Ultrastructure of the Vesicular Component in the Intramural Nervous System of the Guinea-Pig's intestines*

By W. L. TAFURI

From the Department of Pathology (Prof. L. BOGLIOLO), Minas Gerais University, School of Medicine, Belo Horizonte, Brazil

(Z. Naturforschg. **19 b**, 622—625 [1964]; eingegangen am 22. Januar 1964)

In the present paper, a systematic study of the vesicular component found in the unmyelinated nerve fibers of the intestinal nervous plexus from the serosa to the mucosa of the guinea pig was carried out. This vesicular component containing dense granules with diameters from 300 to 1100 Å was distinguished from the synaptic vesicles of 100 to 500 Å. The distribution of granules and vesicles of the myenteric plexus of the duodenum and colon and extraganglion nervous fibers of the mucosa, submucosa and muscularis was studied. These results suggest that the vesicles can be the site of synthesis and storage of the substance P and 5-hydroxytryptamine.

HAGER and TAFURI¹ demonstrated the presence of dense granules and clear vesicle measuring 1200 to 1500 Å, within the intraganglionic unmyelinated fibers of the myenteric plexus of the guinea-pig's colon. Such granules and vesicles are surrounded by a well defined membrane. Both these components are morphologically similar to those found in the neurohypophysis (GREEN and VAN BREEMEN²; PALAY³; BARGMANN and KNOOP⁴; BARGMANN et al.⁵; FUJITA⁶; HARTMANN⁷; BRETSCHNEIDER⁸; GREEN and MAXWELL⁹; GERSCHENFELD et al.¹⁰).

Similar but larger vesicles were found in the chromaffin cells of the intestines and adrenals (SjöSTRAND and WETZSTEIN¹¹; DE ROBERTIS and VAZ FERREIRA¹²; TAFURI¹³).

The reduction of the osmic acid in such vesicles, however, seems to be more intense than in the smaller vesicles mentioned above.

Furthermore, in the pineal gland and in the anterior hypothalamus of rats DE ROBERTIS and PELLE-

GRINO DE IRALDI¹⁴ and PELLEGRINO DE IRALDI et al.¹⁵ found vesicles, which strongly reduce osmium tetroxide.

It is known that adrenaline and noradrenaline, as well as other catecholamines, possess a strong reducing action on the osmium tetroxide in fixation (BENNETT¹⁶). The discovery of vesicles with deposits of reduced osmium suggests the presence of catecholamines in such structures. Recent ultrastructural, biochemical and pharmacological findings corroborate such a hypothesis (DE ROBERTIS and PELLEGRINO DE IRALDI¹⁴; PELLEGRINO DE IRALDI et al.¹⁵).

This paper deals with a morphological study of vesicular component in the several layers of the guinea pig's intestines.

Techniques

Guinea-pigs weighings 300 to 400 grams were used. After ether anesthesia the abdominal cavity was opened and fragments of the duodenum, the ileum and the

* Visiting Professor, supported by the Rockefeller Foundation, at the Instituto de Anatomia General e Embriologia, Faculdade de Ciencias Medicas, Buenos Aires, Argentina, from October, 1961 to January, 1962. The author wishes to express his gratitude to Prof. E. DE ROBERTIS, Dr. A. PELLEGRINO DE IRALDI and Prof. W. T. BERALDO for reading and for valuable discussions of this paper. My sincere thanks are due to Miss L. LEVI for her technical assistance.

¹ H. A. HAGER and W. L. TAFURI, *Naturwissenschaften* **9**, 332 [1959 a].

² J. D. GREEN and V. L. VAN BREEMEN, *Amer. J. Anatomy* **97**, 177 [1955].

³ S. L. PALAY, *Anatom. Rec.* **121**, 348 [1955].

⁴ W. BARGMANN and A. KNOOP, *Z. Zellforsch. mikroskop. Anatom.* **46**, 242 [1957].

⁵ W. BARGMANN, A. KNOOP, and A. THIEL, *Z. Zellforsch. mikroskop. Anatom.* **47**, 114 [1957].

⁶ H. L. FUJITA, *Arch. histol. jap.* **12**, 165 [1957].

⁷ J. F. HARTMANN, *Z. Zellforsch. mikroskop. Anatom.* **48**, 291 [1958].

⁸ H. BRETSCHNEIDER, *Z. mikr. anat. Forsch.* **64**, 575 [1958].

⁹ J. D. GREEN and D. S. MAXWELL, in: *Comparative Endocrinology*, A. Gorbman, ed., pp. 368—392, Wiley, New York, 1959.

¹⁰ H. M. GERSCHENFELD, H. J. TRAMEZZANI, and E. DE ROBERTIS, *Endocrinology* **66**, 5, 741 [1960].

¹¹ F. S. SjöSTRAND and R. WETZSTEIN, *Experientia [Basel]* **12**, 5, 196 [1956].

¹² E. DE ROBERTIS and A. VAZ FERREIRA, *Exp. Cell Res.* **12**, 568, 575 [1957].

¹³ W. L. TAFURI, to be published.

¹⁴ E. DE ROBERTIS and A. PELLEGRINO DE IRALDI, *J. biophysic. biochem. Cytol.* **10**, 3, 361 [1961].

¹⁵ A. PELLEGRINO DE IRALDI, H. F. DUGGAN, and E. DE ROBERTIS, *Anatom. Eec.* **145**, 521 [1963].

¹⁶ S. H. BENNETT, *Amer. J. Anatomy* **61**, 333 [1941].

colon were taken out, washed in the fixative solution and prepared according to the technique described by TAFURI¹⁷. After the fixation in cold 1% OsO₄, in Peryston Beyer at pH 7.2–7.4, for 2 hours (TRUJILLO CENOZ¹⁸) fragments of different layers of the intestine (whole intestinal wall, whole wall without the mucosa, external muscular layer with myenteric plexus, submucosa with Meissner's plexus and isolated mucosa) were embedded in a prepolymerized mixture of butyl- and methyl methacrylate (9 : 1, 8.5 : 5, and 8 : 2).

The sections were made with a Porter-Blum microtome and observed under an EMU 2 RCA electron microscope or a Siemens Elmiscop I.

The localization of Auerbach's ganglia in the whole wall and external muscular layer of the intestine was done according to HAGER and TAFURI¹⁹.

Results

Vesicular component of the intraganglionic nerve fibers of the myenteric plexus in the duodenum and colon

Intraganglionic unmyelinated fibers cut at different angles and presenting various diameters are shown in Figs. 1*, 2 and 3. They are enveloped by cytoplasmic processes of Schwann cells; therefore, it is not rare to find the presence of mesoaxons. In great number of fibers we can also find mitochondria, neuroprotofibrils and a characteristic vesicular component in varying amount. This vesicular component contains: 1. clear synaptic vesicles from 100 to 500 Å; 2. vesicles from 300 to 1100 Å each containing a dense granule varying in size from 100 to 800 Å and presenting variable shapes, internal structure, density and distribution; 3. clear round or oval vesicles lacking dense material*. All vesicles with or without granules are limited by a single membrane 40 to 50 Å thick.

In order to evaluate the vesicular component in the duodenum and colon only those fibers perpendicularly cut were selected for study. The larger diameter of 18 fibers from the duodenum and 33 from the colon was measured. In each of these fibers the diameter of the vesicular components was also measured and all the vesicles were counted. Figs. 4 and 5 summarize these results.

Fig. 4. Histogram showing the percentage distribution of the size of synaptic vesicles and vesicular component in the colon.

Fig. 5. Histogram showing the percentage distribution of the size of synaptic vesicles and vesicular component in the myenteric plexus of the duodenum.

As can be seen from the histograms the size of both the vesicles with dense granules and the clear large vesicles is around 800 Å for the duodenum and 900 Å for the colon, but this difference has no statistical significance.

* Figs. 1, 2, 3 and 7 see table p. 624 a–c.

¹⁷ W. L. TAFURI, Acta Anat. **31**, 522 [1957].

¹⁸ O. TRUJILLO CENOZ, Z. Zellforsch. mikroskop. Anatom. **46**, 272 [1957].

¹⁹ H. A. HAGER u. W. L. TAFURI, Arch. Psych. Zeitschr. f. d. ges. Neural. **199**, 437 [1959 b].

* Since according to DE ROBERTIS²⁰, the synaptic vesicles have a average diameter from 250 to 750 Å in the present work vesicles exceeding 500 Å were considered large clear vesicles.

²⁰ E. DE ROBERTIS, Internat. Rev. Cytol. **8**, 61 [1959].

Vesicular component in the extraganglionic nerve fibers of the myenteric plexus in the muscular layer, in the fibers of the submucous plexus and in the peripheral plexus of the duodenum

Twenty three nerve fibers from above mentioned regions (muscularis, submucosa and mucosa) cut perpendicularly were studied. In each fiber the large diameter and the vesicular components were measured and counted. Fig. 6 illustrates the results of

Fig. 6. Histogram showing the percentage distribution of the size of synaptic vesicles and granulated vesicles in extraganglionic nerve fibers of the myenteric and submucous plexus, and in the peripheral plexus of the duodenum.

these observations. As can be seen, the size of the vesicles containing a dense granules is around 1000 to 1100 Ångström.

As the variation factor of the vesicles could be related to the fiber diameter, we decided to test this point. The relation index ($r=0.18$) demonstrated the absence of any such relationship.

Although technical difficulties did not allow us to collect sufficient material for a statistical analysis, we were at least able to find vesicles of 300–400 Å containing irregular granules (100 Å) within some unmyelinated fibers of the colon serosa (Fig. 7).

Discussion

Recently GRILLO and PALAY²¹ have confirmed in the rat observations of HAGER and TAFURI^{1, 19} in the ganglia and nerves of the autonomic nervous system of the guinea pig intestine. Based on the internal structure, size and density of the dense granules the authors distinguish three types of granulated vesicles.

Our observations in the guinea-pig's intestine suggest the existence of only two different types of granulated vesicles and one of clear vesicles, larger than synaptic vesicles. The first type which includes vesicles from 400 to 1100 Å, containing a large granule with an electron opacity to that of neurosecretory granules of the neurohypophysis was found in the intra- and extraganglionic nerve fibers of the colon and duodenum (Figs. 2 and 3). The second type comprises vesicles of 300 to 400 Å, having a very dense and osmiophilic granule of 100 to 200 Å found in the colon serosa (Fig. 7).

The granular and large clear vesicles have never been seen outside the fibers. They are distributed, in variable quantity, along the fiber, and the concentration does not increase in the region of axosomatic synapses. These findings suggest that such components are being formed along the axon. A similar view concerning to secretion of noradrenaline by the adrenal nerves was presented by VON EULER²². GERSCHENFELD et al.¹⁰ showed that the neurosecretory granules found in the unmyelinated fibers of the preoptico-neurohypophyseal tract of the toad are synthesized within the preoptic nucleus and increase gradually in size along the entire preoptico-neurohypophyseal tract. In the intestine the synthesis of the vesiculated material is probably also made along the axon.

Our observations suggest that the granule material is synthesized and stored inside the vesicular membrane which isolates it from the surrounding cytoplasm. VON EULER et al.²³ using the cell fractionation technique, were able to separate a microgranular fraction rich in noradrenaline from the splenic nerve. According to these authors noradrenaline is stored inside the membrane. It seems possible that the vesicular component of the intramural nerve fibers of the intestine might be isolated with a technique similar to that used by VON EULER, permitting direct biochemical analysis.

The large vesicles which are distinguished from synaptic vesicles by their size are also found in the preoptico-neurohypophyseal tract, according to PALAY³ and GREEN and MAXWELL⁹. It is possible that they derive from the larger dense vesicles after the release of the granulated material.

²¹ M. A. GRILLO and S. L. PALAY, Fifth Int. Congr. Electr. Micr. 2, vol. 1 [1962].

²² U. S. VON EULER, in: Neurophysiology and Physiol. 1 [1959].

²³ U. S. VON EULER and N. A. HILLARP, Nature [London] 177, 4497, 44 [1956].

Fig. 1. Low power electronmicrograph of part of the myenteric plexus ganglia of the guinea-pig's colon. Unmyelinated nerve fibers containing a vesicular component (vc), mitochondria (mi); pericaryon of nerve cell (p), axosomatic synapses (as), capsule of the ganglion with fibroblast (f), elastic (e) and collagenous fibers (co). X 20.000.

Figs. 2. and 3. Electronmicrographs of unmyelinated nerve fibers of the myenteric plexus of the guinea-pig's colon showing numerous synaptic vesicles (sv) and vesicles with a dense granule (dv). Arrows point out some large clear vesicles probably resulting from evacuation of the content. X 60.000.

Fig. 3.

Fig. 7. Serosa of the guinea-pig's colon. Unmyelinated nerve fibers containing vesicles with dense granules (dv), synaptic vesicles (sv) X 60.000.

Regarding the possible nature of the two types of granular vesicles at present we may only speculate.

It is known that intestine is rich in serotonin (5-hydroxytryptamine, enteramine) and in substance P (GADDUM²⁴). It has been suggested that substance P is related to Meissner's plexus (GADDUM²⁴). In support of this EHRENPREISS and PERNOW²⁵ found low concentrations of substance P in the inactive and aganglionic part of the gut, in Hirschsprung's disease and abnormally large amounts in the intestine above this point. In an unpublished

study (TAFURI¹³) we have found serotonin in the mucosa and muscularis and a substance P-like activity in the submucosa of the guinea-pig's colon. On the basis of these experiments it is not unreasonable to think that the granules found in the intra- and extraganglionic unmyelinated fibers of Meissner's plexus of the guinea-pig's intestine, might be the site of synthesis and storage of substance P. On the other hand, the possibility exists that the granulated vesicles of Auerbach's plexus might contain 5-hydroxytryptamine.

²⁴ J. H. GADDUM, in: Polypeptides, vol. 4. London, Symposium Publications Division, Pergamon Press Oxford 1959.

²⁵ T. EHRENPREISS and B. PERNOW, Acta physiol. scand. **27**, 380 [1953].

Die Wirkung von Natriumnitrit auf Nucleinsäurebasen und ihr Verhältnis zur Inaktivierung von Hefezellen

II. Die Inaktivierung von *Saccharomyces*-Zellen verschiedenen Ploidiegrades

VON WERNER STEIN UND ERNST-RANDOLF LOCHMANN

Aus dem Institut für Biophysik der Freien Universität Berlin

(Z. Naturforschg. **19 b**, 625—633 [1964]; eingegangen am 3. Februar 1964)

The inactivation of homozygous yeast strains of different ploidy by NaNO_2 was investigated. The influence of pH, concentration of NaNO_2 , time and temperature of treatment, and ploidy have been studied. On the whole there is a remarkable similarity with certain features of UV-inactivation. A critical comparison with the results of part I of this paper leads to the conclusion that the deamination of DNA-bases is the essential biochemical process which causes the biological inactivation.

In einer vorangegangenen Arbeit¹ wurde die Desaminierung von Nucleinsäurebasen in vitro und vivo untersucht und diskutiert. In diesem 2. Teil der Arbeit sollen Ergebnisse der NaNO_2 -Inaktivierung von Hefezellen verschiedenen Ploidiegrades untersucht, diskutiert und mit den erwähnten biochemischen Ergebnissen verglichen werden. Für solche Analysen stehen weitestgehend isogene und homozygote Hefestämme zur Verfügung, wie sie von LASKOWSKI aufgebaut^{2,3} und von STEIN und LASKOWSKI für die Analyse der Strahleninaktivierung benutzt wurden^{4,5}. Auch die Inaktivierung durch organische Peroxyde wurde mit diesen Stämmen untersucht⁶.

Mit solchen Stämmen läßt sich insbesondere die Ploidiegrad-Abhängigkeit induzierter biologischer Effekte untersuchen, woraus sich wiederum Schlüsse auf den Wirkungsmechanismus ziehen lassen. Für die Unterscheidung zwischen genetischen und nicht-genetischen Wirkungen, und bei ersteren zwischen rezessiven und dominanten Ausprägungen, ergeben sich auf diesem Wege wichtige Anhaltspunkte. Im Rahmen dieser Arbeit interessierte weiter die Frage, ob sich die biologischen Wirkungen auf die biochemisch nachgewiesenen Veränderungen der Nucleinsäurebasen zurückführen lassen.

¹ E. R. LOCHMANN u. W. STEIN, Z. Naturforschg. **18 b**, 809 [1963]. In Teil I der Arbeit muß bei den Abb. 2, 3, 7 [Mol·l⁻¹·min] statt [mol·min] stehen.

² W. LASKOWSKI, Z. Naturforschg. **15 b**, 495 [1960].

³ W. LASKOWSKI, Z. Naturforschg. **17 b**, 93 [1962].

⁴ W. STEIN u. W. LASKOWSKI, Z. Naturforschg. **15 b**, 734 [1960].

⁵ W. STEIN, Z. Naturforschg. **17 b**, 179 [1962].

⁶ W. STEIN u. W. LASKOWSKI, Zbl. Bakteriol. Parasitenkunde, Infektionskrankh. Hyg., I. Abt., Orig. **180**, 390 [1960].