

they represent an intermediate stage between typical polytene chromosomes. As follows from the foregoing account, these manifest several clear cut structural modifications on successive days of the prepupal period. The most relevant phenomenon is a gradual process of decondensation of the chromosomes as the maturation of the larvae progresses. In fact during the 3 first days of the period studied, the chromosomes are almost entirely heterochromatic and condensed, and shortly thereafter, on the 7 day of cocoon formation, when the process of building of the common capsule takes place, they become more diffuse and light stained. At the same time they show several areas of close adherence with the nuclear membrane, sometimes with a typical puff-like granular appearance (Figure 1b). Similar changes were induced in the 4th day of cocoon formation by means of hormonal and temperature treatments. Apparently both experimental procedures provoke an acceleration of the decondensation process, as well as a phenomenon of fusion with the nuclear membrane. A number of investigations on the relation of chromosome differentiation to gene activity suggest that euchromatin should represent the stage at which the genetic information carried in the chromosome is being transcribed¹¹. Conversely the heterochromatin should be the morphological counterpart of the diminution or suppression of the genetic activity. If we can extend to these chromosomes that theory, then, whatever genetic messages they carry out must be transcribed mostly in the latter days of the stage studied, while the chromatin is in the decondensed state. On the other hand, it has been pointed out that many condensed chromosomes are composed of blocks of material with genetic function, which are not heterochromatic at all stages of the life cycle¹¹. The same would be the case for the nucleolus

associated heterochromatin blocks appearing in nerve cells of several avian species which seems to pass through a sort of cycle of condensation-decondensation¹². These studies and others¹³ would suggest that heterochromatin and euchromatin are reversible states, and that chromosomal regions traditionally called heterochromatic are at least in some cases merely stages of transitory chromosomal differentiation.

Résumé. Les auteurs décrivent quelques aspects structuraux des chromosomes dans les tubes de Malpighi de *Rhynchosciara americana* (Diptera). L'apparition de modifications dans la structure chromosomique au cours du développement larval normal, et après traitement hormonal et thermique est suivie. La signification fonctionnelle possible de ces changements structuraux est discutée.

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Comisión de Invest., Cient. de la Provincia de Buenos Aires, Calle 526 entre 10 y 11, La Plata (Argentina), 12 June 1973.

¹¹ K. W. COOPER, *Chromosoma* 10, 535 (1969).

¹² T. P. PESSACQ, *Cytologia* 34, 375 (1969).

¹³ J. J. YUNIS and W. G. YASMINEH, *Science* 174, 1200 (1971).

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Degeneration of Noradrenergic Nerve Terminals in Submucous Ganglia of the Rat Duodenum Following Treatment with 6-Hydroxydopamine

Since it was first shown that administration of 6-hydroxydopamine (6-OHDA) results in selective degeneration of noradrenaline-containing nerve terminals¹, there have been a number of studies in which its effect at neuroeffector sites have been examined²⁻⁸. Fluorescence histochemical studies have established that noradrenaline-containing nerve terminals are present in mammalian enteric plexuses^{9,10}, and ultrastructural studies have demonstrated the presence of axosomatic synapses in the myenteric plexus of the guinea-pig^{11,12}. The distribution of noradrenaline-containing nerve terminals in ganglionated plexuses of the rat duodenum, however, has not been determined. In this study the distribution of degenerating nerve terminals found in submucous ganglia of the duodenum of the rat following administration of 6-OHDA has been examined using electronmicroscopy.

Rats were given a single i.v. injection 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. Subcutaneous heparin (1,000 U) was administered to alternate rats in each of the treated and control series 1 h before the animals were killed. 2, 4, 6, 12 and 19 h after injection of 6-OHDA, the duodenum was fixed by vascular perfusion with a solution containing 2% formaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 0.5 mg/l CaCl₂. After perfusion the duodenum was distended slightly by intraluminal injection of fresh fixative. A short segment of gut was

removed, cut into thin rings, post-fixed in osmium tetroxide and embedded in Araldite. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined in an Hitachi HS-8 electron microscope.

In the submucous ganglia of untreated rats, noradrenergic axons were identified by their content of small vesicles (about 50 nm in diameter) which contained an

¹ J. P. TRANZER and H. THOENEN, *Experientia* 25, 155 (1968).

² T. BENNETT, G. BURNSTOCK, J. L. S. COBB and T. MALMFORS, *Br. J. Pharmac.* 38, 802 (1970).

³ J. B. FURNESS, G. R. CAMPBELL, S. M. GILLARD, T. MALMFORS, J. L. S. COBB and G. BURNSTOCK, *J. Pharmac. exp. Ther.* 174, 111 (1970).

⁴ T. B. CHEAH, L. B. GEFFEN, B. JARROTT and A. OSTBERG, *Br. J. Pharmac.* 42, 543 (1971).

⁵ J. L. S. COBB and T. BENNETT, in *6-Hydroxydopamine and Catecholamine Neurons* (North-Holland Co., Amsterdam, London 1971) p. 33.

⁶ J. P. TRANZER and J. G. RICHARDS in *6-Hydroxydopamine and Catecholamine Neurons* (North-Holland Co., Amsterdam, London 1971) p. 15.

⁷ J. A. GOSLING and J. S. DIXON, *J. Cell Sci.* 10, 197 (1972).

⁸ T. HÖKFELT, G. JONSSON and C. H. SACHS, *Z. Zellforsch.* 131, 529 (1972).

⁹ G. GABELLA and M. COSTA, *Experientia* 24, 706 (1968).

¹⁰ M. COSTA and G. GABELLA, *Z. Zellforsch.* 122, 357 (1971).

¹¹ G. GABELLA, *Experientia* 27, 380 (1971).

¹² G. GABELLA, *J. Anat.* 111, 69 (1972).

electron dense core of variable size¹³⁻¹⁵; axons containing these small granular vesicles comprised only a small proportion of axons seen. Noradrenergic axon profiles also contained small electron-lucent vesicles about 50 nm in diameter, a few scattered large dense-cored vesicles about 100–150 nm in diameter, and mitochondria and occasional glycogen granules. Noradrenergic axons were separated from the perikarya and dendrites of sub-mucous ganglion cells by glial processes in most cases, but some were separated from the neurons by a gap of only 20–30 nm; obvious membrane specialization consisting of thickened apposed membranes with subjacent dark deposit was rarely seen at this site (Figure 1). Such configurations were considered to represent noradrenergic axosomatic synapses. Numerous axon profiles containing electron-lucent vesicles and large granular vesicles were also seen; some of these made synaptic contact with ganglion cells. These axon profiles were not considered to represent noradrenaline-containing nerve terminals.

Degenerating axons were seen in animals killed at all time intervals after injection of 6-OHDA. In the animals killed two or 4 hours following treatment with 6-OHDA

a few axons which contained small granular vesicles showed increased cytoplasmic density suggestive of early axon degeneration (Figures 2 and 3). Most degenerating axons consisted of an osmiophilic mass of irregular outline which, in some cases, appeared to have a definite substructure. These osmiophilic structures, although infrequent, were easily observed in the animals killed 6, 12 or 19 h following injection of 6-OHDA. The frequency and distribution of degenerating axons in animals injected with 6-OHDA was similar to the frequency and distribution of axon profiles containing small granular vesicles seen in control rats. Most of the degenerating axons were not in contact with ganglion cells. A degenerating axon terminal (identified by an axo-somatic contact in which the apposed membranes showed specializations) was rarely seen (Figure 2).

¹³ E. DE ROBERTIS and A. PELLEGRINO DE IRALDI, *J. Biophys. biochem. Cytol.* 10, 361 (1961).

¹⁴ F. E. BLOOM, *Int. Rev. Neurobiol.* 13, 27 (1970).

¹⁵ L. B. GEFFEN and B. G. LIVETT, *Physiol. Rev.* 51, 98, (1971).

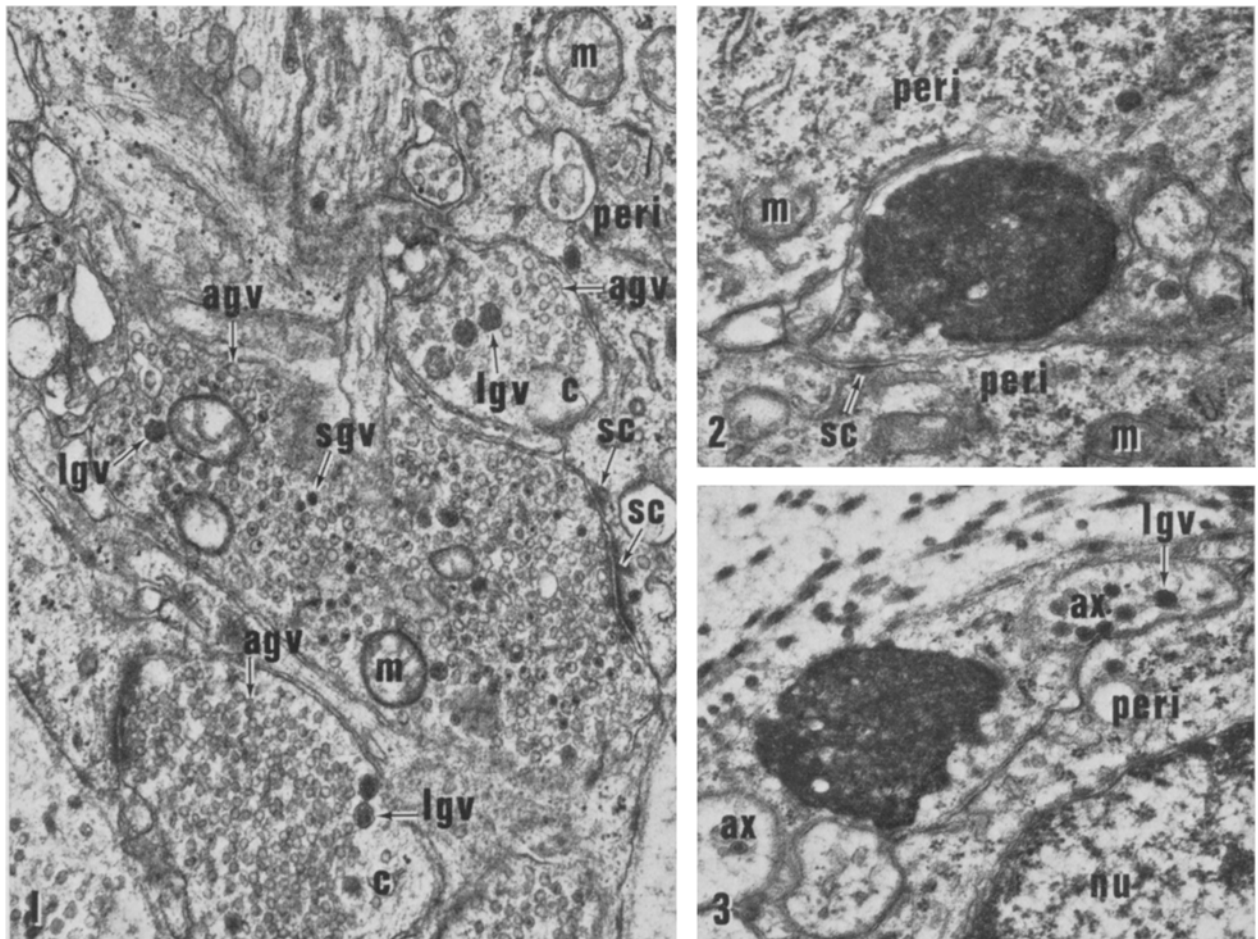


Fig. 1. A nerve terminal, from a control animal, which contains small granular vesicles. The terminal is identified by the synaptic contact with a ganglion cell body. Axon profiles containing electron lucent and large granular vesicles are seen above and below the terminal. m, mitochondria; peri, perikaryon; agv, agranular vesicles; lgv, large granular vesicles; sc, synaptic contact with membrane thickening; sg, small granular vesicles. $\times 30,000$.

Fig. 2. A degenerating nerve terminal from a treated animal (6 h, 100 mg/kg), in contact with a ganglion cell body. peri, perikaryon; m, mitochondrion; sc, synaptic contact with membrane thickening. $\times 36,000$.

Fig. 3. A degenerating axon profile from a treated rat (6 h, 100 mg/kg) in contact with a ganglion cell body. Adjacent axons (ax), containing large granular vesicles, can be seen. lgv, large granular vesicles; peri, perikaryon; nu, nucleus of ganglion cell. $\times 30,000$.

Ultrastructural studies which have examined the effect of 6-OHDA on the intestine of the guinea-pig have led to the view that noradrenergic synapses are entirely absent in the myenteric plexus^{16,17} even though the fluorescence histochemical study of COSTA and GABELLA¹⁰ showed that the submucous and myenteric ganglionated plexuses in the intestine of the guinea-pig are richly innervated with noradrenergic fibres.

The results of the present study show that in the submucous ganglia of the rat duodenum there is correspondence in frequency and distribution between axons containing small granular vesicles in untreated rats and the degenerating axons in animals treated with 6-OHDA. It seems reasonable to suppose that, although noradrenaline-containing axons form only a small proportion of the total nerve terminal population within submucous ganglia, noradrenaline-containing axons do occasionally innervate submucous ganglion cells.

The present study has also shown that noradrenergic nerve terminals in the submucous ganglia of the rat duodenum, like noradrenergic nerve terminals elsewhere in the autonomic nervous system, are able to accumulate sufficient 6-OHDA to initiate nerve terminal degeneration.

Zusammenfassung. Nachweis adrenerger Synapsen an Zellkörpern der duodenalen submukösen Ganglien von Ratten nach 6-Hydroxydopamin-Behandlung.

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¹⁶ H. G. BAUMGARTEN, A. F. HOLSTEIN and C. H. OWMAN, *Z. Zellforsch.* 106, 376 (1970).

¹⁷ L. L. ROSS and M. D. GERSHON, *J. Cell Biol.* 47, 175a (1970).

¹⁸ Part of this work was done while I was on sabbatical leave in the Department of Anatomy, Monash University, in 1972, and I thank Professor G. C. SCHOFIELD for his kindness and encouragement during my stay there. Mr. H. L. CHAN rendered invaluable technical assistance.

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An Initial Report on Interstitial Cells of Testicular Type (Leydig Cells) in the Ovary of *Camelus dromedarius*

BERGER¹ was the first to note the presence of particular cells in the ovary of the human species. Initially, this author maintained that they were 'neurotropic' cells. Later, however, they were likened by KOHN² to the interstitial Leydig-type cells of the testicle. This appears to be the opinion generally accepted today, also according to other research-workers³⁻⁸, and seems to be chiefly based on the presence of lipoids and Reinke crystals in the cytoplasm. These crystals, as is known, are characteristic of the Leydig cells of the human species.

Further arguments in favour of this analogy have been offered by later authors⁹⁻¹², who have established a whole series of extremely significant facts. Among these, we may mention the virilization of women suffering from hyperplasia of these cells, the presence of ketosteroids in the

same cases, and their hyperplasia following administration of gonadotrophins.

According to certain authors^{13,14}, the Leydig-type interstitial cells are also present in the ovary of the cat, the dog, the wolf and *Pithecus fascicularis mordax*. Among domestic animals of economic importance, they are found only in the female of *Sus scrofa*¹⁵.

In the course of observations that we carried out on the structure of the ovary of *Camelus dromedarius*, we ascertained the presence of Leydig-type interstitial cells in 4 cases out of 5 examined. The animals were sexually mature, between the ages of 7 and 11 years old. The ovaries were fixed in buffered 10% formalin and in Bouin Hollande and were embedded in paraffin. They were obtained from the public slaughter-house in Mogadiscio, in the course of a period of teaching in which one of us was engaged at the Agricultural Faculty of the National University of Somalia.

The cells in question occupy different sites, from one case to another. In fact they can be found in the hilus among the large blood vessels, in the connective tissue

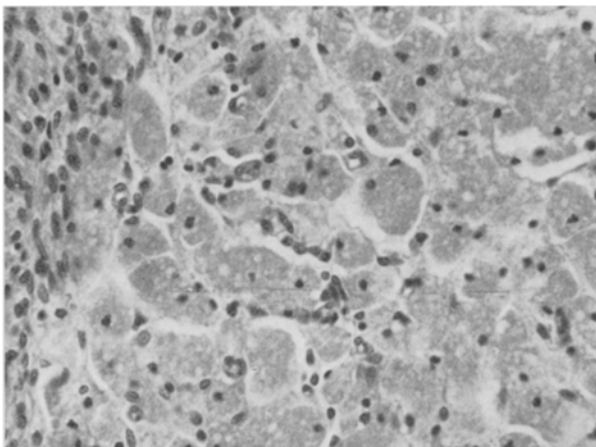


Fig. 1. Group of very large Leydig cells, vacuolized and lacking in granulations. On the left may be seen the ovarian stroma. Hematoxylin. $\times 300$.

¹ L. BERGER, *C.r. Acad. Sci., Paris* 175, 907 (1922).

² A. KOHN, *Endocrinology* 1, 3 (1928).

³ D. BRANNAN, *Am. J. Path.* 3, 343 (1927).

⁴ A. PRIESEL, in *Handbuch der pathologischen Anatomie* (Ed. HENKE-LUBARSCH; Springer, Berlin 1931), vol. 4, p. 3.

⁵ C. WIESER, *Endokrinologie* 8, 321 (1931).

⁶ H. STIEVE, *Z. mikrosk.-anat. Forsch.* 22, 591 (1930).

⁷ J. NOVAK, *Gynäk. Rdsch.* 17, 769 (1930).

⁸ W. H. STERNBERG, *Am. J. Path.* 25, 493 (1949).

⁹ B. A. SACHS and D. SPIRO, *J. clin. Endokr.* 11, 878 (1951).

¹⁰ J. TALLAFERRO, E. J. WALLS, S. KAY and R. H. HOGE, *Obstet. gynec. Survey* 8, 873 (1953).

¹¹ G. DHOM, *Geburtshilfe* 142, 182 and 289 (1954-55).

¹² G. BARTOLOMEI, *Atti Soc. med.-chir. Padova* 31, 162 (1954).

¹³ C. WIESER, *Endokrinologie* 10, 13 (1933).

¹⁴ R. JOACHIMOVITS, *Zbl. Gynäk.* 1937, 2697.

¹⁵ M. WATZKA and J. ESCHLER, *Z. mikrosk. Anat.* 34, 238 (1933).