Some Interesting Points on the Histochemistry of the Cytoplasmic Inclusions of the Ageing Spinal Neurones of Reptiles, Birds and Mammals

The morphology of the cytoplasmic inclusions of the neurones of various animals, vertebrates and invertebrates, has been discussed thoroughly in the past decades by a host of workers. Literature on the histochemistry of neurones in general is, however, quite sparse. A detailed histochemical analysis of the neurones of ageing fowl and wall-lizard, adult pigeons and *Uromastix*, young and adult rats, and immature and adult rabbits, has been made to find out the chemistry of the cytoplasmic inclusions in the ageing animals by the use of the latest histochemical techniques for lipids, carbohydrates, proteins, nucleic acids, pigment bodies, etc. Below are given some interesting points which, the authors feel, are the common features of the neurones of all the above-mentioned animals. Except for a few differences as regards pigment bodies and neurofibrillae, the other cytoplasmic inclusions of the neurones of reptiles, birds, and mammals show a more or less similar plan histochemically.

The mitochondria, as studied in the various histochemical preparations, are in the form of granules, short rods, and filaments. They show a persistent lipo-protein nature, except for the neurones of wall-lizard in which they show a slight amount of RNA as well.

The lipid spheres corresponding to the lipochondria of CASSELMAN and BAKER[1] are of varying sizes, shapes, and histochemical nature. In the young animals, pure phospholipid bodies, together with some lipo-protein particulates (lipid part being phospholipid), are abundant. With age, the neutral lipids (triglycerides) start accumulating until, in the old animals, the lipid bodies are recognized as (I) a mixture of phospholipids and triglycerides, (II) pure phospholipids, and (III) pure triglycerides. The pure triglyceride bodies are quite abundant in the neurones of adult fowls and adult rats, whereas they are absent from those of pigeon, rabbit, and *Uromastix*. Rarely, a few triglyceride bodies in the lizard neurones show scale-like phospholipid ensheathments attached to their surfaces.

Some of the lipid bodies exhibit 'crescentic' appearances in certain fixed preparations, which, in conformity with the views of GUPTA and SHARMA[2], have been proved to be the artifacts of fixation. Such appearances are scanty in the neurones of *Uromastix*, while they are totally absent from those of the rabbits.

It is interesting to note that in the neurones of the young animals under investigation all the lipid bodies are more or less uniformly dispersed in the cytoplasm (Figure 1). With age the lipid particulates begin to move towards the periphery and thence to the axonal pole of the cell, until in the old animals they constitute definite axonal aggregations (Figure 2). It should, however, be noted that the neurones of old wall-lizard and *Uromastix* do not show any regularly occurring axonal aggregations.

In the neurones of the wall-lizard, a quantitative increase in some categories of lipid bodies, particularly the pure triglyceride bodies, is seen with age and change of seasons starting from the neurones fixed during the summer months to those fixed during winter. A reverse case has been found in the *Uromastix* neurones where the neutral lipids are totally absent in the winter neurones but increase gradually as summer approaches. Pure triglyceride bodies have never been seen to make an appearance.

Characteristically, persistent binary spheroids (corresponding to the mulberry spheroids of THOMAS[3]) occur in the neurones of all the adult and old animals under study; they are quite rare in the neurones of the young animals. These bodies have phospholipid 'satellite' granules attached to their thin phospholipid-triglyceride cortices of irregular contours; the cores have probably some pigment in them.

A characteristic as well as interesting feature of the neurones of wall-lizard and *Uromastix*, especially those of the adult and old animals, is the presence of dirty yellow to dark brown pigment bodies of varying shapes and sizes. A detailed histochemical analysis reveals that these bodies are the lipopigments (lipofuscins)[4].

The assumption of MOUSSA and BANHAWY[5] that the lipid spheroids are completely absent from the neurones of the young animals, and that those found in the neurones of the adult animals originate from the integrate canalicular Golgi reticulum found in the neurones of tadpoles and young toads, seems unsound in view of the present investigations, since the neurones of the young chickens, rats, lizards, and rabbits studied in the living condition with phase contrast microscopy and with certain basic dyes used supervitally, and in the various fixed preparations, do not reveal any 'canalicular Golgi apparatus' or even the canalicular spaces; contrarily, the young neurones abound in the lipid particulates only. Moreover, it is only in the neurones of adult and old animals that a system of discrete, ramifying canalicular spaces has been observed in addition to the usual lipid particulates. Such a system of canalicular spaces can, in no way, be homologized with the canalicular Golgi apparatus, since the classicists claim its integrate occurrence in the young animals only.

RNA rich Nissl bodies in the form of well-defined composite patches and numerous irregularly elongate, large bodies have been seen in the neurones of all the animals studied. The Nissl substance seems to proliferate with age because of its sparseness in the neurones of the young animals and abundance in those of the adult and particularly the old ones.

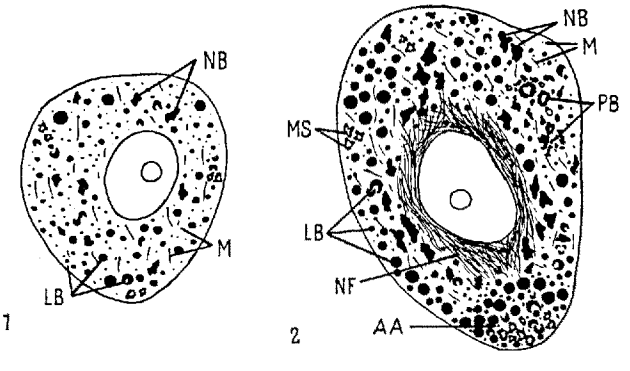

Fig. 1. Diagrammatic representation of the neurone of a young animal showing the lipid bodies (LB), granular and filamentous mitochondria (M), and a few Nissl bodies (NB). Fig. 2. Diagrammatic representation of the neurone of an old animal showing the lipid particulates (LB; MS) of different sizes and shapes, the granular and filamentous mitochondria (M), the pigment bodies (PB), the Nissl bodies (NB), and the circumnuclear neurofibrillae (NF). An axonal aggregation (AA) constituted by different kinds of lipid bodies is also shown.

[1] W. G. B. CASSELMAN and J. R. BAKER, Quart. J. micr. Sci. *96*, 49 (1955).
[2] B. L. GUPTA and S. P. SHARMA, Res. Bull. (N.S.) Panjab Univ. *10*, 267 (1959).
[3] O. L. THOMAS, Quart. J. micr. Sci. *89*, 333 (1948).
[4] S. P. SHARMA, Exper. *17*, 125 (1961).
[5] T. A. A. MOUSSA and M. BANHAWY, J. R. micr. Soc. *74*, 162 (1954).

Finally, there are some neurofibrillae found in the neurones of birds and mammals whose histochemical nature remains undetermined. Characteristically they are absent from the neurones of wall-lizard and *Uromastix*.

Résumé. L'analyse histochimique comparée des neurones des Reptiles, des Oiseaux et des Mammifères révèle que les divers corps qui y sont inclus ont une histochimie identique. Les sphéroïdes lipides abondent dans les phospholipides des jeunes neurones qui sont transformés en corps triglycérides chez les adultes. Les corps lipides ne sont pas un produit de sécrétion de l'appareil canaliculaire de Golgi dont l'homologue a été observé seulement dans les neurones adultes. La substance de Nissl est pleine de RNA.

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Effect of Paraoxon (Diethyl 4-nitrophenyl phosphate) on Axone Reflex Sweating Produced by α-Lobeline

The physiological significance of axone reflex sweating remains obscure despite a considerable amount of information available on its characteristics, as recently reviewed by WADA[1], who, with KUNO[2], has suggested that this mechanism may play a role in local temperature regulation. As yet, however, there is no evidence that the axone reflex occurs under any physiological conditions.

Nicotinic agents, including acetylcholine, can elicit the response when injected intradermally in appropriate concentrations. This suggests that endogenous acetylcholine, normally liberated at sudomotor endings in the skin, may be capable of initiating an axone reflex. This possibility was tested by MCLAUGHLIN and SONNENSCHEIN[3] who studied the effects of a cholinesterase inhibitor, paraoxon (diethyl 4-nitrophenyl phosphate), on human sweat glands and the sympathetic axone reflex. Intradermal injection evoked local sweating which was definitely not of axone reflex nature. The failure of paraoxon to produce axone reflex sweating was thought possibly to be due to an inadequate enzyme inhibition and subsequent accumulation of a subthreshold concentration of endogenous acetylcholine. On the other hand, paraoxon did facilitate the action of injected acetylcholine in producing the axone reflex. This observation did not, however, allow direct inference of the role of endogenous acetylcholine, for it was not possible to distinguish between the effects of paraoxon on this and on the exogenous acetylcholine.

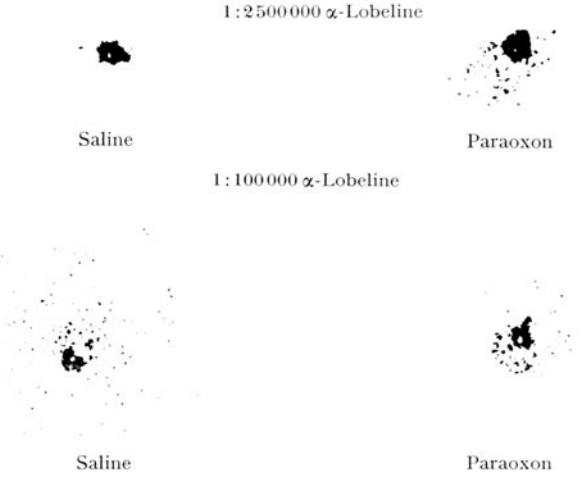
Resolution of this problem was attempted in the present study by determination of the effect of paraoxon on the action of a nicotinic agent, α-lobeline, which is not subject to enzymatic hydrolysis by acetylcholinesterase. It was reasoned that should facilitation of the axone reflex producing action of α-lobeline occur, this would be a result of the increase in local concentration of endogenous acetylcholine acting additively with α-lobeline. Such a finding would support the hypothesis that endogenous acetylcholine can act in the initiation of the axone reflex.

Methods. Paraoxon and α-lobeline were dissolved in sterile 0.9% sodium chloride. Intradermal injections were made into the skin of the volar surface of the forearm in normal human subjects with sterile 0.5 ml tuberculin syringes and $^1/_2$ inch No. 26 needles. 0.3 ml of α-lobeline solution was injected intradermally in the center of an area where 0.5 ml of the paraoxon solution had been injeced intradermally 5–7 min previously. This intervalt allowed for the manifestation of the local sudorific effect of paraoxon[3]. A control was made for comparison simultaneously on the opposite arm where 0.3 ml of the same concentration of α-lobeline was similarly injected into an area previously injected with 0.5 ml of 0.9% saline. Room temperature was maintained constant at 24.1–24.8°C; relative humidity was 56–62%. Sweating was detected by RANDALL's[4] method; 2% tincture of iodine was applied to the skin and allowed to dry, after which a piece of heavy bond paper, already containing starch, was held firmly on the skin for an appropriate length of time, generally 20 sec. The sweat prints so obtained were in-

Effect of paraoxon (1.5×10^{-4} *M*) on axone reflex sweating[a] produced by α-lobeline

Concentration of α-lobeline (w/v)	Number of experiments			
	Total	Facilitation	Inhibition	No effect
1:50000 to 1:125000	17	6 (1)[b]	7 (3)[c]	0
1:500000 to 1:1750000	19	7	8 (1)	3
1:2000000 to 1:2500000	34	24 (3)	3	4

[a] In 6 experiments, the sweating produced by α-lobeline was apparently local.
[b] Numbers in parentheses are experiments whose results were doubtful.
[c] Includes 2 experiments using 1.5×10^{-3} *M* paraoxon.



Effect of intradermal paraoxon (1.5×10^{-4} *M*) in facilitating the production of axone reflex sweating by a low concentration of α-lobeline and inhibiting that of a high concentration. In each case, sweat prints were taken for the interval 20–40 sec after injection of α-lobeline.

[1] M. WADA, in *Essential Problems in Climatic Physiology* (Nankodo Ltd. Co., Tokyo 1960), p. 185.
[2] Y. KUNO, *Human Perspiration* (Charles C. Thomas, Springfield 1956), p. 291.
[3] J. T. MCLAUGHLIN and R. R. SONNENSCHEIN, Acta pharmacol. toxicol. *17*, 7 (1960).
[4] W. C. RANDALL, J. clin. Invest. *25*, 761 (1946).