Neurosecretion

The concept of neurosecretion comprises two generalized views: (1) the elaboration of physiologically active substances by certain morphologically specialized neurones having a conspicuous distribution; (2) the 'selective' stainability of such substances with aldehyde fuchsin[1] (AF), Masson's trichrome, and particularly with chrome haematoxylin[2] (CH).

The present communication purports to furnish an outline of the author's work on the problem of neurosecretion in the spinal ganglion cells of fishes (*Periophthalamus* sp., *Heteropneustis fossilis* and *Ophiocephalus striatus*), amphibians (frog, *Rana tigrina* and the toad, *Bufo stomaticus*), and reptiles (lizards, *Hemidactylus* sp. and *Uromastix*, snakes, *Natrix* and *Eryx johnii*, and the turtle, *Lissemys* sp.).

Neither CH nor AF, used for the demonstration of the neurosecretory substances, indicated the presence of any morphologically specialized (neurosecretory) cells or stainable products. However, four types of inclusions have been deemed neurosecretory on the basis of their origin, behaviour, role and final fate in the different materials studied. These inclusions are:

(1) *Giant lipid spheres:* These have not been seen in the nerve cells of the turtle, fishes, *Hemidactylus* and *Uromastix*. They measure 3 μ to 14 μ and even more in diameter and have acidic lipids, neutral lipids, or a mixture of both. Their cortices, being predominantly phospholipids, also have the SH groups, fatty acids, and ferric iron. A few reveal a feeble alkaline phosphatase activity as well.

After their formation from the phospholipid granules, they come to lie at the cell periphery, mostly during the monsoon period (Figure 1). Thence they go into the inter-neuronal spaces. Most of them are consumed during winter.

The secretion products, according to SCHARRER and PALAY (quoted from THOMAS[3]), actually come to lie at the cell periphery before being discharged into the surrounding tissue fluid. Both the intra-neuronal and inter-neuronal lipid spheres may be homologized with the endoerdonous and exoerdonous secretion products of HIRSCH and BAKER (quoted from THOMAS[3]).

Lipofuscin pigment bodies: These have been observed in the nerve cells of all the animals under study. They measure 0.2 μ to 2 μ and even more in diameter and occur as granules and duplex bodies, the latter with dark-brown cortices and sharply refringent pale-yellow medullae. Histochemically these bodies are lipofuscins. They also show esterase and acid phosphatase activities. These bodies appear to arise as a result of total or partial oxida-

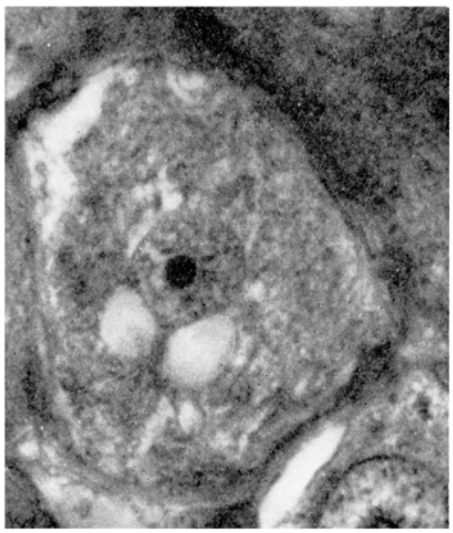

Fig. 2. A cold acetone (4°C)-fixed neurone showing two big hyaline spheres coming out of the nucleus (Old *Natrix*). 1625 ×.

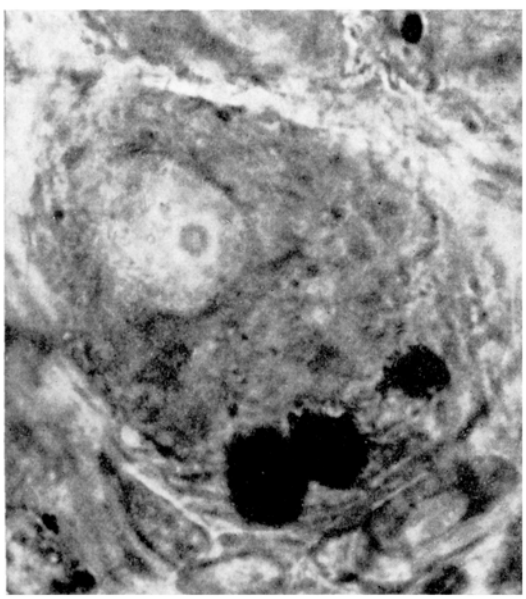

Fig. 1. Formaldehyde-calcium post-chromed (FCa + Pc)/Sudan III and IV (60° C) depicting three giant lipid spheres migrating into the inter-neuronal spaces. A few phospholipid granules adhering to the spheres are also clearly disernible (Adult *Natrix*). 2240 ×.

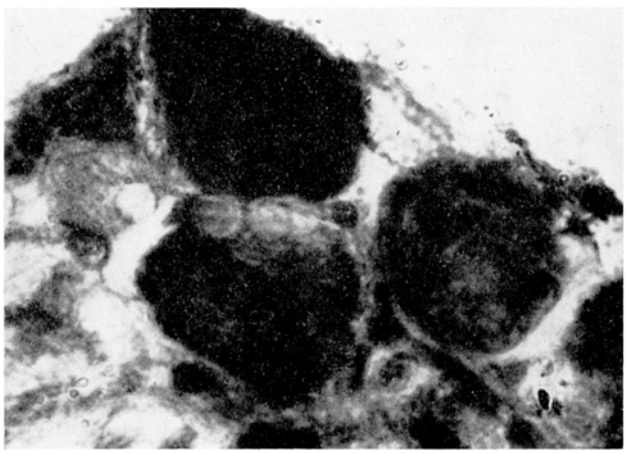

Fig. 3. Neurones fixed in cold acetone (4°C) showing a strong acid phosphatase activity in the cytoplasm. A large number of prominent hyaline spheres lying intra-neuronally as well as iner-neuronally are also present. Several hyaline spheres can be seen going into the surrounding interstitial spaces (Old *Periophthalamus*). 960 ×.

[1] N. S. HALMI, Stain Tech. *27*, 61 (1952).
[2] W. BARGMANN, Z. Zellforsch. *34*, 610 (1949).
[3] O. L. THOMAS, J. comp. Neurol. *95*, 73 (1951).

tion of the lipid or lipo-protein bodies[4]. The present author, in conformity with the views of GATENBY and MOUSSA[5], and ISSIDORIDES and SHANKLIN[6], considers these bodies to be some secretion products (endoerdonous[3]).

(3) *Pale-yellow substance:* This is seen as a pale-yellow diffuse substance in the cytoplasm and in the duplex bodies of the living spinal neurones of the frog, the toad, and rarely in those of *Natrix* and *Eryx*. This substance corresponds to a similar product described by THOMAS[3] and MALHOTRA[7] in the neurones of *Helix* and the frog respectively. The views regarding the elaboration of secretion products in the cores of the duplex lipid bodies have been advanced and bolstered for different animals by HIRSCH[8], BAKER[9], etc.

(4) *Hyaline spheres:* They originate from the nucleus and measure 0.2 μ to 5 μ and even more in diameter. After their discharge into the cytoplasm, they grow (Figure 2.) Although the chemical nature of the hyaline spheres remains undetermined, yet in the *Lissemys* nerve cells, a few spheres, which have in their vicinity an area of very small phospholipid granules, react feebly for the acidic lipids in the cortical sites. Sometimes, as seen in the *Eryx* and *Lissemys* neurones, extremely small phospholipid granules have also been seen attached to the outer borders of these spheres. All this tentatively suggests the participation of lipids as one of the 'building materials' of these bodies. Feeble acid phosphatase activity has been recorded in the cortical sites of a few hyaline spheres of the *Natrix* nerve cells. The hyaline spheres have been seen migrating into the inter-neuronal spaces in the case of the toad, *Lissemys*, *Eryx* and *Periophthalamus* nerve cells (Figure 3). Axonal transportation of these spheres has been demonstrated only in one or two *Lissemys* nerve cells. These bodies have been considered as some secretion products, both endoerdonous and exoerdonous[3]. PALAY[10] has also described the nuclear origin of the secretory products in certain fishes. Secretion in the form of vacuoles has been described by HAMMAR[11], MAZIARSKY[12,13], SMITH[14], GRZYCKI[15], etc.

Although it is not possible, with the prevalent histochemical techniques or with the methods alleged to be 'selective' for the neurosecretory substances, to detect the presence of the hormones – a fair indication of neurosecretion – the various intra-neuronal as well as inter-neuronal inclusions described above, cannot be dismissed as 'ordinary' cytoplasmic inclusions, particularly when they exhibit so many physiological characteristics: some significance, in the sense of their being 'neurosecretory' substances, seems essential.

The view that only those cells which show certain morphological specializations[16,17] can secrete the neurosecretory substances (NSS), seems untenable in the materials studied, although it may hold good for the other actively secretory centres, such as the mid-brain, hypophysis, etc. Also, the various stains – particularly the CH – which are alleged to be 'selective' for the NSS fail to warrant any substantial statement as to the histochemical background of the secretion products. Even the 'selectivity' of these stains is fraught with many pit-falls. Thus, in teleosts, skates, and amphibians, even some chrome haematoxyphobe substances have been labelled as the NSS[16].

In the author's opinion, it is more rational to pursue the problem of neurosecretion in the light of cellular physiological events rather than to attach unqualified importance to certain particular stains.

Résumé. Des recherches effectuées sur les neurones spinaux de quelques Poissons, Amphibiens et Reptiles ont amené l'auteur à considérer comme substances neurosécrétoires les 4 types d'inclusions telles que les sphères lipides, les sphères hyalines, la substance jaune-pâle et les corps pigmentés dits lipofuscins en se basant sur leurs caractères originels, leur foncton vraisemblable, leur rôle probable et leur destin final. Le chrome hématoxyphile ou l'aldéhyde fuchsine positive n'ont pas été observés.

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PRO EXPERIMENTIS

Determination of Mass Spectra of Non-Volatile Substances[1]

The main restriction to the wide application of mass spectrometry in organic chemistry has been the inability to volatilize in undecomposed form many organic substances. The use of heated inlet systems[2,3] (preferably all-glass) has greatly increased the use of this physical tool in the past few years as demonstrated especially by the very recent applications in the field of natural products[4–6]. Nevertheless, there exists a large number of interesting organic compounds which have resisted until now mass spectrometric investigation because of their non-volatility even in such heated inlet systems.

One way around this difficulty is the direct insertion of the substance near the electron beam. Such a system is available[3] in the Bendix Time-of-Flight mass spectrometer, but the latter suffers from the serious limitation

[1] This paper represents part XXII in the series *Mass Spectrometry in Structural and Stereochemical Problems*. For preceding article, see E. LUND, H. BUDZIKIEWICZ, J. M. WILSON, and C. DJERASSI, J. Amer. Chem. Soc., in press.
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