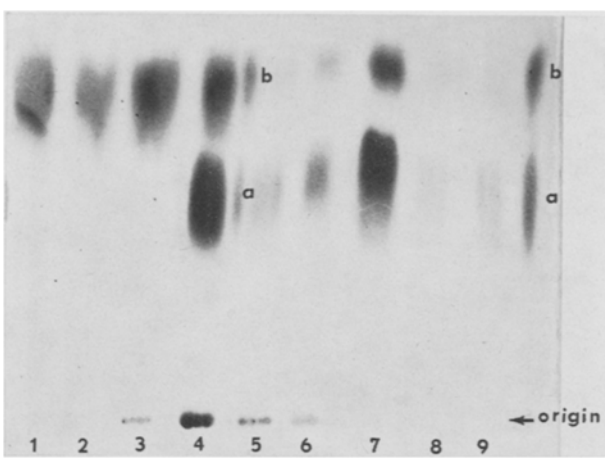
the aqueous phase, evaporated under vacuum and the excess lipid (non complexed) was separated washing 3 times with ethanol-diethyl ether (3:1 by volume). The insoluble complex was dissolved in concentrated formic acid and partitioned between the organic and aqueous layers of chloroform-methanol 1 *N* HCl (200:100:75 by volume) as described elsewhere[5]. Samples of the non complexed lipid, complex dissolved in chloroform and partition lipids remaining in the organic layer of the partitioned complex were analyzed by thin-layer chromatography on basic plates of silica gel without calcium sulfate binder, using chloroform-methanol-glacial acetic acid-water (50:25:7:3 by volume) as solvent[6]. Pure standards of PS and PE were chromatographed on the same plate. The spots were located, using ninhydrin reagent (Figure). The analysis indicates that practically the total PS and part of the PE are present in the tumor as phospholipid-calcium phosphate complex (Figure, 4, 5 6). These complexes show a non-migrating moiety which disappears by partition (Figure, 7, 8 and 9). Phosphorus was determined in the partition aqueous layer (inorganic phosphate) as well as in the organic partition layer and non complexed extract after hydrolysis with $HCLO_4$ by the method of MARTIN and DOTY[7]. Calcium was determined in the aqueous partition layer according to SOBEL and HANOK[8]. For Ehrlich ascites the molar ratio found was 64:8:15:15 (*P* as non complexed phospholipid: *P* as complexed phospholipid: *P* as inorganic phosphate: Ca).

Since it has been postulated that phospholipids may be involved in calcification at sites of primary mineralization[5], the importance of these experimental results in the interpretation of the tumor calcification process and of the carcinogenesis problem itself is clear when approached from the membrane phenomena point of view[9].



Thin-layer chromatography and ninhydrin detection of: uncomplexed phospholipids (1,2,3); complexed phospholipids (4,5,6) and partitioned complex (7,8,9); Ehrlich ascites: 1, 4 and 7; lymphatic leukemia BW5147: 2, 5 and 8; lymphosarcoma 6C3HED: 3, 6 and 9. a) Standard of phosphatidyl serine. b) Standard of phosphatidyl ethanolamine.

Zusammenfassung. Die chromatographische Trennung und chemische Analyse der Phospholipide von Versuchstumoren zeigte, dass die grösste Menge von Phosphatidylserin und ein Teil des Phosphatidylaethanolamin mit Kalzium und Phosphat-Ion komplexiert sind. Dieses Ergebnis beweist die mögliche Bedeutung dieser Komplexe in der Kalzifikation des Tumors und in den Eigenschaften der Zellenmembrane.

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Distribution of Particulate Elements at the Axolemma of Preterminal and Terminal Vegetative Nerve Fibers as Revealed by Freeze-Etching

Freeze-etching has proved to be a useful tool for studying the surfaces of biological membranes. Most of the membranes investigated by this method showed surfaces carrying particles of different means and amounts. It has been suggested that these particles may represent globular proteins located in the internum of the biomembranes[1-3]. The application of freeze-etching to nervous tissues has proved to be successful when studies of the synaptic region of the CNS were carried out[4-6]. No data are available about membrane faces of the peripheral autonomic tissue, especially of the terminal 'efficient part'[10]. Previous investigations of the myelin of peripheral nerves demonstrated a rather fine granulation of the single lamella at its exterior surface, which is explained by the metabolic inactivity of the myelin[7,8]. The present study was intended to clarify whether there is a significant change of the axolemma of autonomic nerves in their course from preterminal to terminal regions.

Ductus deferens obtained from adult white rats were dissected immediately after preparation and treated with phosphate buffer at pH 7.4, containing 25% glycerol. After having been frozen in liquid nitrogen, the specimens were freeze-etched and replicated in a Balzers high vacuum unit (BA 360 M). Measurements of the replicas were made by a quarz crystal thin film oscillator. The thickness of the replicas varied between 20–30 Å for Pt/C and 200–300 Å for carbon alone. All the specimens studied were taken from unfixed tissue. The fracture exposes the intracellular faces of the axolemma of preterminal autonomic nerves and of the cytolemm of the Schwann cell. The axolemma of the preterminal autonomic nerves does not differ very much from the neurilemm of myelinated nerves. Particles

are sparcely distributed over the whole membrane which itself has a smooth appearance (Figure 1).

There was no difference in the distribution and amount of particles between the interior and exterior surface of the preterminal axolemma. This statement is of particular interest because most of the cell membranes are known to show dissimilar numbers of membrane-associated particles at their intra- and extraplasmatic faces. In this respect the preterminal axolemma seems to be an exception. Furthermore it is worthwhile to note that the inner aspect of the cytolemm of the Schwann cell does not show the 'humped' projections as was described for the myelin sheet, whereas the surface facing the interstitial space shows an increase of particulate elements in comparison to the inner aspect of the Schwann cell cytolemm. The axoplasma of the preterminal vegetative axons appears

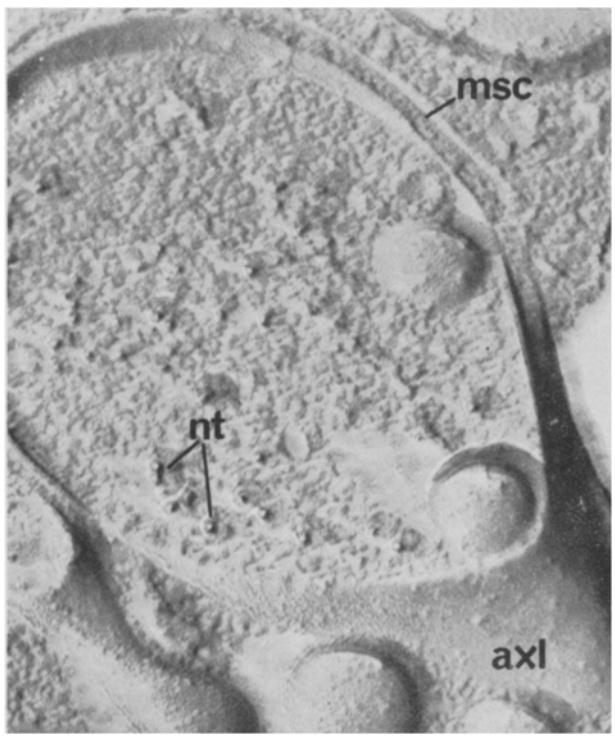

Fig. 1. Preterminal axon. Exterior surface of the axolemma shows randomly distributed particles. nt, neurotubuli; axl, axolemma; msc, mesaxonal cleft; sc, Schwann cell. ×153,000.

Fig. 2. Exterior surface of a terminal axolemma facing the interstitial space. Aggregations of particles lie in flat impressions of the axolemma. Protrusion of the axolemma (→). axl, axolemma; im, impression. ×140,000.

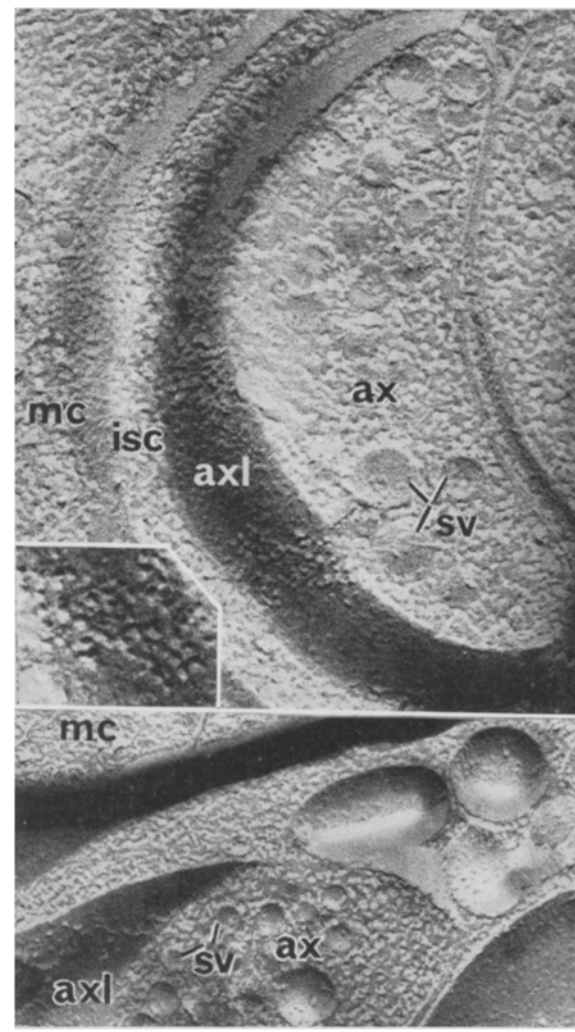

Fig. 3. Neuromuscular junctions of terminal vegetative axons. a) plaque-like aggregations of particles at the exterior face of the 'presynaptic membrane'. ×155,000. Inset: Circular arrangement of particles at higher magnification. ×248,000. b), Axoplasmatic aspect of the axolemma, that is in close relation with a smooth muscle cell. ax, axon; axl, axolemma; sv, synaptic vesicles; isc, intersynaptic cleft; mc, smooth muscle cell. ×93,000.

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undifferentiated as it is known from conventional electron micrographs. It appears granular containing a few mitochondria and profiles of vesicles. Neurotubuli when broken crosswise showed a central lumen only in a few cases.

Terminal axons are frequently found by ultra thin sections. In freeze-etched replicas it is rather difficult to identify vegetative axons because of the absence of the characteristic envelope by the SCHWANN cell and the resulting similarity to muscle cell processes. The range of diameter of the vesicles varied between 320–1100 Å in the unfixed specimen, including clear vesicles as well as granular ones. The data coincide with the measurements obtained by conventional electron micrographs and confirm the observation made in the CNS by the use of the freeze-etching method. The terminal axolemma when exposed to the interstitium showed an increase of particulate elements at its exterior surface sometimes congregated in a rosette-like arrangement and lying in flat impressions of the membrane (Figure 2a).

In some cases we were able to obtain synaptic contacts of unmyelinated axons and smooth muscle cells. The striking observation in these 'active regions' are particles arranged in plaques at the exterior face of the presynaptic membrane (Figures 2, 3a and b). Some of these membrane-associated particles were just as grouped in the rosette-like formation as mentioned above (Inset). A similar distribution of the particles could be found at the interior presynaptic face, but they do not give the impression of such a circumscribed localisation as occured at the exterior one (Figure 3b).

Considerable evidence indicates that these areas may represent the 'efficient zones' of the peripheral autonomic axons. The particulate elements may probably represent active protein complexes involved in the releasing mechanism of the transmitter substances. The terminal axolemma occurs in cross fractures as well as in the 'en face' aspect rather flat without irregularities like invaginations or undulations that are common in conventional electron micrographs. Sometimes, however, when the axolemma was facing the interstitial space we found it bulged as if protruding from an underlying vesicle (Figure 2, →). Synaptopore-like formations that were found in synapses of the CNS and believed to be an essential feature of the presynaptic region were not definitely identified[9].

Evidently, a detailed study with special reference to this problem should be carried out before datas of the synaptic region of the CNS and the peripheral autonomic nerves can be successfully compared.

Zusammenfassung. Erstmals werden mit Hilfe der Gefrierätzung die Synapsen im Ductus deferens dargestellt, und es kamen dabei partikuläre Auflagerungen auf das Axolemm erstmals zur Darstellung.

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Activité Cholinestérasique dans le rein du rat

Une activité butyrylcholinestérasique a pu être localisée dans la paroi des vaisseaux cérébraux chez le rat (DELBARRE et al[1]). Dans un travail récent, CONSTANTINIDIS et al.[2] ont montré que la barrière capillaire pour la DOPA était identique dans le cerveau et dans le rein. Il nous a donc paru intéressant de rechercher l'existence d'une activité cholinestérasique dans le rein du rat.

Matériel et méthode. Nous avons utilisé des rats de souche Charles-River dont le poids variait entre 200 et 400 g. Nos expériences ont porté sur 26 rats. L'animal est anesthésié avec du penthobarbital à la dose de 40 mg/kg par voie i.p. Par voie intracardiaque, on perfuse une solution isotonique de $Na_2\ SO_4$ (16,7 g/l) suivie d'une solution isotonique de $Na_2\ SO_4$ contenant du formol à 10% (30% p/v).

Le rein est alors rapidement prélevé et placé dans une solution isotonique de $Na_2\ SO_4$-formol 10% pendant 4 h, à la température de 4 °C. Le rein est ensuite lavé à l'eau distillée puis déposé dans une solution d'éthanol à 20% pendant au moins 24 h. La méthode de mise en évidence des cholinestérases est celle de KOELLE-FRIEDENWALD[3]. L'incubation est de 2 heures à 37°, pH 5,5. Les substances que nous avons utilisées sont les suivantes: Comme substrats: l'acétylthiocholine-iodide (Calbiochem) qui met en évidence l'activité cholinestérasique totale: acétylcholinestérases et butyrylcholinestérases. La butyrylthiocholine-iodide (Calbiochem) qui révèle spécifiquement l'activité des butyrylcholinestérases. Comme inhibiteurs: l'iso-OMPA: tetra-isopropyl pyrophosramide (Koch-Light Laboratories) qui inhibe spécifiquement les butyrylcholinestérases. Le BW 284 C 51:1,5 *bis* (4 alkyl diméthyl ammonium pentan-3-one-dibromide) (Welcome Research Laboratories) qui inhibe spécifiquement les acétylcholinestérases.

Avec les inhibiteurs, une période de préincubation d'une demi-heure est réalisée, le pH étant ajusté à 5,5.

Nous avons réalisé les protocoles suivants: 1. acétylthiocholine; 2. butyrylthiocholine; 3. acétylthiocholine + iso-OMPA; 4. butyrylthiocholine + iso-OMPA; 5. acétylthiocholine + BW 284 C 51; 6. butyrylthiocholine + BW 284 C 51.

Résultats. Nous n'avons trouvé aucune activité acétylcholinestérasique au niveau du parenchyme rénal. La réaction à l'acétylthiocholine, révélatrice de l'activité des acétylcholinestérase et ces butyrylcholinestérases est positive dans la paroi de certains vaisseaux, mais elle est négative en présence d'iso-OMPA, inhibiteur spécifique des butyrylcholinestérases et par contre, elle reste entière-

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