of the pellet (Figure 1). The average diameter of the vesicles is between 750 and 800 Å. A higher magnification of the vesicles is shown in Figure 2. It demonstrates their single unit membranes about 70 Å in width (arrowheads) and the fine granular matrix in most of the vesicles.

The pure upper layer merges into a less pure zone amounting to about 70% of the pellet depth. It contains large numbers of vesicles mixed with a few small mitochondria and other membrane profiles. Only in the bottom zone amounting to 5–10% of the pellet are the vesicles relatively sparsely distributed compared with the contaminants. Of the vesicles present here, many have a larger range in diameter, 1000–2000 Å. The bottom zone usually appears compressed and is composed of amorphous material scattered between mitochondria, of which many are empty or broken. Also numerous large empty membrane profiles are present and a few Golgi membranes may be identified.

Various sized dense osmiophilic granules can be seen within some of the vesicle matrices and in areas between the vesicles. The most prominent dark granules are about 200 Å in diameter (Figure 1, fine arrows). Smaller granules of 30–60 Å diameter are also common in many vesicles. The 200 Å granules often cluster together and give the appearance of a single large black granule or 'core' (Figures 1 and 2, thick arrows). Identical dark granules occur free, both singly and in various sized clusters. In general, the clusters are distributed according to size, the larger occur nearer to the bottom of the pellet. The free granules are believed to originate from ruptured vesicles. It is not uncommon to find discontinuities in vesicle membranes and their granular content emerging. We interpret the dark granules to represent polymerized and/or contracted vesicle matrix material, which probably results from loss of membrane integrity leading to degenerative changes in the vesicle and to the strong osmiophilic reaction. The electron density of the granules is not dependent on uranyl acetate or lead citrate staining, as it is obvious also in unstained material.

On the basis of electron microscopic examination, we feel that the original estimate of about 20% purity based on biochemical data is probably too low and conservatively could be doubled. A more comprehensive study of vesicle appearance after various treatments is in progress.

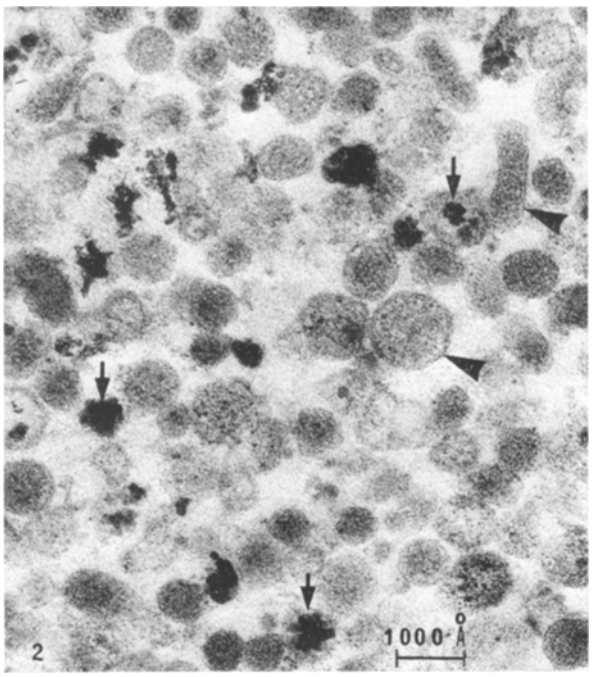

Fig. 2. Same as in Figure 1. Arrowheads indicate 70 Å unit membranes of the NA storage vesicles. Thick arrows indicate clusters of 200 Å granules giving appearance of a dense 'core'. × 100,000.

Résumé. Les vésicules de la NA du nerf splénique de Bœuf ont été obtenues par gradient de centrifugation («sucrose-heavy water»). Le rapport NA/protéine observé est de 4 à 7 fois plus élevé que celui qui a été mentionné précédemment. L'examen préliminaire de cette fraction, par microscopie électronique, révèle une couche importante et pratiquement pure de vésicules à la surface du sédiment.

Å. Thureson-Klein[8], R. L. Klein[8,9], H. Lagercrantz and L. Stjärne[10]

Department of Physiology I, Karolinska Institute, Stockholm (Sweden), 23 April 1970.

[1] H. Lagercrantz, R. L. Klein and L. Stjärne, Life Sci. *9*, 639 (1970).
[2] U. S. von Euler, Acta physiol. scand. *43*, 155 (1958).
[3] R. H. Roth, L. Stjärne, F. Bloom and N. J. Giarman, J. Pharm. exp. Ther, *162*, 207 (1968).
[4] H. J. Schümann, K. Schmidt and A. Philippu, Life Sci. *5*, 1809 (1966).
[5] A. Burger, A. Philippu and H. J. Schümann, Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak. *262*, 208 (1969)
[6] H. Hörtnagel, Heide Hörtnagel and H. Winkler, J. Physiol., Lond. *205*, 103 (1969).
[7] U. S. von Euler and F. Lishajko, Acta physiol. scand. *59*, 454 (1963).
[8] Permanent address and reprint requests: Dept. Pharmacology and Toxicology, University Mississippi Medical Center, Jackson (Ms. 39216, USA).
[9] Supported by Research Career Program Award No. 5-K03-HE-0592 from the Nat. Heart and Lung Inst. and Research Grant No. 5-R01-GM15490 from the Nat. Inst. Gen. Med. Sci., U.S.P.H.S.
[10] Supported by the Swedish Medical Research Council No. K70-14X-2479-03A and Knut and Alice Wallenbergs Foundation.

Heterogeneity in the Fibre Composition in the Flight Muscles of *Periplaneta americana* and *Belostoma* sp.[1]

The structural and functional significance of heterogeneity in the fibre composition of vertebrate muscles is well known[2]. Heterogeneity in fibre composition in insects was first observed by Bhat[3] in the flight muscles of the dragonfly *Pantala flavescens*. Recently Kallapur[4] has also reported the prescence of some specilized large fibres in the leg muscles of 2 species of cockroaches, *Blatella germanica* and *Periplaneta australasiae*. He further reported that neither the leg nor the flight muscles of *Periplaneta americana*, *Cybister confusus*, *Ranatra elongata*, *Atractomorpha crenulata* and *Cyrtacanthacris ranacea* showed such specilized fibres. This communication

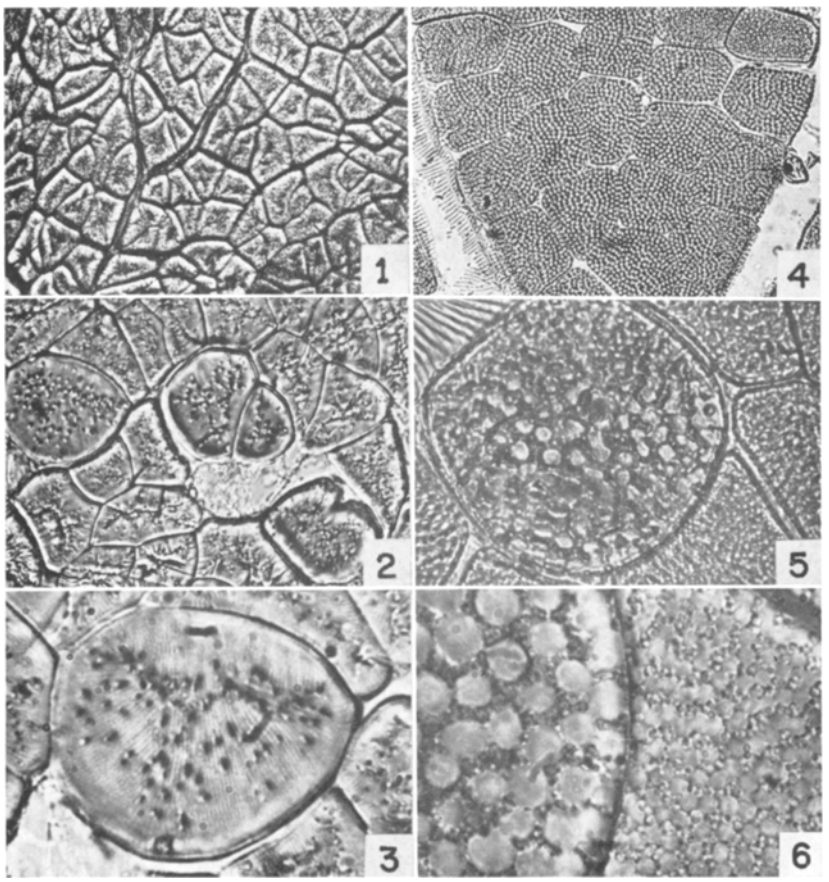

Fig. 1–3. Cross section of flight muscles of *Periplaneta americana*, stained with Sudan black B. 1. Normal fibres. × 100. 2 and 3. Large fibres surrounded by normal fibres. × 450 and × 1000.

Fig. 4–6. Cross section of mesothoracic dorsal longitudinal flight muscle of *Belostoma* sp. stained with Sudan black B.

Fig. 4. Normal fibres. × 100.

Fig. 5. A large fibre surrounded by normal fibres. × 450.

Fig. 6. A part of normal and large fibres showing the comparative size of their fibrils. × 1000.

presents observations on the structure of flight muscle fibres of *Periplaneta americana* (Dictyoptera: Blattidae) and *Belostoma* sp. (Hemiptera: Belostomidae), showing heterogeneity in their fibre composition.

The insects used in the present study were collected alive from the field. The thorax was first separated from the head and the abdomen, and bisected under cold (4 °C) insect saline. It was then fixed in cold (4 °C) Baker's formal-calcium for 1 h, embedded in gelatin and sectioned on a freezing microtome. The sections, which were 15 to 20 μ thick were stained with Sudan black B for further microscopical studies.

In *Periplaneta americana* the general organization of the fibres in the flight muscles was observed to be the same as described by TIEGS[5] (Figure 1). In a cross section, the diameter of most of the fibres ranged from 20 to 30 μ. There were, however, some muscle fibres which were significantly larger than the rest (Figures 2 and 3). These ranged from 40 to 85 μ in diameter, with a circular or avoid outline, and were usually found towards the periphery of the muscle bundle, either singly or in groups of 2 to 4. These large fibres had a few small Sudan black B positive bodies scattered in between the fibrils. The central core of sarcoplasm in these larger fibres was reduced or absent (Figure 3). Such large fibres were observed in many of the thoracic flight muscles.

The general organization of fibres and fibrils in the flight muscles of *Belostoma* sp. was observed to be similar to that of other Hemiptera described by TIEGS[5] (Figure 4). In a cross section these fibres measured from 40 to 70 μ in diameter and their fibrils were less than 1 μ thick. Some of the fibres in the mesothoracic dorsal longitudinal flight muscle were much larger than the rest (Figures 5 and 6). These were generally found towards the periphery of the muscle bundle, and appeared circular or avoid in a cross section. They measured from 100 to 155 μ in diameter with their fibrils almost 3 μ thick (Figure 6). These large fibres had comparatively larger and darker mitochondria packed in between the fibrils.

The larger fibres observed in the flight muscles of the dragonfly *Pantala flavescens*[3] stained much lighter with Sudan black B than the other fibres, indicating lesser fat content in them, whereas, the larger specialized fibres observed in the leg muscles of two species of cockroaches[4], *Blatella germanica* and *Periplaneta australasiae* were more sudanophilic than the other fibres, showing higher lipid content in them. In the present study, on the flight muscles of *Periplaneta americana* and *Belostoma* sp. the larger fibres did not show any difference in the intensity of staining with Sudan black B in comparison with other fibres, indicating no striking difference in their fat content. It is interesting to note that these larger muscle fibres observed in different insects differ markedly in

[1] The work was carried out during the year 1965/66 when the author was the recipient of a U.G.C. scholarship under Dr. J. C. GEORGE.

[2] J. C. GEORGE and A. J. BERGER, *Avian Myology* (Academic Press, New York 1966).

[3] U. K. M. BHAT, Curr. Sci. *37*, 207 (1968).

[4] V. L. KALLAPUR, Curr. Sci. *38*, 245 (1969).

[5] O. W. TIEGS, Phil. Trans. R. Soc. *238 B*, 221 (1955).

their lipid content. BHAT[6] recently reported that in the flight muscles of the dragonfly *Brachythemis contaminata* fat was confined mostly in the fibres present towards the centre of the muscle bundle, whereas glycogen was found to be concentrated in the peripheral fibres of the same muscle bundle. The functional significance of this variation in the size and the content of metabolites in the muscle fibres of these insects is not yet clearly understood. In fact, very little information is available on the metabolism in these different types of muscle fibres. Further experimental studies would be necessary before drawing any definite conclusions in this regard.

Zusammenfassung. In Flugmuskeln von *Belostoma* sp. und von *Periplaneta americana* kommen neben den normalen Fasern auch abnorm grosse Fasern vor. Diese Riesenfasern zeigen gegenüber Sudanschwarz B das gleiche färberische Verhalten wie die Normalfasern.

U. K. M. BHAT[7]

Department of Zoology, M.S. University, Baroda (India), 3 March 1970.

[6] U. K. M. BHAT, Ann. ent. Soc. Am. *61*, 1033 (1968).
[7] Present address: Virus Research Centre, 20A, Wellesley Road, Poona (India).

The Effect of Experimental Removal of the Tensor Tympani Muscle on the Ipsi- and Contralateral Mesencephalic Nuclei of the Trigeminal Nerve in the Albino Rat

Silver impregnation reveals pseudounipolar (T-shaped process) cells along the nerve serving the tensor tympani muscle in *Epimys norvegicus var. albina* (Erxl.) and *Erinaceus europaeus* (L.)[1]. The observation of annular and spiral free nerve endings on the perimeter of some muscle fibres in these two species, and the absence of typical neuromuscular spindles[1,2], have suggested that these cells are proprioceptive in function.

The site of these sensory neurons is atypical, and it may be that they are Gasserian ganglion cells that have migrated during development[1]. Yet the well-known proprioceptive nature of the mesencephalic nucleus of the trigeminus makes it equally likely that this may be the site from which such migration began; cell migration from this nucleus both within and outside the brain stem has been reported by ALLEN[3], WEINBERG[4], and others.

On the other hand, the fact that tensor tympani nerve cell numbers may vary in different individuals of the same species suggests that nerve cells having the same peripheral distribution territory are able to maintain their original central position. This would agree with the data concerning ipsilateral trigeminus mesencephalic nucleus cell chromatolysis observed by WILLEMS[5] following tensor tympani nerve section in the rabbit.

The aim of the present research was to discover whether removal of the rat tensor tympani muscle was followed by secondary effects on the mesencephalic nucleus of the trigeminus. It was considered that the observation of such effects would indicate that this muscle possesses sensory innervation, and that its internal neuromuscular devices can be classed as proprioceptive.

30 adult albino Sprague-Dawley rats (*Epimys norv., var. albina* Erxl.) with disease-free ears were employed. Following narcosis with ether, the left tensor tympani was resected. Access to the middle ear was obtained via an opening in the lower wall of the 'bulla tympani'; the muscles in the operative area were left intact. The pons and mesencephalon were removed on the 14th to 17th day and fixed in Bouin's fluid or mercuric chloride. After embedding in paraffin, serial sections were taken and stained with an 0.5% aqueous solution of toluidine blue. The pons and mesencephalon of normal control animals were fixed in 12% neutral formalin and treated with Bielschowsky-Boeke's nervous tissue silver impregnation method. This showed that the mesencephalic nucleus was in fact formed of pseudounipolar cells (Figure 1).

3–5 cells in certain total chromatolysis (Figure 2, a) were observed in ipsilateral mesencephalic nucleus sections from 20% of the series, usually in the caudal half of the cell column. These cells had a size range of 46×28 to 28×20 µ. The same subjects also presented chromatolytic cells in the contralateral nucleus (Figure 2, b), though here cell numbers were constantly less (2 per animal). Site and size data, however, were unchanged.

These results indicate that sensory innervation of the rat tensor tympani muscle may be dependent on neurons situated in the mesencephalic nucleus of the trigeminal nerve. The low percent of nuclear reactions observed in our series may be attributable to the fact that tensor

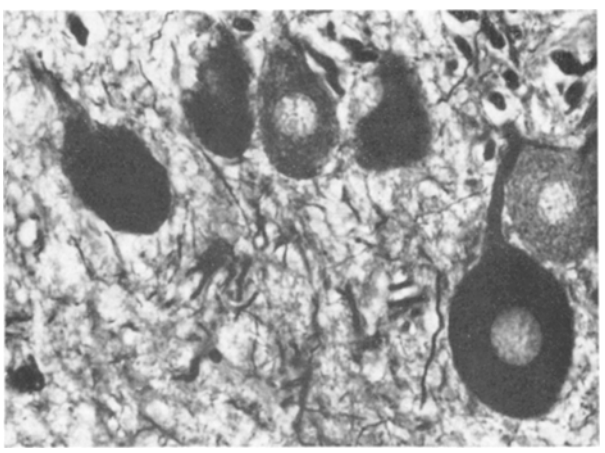

Fig. 1. *Epimys norv. var. albina* (Erxl.). Control animal. Mesencephalic nucleus of the trigeminal nerve: oval cell body with regular border due to absence of dendrites; note that only one process is extended (pseudounipolarity). Silver impregnation according to Bielschowsky-Boeke. × 600.

[1] L. CANDIOLLO and R. GUGLIELMONE, Anat. Anz. *125*, 161 (1969).
[2] L. CANDIOLLO, Z. Zellforsch. mikrosk. Anat. *67*, 34 (1965).
[3] W. F. ALLEN, J. comp. Neurol. *38*, 349 (1925).
[4] E. WEINBERG, J. comp. Neurol. *46*, 249 (1928).
[5] E. WILLEMS, Névraxe *12*, 1 (1911).