off from the tubules, it is temping to relate the tubules to amine storage, maybe as precursors of storage vesicles. Our findings would thus favour the possibility of a local formation of storage vesicles from the ASER as suggested in previous studies[17–19, 26]. In other studies formation of storage vesicles from microtubules has been described[8]. However, since microtubules are not visualized in $KMnO_4$ fixed tissue[27] and since the tubules and 'elongated' vesicles in the present study clearly have a typical triple-layered membrane structure, considerable transformational changes would have to occur during such a process.

It should be emphasized that the present data do not exclude the formation of storage vesicles in the cell body. It has been clearly shown that both small and large DCV are present in the cell bodies of peripheral[4] and central (to be published) NA neurons as well as in the axons[4]. Thus, vesicles produced in the cell body and transported down with the axoplasmic flow in all probability contribute to the vesicular population in the varicosities[28]. It is at present impossible to evaluate the relative importance of these two possible sources of vesicles, i.e. such produced in the cell body and those locally in the nerve endings from ASER. Furthermore, other sites of origin may also exist as discussed above[29].

Zusammenfassung. Die adrenergen Nervenendigungen der von Ratten, die während 6 Tagen Reserpin Iris erhalten haben, enthalten eine höhere Anzahl von länglichen synaptischen Vesikeln und Tubuli als diejenigen unbehandelter Kontrolltiere. Die Möglichkeit, dass die adrenergen Bläschen unter gewissen Umständen in den Nervenendigungen gebildet werden könnten, wird diskutiert.

T. HÖKFELT

Department of Histology, Karolinska Institutet, S–104 01 Stockholm (Sweden), 12 January 1973.

[22] A. DAHLSTRÖM, K. FUXE and N.-Å. HILLARP, Acta pharmac. toxic. *22*, 277 (1965).
[23] A. DAHLSTRÖM and J. HÄGGENDAHL, J. Pharm. Pharmac. *21*, 633 (1969).
[24] H. THOENEN, R. A. MUELLER and J. AXELROD, Nature, Lond. *221*, 1264 (1969).
[25] P. B. MOLINOFF, S. BRIMIJOIN, R. WEINSHILBOUM and J. AXELROD, Proc. natn. Acad. Sci. USA *66*, 453 (1970).
[26] A. HERVONEN, L. KANERVA, R. LIETZEN and S. PARTANEN, Acta physiol. scand. *85*, 283 (1972).
[27] E. G. GRAY, in *Electron Microscopy Anatomy* (Ed. S. M. KURTZ; Academic Press, New York 1964), p. 369.
[28] A. DAHLSTRÖM, in *New Aspects of Storage and Release Mechanisms of Catecholamines* (Ed. H. J. SCHÜMANN and G. KRONENBERG; Springer-Verlag, Berlin, Heidelberg, New York 1970), p. 20.
[29] This study was supported by grants from the Swedish Medical Research Council No. B73-04X-2887-04C, by a grant from Karolinska Institutets forskningsfonder and Magn. Bergvalls Stiftelse. The generous supply of Serpasil® from Swedish CIBA, Stockholm, is gratefully acknowledged.

Electron and Fluorescence Microscopy of the Hamster Atrium After Administration of 6-Hydroxydopamine

6-Hydroxydopamine (6-OH-DA) can induce selective degenerative lesions in terminal adrenergic nerves, as demonstrated by electron microscopy[1], by the rapid disappearance of monoamine fluorescence in these nerves, and by a pronounced decrease in the norepinephrine content of various tissues[2]. The catecholamine content of the adrenal medulla, after administration of 6-OH-DA, remains normal but a marked increase in the activity of tyrosine hydroxylase, dopamine-B-oxidase and phenylethanolamine-N-methyl transferase suggests enhanced secretion of adrenal catecholamines[3]. The present work was undertaken to observe the behavior of the atrial specific granules[4] in the hamster heart after chemical sympathectomy with 6-OH-DA.

Materials and methods. Forty male MHA/SsLAK hamsters (Lakeview Hamster Colony, Newfield, New Jersey, USA) were used for two experiments. Each experiment consisted of 20 animals, half of which served as controls. The hamsters were kept in an air conditioned room and were maintained on Purina Laboratory Chow and tap water ad libitum. 6-Hydroxydopamine hydrobromide(6-OH-DA) (Regis Chemical Co., Chicago, USA) was dissolved in cold (0 °C) demineralized water containing 1% NaCl to which had been added 1% ascorbic acid (Fisher Scientific Co., Montreal, Canada) to decrease oxidation, and injected slowly (1 min) into the jugular vein under light ether anesthesia in a volume of 0.5 ml. In the first experiment, hamsters avering 60 g (range: 50–70 g) received 10 mg of 6-OH-DA on the first day and were sacrificed 24 h later together with the controls. In the second experiment, the hamsters averaged 135 g (range: 120–145 g) and were given 6-OH-DA at the dose of 10 mg per 100 g body weight on the 1st, 6th, 12th and 18th day. Experimental and control animals were killed together 24 h after the last injection.

For electron microscopy, 8 animals from each group were anesthetized with ether and the chest opened by an anterior midline incision. The right atrium was perfused with 2% cold glutaraldehyde buffered with cacodylate HCL (0.1 *M* at pH 7.1) using a needle inserted into the right ventricle[5]. During perfusion, the same fixative was dripped onto the surface of the right atrium. After perfusion, thin fragments of the anterior portion of the right atrium were placed in the fixative for 1 h, then washed for 3 periods of 15 min each in cacodylate to which 2% sucrose had been added. Subsequently, the specimens were left in the cacodylate solution for 12 h, post-fixed in osmium tetroxide buffered with veronal acetate for 1 h, and embedded in Araldite. Thick sections were stained with toluidine blue for orientation and thin sections were mounted on grids coated with formvar and carbon, stained with uranyl acetate and lead citrate, and examined with a Philips 200 electron microscope.

Monoamine fluorescence was assessed on two controls and two 6-OH-DA-treated animals at the end of each experiment. After sacrifice, the right atrium was immediately removed, rapidly quenched in liquid nitrogen-cooled isopentane and freeze-dried for 3 days. The tissues were exposed to paraformaldehyde vapors of optimal humidity for 1 h at 80 °C, followed by vacuum infiltration in paraffin for 15 min and sectioning at 8 µm. The fluorescence microscope was equipped with an Osram

[1] J. P. TRANZER and H. THOENEN, Experientia *24*, 155 (1968).
[2] J. DE CHAMPLAIN and R. NADEAU, Fedn. Proc. *30*, 877 (1971).
[3] H. THOENEN, R. A. MUELLER and J. AXELROD, Nature *221*, 1264 (1969).
[4] J. C. SOSA-LUCERO, F. A. DE LA IGLESIA, G. LUMB, J. M. BERGER and S. BENCOSME, Lab. Invest. *21*, 19 (1969).

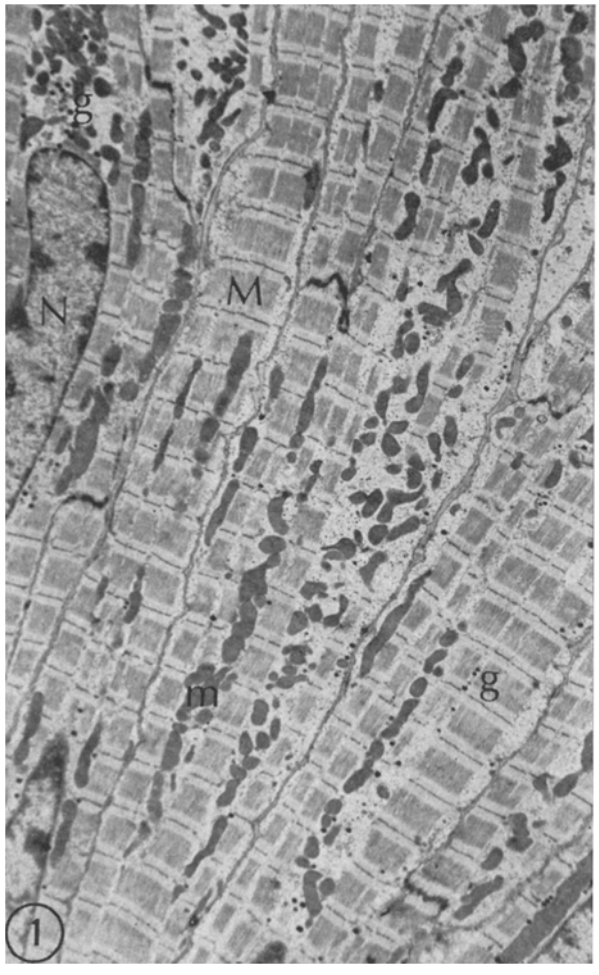

Fig. 1. Atrial cardiocytes of hamster chronically treated with 6-hydroxydopamine. Nucleus (N), myofilaments (M) and mitochondria (m) appear normal. Specific granules (g) can be seen in the juxtanuclear area and between the myofilaments. × 5200.

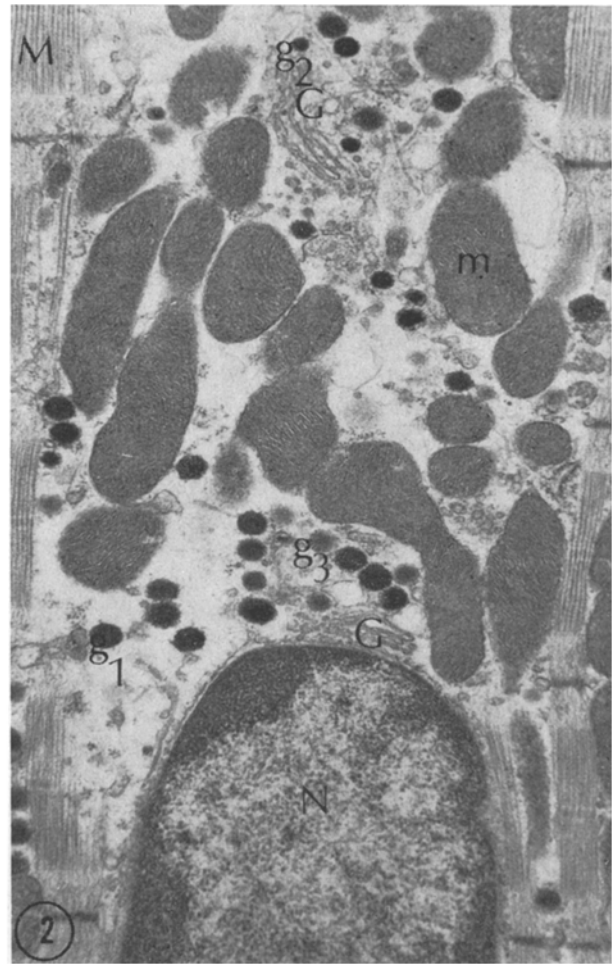

Fig. 2. Atrial cardiocyte of hamster chronically treated with 6-hydroxydopamine. The Golgi complex (G), the myofilaments (M), the mitochondria (m) and the 3 types of specific granules [A-granules (g_1), D-granules (g_2) and B-granules (g_3)] have a normal morphology. ×17,300.

HBO-200 high pressure mercury vapor lamp and a dark field condensor. A Shott BG-12 exciting filter was used in combination with a Shott OG-1 barrier filter[5,6].

Results. Electron microscopy. No difference in the fine structure of atrial cardiocytes could be detected between control and 6-OH-DA treated animals in either experiment. The number, size and distribution of the 3 types of specific granules (dense A-granules and pale B-granules located in the paranuclear zone; small, dense D-granules located along the sarcomeres) were similar in all groups. There was no increase or change in morphology of residual bodies or C-granules (Figures 1 and 2).

Fluorescence microscopy. The atria of control hamsters, in both experiments, characteristically showed numerous noradrenergic nerve fibers, as judged by their bright green fluorescence (Figure 3). This fluorescence was not present after acute or chronic treatment with 6-OH-DA (Figure 4).

Discussion. Our results indicate that in hamsters, as in other species[2], 6-OH-DA produces a chemical sympathectomy in the atrium, as demonstrated by the loss of fluorescence of adrenergic nerve fibers. Since the doses of 6-OH-DA in our experiments were the maximum tolerated by hamsters of the weights used, it is probable that administration of 6-OH-DA at 6-day intervals maintained the animals in a permanent state of more or less complete sympathectomy, by preventing regeneration of adrenergic nerve terminals[7]. As in the cat after bilateral surgical sympathectomy[8], chemical sympathectomy in the hamster is not followed by any change in the specific granules of the atrial cardiocytes. Since surgical and particularly chemical sympathectomy with 6-OH-DA induce a pronounced decrease (up to 95%) of norepinephrine in the atrium[7], it is doubtful whether the specific granules account for any large fraction of norepinephrine. It has been assumed that, because of their morphologic similarity to the granular vesicles of sympathetic axons and their silver-positivity[8], the small atrial specific D-granules, seen mainly among the myofibrils, possess the same function as their axonal counterpart, namely, the storing of catecholamines[9]. However, the absence of fluorescence of these specific granules in our study indicates that, if

[5] M. Cantin and R. Veilleux, Lab. Invest. *27*, 495 (1972).
[6] B. Falck and C. Owman, Acta Univ. lund. *11*, 7, 1 (1965).
[7] J. De Champlain, Can. J. Physiol. Pharmac. *49*, 345 (1971).
[8] S. Bencosme and J. M. Berger, in *Method and Achievements in Experimental Pathology* (Eds. E. Bajusz and G. Jasmin; Karger, Basel 1971), vol. 5, p. 173.
[9] W. G. Nayler and N. C. R. Merrillees, J. Cell Biol. *22*, 533 (1964).

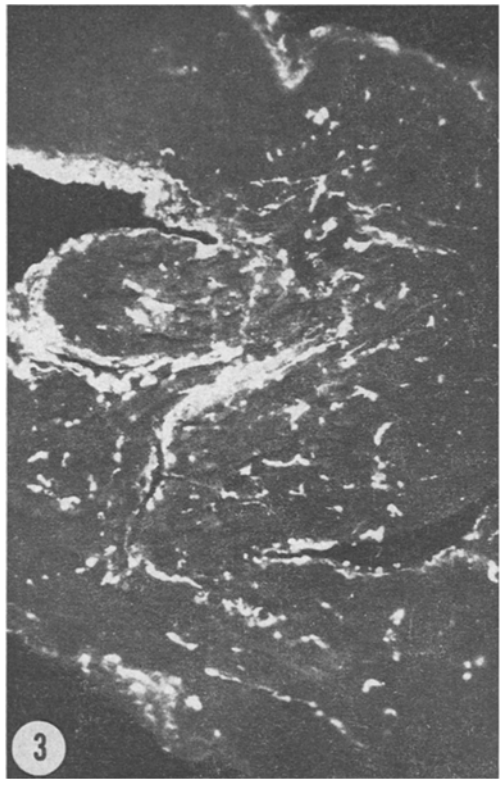

Fig. 3. Fluorescence of noradrenergic nerves in the atrial wall of control hamster. × 280.

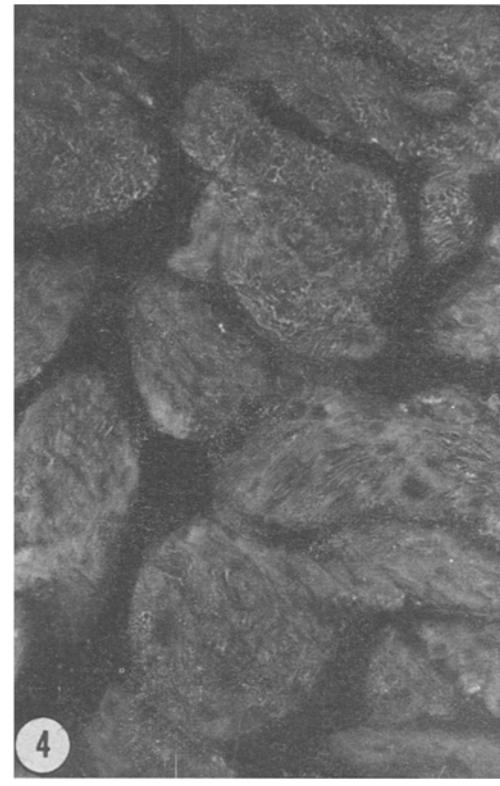

Fig. 4. Atrium of hamster chronically treated with 6-hydroxydopamine. The fluorescence of noradrenergic nerves has totally disappeared. × 280.

these granules do store catecholamines, they do so in a manner quite unrelated to axonal endings of adrenal medulla. Finally, the absence of lesions in atrial cardiocytes is not surprising since the damage caused by 6-OH-DA is strictly limited to adrenergic nerve endings, leaving Schwann cells, smooth muscle cells and cholinergic fibers intact[1].

Résumé. L'administration aiguë ou chronique de doses importantes de 6-hydroxydopamine au hamster induit la disparition de la fluorescence spécifique des fibres adrénergiques au niveau de l'oreillette droite mais ne produit aucun changement dans l'ultrastructure des cellules cardiaques. Le nombre, la taille, la disposition, la morphologie des granules spécifiques de ces cellules ne sont pas altérés.

M. CANTIN, R. VEILLEUX and M. HUET[10]

Département de Pathologie et Département d'Anatomie, Université de Montréal, Case postale 6128, Montréal 101 (Québec, Canada), 17 October 1972.

[10] Supported by a grant from the Quebec Heart Foundation and the Medical Research Council of Canada.

Parathymische Lymphknoten der Maus und ihre Reaktion auf Thymektomie[1]

Über parathymische Lymphknoten liegen bisher nur wenige Mitteilungen vor. DUNN[2] fand bei Mäusen 3 bis 4 kleine, dem Thymus dorsal anliegende Lymphknoten. Ebenso hat MILLER[3] kleine thoracische Lymphknoten der Maus nachgewiesen, welche in oder dicht an der Thymuskapsel lokalisiert sind. Auch bei Ratten grenzen mehrere Lymphknoten an den Thymus[4,5].

Bei eigenen Untersuchungen an neonatal thymektomierten Ratten und Mäusen ist eine Zunahme von Gewicht und Anzahl der parathymischen Lymphknoten aufgefallen, was auf eine wechselseitige Beziehung zwischen dem Thymus und den ihn umgebenden Lymphknoten hinweisen könnte. Hierzu wird deshalb an NMRI-Mäusen geprüft, ob quantitative Beziehungen zwischen Anzahl und Grösse der parathymischen Lymphknoten und dem Ausmass der Thymektomie bestehen.

Material und Methoden. Tiermaterial: Hochschwangere NMRI-Mäuse stammten vom Zentralinstitut für Versuchstierzucht in Hannover. Die Tiere wurden in Einzelkäfigen konventionell gehalten. Nach dem Werfen wurden dem Trinkwasser 30 mg/l Achromycin zugesetzt.

[1] Herrn Prof. Dr. O. WESTPHAL zum 60. Geburtstag.
[2] T. B. DUNN, J. natn. Cancer Inst. *14*, 1281 (1954).
[3] J. F. A. P. MILLER, Br. med. J. *2*, 459 (1963).
[4] W. W. BONNEY and J. D. BATTENBERG, Transplantation *5*, 544 (1967).
[5] J. N. BLAU and J. M. GAUGAS, Immunology *14*, 763 (1968).