(J = 10 c/s). The alkaloid is thus unambiguously shown to be 19-dehydroyohimbine (VI) and to represent the third member of the rare class of 19-dehydroyohimbinoid alkaloids, the other two representatives being deserpideine[14] and raujemidine[15,16].

Zusammenfassung. Ausser den bekannten Alkaloiden Ulein (I), Appariein (II), Demethylaspidospermin (III), Yohimbin (IV) und β-Yohimbin (V) wurde noch ein unbekanntes Alkaloid aus der Rinde von *Aspidosperma pyricollum* Muell.-Arg. isoliert, dessen Struktur als 19-Dehydroyohimbin (VI) chemisch sowie spektroskopisch bewiesen wurde.

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Electron Microscopic Observations in the Substantia nigra of Mouse during Reserpine Administration

Distribution of catechol amines in the brain has been demonstrated histobiochemically by many investigators[1–4]. Catechol amine-containing cells in the Substantia nigra and neostriatum (putamen and caudate nucleus) were described by HILLARP et al.[5,6]. Recently WOOD and BARNETT[7] reported the appearance of catechol amine-containing granules in the ventromedial nucleus of the hypothalamus at a fine structural level. The storage of catechol amine in cytoplasmic granules in a bound state was reported by BERTLER, HILLARP, and ROSENGREN[8]; one can regard the storage as the final step in the formation of amines, making them available for physiological need. And it can be assumed that norepinephrine, 5-hydroxy-tryptamine, and dopamine are directly formed in the brain, judging from the relative impermeability of the blood-brain barrier to these amines[9].

Since reserpine blocks the storage of catechol amines in the brain[10], the present experiment was focused on further study of effects of reserpine on granules of brain monoamines, their morphological features and distribution at a fine structural level.

Materials and method. Twenty mice, six months of age, weighing an average of 30 g, were used in the present experiment. One group of animals was treated with reserpine (sedaraupin) 0.5–1 mg/kg i.p. daily. The animals showed clear-cut symptoms of sedation. 5 mice were sacrificed after 24 h, and 5 after one week for the purpose of electron microscopy. Another group of 10 untreated mice was used as controls.

The whole brain was removed as quickly as possible from the subjects, cut transversely through the Substantia nigra, and fixed in 1% OSO_4 fixative buffered with 0.14 ml veronal acetate (pH 7.4) containing 0.9 m of sucrose per ml for 4 h. The tissue was dehydrated with graded ethanol and propylene oxide; during dehydration, regions of the Substantia nigra were harvested in small pieces under the dissecting microscope. These were then embedded in Epon 812 according to the method of LUFT[11], polymerized for several days at graded temperatures, and sectioned with a LKB microtome. Sections were stained with uranyl acetate and examined in a Siemens Elmi 1.

Observations. Polymerized blocks were reoriented to include only the region of Substantia nigra under the phase microscope. The basis pedunculi was used as a reference in identifying the cells in Substantia nigra, when 10 to 20 μ sections were examined under the phase microscope.

Polygonal-shaped nigra cells ranging from 11 to 15 μ showed small ovoidal mitochondria distributed throughout the cytoplasm.

There were numerous vesicles with electron dense core and less dense periphery ranging from 0.1 to 0.3 μ in diameter at the periphery of cytoplasm (Figure 1), in the axoplasm and axonterminals (Figure 2). In control animals, not all the cells contain such granular vesicles in cytoplasm, which may indicate the existence of functionally different cells or the active metabolic use of amine-containing granules.

In addition to these granular vesicles, there were clear vesicles ranging from 0.08 to 0.1 μ in diameter seen evenly distributed in the axonterminals (Figure 3).

There were evenly distributed polysomes, and the prominent nucleus had an indentation in which presumably the cytoplasmic centre lies. The structure of nucleolus in these cells showed the characteristic presence of several vacuoles.

A notable aspect in these cells is the appearance of an oval osmiophilic body without membrane in the cyto-

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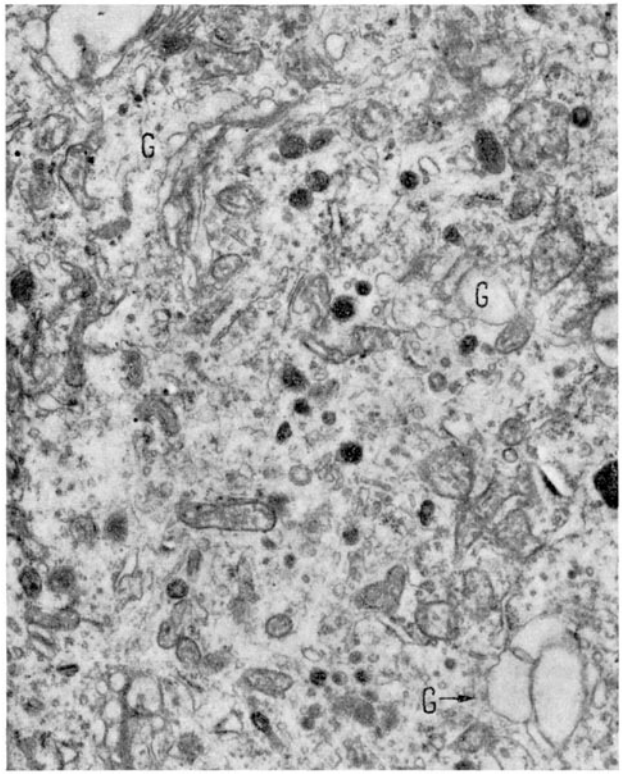

Fig. 1. A low-power micrograph showing peripheral portion of the cytoplasm of nigra cell containing numerous granular vesicles with electron dense core and less dense periphery. These variously dense granular vesicles are crowded at the Golgi apparatus (G), suggesting that these granules may be developed at the Golgi zone. × 12,500.

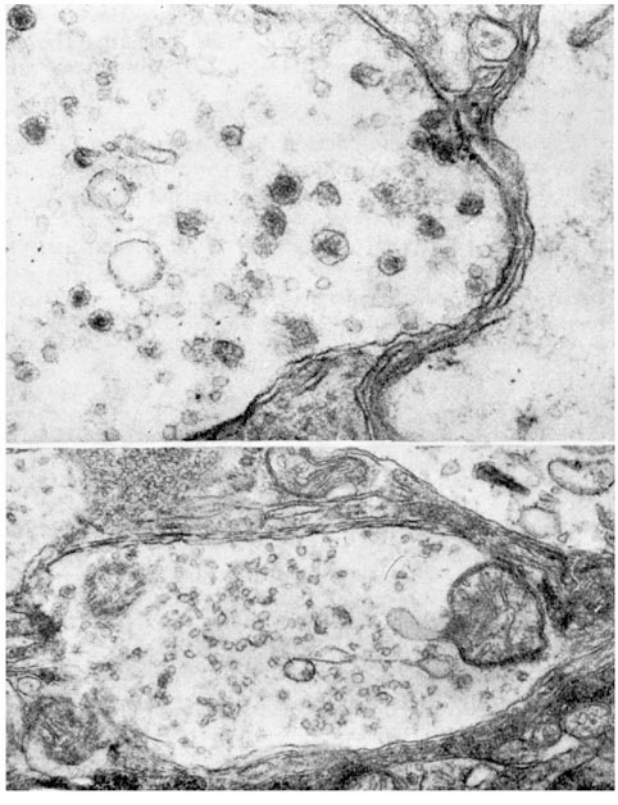

Fig. 2. Above: A highly magnified micrograph showing the axonterminal in the Substantia nigra which are filled with catechol amine granules. The density and size of the granules are uniform compared to those granules shown in Figure 1. × 48,000. Below: Another type of axonterminal in Substantia nigra which are filled with clear vesicles. × 40,00.

plasm, ranging from 0.7 to 2.5 μ in diameter. Some polysomes are in contact with the osmiophilic bodies. The latter appear to be finely granular or filament-like in structure and differ from lysosomal structures in their size.

The most dramatic alteration seen in the cytoplasm of nigra cells following reserpine treatment is in the disappearance of the granular vesicles and the osmiophilic membraneless bodies, whereas lysosomal granules are still present. Additionally, the normally poorly developed endoplasmic reticulum becomes swollen in shape and randomly distributed throughout the cytoplasm. This can be interpreted as resulting from depletion of amines in the cells.

Otherwise, the cytoplasm seems to be without granular vesicles other than the numerous free polysomes, ribosomes, and a few mitochondria which are slightly changed by loss of the mitochondrial matrix. In axoplasm there is hardly any evidence of granular vesicles, which are obvious in control cells.

Discussion. The results described here indicate that catechol amines are present in polygonal-shaped nigra cells in the form of granular vesicles, the fine structures of which are similar to the norepinephrine granules observed

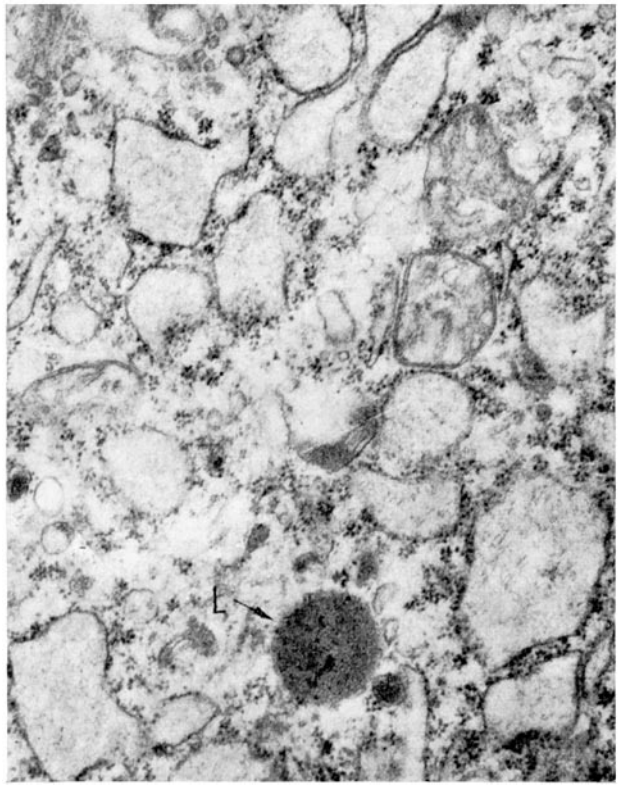

Fig. 3. A micrograph showing a remarkably swollen endoplasmic reticulum following reserpine treatment. In treated cells one can hardly find any presence of catechol amine type granules, whereas lysosomal granule is still present (L). × 48,000.

by DE ROBERTIS in the adrenal medulla[12]. In control cells, various sizes of vesicles which differ in density are seen in the cytoplasm, whereas only dense vesicles of a uniform size are present in axoplasm and axonterminals. Electron micrographs of normal cells show clearly that granular vesicles of variable density are crowded at the Golgi apparatus, suggesting that these granules originate at the Golgi apparatus and later develop into catechol amine-containing granules, forming a storage pool which may then flow into the site of physiological need. The hypothesis that these granules are catechol amine is supported by BARNETT's demonstration of a positive reaction with potassium dichromate oxidizing medium[7], by the monoamine fluorescence technique[6], and by the present observations in the Substantia nigra of mouse during reserpine administration. It is not clear how these electron-dense granular vesicles relate to the clear vesicles which probably contain acetylcholine and are found in the same synaptic endings.

In addition, oval osmiophilic bodies which are unusually large and sensitive to reserpine are often encountered in control cells. Our observation that reserpine-treated cells show a remarkably swollen endoplasmic reticulum suggests that this may be due to either compensatory overproduction or a failure of release of monoamine precursors.

The axonterminals in the nigra, which are filled with catechol amine granules, may be short axon collaterals from nigra cells. It has already been shown that long catechol amine-filled processes extend into the hypothalamus and striatum[7]. One can speculate whether such short axon collaterals represent a feed-back system on other nigra cells.

Zusammenfassung. Reserpin bewirkte an den Zellen der Substantia nigra der Maus Verschwinden der Katecholamin-haltigen Granula und Anschwellen des endoplasmatischen Retikulums. Das Hauptmerkmal von normalen Kontrollzellen bildeten elektronenoptisch dichte Körper im Cytoplasma.

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In vitro Effects of Different Rat Tissues on Radioiodinated 4-Iodoantipyrine

Radioiodinated 4-iodoantipyrine has been used in the measurement of total body water[1] as well as cerebral[2] and coronary blood flow[3]. STRAUB et al.[4,5] have stated the invalidity of the total body compartment measures because, when it is metabolized, it produces more diffusible forms and a large amount of the plasma activity is found as free radioiodide. The in vitro action of different tissues on radioiodinated 4-iodoantipyrine has been studied in this experimental work.

200 mg of the Wistar rat tissues tested were finely minced and suspended in 4 ml of saline. Then 10 μC of radioiodinated 4-iodoantipyrine were added to the tissue suspension and incubated at 37°C with occasional stirring, avoiding any evaporation.

After 6 h and 15 h, samples were taken of the supernatant, after centrifugation at 2500 rpm, and analysed by paper electrophoresis on Whatman paper 3 mm, using as a buffer 0.2% sodium bicarbonate solution and a voltage gradient of 15 V/cm. Under these experimental conditions, after 1 h of electrophoresis, the radioiodinated 4-iodoantipyrine remained at the starting line and the free-radioiodide migrated. The electrophoregrams were scanned using a thin-window Geiger-Müller tube with an automatic graphic recorder. The percentage of the total activity corresponding to each peak was determined by integration.

Kidney, lung, stomach, muscle and blood show no action in vitro. Intestine produces a small amount of free radioiodide (3.0%) after 15 h incubation. The deiodinising activity has been observed only with the liver tissues; 32.9% of radioiodide at 6 h and 63.7% at 15 h.

All these findings corroborate CHAIKOFF's[6] and STRAUB's[5] observations that rat liver is rich in a 'non-specific deiodinase' capable of splitting the radioiodine tag from labelled thyroid hormone intermediates and from radioiodinated 4-iodoantipyrine. Also, the in vitro specificity showed by the liver tissue confirms the reliability of the radioiodinated 4-iodoantipyrine liver function test[7].

Résumé. On a étudié l'action de differents tissus du rat sur la 4-iodoantipyrine radioiodée. On a observé une activité déiodante seulement avec le tissu hépatique.

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