size and shape. Their outward difference was due to the irregular deposit of phospholipines.

There was no evidence to show the protoplasmic nature of the Golgi bodies or their multiplication by division. On the contrary, the observations suggest that they are separate objects which originate in the cytoplasm and have a nutritive function.

REFERENCES

Baker, J. R., 1944. Quart. J. micr. Sci., **85**, 1.
—— 1946. Ibid., **87**, 441.
—— 1947. Ibid., **88**, 463.
Bensley, R. R., and Gersh, I., 1933. Anat. Rec., **57**, 217.
Cain, A. J., 1947*a*. Quart. J. micr. Sci., **88**, 383.
—— 1947*b*. Ibid., **88**, 467.
—— 1948. Ibid., **89**, 429.
Feigl, F., 1947. *Qualitative analysis by spot tests*. Translated by Ralph E. Oesper. Elsevier Publishing Co. (N.Y.).
Gatenby, J. B., and Painter, T. S., 1946. *The microtomist's vade-mecum*. Churchill (London).
Krishna, D., 1950. Proc. Nat. Acad. Sci. India, **20**, 60.
Lison, L., 1936 and 1953. *Histochimie et cytochimie animales*. Gauthier-Villars (Paris).
McFarlane, A. S., 1942. Nature, **149**, 439.
Sahyun, M., 1944. *Outlines of the amino acids and proteins*. Reinhold Publishing Corporation (N.Y.).
Tiselius, A., and Erikson-Quensel, I., 1939. Bioch. Journ., **33**, 1752.