Some observations concerning the supposed identity of the Golgi apparatus and Nissl substance of neurones

By OWEN LEWIS THOMAS

(From the Department of Biology, Royal University of Malta, Valletta, Malta, G.C.)

With two plates (figs. 1 and 2)

Summary

In nerve-cells the cytoplasmic inclusions commonly described as the Golgi apparatus are not identical with the basiphil Nissl substance.

Introduction

CONFLICTING statements have recently been published concerning the identity of Golgi apparatus and Nissl substance of the neurones of vertebrates. Malhotra (1959, 1961) and David and his colleagues (1960, 1961) believe that the 'Golgi apparatus' results from a deposit of silver or osmium on the basiphil grumes of Nissl. Legendre (1910) proposed a similar hypothesis. Contrary views have been expressed by the author (Thomas, 1961).

Although electron-microscope studies have been used to support both opinions, such evidence cannot be in itself conclusive since the precise identity of the Golgi apparatus in electron micrographs of the nerve-cell remains a matter for conjecture. On the other hand, since both Nissl substance and Golgi apparatus can readily be studied in classical preparations by the light microscope, the problem is better resolved by this means, especially as techniques exist whereby the same cells can be compared after contrasting methods of staining (Thomas, 1949). In this paper the results of such procedures are recorded as a series of photomicrographs (figs. 1 and 2).

Methods and results

The dorsal root ganglia of neo-natal kittens (6 to 10 days old) were fixed in Zenker-formol (Maximow's fluid) for 24 h, washed briefly, and postchromed at 37° for 3 to 6 days. Paraffin sections 3 to 7 μ thick were mounted on slides and after down-grading to 70% alcohol, coloured with a saturated solution of Sudan black in 70% alcohol for 1 h, rinsed briefly in 50% alcohol, then rinsed in water and mounted in Apáthy’s medium.

Fig. 1, A shows a neurone from such a preparation. The nucleus n is distinct and contains an unstained nucleolus nl. The cytoplasm shows portions of the Golgi network (black). Note a typical tubular myelin Golgi segment similar to the forms described by Palade and Claude (1949). Other portions of the network show this character. As thin sections are necessary for the sake of clarity at high magnification, only isolated segments of the three-dimensional network are visible. Nerve-fibres at b show the myelin...
sheath and axoplasm clearly differentiated. Note that the satellite nuclei (s) are pale, with indistinct broken outlines.

Fig. 1, B is a photomicrograph of the same cell. The coverglass was removed, Apáthy’s medium dissolved away in water, and the Sudan removed by leaving the slide in 96% alcohol for 5 min. The preparation was then rehydrated and stained, according to the Nissl method of Fernstrom (1958), with cresyl-echt-violett. Fernstrom’s method includes (as step 5) the extraction of the section with chloroform. This is a valuable but not essential step in the procedure. Tress and Tress (1935), apparently, were the first to use chloroform in a Nissl method; they found that it cleared the background. Its preliminary use in restaining experiments would certainly help to assure complete extraction of sudanophil substances from the sections.

Note that the nucleolus nl is now the most densely staining structure of the cell. As precision in refocusing of the restained cell is important in order to obtain a high degree of accuracy in registration of the two photographs, the same nucleolus is taken in each case as the central focusing point. It should, if focus is precise, be of the same diameter in optical section (compare nl in fig. 1, A). The Nissl substance is now visible in the form of scattered masses, evenly distributed throughout the cell. According to Nissl’s classification the cell is a somatochrome neurone of the second type (arkyochrome). Note that the sudanophil tubular structures (Golgi in fig. 1, A) are not visible in the restained cell, and do not register with individual Nissl grumes (Nissl). The capsule nuclei (s) are clearly stained, and the nerve-fibres (b) are faintly shown in outline (compare fig. 1, A).

Fig. 2, A shows a group of smaller neurones from the dorsal root ganglion of a kitten, similarly fixed in Zenker-formol, postchromed, and coloured with Sudan black. Lipoidal structure is well shown as twisted strands which tend to imbricate to form a typical Golgi network. The net-like appearance is emphasized as the section is a thicker one than fig. 1, A.

After destaining and restaining with Fernstrom’s stain the cells show a peripheral arrangement of Nissl substance (fig. 2, B). They are somatochrome neurones of type 4 (perichrome) of Nissl. The nucleoli show that the register is accurate. Note that the sudanophil networks (Golgi) tend to occupy the more central area of the cytoplasm, which remains a clear space in the Nissl preparation, and conversely.

Classical urano-formol silver impregnations were also bleached and re-

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Fig. 1 (plate). A, dorsal root ganglion cell of kitten. Maximow’s fluid, postchromed, Sudan black, 3 μ paraffin section. n, nucleus; nl, nucleolus; Golgi, Golgi apparatus (tubular myelin form); b, nerve processes; s, nucleus of satellite cell.

B, same cell as A, Sudan removed, restained with cresyl-echt-violett. Register accurate; note densely stained nucleolus (nl). Nissl substance (Nissl) does not register with sudanophil Golgi of A. Satellite nuclei (s) have clear details; fibres (b) faintly shown.

c, dorsal root ganglion cell of kitten. Cajal’s urano-formol silver method. Golgi, classical Golgi apparatus; n, nucleus; nl, nucleolus.

d, same cell as c. Note accurate register; nl, nucleolus. Nissl substance N is low in amount and restricted to periphery of cell (perichrome position). n, nucleus.
FIG. 1
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Fig. 2

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stained with Fernstrom's method. Fig. 1, c is a dorsal root ganglion cell of a kitten, showing a classical Golgi apparatus impregnated by Ramón y Cajal's silver method and mounted in balsam. The preparation was subsequently down-graded to water and treated with 4% iron alum for 10 min in order to remove the silver deposit completely; after rinses in several changes of distilled water, Fernstrom's method was applied to the sections. Fig. 1, d shows the same cell restained and mounted in balsam. Nissl grumes are present, arranged in the perichrome position (Nissl somatochrome, type 4). The area of cytoplasm previously occupied by the Golgi apparatus is clear of basiphil grumes and shows only a fine granulation. The Golgi apparatus is unstained and is therefore not basiphil.

Osmium fixation methods, although ideal for the impregnation of the classical Golgi apparatus, do not lend themselves to the subsequent application of basic aniline stains. In some way the basiphilia of the cytoplasm is altered. It has recently been shown, however, that this basiphilia can be restored to the cells by the application of peracetic acid before staining (Munger, 1961). Fig. 2, c shows a Kolatchev preparation of a dorsal root ganglion cell of the frog. Portions of the Golgi network are shown impregnated by the osmium. The section is about 3 μ thick. Fig. 2, d shows the same cell after being treated for 1 min in the peracetic acid reagent (Lillie, 1952) diluted 1 in 20 with distilled water, then rinsed and restained by Fernstrom's method. The individual portions of the Golgi net visible in fig. 2, c can still be recognized in the same cell in fig. 2, d, since bleaching was not allowed to progress to completion. The Nissl grumes are now rendered visible and have been stained with cresyl-echt-violett. They occupy positions in the cell quite distinct from the Golgi material. Visually these preparations are very striking and in my opinion are the most convincing to support my views. The Nissl grumes are fluffy structures stained a purplish pink colour, while the compact osmiophil strands of the Golgi apparatus, in contradistinction, are densely blackened. It will be noted that the cell is Nissl's somatochrome, type 2 (arkyochrome). The slide from which fig. 2, d was made will be sent to the Depository for Cytological Slides, Institute Carnoy, Louvain, Belgium, where it can be inspected by interested persons.

Discussion

These results show that the object described as the Golgi apparatus and revealed by classical methods is not identical with the basiphil Nissl substance. The views expressed by Malhotra (1959, 1961) and David and his colleagues

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Fig. 2 (plate). A, group of neurones, dorsal root ganglion of kitten, Maximow's fluid, Sudan black. nl, nucleolus.
B, same cells as A restained with cresyl-echt-violett. Note peripherally arranged Nissl substance (perichrome type). Golgi nets are not stained. nl, nucleolus.
C, dorsal root ganglion cell of frog, Kolatchev's osmium method, 3 μ section.
D, same cell as C treated with peracetic acid to restore basiphilia of the cytoplasm. Nissl grumes now visible and distinct from Golgi segments. nl, nucleolus; n, nucleus.
(1960, 1961) that the apparatus of Golgi in the neurones of vertebrates results from a deposit of silver or osmium of the Nissl grumes is not substantiated. The reader may compare figs. 1 and 2 of this paper with those published by authors expressing contrary views.

Experimental error may affect staining procedures of this nature, and it is essential that certain criteria be made applicable to all illustrations intended to show Nissl substance and Golgi apparatus in the same nerve-cells, viz.:

1. The register of the two photographs under comparison must be accurate. Cells should be selected with clear nucleoli, and the diameter of the identical nucleoli should agree in the two pictures.

2. Since Sudan black may be incompletely removed and appear in the Nissl preparation, where it would be photographically indistinguishable from Nissl substance, the Nissl preparations should be finally mounted in resinous media in the usual manner after dehydration in alcohol and clearing in xylene. Whether or not this has been done is usually discernible in the final photomicrographs; preparations mounted in balsam show clearer details, particularly nuclear outline and contents. The cells appear fuzzier and paler when mounted in watery media.

3. Incomplete or faulty staining of the Nissl substance may occur. As a check that Nissl’s methods have been successfully applied, the neurones depicted should show strong nucleolar staining. The capsule nuclei should have sharp outlines and contain basiphil chromatin granules. On the other hand, nerve-fibres will be only feebly stained. The converse will be true for Sudan preparations (figs. 1, a; 2, a).

The staining experiments described by David and others (1961, p. 491) require comment. The Nissl substance in their fig. 7, c appears to be adequately preserved and stained with a Nissl stain. The silver-on-the-slide method colours these Nissl grumes in the subsequent staining experiment (fig. 7, b), but have they in fact revealed the Golgi apparatus? They admit that their silver method is not specific and will in fact colour a number of unrelated intracellular objects. It is more than possible that the Nissl substance has been impregnated by silver in their fig. 7, c.

Finally, it must be stressed that in all inquiries concerning the ‘Golgi apparatus’ it is important to establish very clearly that one is dealing with the classical object as originally described in neurones by Golgi in 1898. This can only be achieved by following the specific methods used by Golgi and his contemporaries. Results based on silver staining alone are insufficient and should always be controlled by both osmium and Sudan methods. Fortunately, all three techniques lend themselves to analysis by the methods described above.

References

— — — — 1961. Ibid., 102, 481.
—— 1961. Ibid., 102, 387.