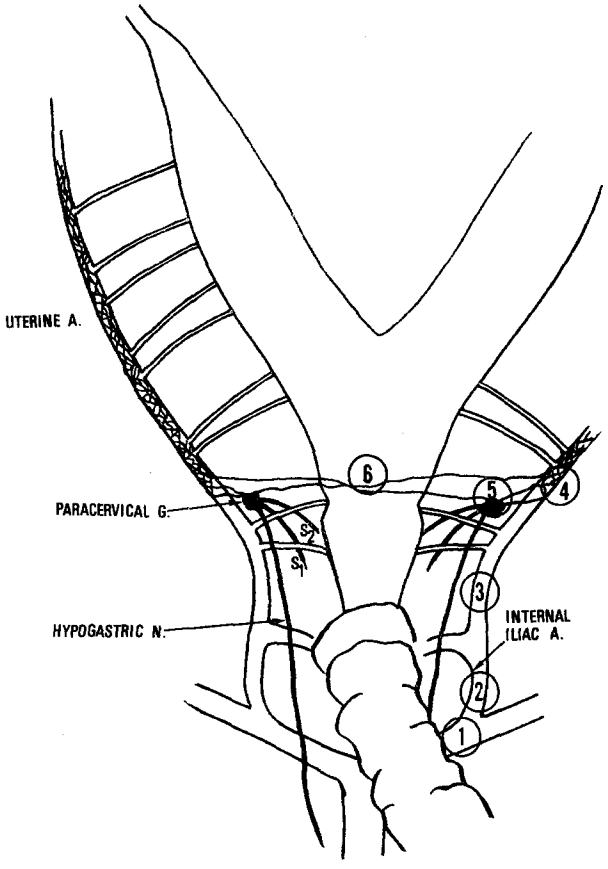
of the adrenergic vasomotor supply to the uterine artery.

These results demonstrate that the cholinergic vasodilator nerves which supply the uterine artery in the guinea-pig originate entirely from the paracervical ganglia. As no degeneration of vasomotor fibres was noted following lesion of either the hypogastric or sacral nerves it appears that these fibres arise at least in the main from paracervical ganglion cells, rather than merely travelling through the ganglion as postganglionic nerves.

The spinal origin of the nerves remains uncertain. It is well known that the hypogastric nerve synapses in pelvic ganglia[10,11] and the sparse cholinergic innervation to the vas deferens of the guinea-pig, which travels through these ganglia[12], has been thought to be hypogastric in origin[13]. On the other hand coagulation of the paracervical region with phenol, which greatly assists in visualizing fine nervous elements, reveals that the first and second sacral nerves send elements into the paracervical ganglion. Certainly a parasympathetic origin would be in accord with the majority of data for cholinergic dilator supplies[14]. In this regard it may be noted that degeneration studies on the rat paracervical ganglion have revealed the presence of presynaptic endings of both hypogastric and pelvic origins[15].

The fact that the cholinergic dilator supply to the parametrial vasculature can be selectively destroyed by ablation at a ganglionic synapse means that regeneration is unlikely. This is supported by the fact that no intact AChE-positive fibres were seen in the uterine artery of an animal killed 6 months after bilateral ganglionectomy. Thus this technique affords a method by which the progress of pregnancy in the absence of local cholinergic dilator influences can be studied.



The anatomical course of the cholinergic dilator nerves supplying the parametrial artery of the guinea-pig. Note that the preganglionic fibres may be of sacral or of hypogastric origin. The ringed numbers denote sites of phenol-induced coagulation.

Résumé. Les nerfs cholinergiques vasodilatateurs de l'artère utérine du cobaye ont leur origine dans les ganglions paracervicaux. L'ablation de ces ganglions permit d'évaluer l'importance des nerfs vasodilatateurs dans le cas d'une hyperémie de l'uterus apparaissant durant la grossesse.

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[17] This study was supported by the National Heart Foundation of Australia.
[18] Terramycin® was kindly donated by Pfizer Pty. Ltd.

The Package of Vesicle Content in *Octopus* Synapses as Revealed by Unbuffered Zinc Iodide-Osmium

The zinc iodide-osmium (ZIO) method was first introduced in invertebrates by MARTIN et al.[1] The method turned out to react with several types of synaptic vesicles in the *Octopus* brain, causing a homogeneously black precipitate within the vesicle membrane. The substance which reacts with ZIO has not been determined as yet. In all investigations reported so far, the ZIO-impregnation of synaptic vesicles proceeds in an all-or-none fashion: the content of a vesicle either reacts completely or not at all. This type of reaction can be modified by the use of unbuffered ZIO.

Medium-sized animals were anaesthetized in urethane (3% in seawater) and fixed by perfusion through the cephalic arteria with glutaraldehyde (3.5% in seawater, pH adjusted to 7.2 with NaOH 0.1 *N*). Small tissue slices (0.5–1 mm thick) were dissected from the perfused brain lobes and the iris and washed 3×10 min in an unbuffered stock solution containing 0.57 *M* NaCl, 0.0054 *M* $CaCl_2$ and 0.0015 *M* $MgCl_2 \cdot 6\ H_2O$, (pH 5.7). Then the tissue was ready for incubation in ZIO. 1 h before incubation 2 solutions were prepared, A) and B). A) contained 5% OsO_4 and B) 7.5% Zn-powder + 2.5% iodium bisublimate in the unbuffered stock solution. B) was shaken and

[1] R. MARTIN, J. BARLOW and A. MIRALTO, Brain Res. *15*, 1 (1969).

filtered immediately before use. A) and B) were then mixed in a ratio of 2:3 and finally the tissue slices were incubated in this ZIO-mixture for 13 h at 4 °C. Controls were 1. incubated at the same time in the unbuffered stock solution and 2. incubated in ZIO buffered with *tris* at 7.2. The slices were dehydrated with EtOH and embedded in Taab resin[2]. The thin sections were stained with lead citrate and uranyl acetate.

Synaptic vesicles of 3 brain lobes and of the nerve-muscle junctions in the iris were investigated. The following synapses contain ZIO-reacting vesicles: 1. Synapses of afferent fibres from the lateral basal to the chromatophore lobes, 2. optic afferents, ending in the lateral basal lobe and 3. nerve terminals of the iris sphincter and chromatophore muscles. All the vesicles in these synapses apparently belong to the same class, 38 nm in diameter and electron-transparent in tissue fixed with osmium or glutaraldehyde without subsequent ZIO incubation. The proportion of ZIO-positive vesicles per synapse is always between 85 and 100%.

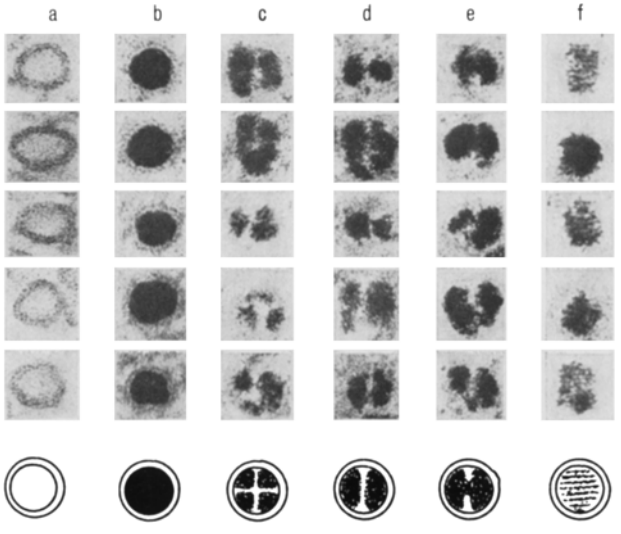

Fig. 1. Profiles of synaptic vesicles in the *Octopus* brain after ZIO-incubation. ×180,000. a) control, incubated in unbuffered stock solution; b) control, incubated in buffered ZIO, following[1]; c)–f) incubated in unbuffered ZIO, pH ca. 4; the reaction product appears divided into 2, 3 or 4 lobes. In f) the precipitate shows a striation.

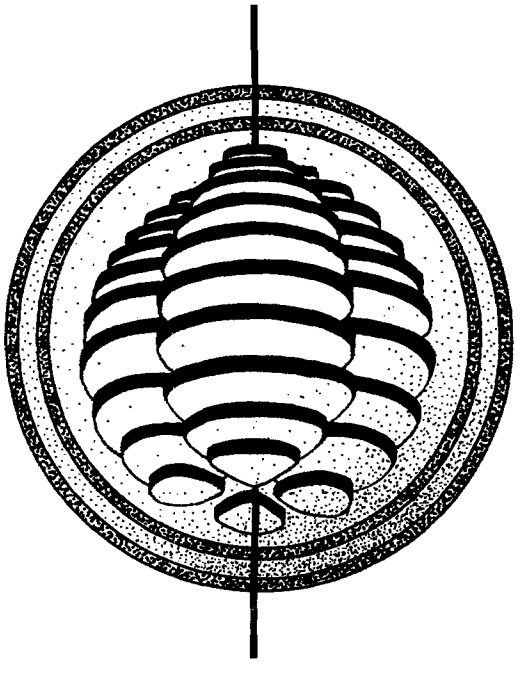

Fig. 2. The spatial structure of vesicular content after the incubation in unbuffered ZIO. It results from combining the profiles c)–f) in Figure 1. The diameter of the vesicle is about 38 nm.

Vesicles showing an entirely electron-dense content after normal ZIO-treatment[1] (pH 7.2) exhibit incomplete impregnation by using unbuffered ZIO (pH ca. 4). Moreover, the reaction product of unbuffered ZIO gets arranged in a regular fashion within the membrane of the vesicle. The most frequent patterns of vesicle content obtained in thin sections are shown in Figure 1. The spatial structure of the reaction product (Figure 2.) is demonstrated by combining the sectional patterns. Thus a reacted vesicle consists of a spherical membrane which envelops 4 lobes arranged around a central axis. The lobes are shaped like orange segments and each one consists of about 8 to 10 lamellae.

The ZIO-method was discovered in 1962 by MAILLET[3], who modified the old technique of CHAMPY[4]. The first scientist who tested the method with the EM was GARRETT[5]. Since then, a great amount of work has been done using this technique; however the discussion about the nature of the reactive substances is still going on. While investigations on the specifity of several transmitters did not provide convincing results[6,7], the hypothesis of MAILLET[3] and NIEBAUER[8] still has not been rejected; they suppose lipoproteins to be the site of reaction. Their best pieces of evidence are 1. the failure of the ZIO-impregnation after the use of lipid solvents and 2. theoretical considerations about the ability of ZIO to uncouple lipids from protein and to deposit metal on the free lipids.

The present investigation shows that the homogeneous black vesicle content of the normal ZIO-technique[1] can be made to exhibit a particular pattern by the use of unbuffered solutions. This suggests that the configuration of the vesicle content is pH-dependent: at pH 7.2 the precipitate is amorphous and at pH ca. 4 crystalline. The hypothesis[3,8], that the site of ZIO-reaction is probably either a protein or a lipoprotein, is supported by the present result, since the configuration of these substances is also pH-dependent. However, the question of the origin and the nature of the reactive substance and its role in the fate of synaptic vesicles needs further work.

Zusammenfassung. Die Inkubation synaptischer Bläschen von *Octopus* in ungepuffertem Zink Jodid-Osmium lässt eine regelmässige Strukturierung des Bläscheninhalts erkennen.

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