2 control groups, an interesting fact was noted: in the hearts perfused with an insulin-containing medium, in agreement with the functional findings, no damage to the muscle cells or enlargement of the sarcoplasmatic reticulum (T-tubuli) was found as seen in long-time Langendorff-preparations[15].

Our findings may be interpreted thus: The formation of cardiac edema is intensified by the ligation of lymph vessels in the isolated working heart. Moreover, an interference with microcirculation due to edema, swelling of the endothelial cells and closed or collapsed blood capillaries is increased by lymphostasis. Therefore, the irreversible damage to the myocardium is not only the result of the lymphedema but also due to ischemia, according to other investigators[16].

The reduced lymph flow causes an impairment of metabolism because oxygen transport, substrate utilization, and removal of metabolites may be hindered. If insulin is added to the medium, the substrate utilization is considerably improved, so that the disturbance of microcirculation is compensated for a certain period of time. Recent investigations[17-20] have shown that glucose and insulin have a protective effect on hypoxically induced injury of the heart. Whether a similarly favourable effect occurs in lymphostasis will have to be shown in further experiments.

Zusammenfassung. Die experimentelle Lymphstauung bewirkt am isolierten, arbeitenden Rattenherzen trotz konstantem Koronarfluss eine progressive Abnahme der Auswurfleistung des linken Ventrikels, die morphologisch unter anderem mit einem interstitiellen und intrazellulären Ödem, insbesondere der Herzmuskelzellen und Kapillarendothelien sowie mit partiellen Herzmuskelzellnekrosen einhergeht.

H. Guski, P. Buntrock, H. Braselmann and I. Marx[21]

Pathologisches Institut des Bereichs Medizin, Rudolf-Virchow-Haus der Charité, Humboldt-Universität Berlin, Schumannstrasse 20/21, 104 Berlin (German Democratic Republic, DDR), 24 June 1974.

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Degeneration in the Adult Rat Spinal Cord Following Systemic Treatment with 6-Hydroxydopamine. Electron Microscopic Study

While there is now considerable evidence at the ultrastructural level to show that the systemic administration of 6-hydroxydopamine (6-OHDA) in adult animals causes degeneration of peripheral noradrenergic (NA) nerve terminals[1-9], the effect of the drug via this route on the central NA systems would appear to be much more severe if the animals treated were neonates than if they were adults[10-15]. This has been attributed to the greater permeability of 6-OHDA across the blood-brain barrier in the former than in the latter (see Thoenen and Tranzer[16] for discussion). On the other hand, in adult animals, when appropriate doses of the drug were injected either directly, intracisternally or intraventricularly, in several nuclei that are known to have a rich NA innervation, electron microscopy showed degeneration of nerve terminals[17-22]. This suggests that 6-OHDA can cause selective degeneration of central NA nerve terminals as well as peripheral ones. Recently, a review on the central effects of 6-OHDA suggests that in adult animals a little of the drug may cross the blood-brain barrier[23]. Whether this amount of diffusion of 6-OHDA would cause a sufficient degree of degeneration of central NA nerve terminals that can be detected ultrastructurally is not known. The present paper describes an electron microscopic study of this problem in the intermediolateral column (ILC) of the adult rat spinal cord, a nucleus that is known from fluorescence histochemical studies to be richly innervated by NA nerve terminals[24].

Adult albino rats weighing between 250–300 g were given a single i.v. injection through the saphenous vein of 100 mg/kg 6-OHDA HCl (25 mg/ml 6-OHDA HCl dissolved in a solution containing 1 mg/ml ascorbic acid). Control rats were injected i.v. with an equivalent volume of ascorbic acid. At 24, 48 and 72 h after injection of 6-OHDA, the spinal cord was fixed by intracardiac perfusion with a solution containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 *M* cacodylate buffer containing 0.5 mg/l $CaCl_2$. The perfusion time was about 20 min. A short segment of the mid-thoracic spinal cord was then carefully dissected out and further sliced transversely into thinner segments. The latter were kept overnight in ice-cold fresh fixative, after which they were post-fixed in 1% osmium tetroxide[25], dehydrated with acetone and embedded in araldite. Semi-thin transverse sections of the spinal cord were stained with methylene blue and prepared for light microscopy. Ultra-thin transverse sections were stained with aqueous saturated uranyl acetate[26] and lead citrate[27] and examined in a Hitachi HS-8 electron microscope.

The observations were confined to the ILC of the adult rat spinal cord (Figure 1, inset). The results will be expressed in terms of those positive features that were present in the experimental material but were absent in the controls. The evaluation of the presence of degeneration was aided by reference to the paper of Glees and Hasan[28] and to previous studies on the degeneration of central NA nerve terminals following treatment with 6-OHDA[17-22].

A study of the 24 h material showed that scattered sparsely in the generally normal-looking neuropil were nerve terminals that were more electron-dense than others that were present (Figure 1). These electron-dense nerve terminals appeared collapsed with crowding of vesicles, the majority of which were of the small spherical

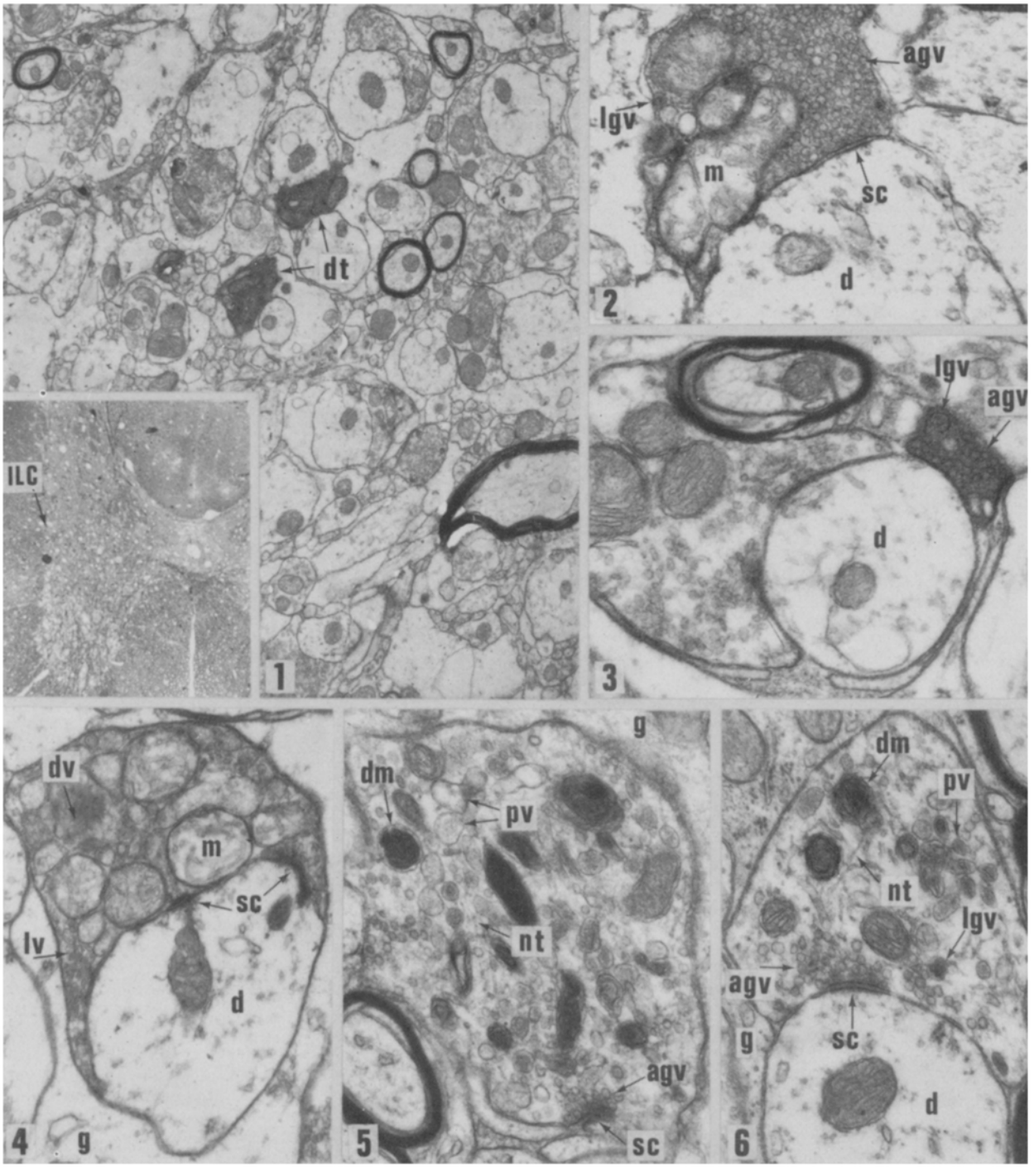

Fig. 1. A low power electron micrograph of neuropil from a treated animal (24 h, i.v. 100 mg/kg) to show 2 isolated degenerating electron dense nerve terminals (dt). ×10,500. Inset, a light photomicrograph of a semi-thin transverse section of thoracic spinal cord from a treated animal (72 h, i.v. 100 mg/kg) to show the area observed for degeneration. ILC, intermediolateral column. ×55.

Fig. 2. A degenerating electron dense terminal forming an axo-dendritic synapse from a treated animal (24 h, i.v. 100 mg/kg). Note crowding of agranular vesicles (agv). d, dendrite; lgv, large granular vesicle; m, mitochondrion; sc, synaptic contact.

Fig. 3. A degenerating electron dense nerve terminal from a treated animal (48 h i.v. 100 mg/kg). Note crowding of agranular vesicles (agv) and also the normal looking axo-dendritic synapse adjacent to it. d, dendrite; lgv, large granular vesicle. ×45,000.

Fig. 4. A nerve terminal forming an axo-dendritic synapse in an advanced state of degeneration from a treated animal (48 h i.v. 100 mg/kg). Note that some of the lucent vesicles (lv) are discoid; this is unusual. d, dendrite; dv, disintegrated vesicles; g, astroglial process; m, mitochondrion; sc, synaptic contact. ×27,500.

Fig. 5. A nerve terminal forming an axo-dendritic synapse in an advanced state of degeneration from a treated animal (72 h, i.v. 100 mg/kg). Note variety of inclusions within the nerve terminal. agv, agranular vesicle; dm, degenerating mitochondrion; g, astroglial process; nt, neurotubule; pv, pleomorphic vesicles; sc, synaptic contact. ×37,500.

Fig. 6. A nerve terminal forming an axo-dendritic synapse in an advanced state of degeneration from a treated animal (72 h, i.v. 100 mg/kg). Note variety of inclusions within the nerve terminal. agv, agranular vesicle; d, dendrite; dm, degenerating mitochondrion; g, astroglial process; lgv, large granular vesicle; nt, neurotubule; pv, pleomorphic vesicles; sc, synaptic contact. ×37.500.

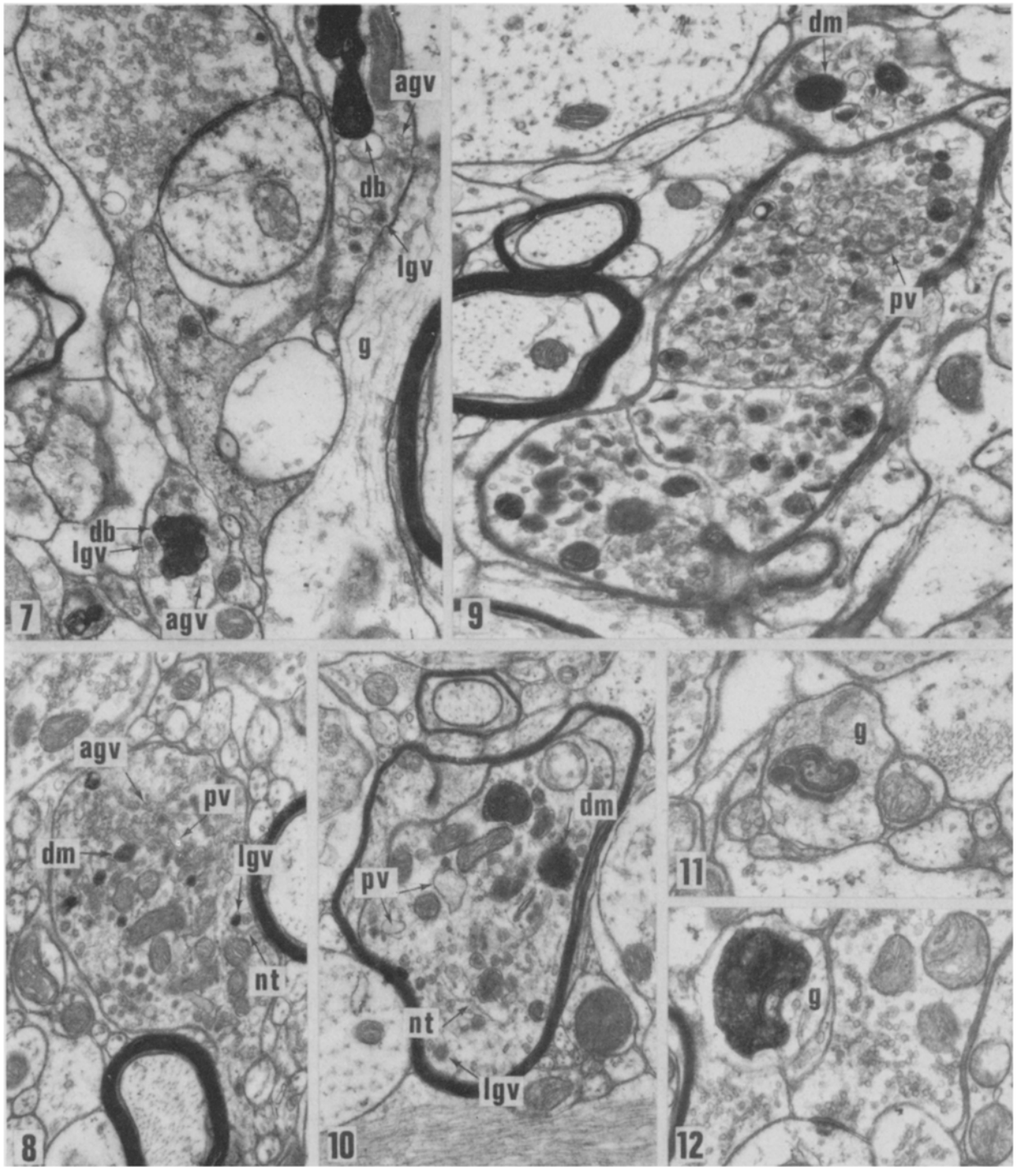

Fig. 7. Two degenerating unmyelinated axons flanking a normal looking axo-dendritic synapse from a treated animal (48 h, i.v. 100 mg/kg). agv, agranular vesicle; db, dense body; g, astroglial process; lgv, large granular vesicle. ×27,5000.

Fig. 8. A degenerating unmyelinated axon, possibly a preterminal from a treated animal (72 h, i.v. 100 mg/kg). Note variety of inclusions within the axon, agv, agranular vesicle; dm, degenerating mitochondrion; lgv, large granular vesicle; nt, neurotubule; pv, pleomorphic vesicle. ×22,000.

Fig. 9. A group of degenerating myelinated axons from a treated animal (72 h, i.v. 100 mg/kg). Note normal-looking axons adjacent to them. dm, degenerating mitochondrion; pv, pleomorphic vesicles. ×27,500.

Fig. 10. A degenerating myelinated axon from a treated animal (72 h, i.v. 100 mg/kg). Note variety of inclusions within the axon. dm, degenerating mitochondrion; lgv, large granular vesicle; nt, neurotubule; pv, pleomorphic vesicles. ×22,000.

Fig. 11. A degenerating nerve terminal completely engulfed by astroglial processes (g) from a treated animal (48 h, i.v. 100 mg/kg). ×37,500.

Fig. 12. A degenerating nerve terminal completely engulfed by astroglial processes (g) from a treated animal (72 h, i.v. 100 mg/kg). Note the normal-looking axo-dendritic synapse adjacent to it. × 27,500

agranular type (agv) with occasional large granular vesicles (Figure 2). The outline of the agv, though not distinct, could still be made out and there was no loss of vesicles in all the electron-dense nerve terminals observed. The majority of the mitochondria in these nerve terminals at this stage appeared normal, though some could be considered to be degenerating. Such electron-dense nerve terminals were rarely seen in the 48 h material (Figure 3) and not at all in the 72 h specimens. Degenerating nerve terminals encountered in these stages appeared swollen and contained within them a variety of inclusions not met with in normal nerve terminals (Figures 4–6). In all the degenerating nerve terminals studied in the 48 and 72 h specimens, at least a proportion of the mitochondria were degenerating and there was loss of agv. In some degenerating nerve terminals there were accumulations of pleomorphic vesicles of different sizes and tubular profiles that resembled smooth endoplasmic reticulum (Figures 5 and 6). Though the degenerating nerve terminals were surrounded by astroglial processes, the paramembranous structures at the synaptic sites were distinguishable. In all the stages studied so far, the degenerating nerve terminals have been observed to form only axo-dendritic synapses and they did not show any neurofilamentous accumulation.

In the 48 and 72 h specimens, not infrequently, some unmyelinated axons and possibly preterminals as well were obviously degenerating and contained within them a variety of inclusions not seen in normal axons (Figures 7 and 8). Some degenerating myelinated axons showed accumulations of neurotubular elements and a host of pleomorphic vesicles of all sizes, some of which had very large dense cores (Figures 9 and 10). Occasionally dense osmiophilic masses with some recognizable internal structure were found completely engulfed by astroglial processes (Figures 11 and 12). These were diagnosed as degenerated, shrunken and phagocytosed nerve terminals.

The results of the present study which suggest that, following systemic treatment with 6-OHDA in adult rats, there is degeneration in the ILC of the spinal cord, need to be interpreted cautiously, expecially since it has been found that, even in normal neuropil, spontaneous degeneration of nerve terminals and axons may occur[29,30]. Notwithstanding, the present findings indicate that in the experimental material the degeneration observed was sequential and progressive, and the evidence agrees with previous studies on the degeneration of central NA nerve terminals following treatment with 6-OHDA[17–22]. Moreover, such features were not observed in the control neuropil. It was therefore highly probable that the degeneration observed resulted from the treatment with 6-OHDA and that some amount of the drug may be postulated to have passed the blood-brain barrier. Our study compares with that of BERTLER et al.[31] which showed that L-Dopa, when given systemically in high doses (80 mg/kg), can cross the blood-brain barrier. Nevertheless, it would be desirable to confirm the present study by introducing the drug via a more direct route, e.g. intracisternal, and this needs to be done.

While the question of the actual distribution of NA nerve terminals in the rat ILC may not be answered with the present evidence (since it is not known if the 6-OHDA that had crossed the blood-brain barrier had acted on all or only a proportion of the NA nerve terminals that were present) yet the fact that all the degenerating nerve terminals so far observed formed only axo-dendritic synapses, suggests that these terminals may have a modulating influence on the pre-ganglionic neurons in the ILC. RÉTHELYI[32], in his study of the normal cat ILC, has suggested that NA nerve terminals may be those with a preponderance of large granular vesicles within them. We have not been able to confirm this in the degenerating nerve terminals in the rat ILC following treatment with 6-OHDA. Indeed, as far as can be ascertained, in all the degenerating nerve terminals observed, agv predominated.

The degeneration of axons following 6-OHDA treatment has been reported previously in electron microscopic[18–22] as well as light microscopic studies[33]. The appearance of these axons resembles that described in the proximal segments of experimentally constricted peripheral NA axons[34,35].

The systemic administration of 6-OHDA has the advantage in that it avoids some of the complications in interpretation that are encountered when a more direct

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route is being used[21]. This approach should prove useful in comparing, at the ultrastructural level, with the results from a recent report derived from fluorescence histochemical studies, which showed that even in the adult rat spinal cord NA nerve terminals may regenerate following previous selective destruction by 6-OHDA treatment[36].

Zusammenfassung. Bei ausgewachsenen Albinoratten gelang nach i.v. Injektion von 6-OHDA (100 mg/kg) der elektronenmikroskopische Nachweis der terminalen und axonalen Degeneration an der Intermedia lateralis des Halsmarkes. Bei genügender Dosierung konnte 6-OHDA die Gehirnblutschranke überschreiten.

W. C. Wong[37] and C. K. Tan[38]

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[38] Present address: Department of Anatomy, University College London, Gower Street, London WC1E 6BT, England.

High-Voltage Electron Microscopy of the Cat Adrenal Medulla

A prominent morphological feature of adrenal medullary cells is a large number of dense-cored vesicles. Using conventional electron microscopy, most vesicles appear spherical with an osmiophilic core separated from an outer membrane by an electron-translucent halo. Although some of the vesicles appear irregular in shape[1], little significance has been attached to these. Generally, it is thought that the vesicles are individual organelles and that each is derived by 'budding' from the Golgi complex before traversing the cytoplasm and releasing their contents by exocytosis[2,3]. It has been demonstrated biochemically that these vesicles contain catecholamines, ATP, and soluble proteins[4,5].

The cells of the cat adrenal medulla have been examined employing high-voltage electron microscopy in a study designed to reinvestigate the relationship of catecholamine-containing vesicles to the cellular membrane systems. The examination of ultrastructure in relatively thick (0.5–2.0 μm) specimens allows for stereoscopic viewing after pairs of micrographs are taken at appropriate stage tilt angles. Thus, three-dimensional analysis of cell organelles is possible and more information about the relationships of cellular constituents is gained.

Cat adrenals were perfused in situ with a dilute aldehyde solution as described by Smith and VanOrden[3]. Fixation was continued in osmium tetroxide and the adrenal medulla was embedded in Epon. Representative sections (0.5 to 2.0 μm in thickness) from 4 animals were mounted on copper grids coated with Formvar and carbon, and stained with uranyl acetate and lead citrate for 30 min to 2 h. Specimens were examined at 1000 KV with the electron microscope at the United States Steel Research Laboratory in Monroeville, Pennsylvania. Stereo-pair micrographs were taken at 10,500 × magnification at tilt angles from 6° to 36°, and were analyzed using a lens-mirror stereoscope.

In both types of chromaffin cells (epinephrine and norepinephrine), most of the catecholamine-containing vesicles were spherical. Each vesicle was enclosed in its own limiting membrane. Some membranes were observed to taper from the electron-dense core for a short distance

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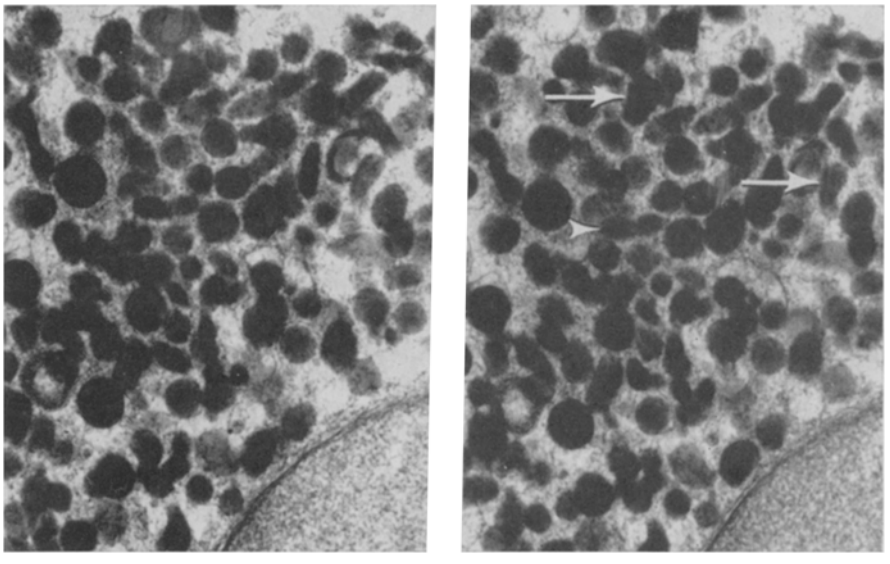

This stereo-pair micrograph illustrates tubular (arrows) and double condensation (arrowhead) catecholamine-containing vesicles. A small stereo viewer is needed to obtain a three-dimensional image. × 21,000.