A Comparative Histochemical Study of the 'Golgi Apparatus'

By S. K. MALHOTRA

(From the Cytological Laboratory, Department of Zoology, Oxford)

SUMMARY

The purpose of this investigation was to find out the responses of Golgi's net in the neurones of vertebrates to various dyes and histochemical reagents, and to compare these responses with those given by the 'dictyosomes' of the cells of invertebrates. Dictyosomes are regarded by many cytologists as the homologue of Golgi's net. The young oocyte of *Limnaea stagnalis* was chosen as a cell that possesses typical dictyosomes, which have recently been examined by histochemical methods.

The object that presents the characteristic Golgi pictures in the neurones of vertebrates is essentially different, not only in ultrastructure but also in its chemical composition, from those that represent in life the 'dictyosomes' of invertebrates. The dictyosomes of the oocyte react positively to tests for phospholipid and cerebroside, whereas Golgi's net is negative to these tests. Tests for arginine and for ribonucleic acid are positive for Golgi's net, but not for the dictyosomes of the oocyte. The dictyosomes are intensely stained by Rawitz's inversion staining technique and also by acid fuchsine (Metzner) and by iron haematoxylin, but these techniques do not show the net in the neurones of vertebrates. Golgi's net is resistant to embedding in paraffin after fixation in Clarke's (Carnoy's) fluid, but the dictyosomes are not. Neutral red is taken up during life by the object representing the dictyosomes, but not by the net of Golgi.

INTRODUCTION

IT is well known that when 'Golgi' impregnation methods (silver nitrate or osmium tetroxide) are applied to the neurones of vertebrates, a black network is seen; this is commonly called the 'Golgi apparatus' after its discoverer (Golgi, 1898). In many other kinds of cells the Golgi techniques do not produce a network; but instead small, separate rods and crescents, usually associated with a chromophobe substance, are seen. This is regarded by many zoologists as the typical form of the Golgi apparatus in most cells of invertebrates. These rods and crescents ('dictyosomes') are believed by many workers to represent, in a somewhat distorted form, the spherical or subspherical bodies that colour in life with neutral red (Parat, 1928; Young, 1953, 1956; Chou, 1957*a*; Baker, 1959; Malhotra & Meek, 1961). These neutral red bodies often show a differentiation into a surface layer of lipid nature and a non-lipid interior (Baker, 1959; Malhotra, 1960*b*). It is this lipid surface that selectively reduces osmium tetroxide or silver nitrate in Golgi methods. The blackening of the surface layer of the dictyosomes has led many zoologists to homologize these bodies with the network seen in the neurones of vertebrates, and indeed the word *dictyosome* (net-body) begs the question by asserting this homology. As a result, many cytologists consider that a homologue of Golgi's net occurs in all animal cells.

[Quarterly Journal of Microscopical Science, Vol. 102, part 1, pp. 83–87, 1961.]

It is the purpose of this paper to consider whether the 'dictyosomes' of the cells of invertebrates are in fact similar in their reactions to Golgi's net. It is thought best to select a particular cell that contains typical dictyosomes, and to compare their reactions to various dyes and histochemical reagents with those given by the object that is blackened to produce Golgi's net in the neurones of vertebrates. The cell selected for this comparison is the young oocyte of the pond-snail, *Limnaea stagnalis*. The structure and chemical composition of the 'dictyosomes' in this cell have recently been investigated in some detail (Malhotra, 1960*c*).

Results

The neurones of the dorsal root ganglia of the mouse show a typical net (apparatus) of Golgi.

The object that other authors (Gatenby, 1919; Bretschneider & Raven, 1951; Raven, 1958) have called the Golgi apparatus ('dictyosomes') in the early oocyte of the pond-snail, *L. stagnalis*, consists of small, separate objects dispersed in the cytoplasm. In Golgi preparations they mostly appear in the form of rods and crescents, in association with chromophobe substance. Attention has been concentrated on early oocytes in which the yolk has not yet appeared.

If the tissue is fixed in Helly's (1903) fluid and postchromed, the dictyosomes of the oocyte can be intensely stained by acid fuchsine in Metzner's technique for mitochondria (1928), or by iron haematoxylin. These techniques, however, do not show the apparatus in the neurones of most vertebrates (Malhotra, 1959; Casselman & Baker, 1955). The dictyosomes are also strongly stained by basic fuchsine, used after mordanting in tannic acid and potassium antimonyl tartrate according to Rawitz's 'inversion staining' method (Przełęcka, 1959). The apparatus of the neurone is not generally shown by this method (Baker, 1959); it may occasionally be very feebly coloured.

The dictyosomes are coloured by Sudan black (Baker, 1949). They react positively to the acid haematein (AH) test, which becomes negative after extraction with pyridine (Baker, 1946); the results thus indicate the presence of phospholipid. Though one would expect the presence of lipid in an object that has a remarkable affinity for silver or osmium, the apparatus of the neurone is not coloured by Sudan black nor by the AH test (Casselman & Baker, 1955; Malhotra, 1959). Even when a powerful unmasking agent, such as cadmium chloride or mercuric chloride (Clayton, 1959), was used as a fixative in place of formaldehyde/calcium in the standard AH test, the apparatus did not show; it was coloured only very feebly with Sudan black. It is difficult to demonstrate lipid in this object by *in situ* histochemical methods.

The dictyosomes react positively to the periodic acid/Schiff (PAS) test of McManus (1948); the reaction is strong whether the test is applied to paraffin sections of tissue fixed in Zenker (1894) or gelatine sections of ovotestis fixed in formaldehyde/saline (Baker, 1949). The PAS reaction becomes weaker after digestion in saliva. The PAS test is also positive after extraction

with cold or hot acetone (Casselman & Baker, 1955), though it becomes weaker when hot acetone is used. Sudan black also colours the dictyosomes more intensely after fixation in cold acetone than in hot. The apparatus of the neurone also responds positively to the PAS test, but the reaction is much weaker than in the dictyosomes of the oocyte. The reaction is also positive, though very weak, after saliva treatment. No positive reaction is seen in the apparatus of the neurone when the PAS test is applied to tissue fixed in acetone, hot or cold; Sudan black also does not colour the apparatus after this fixation.

The dictyosomes are blued by the Nile-blue test (Cain, 1947), while the apparatus of the neurone is not coloured at all.

The dictyosomes resist embedding in paraffin after fixation in Zenker; this suggests the presence of protein. However, when the oocyte is subjected to tests for amino-acids, namely, Sakaguchi (Baker, 1947) for arginine and Hg/nitrate (Baker, 1956) for tyrosine, a positive reaction is seen throughout the cytoplasm, but the dictyosomes cannot be differentiated. The apparatus of the neurone, on the contrary, is positive, though weakly, to the Sakaguchi test, but not to the Hg/nitrite test.

The apparatus of the neurone is intensely coloured by basic dyes (Malhotra, 1959, 1960*a*). The basiphil material can be extracted by ribonuclease (Bradbury, 1956) or trichloroacetic acid (Pearse, 1954). In contrast to this, the dictyosomes are not stained by basic dyes.

If Clarke's fluid (Clarke, 1851; Carnoy, 1886) is used as fixative and the ovotestis embedded in paraffin, the dictyosomes of the oocyte are not revealed in the sections. On the contrary, the apparatus of the neurone is resistant to such a treatment and can be demonstrated by colouring with basic dyes.

Neutral red is taken up by the dictyosomes of living cells from salt solutions. When this vital dye is used on the neurone of vertebrates, it appears in the small spherical bodies dispersed throughout the cytoplasm (Baker, 1944; Thomas, 1948; Malhotra, 1959), but the apparatus is not dyed.

Discussion

These results indicate that the objects commonly described as Golgi apparatus (dictyosomes) in the oocyte of the pond-snail are very different in their chemical composition from the structure that bears this name in the neurones of vertebrates. The objects representing in life the dictyosomes of the oocyte are similar in their form and chemical composition to the lipid globules of the neurones of vertebrates (Casselman & Baker, 1955). The lipid globules of these neurones are quite different from the structure that is blackened by Golgi methods to produce Golgi's net (Malhotra, 1959). In the neurones of invertebrates, however, the objects that produce the appearances of dictyosomes are the lipid globules that colour in life by neutral red (Parat, 1928; Young, 1953, 1956; Shafiq, 1954; Chou, 1957*a*; Chou & Meek, 1958; Malhotra & Meek, 1961). These are also histochemically similar to the dictyosomes of the oocyte of the pond-snail (Shafiq & Casselman, 1954;

Chou, 1957*b*; Malhotra, 1960*b*, though there may be slight differences in the chemical composition of the lipid globules in different cells.

The Golgi apparatus of the neurone of vertebrates differs from those of other cells not only in chemical composition, but also in its ultrastructure (Malhotra & Meek, 1960). Many electron microscopists consider that the Golgi apparatus of all kinds of cells is represented in micrographs by a system of closely packed, smooth-surfaced membranes, arranged in pairs (γ-cytomembranes of Sjöstrand, 1956), often seen in relation with vacuoles or vesicles (see Palay, 1958; Oberling, 1959, for references). Though elements of this system have been described in electron micrographs of the neurones of vertebrates (Palay & Palade, 1955; Hess, 1955; Malhotra & Meek, 1960), it remains to be proved that they form a reticulum corresponding to what is seen in this cell by routine Golgi techniques for light microscopy. This system is very sparse in electron micrographs and not nearly abundant enough to produce the massive net of Golgi (1898). Recently it has been suggested that this net is more likely to be formed by a deposit of silver on the membranes of the endoplasmic reticulum (Malhotra & Meek, 1960). The small ribonucleo protein particles (Palade & Siekevitz, 1956) present on and in between the membranous cisternae of the endoplasmic reticulum are presumably responsible for the basiphil nature of the Golgi apparatus in these neurones (Malhotra, 1959; David & others, 1960).

It would thus appear that the structures that are commonly called Golgi apparatus in the neurones of vertebrates and in the oocyte of *L. stagnalis* have nothing in common, except that they reduce osmium tetroxide or silver nitrate in arbitrarily devised techniques that are devoid of histochemical validity.

I am grateful to Dr. J. R. Baker, F.R.S., for the help and valuable advice given during the course of this work. Professor Sir A. C. Hardy, F.R.S., has been very kind in providing laboratory facilities.

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