The Chemical Composition of Lipid Globules in the Neurones of *Helix aspersa*

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SUMMARY

1. The three kinds of globules that can be recognized in the living neurone of *Helix aspersa* are sharply distinguishable from one another by histochemical tests.

2. One kind of globule contains phospholipid; another appears to consist of triglyceride; the third is complex chemically, containing mixed lipids with some protein and carbohydrate.

3. When osmium techniques for the 'Golgi apparatus' are applied to this particular cell, phospholipid is blackened.

IN an earlier paper (Chou, 1957) it was shown that there are three kinds of lipid globules in the cytoplasm of the neurones of *Helix aspersa*. They are shown here in fig. 1. These three kinds react quite differently to vital dyes. One kind is readily coloured blue by Nile blue, methylene blue, and brilliant cresyl blue: I therefore call these 'blue' globules, though they are colourless in life. Another kind cannot be coloured by any vital dye, so far as is known: I call these colourless globules. The third kind is in life pale yellow, and I call these yellow globules. They appear green when coloured by the blue vital dyes. The colourless and yellow globules are more refringent in life than the 'blue' ones. The purpose of the present investigation was to find whether there were histochemical differences between the three kinds of globules.

In fixed preparations it is easy to distinguish the colourless globules because many of them lie in nearly straight rows at the base of the axon. The yellow globules are generally the largest; they are irregular in shape, and many of them are grouped in or near the axon hillock, though they are not confined to this region. The 'blue' globules are recognized by their being spherical, nearly uniformly distributed throughout the cytoplasm, and different in their reactions from the colourless globules.

The techniques used are set out in tabular form in the appendix (p. 64). The cerebral ganglion was used, or sometimes the sub-oesophageal ganglion-mass. The neurones of all the ganglia are similar in their cytoplasmic inclusions, though there is considerable difference in size.

RESULTS

All three kinds of globules react positively to Sudan IV and Sudan black. It is clear that they contain or consist of lipid.

None of the three kinds reacts positively to Fischler's test for fatty acids,

[Quarterly Journal of Microscopical Science, Vol. 98, part 1, pp. 59–64, March 1957.]

Windaus's for cholesterol, or Sakaguchi's for arginine, &c. The globules are not basiphil nor chromotropic. Feulgen's test is negative.

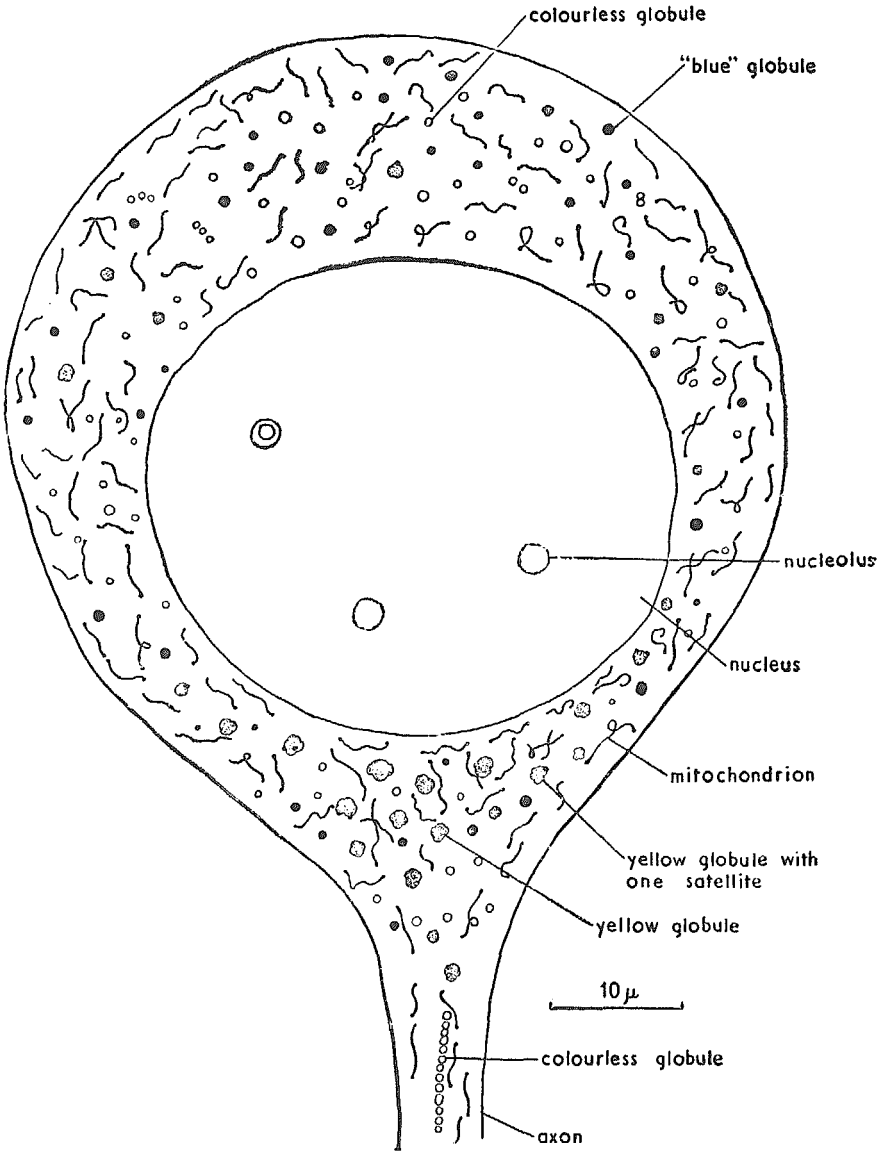

FIG. 1. Diagram showing the cytoplasmic inclusions present in the neurones of the cerebral ganglion of *Helix aspersa*. From Chou (1957).

The three kinds of globules are distinguishable by the tests enumerated below.

'Blue' globules. These are the only globules that react positively to the acid haematein (AH) test. The test was negative after pyridine extraction. The globules therefore contain phospholipid. When Metzner's method for

mitochondria is applied, the acid fuchsine dyes the globules strongly. There is often a correlation between red staining with Metzner's method and the presence of phospholipid.

Apart from the Sudans and AH, no histochemical test used in this investigation gave a positive result with the 'blue' globules.

The low refringence of these globules suggests that the phospholipid may be associated with water.

Colourless globules. These are negative to all the tests except the Sudans (Ciaccio's test is positive). The facts suggest but do not prove that the globules contain triglyceride. The negative result with Nile blue (neither blue nor red reaction) is difficult to interpret. It is unfortunate that there is no positive test by which the presence of the triglycerides can be proved. The high refringence of these globules suggests that they may consist entirely of lipid.

Yellow globules. These are the only globules that have a non-lipid as well as a lipid content. They are considerably more complex histochemically than the 'blue' and colourless globules.

Cain (1948) showed, by the application of concentrated sulphuric acid, that the pigment in these globules is carotenoid. This was confirmed also in the present investigation by the Carr–Price reaction (Carr and Price, 1926), but the reaction was feeble. The globules are of a much paler colour than the corresponding ones in *Limnaea stagnalis* and *Planorbis corneus* (Cain, 1948). AH gives a feeble reaction on the surface only of the globules. The test for plasmalogen is positive. Liebermann's test for cholesterol and its esters is positive (though weakly), provided that the sections are exposed to as much direct sunlight as possible for about 10 days. (Oxidation by iron alum is not successful.) The positive reactions with the PFAS and PAAS tests suggest unsaturation of the lipids.

The periodic acid / Schiff test gives a positive result. After digestion with saliva the reaction is weaker (though it still exists). This suggests the presence of a carbohydrate. If a ganglion is fixed in cold acetone and sections are coloured with Sudan black, the globules become pale grey; this is not so if hot acetone in a Soxhlet apparatus is used. The facts suggest the presence of cerebroside (Casselman and Baker, 1955), and would account in part for the positive PAS reaction. There is no evidence of the existence of mucopolysaccharides, acidic or netural.

The Hg/nitrite test (Baker, 1956) gives a weakly positive result, presumably indicating the presence of tyrosine in protein; but, as remarked before, the Sakaguchi test gave a negative result. There is not enough protein to leave a stainable 'granule' in fixed preparations.

No general enzymological study was undertaken, but Gomori's (1952) test for alkaline phosphatase was performed. This gave a positive result on the surface of the yellow globules; the latter were covered with tiny black dots. It is interesting to notice that the phosphatase appears in the same position as phospholipid.

Discussion

It is clear that the three kinds of globules distinguishable in life in the neurones of *Helix aspersa* are sharply different in chemical composition. The facts may be briefly summarized thus:

'Blue' globules. These contain phospholipid.

Colourless globules. These probably consist of triglyceride.

Yellow globules. These are complex chemically. They appear to contain mixed lipids, with some carbohydrate and protein; the colour is due to carotenoid.

In osmium preparations for the 'Golgi apparatus', the phospholipid of the 'blue' globules is precipitated by the fixative on one side of the sphere, and then blackened by reduced osmium. The resulting crescent- or cap-shaped body is commonly called a 'dictyosome' (net-body). Osmium also blackens the surface of the yellow globules, and indeed Moussa (1950), in his description of the neurones of *Limnaea stagnalis*, regards the blackened surface of the yellow globules as the 'dictyosome' and the rest of the globule as 'Golgi product'.

The phospholipid of the yellow globules is situated at their surface in fixed preparations; this was shown by Thomas (1947) and confirmed in the present investigation. It is not shown whether during life the phospholipid is at the surface or distributed throughout the globule.

It is of interest to note that in these cells osmium tends to be deposited, in 'Golgi' preparations, wherever there is an accumulation of phospholipid. In the neurones of mammals osmium is deposited in the form of the characteristic Golgi 'network', but no one has described a phospholipid network that could be responsible for this appearance. It seems best to use ordinary chemical nomenclature whenever possible, in preference to ill-defined words like 'Golgi apparatus'. Lipid globules do indeed occur in the neurones of mammals, and these contain phospholipid (Casselman and Baker, 1955). They may perhaps be the same as the *plaquettes* seen by Golgi (1898) in his network.

In Aoyama preparations silver is deposited on the surfaces of all three kinds of lipid globules.

I have great pleasure in acknowledging my debt to Dr. J. R. Baker for suggesting and supervising this investigation, and to Professor A. C. Hardy, F.R.S., for providing me with facilities for working in his department.

The work was carried out during tenure of an Inter-University Council Fellowship through the Carnegie Corporation of New York, and study leave from the Department of Zoology, University of Hong Kong.

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APPENDIX

A summary of the histochemistry of the lipid globules in the neurones of Helix aspersa

Test applied					*Results obtained with the three kinds of lipid globules*		
Name of test	*Fixation*	*Embedding medium*	*Thickness of sections in μ*	*Reference*	*Yellow globules*	*'Blue' globules*	*Colourless globules*
Standard Sudan black	FCa+PC Fs+PC	G	10	Baker, 1944, 1949	+++	+++	+++
Sudan IV	FCa+PC Fs+PC	G	10	Herxheimer, 1901	+++	+++	+++
Windaus's	Fs; FCa	G	10	Lison, 1953	O	O	O
Liebermann's	Fs	G	10	Lison, 1953	+	O	O
Fischler's	F; FCaS	G	10	Pearse, 1954	O	O	O
Acid haematein	FCa+PC	G	10	Baker, 1946	+	++	O
Acid haematein, pyridine extraction	WB+PE	G	10	Baker, 1946	O	O	O
Ciaccio's	CF+PC (6 days)	P	8	Lison, 1953	+/++	+++	+++
PAAS	Z	P	8	Pearse, 1954	+/++	O	O
PFAS	Z	P	8	Pearse, 1954; Lillie, 1952	+/++	O	O
Casselman and Baker's for cerebroside	CA	G	10	Casselman and Baker, 1955	+	O	O
Casselman and Baker's for cerebroside, control	HA	G	10	Casselman and Baker, 1955	O	O	O
Nile blue	FCa	G	10	Cain, 1947	O	O	O
PAS	Z	P	8	Pearse, 1954	+++	O	O
PAS, control	Z	P	8	Pearse, 1954	O	O	O
PAS after saliva digestion	Z	P	8	Pearse, 1954	++	O	O
Feulgen's	Z	P	8	Feulgen and Rossenbeck, 1924	O	O	O
Feulgen's, control	Z	P	8	Feulgen and Rossenbeck, 1924	+	O	O
Plasmal	freshly teased			Pearse, 1954	+	O	O
Cain's for carotenoids	freshly teased			Cain, 1948	+	O	O
Carr and Price test	freshly teased			Carr and Price, 1926	+	O	O
Metachromasy	Z	P	8	—	O	O	O
Bignardi's for neutral polysaccharide	H	P	8	Bignardi, 1940	O	O	O
Sakaguchi	Z	P	8	Baker, 1947	O	O	O
Hg/nitrite	Fs	C	15	Baker, 1956	+	O	O
Gomori's for alkaline phosphotase	Alc/Ac	freshly teased		Gomori, 1952	+	O	O
Metzner's	Alt	P	4	Metzner and Krause, 1928	+	++	O
Basiphilia	Z	P	8	—	O	O	O

KEY: Alc/Ac = absolute alcohol / acetone mixture; Alt = Altmann's fluid; C = celloidin; CA = cold acetone; CF = Ciaccio's fixative; F = neutral formaldehyde; FCa = formaldehyde calcium; FCaS = neutral formaldehyde with saturated calcium salicylate; Fs = formaldehyde saline; G = gelatine; H = Hermann's fluid; HA = hot acetone; P = paraffin. +PC = with postchroming; WB+PE = weak Bouin followed by pyridine extraction; Z = Zenker's fluid; +++ = strong reaction; ++ = moderate reaction; + = weak reaction; O = negative.