The Cytoplasmic Inclusions of the Neurones of Certain Insects

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With one plate (fig. 3)

SUMMARY

A comprehensive study of the thoracic neurones of fifth instar and immature adults of the locust, *Schistocerca gregaria* Forsk., and of adults of the water-bug, *Laccotrephes rubra* Fabr., has been made by employing the latest cytological techniques and phase-contrast microscopy. The mitochondria are seen as granules stainable in life with Janus green. Alignment of granules into filamentous mitochondria has also been observed in fixed preparations. The Golgi bodies (lipochondria of Shafiq) are sudanophil, osmiophil, and argentophil spheroids. The bigger spheroids show a duplex structure. There is a chromophil, cortical, lipid component, which may be in the form of a complete ring (*Schistocerca*) or in the form of one or two granules or a crescent (*Laccotrephes*), and a chromophobe medulla stainable with the basic dyes, neutral red and methylene blue. The smaller Golgi bodies in *Schistocerca* show a homogeneous structure. The Golgi bodies have not been observed to be engaged in any secretory activity. Neurofibrillae have been observed in the neurones of the insects studied.

INTRODUCTION


Earlier Beams and King (1932) had described similar osmiophil and argentophil bodies as Golgi material in the nerve-cells of the grasshopper. They recorded their observations by using Golgi techniques, and came to the conclusion that the Golgi elements show a double structure, with a thick osmiophil cortex and an osmiophobe medulla. They believed the cortical component of the double Golgi bodies to be the true homologue of the classical Golgi element. Beams and others (1953) revised the previous work of Beams and King (1932) by electron microscopy, and doubted the presence of an osmiophobe medulla in the Golgi body; they could not demonstrate it owing to the opacity of the osmiophil bodies to the electron microscope. But Shafiq (1953) interprets the osmiophobe substance as the lipochondrion, and the osmiophil bodies of Beams and King (1932) as partly, if not entirely, impregnation artifacts.

Further, Shafiq (1953, 1954) observed that the lipochondria are stainable with the basic vital dyes, neutral red and methylene blue, in contrast with the findings of Beams and King (1932), who regarded the neutral red granules as aggregations of dye particles. Gatenby and others (1953) described the neutral red granules in the neurones of vertebrates as senility pigment.

It thus becomes clear that there is a good deal of disagreement regarding the
true form of the Golgi material of nerve-cells. In view of this, I undertook a comprehensive study of the subject at the suggestion of Professor Vishwa Nath.

**Material and Technique**

The neurones of the thoracic ganglia of the fifth instar and immature adults of the locust, *Schistocerca gregaria* Forsk., and the adults of the water-bug, *Laccotrephes rubra* Fabr., were studied. The living animals were dissected in Ringer or in the saline solution recommended by Baker (1944), and the ganglia were taken out immediately for study. The following techniques were employed:

1. **Phase-contrast microscopy.** The living neurones of both the species were studied in Ringer or saline solution under the phase-contrast microscope, and photomicrographed.

2. **Vital staining.** The vital dyes, neutral red, methylene blue, and Janus green, were used in very dilute solution in saline solution.

3. **Golgi techniques**

   a. **Kolatchev.** The material fixed in Champy's fluid for 24 hours was osmicated at 37°C for periods varying from 4 to 12 days.

   b. **Formaldehyde-osmium.** The ganglia fixed in formaldehyde-saline were osmicated at 37°C in 1% osmium tetroxide for periods varying up to 48 hours, as recommended by Baker (1944).

   c. **Aoyama.** The ganglia were fixed in Aoyama's fixative, and the material was treated for varying periods in silver nitrate to study the effect of prolonged treatment with silver.

4. The material was fixed in chrome-osmium, Helly (with post-chroming), or Regaud (with post-chroming). Paraffin sections were stained in iron haematoxylin. Sections of Helly material were also stained in Masson's tricolor stain.

5. **Sudan colouring**

   a. Paraffin sections of material fixed in Helly and post-chromed in a saturated solution of potassium dichromate at 37°C were coloured in a saturated solution of Sudan black in 70% alcohol, and mounted in Farrants's medium (Thomas, 1948).

   b. Smears fixed in Flemming-without-acetic were also coloured with the solution of Sudan black.

   c. The material fixed in Aoyama's fluid, after treatment with silver nitrate and the reducing mixture, was postchromed in 5% solution of potassium dichromate (Lacy, 1954). The paraffin sections were coloured with Sudan black.

   d. Paraffin sections of post-chromed Helly material were also coloured with Sudan IV for the study of triglycerides.

   e. Sudan IV was also used on material fixed in formaldehyde-saline. Sections were mounted in glycerine.

6. Material was fixed in Bouin and Carnoy for control.
OBSERVATIONS

In the neurones of the thoracic ganglia of the two species studied, there did not appear to be any cytological differences between one individual and another at the same phase of the life-cycle, nor between individuals at the different phases that were used in this investigation.

MITOCHONDRIA

In both species the mitochondria can best be studied in material that has been fixed in Helly (with post-chroming) or in chrome-osmium, and stained in iron haematoxylin (fig. 1, F and G; fig. 2, D). They are stainable in life with Janus green, though great difficulty is experienced in working with this dye. The mitochondria are seen to be uniformly dispersed throughout the cytoplasm, and there appears to be no definite pattern of dispersal. The mitochondria appear to be very fine granules, but here and there mitochondrial filaments and chains of mitochondrial granules can be seen. Under the phase-contrast microscope, the mitochondria invariably appear in the form of granules forming a greyish background for the more refringent Golgi bodies (fig. 3, A and B).

GOLGI BODIES

The term Golgi bodies has been used to denote the classical Golgi substance, which is lipoidal, and the chromophobe substance, if present, associated with the lipoidal substance. Shafiq (1953, 1954) has used the term lipochondria for these bodies in the thoracic neurones of the locust, Locusta migratoria.

The Golgi bodies are dispersed in the cytoplasm of the nerve-cells. They are present invariably in all the thoracic neurones of Schistocerca and Laccotrephes. The size of the cells varies considerably in both species, in accordance with the amount of cytoplasm present. In the smallest neurones the Golgi bodies are mostly confined to the middle of the cell, but in the larger cells they are uniformly spread throughout the cytoplasm. The Golgi bodies are of spheroidal form, varying considerably in size even in the same cell. There appears to be no direct relation between the size of the Golgi spheroids and the size of the cell, for the bigger spheroids are often seen in the smaller cells and the smaller spheroids in the larger cells. The number of the Golgi spheroids is, however, generally less in the smaller cells.

Phase-contrast study

Schistocerca. Great difficulty is experienced in studying the living neurones under the phase-contrast microscope. The contents of the neurones are very dense, and the Golgi bodies seem to have almost the same refractive index as the ground cytoplasm. But when the tissue has been well teased and the cells have been well flattened by pressure on the cover glass, the Golgi bodies can be seen scattered throughout the cytoplasm of the neurones. They vary considerably in size. Under positive phase-contrast the bigger Golgi spheroids show a dark cortex and an almost colourless medulla (fig. 3, A). The cortex of
Fig. 1. Neurones of *Schistocerca*. A, fifth instar; Helly/Sudan black. B, fifth instar; from Flemming-without-acetic, Sudan black smear. C, immature adult; Regaud/haematoxylin, showing Golgi bodies and neurofibrillae. D, immature adult; Aoyama (toned), showing Golgi rings and silver deposition. E, immature adult; neutral red. F, fifth instar; Helly/haematoxylin, showing Golgi bodies, and granular mitochondria in the background. G, immature adult; Flemming-without-acetic, haematoxylin, showing Golgi bodies, and granular mitochondria in the background. H, immature adult; Helly/Masson.
the Golgi bodies sometimes appears as a crescent, which must be interpreted as an optical section, for the cortex becomes complete round the medulla when the focus is changed. The smaller Golgi bodies are seen as dark, homogeneous spheroids (fig. 3, A).

With Sudan black techniques the Golgi spheroids show a duplex structure, with a thick sudanophil cortex investing a chromophobe sphere. There is therefore no evidence that the cortex of the Golgi bodies, observed under the phase-contrast, is an optical ‘edge-effect’, as suggested by Shafiq (1953 and 1954).
Laccotrephes. The cytoplasm of the neurones of Laccotrephes is thinner than in Schistocerca, and consequently it is easier to study the cell inclusions in the living neurones of this species under the phase-contrast microscope.

In the living neurones of Laccotrephes the Golgi bodies are scattered throughout the cytoplasm and there is generally one granule (sometimes two) or a crescent associated with the spheroidal medulla. The cortical granular or crescent-shaped component appears dark, while the medulla is bright under positive phase-contrast (fig. 3, B). Sometimes the rounded shape of the medulla is slightly distorted owing to pressure on the cover-glass.

Vital Staining. Neutral red stains the Golgi bodies brilliantly (figs. 1, E, and 3, G), even when it is used supravitally in very low concentrations. If the ganglia are studied after the dye has acted for about 10 minutes, it is seen that the medulla of the Golgi bodies alone is stained with neutral red. But after about 45 minutes the cortex is not seen and the Golgi bodies become homogeneously stained. This process has been clearly observed with methylene blue in the neurones of Schistocerca, and with neutral red in neurones of Laccotrephes examined under the phase-contrast microscope (fig. 3, G). Reference should be made to the use of neutral red in similar studies by Thomas (1947), Baker (1949), Shafiq (1953, 1954), and Roque (1954). When the dye is supravitally used it gives a slight tinge to the mitochondria, and sometimes to the nucleus also. Methylene blue stains the chromophobe substance more lightly in Laccotrephes than in Schistocerca (fig. 3, H). The contents of the chromophobe substance of the Golgi bodies appear to be acidic and watery ('vacuome' of Parat). It is also concluded, in opposition to the findings of Beams and King (1932), that the neutral red bodies are not artifacts.

The Golgi bodies can be demonstrated by short osmication. When living neurones are studied under the ordinary microscope in saline solution to which some osmium tetroxide has been added, the Golgi bodies appear, each with a brownish cortex and colourless medulla.

Chrome-osmium. Helly and Regaud techniques

In chrome-osmium preparations stained with haematoxylin the Golgi bodies are seen as homogeneous spheroids of varying sizes (fig. 1, C) in the neurones of Schistocerca. The cortex of the bigger (duplex) Golgi bodies becomes so intensely stained that it conceals the medulla, but in Laccotrephes the duplex structure is very clearly seen (fig. 2, D), for the cortical component does not completely ensheath the medulla. Paraffin sections of Helly or

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FIG. 3 (plate). Photomicrographs of neurones.
A, immature adult of Schistocerca; living cell, phase-contrast
B, Laccotrephes, living cell, phase-contrast.
C, fifth instar of Schistocerca; Kolatchev. Note flattened platelets.
D, the same cell as in fig. 1, D. The arrow indicates a Golgi ring.
E, the same cell as in fig. 1, A. The arrows indicate duplex Golgi bodies.
F, the same cell as in fig. 2, C. The arrow indicates a Golgi body.
G, Laccotrephes, living cell, neutral red, phase-contrast.
H, Laccotrephes, living cell, methylene blue.
Regaud material, post-chromed, show duplex Golgi bodies (fig. 1, c and r) in both species after staining with iron haematoxylin. Smaller homogeneous Golgi bodies are always also seen in such preparations. Helly preparations have given especially good results with the neurones of *Schistocerca*, for they show almost the same form of the Golgi bodies as in Sudan black preparations.

The Golgi bodies are also stainable with acid fuchsin, when paraffin sections of Helly material, post-chromed, are stained with Masson's tricolor stain (fig. 1, H).

**Golgi techniques**

The essential form of the Golgi bodies is preserved in Golgi preparations (Kolatchev and Aoyama), but there is no doubt that these techniques introduce artifacts. In Kolatchev preparations of the neurones of *Schistocerca*, the Golgi bodies show a thick osmiophil cortex and an osmiophobe medulla (fig. 3, c), but the latter becomes greatly swollen up and the Golgi spheroids are converted into flattened platelets. The osmiophil cortex appears thicker in these preparations than with Sudan black, owing to excessive deposition of osmium. So it appears that the osmiophil cortex seen in these preparations is partly an artifact. Shafiq (1953) regards it partly, if not entirely, an osmium deposit. In some of the Kolatchev preparations Golgi crescents and rods are also seen as optical sections of flattened platelets; and some of the rods seem to have been produced by joining up of the smaller spheroids. In Aoyama preparations of the neurones of *Schistocerca* the Golgi bodies show beautifully as rings in the finished slides (figs. 1, D, and 3, D), but invariably artifacts in the form of black granules appear in or round the Golgi rings.

In the neurones of *Laccotrephes* prepared by the Kolatchev and formaldehyde-osmium techniques, the cortical component of the Golgi body appears granular or crescent-shaped and is associated with a clear medulla. There is generally a single granule or a crescent, but sometimes there may be two granules associated with the medulla. This form of the Golgi bodies is only seen when there is optimum impregnation (40 hours) with osmium (fig. 2, F); but if the impregnation is prolonged the cortical component appears bigger. If the impregnation is further prolonged the osmium is deposited all round the medulla and later in the medulla itself (fig. 2, E). Similar artifacts appear in the silver nitrate technique.

**Sudan black**

The Sudan black preparations show essentially the same form of Golgi bodies as in the techniques described above, or as in the living neurones examined under the phase-contrast microscope.

In the neurones of *Schistocerca* and *Laccotrephes* prepared by the Helly/Sudan black technique, the Golgi bodies appear as heterogeneous spheroids, each showing a sudanophil cortex and a chromophobe medulla (figs. 1, A; 2, A; and 3, E). It has been observed that the cortical component appears smaller than by the long osmication technique. From this it would appear that osmium is deposited on the cortical region of the Golgi body.
Smears of neurones fixed in Flemming-without-acetic and coloured with Sudan black show homogeneously-stained Golgi spheroids in *Schistocerca* (fig. 1, b), but in smears of *Laccotrephes* the double structure can be made out clearly (fig. 2, b). These preparations rarely show an incomplete investing rim to the medulla of the Golgi body in the neurones of *Schistocerca* (fig. 1, b).

Aoyama/Sudan black preparations of neurones of *Laccotrephes* show the cortical component slightly bigger than in other Sudan black preparations, owing to deposition of silver (figs. 2 and 3, f). These preparations show that argentophil bodies are also sudanophil.

Sudan black preparations of *Schistocerca* neurones show, in addition to the bigger duplex Golgi bodies, smaller homogeneously stained Golgi spheroids (figs. 1, A, and 3, E), as seen in Helly's or chrome-osmium/haematoxylin and Helly/tricolor preparations.

The cortical component of the Golgi body appears to be lipid, as it colours with Sudan black. It reduces osmium and silver. It is stained darkly by haematoxylin after chrome-osmium, Helly (post-chromed) or Regaud (post-chromed). It is stainable with acid fuchsin; and it is not seen after Bouin or Carnoy.

Triglycerides seem to be absent from the neurones of insects (*Schistocerca* and *Laccotrephes*), as there is nothing in the neurones that is coloured by Sudan IV.

Neurosecretory products have not been observed in these insects, nor have these Golgi bodies been seen to be engaged in any secretory activity.

**Neurofibrillae**

Beams and King (1932) and Beams and others (1953) described neurofibrillae in the nerve-cells of the grasshopper, but Shafiq (1953, 1954) did not observe any neurofibrillae in *Locusta*.

My Regaud preparations show very thin filaments interwoven into a net-like structure (fig. 1, c). These appear to be neurofibrillae. The mitochondria cannot be mistaken for neurofibrillae, as the former are completely washed out in Bouin and Carnoy, whereas the latter stand out prominently. Moreover, filamentous mitochondria have not been observed in *Schistocerca* and *Laccotrephes*. The neurofibrillae are more clearly seen in *Schistocerca*. However, they could not be seen in the living material of either species examined under the phase-contrast.

**Discussion and Conclusions**

The Golgi bodies of the neurones of the locust, *Schistocerca gregaria*, and the water-bug, *Laccotrephes rubra*, exist as spheroids, each showing a duplex structure, a chromophil, sudanophil, osmiophil, argentophil lipid cortex, and a chromophobe medulla. The cortical component may completely ensheath the medulla (*Schistocerca*), or it may be restricted to one or two granules or a crescent, associated with the medulla (*Laccotrephes*). The latter form of the Golgi body is identical with the ‘binary spheroids’ of *Helix* (Thomas, 1947). The medulla of the Golgi body is not impregnated with osmium or silver.
under optimal conditions of impregnation; it is not coloured by Sudan black, but is stainable with the basic vital dyes neutral red and methylene blue. It is thus concluded, in conformity with Shafiq (1953, 1954) and Roque (1954), that the sudanophil and osmiophil bodies exist in association with bodies that stain with neutral red.

The Golgi bodies described in the neurones of *Schistocerca* by the present writer are certainly identical with the lipochondria of Shafiq (1953, 1954); but there are slight differences which are, I think, only differences of interpretation. Shafiq thinks it possible that the lipochondria may be homogeneous bodies. He suggests that the duplex structure of the Golgi body (lipochondrion) as seen under the phase-contrast microscope may be due to an optical ‘edge effect’, and that the osmiophil cortex may be partly, if not entirely, an osmium deposit. Roque (1954) also describes the duplex structure seen under phase-contrast in the neurones of *Helix* as an optical ‘edge effect’, although this author describes duplex Golgi bodies (paranuclear bodies), in conformity with Nath (1955), in the sperm-forming cells of *Helix*. Shafiq (1954) says: ‘By positive phase-contrast they sometimes appear to be binary in structure, having an outer, dark cortex and a lighter inner medulla; but as with the lipochondria of nerve-cells, it is not possible to assert definitely whether this is an optical illusion or not.’

Beams and King (1932) also described duplex Golgi bodies in the neurones of the grasshopper. But Beams and others (1953) could not clearly demonstrate the osmiophobe substance in the Golgi bodies in the neurones of the grasshopper in their studies with the electron microscope. They doubted the existence of the osmiophobe substance, as ‘the Golgi bodies are relatively opaque to the electrons, but they do not seem to be completely homogeneous as is evidenced by the lighter appearing areas within them’.

My observations, however, lead to the conclusion that the Golgi bodies have a duplex structure in the neurones of insects. I am in agreement with Shafiq (1953) and Baker (1954) that osmium first deposits on the lipoidal bodies, then around them, and finally on other structures. I have observed the effect of osmication on the material initially fixed in Champy or formaldehyde-saline. The material was kept in osmium tetroxide at 37°C for varying periods, and the results were compared with Sudan black preparations. My preparations show that formaldehyde-saline material osmicated for 40 hours gives almost the same picture of the Golgi bodies as the Sudan black preparations. If the impregnation is further prolonged the lipid component appears thicker, and other artifacts also appear. My Sudan black preparations also show the duplex structure of the Golgi bodies.

In Kolatchev material the chromophobe substance (medulla) becomes greatly swollen, and the spheroids become flattened in the neurones of *Schistocerca*. Besides the bigger spheroids, there are also seen crescents and irregular rods as optical sections of flattened spheroids (Nath, 1944; Shafiq, 1953; Roque, 1954), or some of the rods are produced by joining up of the smaller spheroids (Shafiq, 1953). It can, therefore, be reasonably concluded
that osmication techniques do not produce artifacts if used for an optimal period.

The form of the Golgi bodies in the neurones of *Laccotrephes* is identical with the 'binary spheroids' of *Helix* (Thomas, 1947) and the Golgi elements of the vertebrate neurones described by Baker (1949). Generally there are one or two granules or a crescent attached to the medulla. A similar form of the Golgi body has been observed in this laboratory by Sud (1955, 1956) in the sperm-forming cells of the snake, *Natrix piscator*, and the tortoise, *Lissemys punctata*. He has also observed that the cortical component is sudanophil, osmiophil, and argentophil, whereas the medulla is stained by neutral red.

The medulla of the Golgi bodies of the neurones of insects (*Schistocerca* and *Laccotrephes*) is stained in life by neutral red. The medulla of the 'Golgi body' of Baker (1949), 'spheroids' of Thomas (1947), and 'lipochondria' of Shafiq (1953, 1954) and Roque (1954) are also stainable with neutral red.

There is no evidence that 'Golgi nets' exist in the neurones of insects. Monti (1915), as quoted by Shafiq (1953), described a Golgi network in arthropod nerve-cells; and Hosselet (1929) also described a network in insect neurones, formed by the hypertrophy of mitochondria. I believe in conformity with Beams and King (1932) that the 'nets' of Monti (1915) and Hosselet (1929) may be ascribed to the presence of neurofibrillae.

There is no evidence for the belief that these Golgi bodies are a complex of myelin figures (Palade and Claude, 1949, a and b).

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