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.

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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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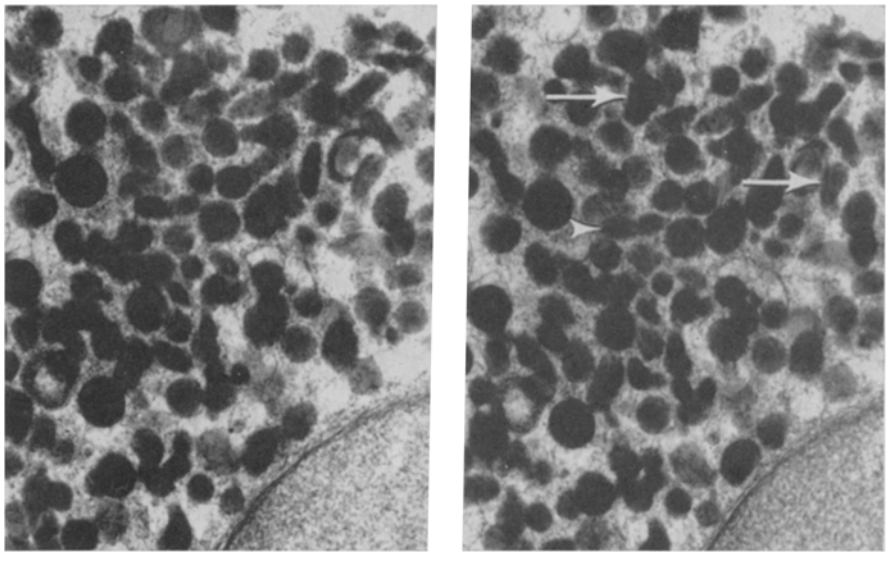

This stereo-pair micrograph illustrates tubular (arrows) and double condensation (arrowhead) catecholamine-containing vesicles. A small stereo viewer is needed to obtain a three-dimensional image. × 21,000.

but appeared to remain continuous. Of the 7,026 vesicles observed, 14.9% appeared tubular by three-dimensional analysis (Figure). The tubular forms consisted of a single membrane-bound dense core while some were individual condensations of electron-dense material surrounded by a single limiting membrane. However, the length of the tubes or their existance in a network could not be determined as they usually extended from the top or bottom surfaces of the section. The tubular forms (both the single and multiple condensation types) appeared with greater frequency near the nucleus. The two types did not appear to be interconnected by a continuous membrane nor were they seen to be connected to the Golgi complex. Spherical vesicles were occasionally seen in a linear array.

The visualization of the tubular forms of vesicles may suggest that the granular matrix which begins to form in the Golgi complex enters a network of tubules, and at the terminal aspects of the system the granules condense and become enclosed by a separate membrane. It seems likely that the vesicles observed in a linear array represent the last stage in the maturation of the spherical granules. On the other hand, it is possible that the tubular vesicles remain as separate forms and may be functionally different from the more frequently encountered spherical vesicles. Perhaps the use of other heavy metal stains will provide better resolution of the membrane systems of the adrenal medulla for three-dimensional analysis and more precise information about the development of the catecholamine-containing vesicles can be obtained.

Comparing these data with that obtained from conventional electron microscopy, it seems likely that the irregular forms of vesicles (ovoid, elongated, comma-shaped, dumbell-shaped) reported by other investigators[6–8] result from tangential sectioning through these tubular forms. In ultrathin sections a recognition of tubular forms would be difficult. Even though interconnections were not found in a freeze-etching study[9] the possibility that these tubular vesicles interconnect should remain open for further investigation.

Zusammenfassung. Nachweis, dass die catecholaminhaltigen Vesikel des Nebennierenmarkes eine sehr spezifische dreidimensionale Form haben, die sich bei der gewöhnlichen Transmissionselektronenmikroskopie nur durch Unregelmässigkeiten bemerkbar macht, jedoch mit Hilfe der Stereo-Elektronenmikroskopie mit Hochspannung sichtbar gemacht werden kann.

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Development of Enterochromaffin and Dopamine Cells in the Gastrointestinal Tract of the Calf

The gastrointestinal (G-I) tract of the cow and some other species contains numerous amine-stroing cells[1]. Enterochromaffin cells (EC) are characterized by 5-hydroxytryptamine (5-HT)[2], whereas what are called 'dopamine cells' (DC) have been shown to contain dopamine[3]. The epithelial origin of the EC has been generally accepted[4,5], whereas it is presumed that DC belong to the mast cell category[6]. It is characteristic of G-I amine-strong cells that they appear at early stages of development, whereas accumulation of amines by these cells was shown to vary in vivo and in vitro experiments[7,8]. Because there are no data concerning the appearance of DC in the G-I tract, an attempt was made to correlate the development of DC with that of other amine-containing structures, using the specific histochemical method to demonstrate monoamines.

Material and methods. 19 embryonal calves of both sexes were studied. The age of the embryos was determined on the basis of body length[9]. The embryos were obtained within about 5 min after shooting the cow and small pieces were cut immediately from the corpus and antrum area of the stomach, the oral third of the duodenum and the jejunum, the ileum terminale and the oral third of the colon. The pieces were immediately frozen in isopentan precooled with liquid nitrogen. The histochemical method to demonstrate monoamines followed the principles outlined by ERÄNKÖ[10] and FALCK et al.[11]. The specimens were freeze-dried at −40 °C in vacuo for 24 to 48 h. Then temperature of the holder in vacuo was gradually increased to above room temperature. The specimens were treated with formaldehyde vapour at 80 °C for 1 h. Paraformaldehyde was equilibrated at 60% humidity. The paraffin sections were cut perpendicularly to the axis of the intestinum and contained all intestinal layers. The number of EC and DC was determined for the duodenum. The total cells counted for each specimen varied from 150 to 250. The volume of 5 µm adjacent sections was determined by planimeter and appropriate corrections were made[12].

Results. EC were first seen in the duodenum and in the other parts of the G-I tract before the 7th embryonic week. The cells were identified by their bright, strong

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