*Results and discussion.* Electron-dense granules of 50–120 Å are visible in the center of the synaptic vesicles; occasionally they are excentrically situated and bound to the vesicular membrane (Figure 1). Additional staining with uranyl acetate and lead citrate increases the electron-density and the size of the granules (Figure 2). Vesicles with a similar dense center are noted scattered throughout the presynaptic part of the synapses. Treatment of the material with trichloroacetic acid, employed in biochemistry for the precipitation of proteins, leads to formation of larger and denser granules, but considerably damages the structure. Single particles are also found within the synaptic cleft, as well as along the mitochondrial membrane, and within the sacs of the smooth endoplasmic reticulum. The adrenergic vesicles observed in substantia nigra showed their typical dense core. Deamination accounts for a strong reduction of the contrast staining of particles in the vesicles up to their full disappearence (Fig. 3), whereas digestion with trypsin, before or after acid hydrolysis, leads to completely negative reaction for dense granules' demonstration. Based on the above data, it may be assumed that it is a matter of protein structures' visualization. Most probably, the effect of acids is reduced to immobilization of the protein substance within the synaptic vesicles, which makes possible its demonstration with $OsO_4$. The failure to demonstrate this presumably low-molecular protein[4] using the routine electron microscopic method, may be attributed to the loss of protein during $OsO_4$ fixation, and during the following dehydration[5]. Recent data reported by WHITTAKER and ZIMMERMANN[6] prove that, in the composition of the acid protein-vesiculin, which plays the role of an acetylcholine carrier substance, a great amount of amino acids participate, such as: glutamine, asparagine, serine, proline and lysine, which react actively with $OsO_4$[7]. It is presumed that acid hydrolysis accounts for splitting of a number of peptide bonds in this protein or in the transmitters of protein character[8], and the amino acid groups liberated by this way enter into reaction with $OsO_4$.

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## Cobalt-Staining of Motor Nerve Endings in the Locust (*Locusta migratoria*)

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*Summary.* A method is described for axonal cobalt-staining of peripheral nerve branches. First experiments were carried out on abdominal muscles of the locust.

It is often desirable to confirm electrophysiological results about the peripheral branching of a particular nerve by histological methods. For the analysis of central projections of identified neurones, intracellular staining with Procion Yellow[1] or cobalt[2] and axonal iontophoresis[3] of these dyes have proved most valuable. In the following paper, it is shown that axonal iontophoresis of cobalt is also applicable to peripheral nerve branches.

In order to permit staining of very short and thin nerve stumps, difficult to handle otherwise, a gap-electrode was developed. The dorsal nerve of the second free abdominal ganglion of *Locusta migratoria* was chosen as a test object for optimal staining conditions. USHERWOOD[4] saline, modified by addition of 5 m*M* sodium-acetate and 5 m*M* glucose was used.

*Procedure.* The abdomen of the locust is opened by longitudinal incisions and pinned out in a chamber containing saline continually bubbled with carbogen. The nerve is sectioned near the respective ganglion. Then, the electrode is positioned above the nerve stump with the aid of an appropriate holder. The electrode basically consists of 2 concentric rings of platinum wire (Figure 1). The outer ring holds a drop of low viscosity liquid paraffin. By means of a fire-polished microcapillary, a drop of distilled water is applied to the inner ring. The nerve

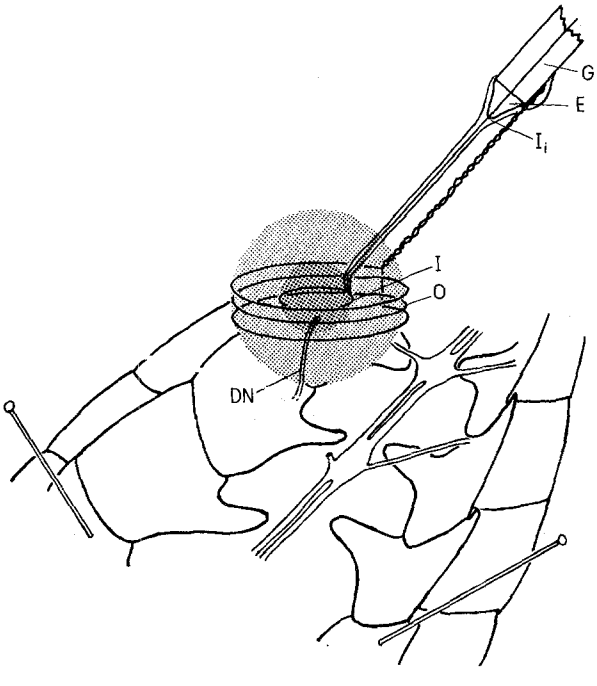


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Fig. 1. Electrode and preparation. I, inner ring (diameter 1 mm) containing $CoCl_2$; O, outer ring (diameter 3 mm) containing liquid paraffin. Dimensions may be adapted for finer nerve branches. $I_i$, silicon gum coating to provide electrical insulation of the inner ring in order to allow passage of current; G, supporting glass capillary (diameter 1.5 mm); E, Epoxi-glue; DN, dorsal nerve.

stump is then sucked into the capillary and transferred to the drop of water. Finally the water is replaced by 1 *M* $CoCl_2$-solution, which is allowed to diffuse down the nerve fibres for approximately 8 h at 29 °C. After that, cobalt is precipitated with ammonium sulphide (0.5% in saline for 15 min). Fixation is in 2.5% glutaraldehyde at pH 7.5, followed by dehydration in ethanol. Muscles are then dissected out and embedded in Caedax as whole mounts.

The method described allows tracing of peripheral nerves. Ramifications down to 3 µm in thickness, possibly being preterminal, were regularly stained, clearly showing multiterminal innervation (Figure 2a). Actual dimensions, however, may be somewhat different, because a certain degree of swelling of the axons as well as glia-staining

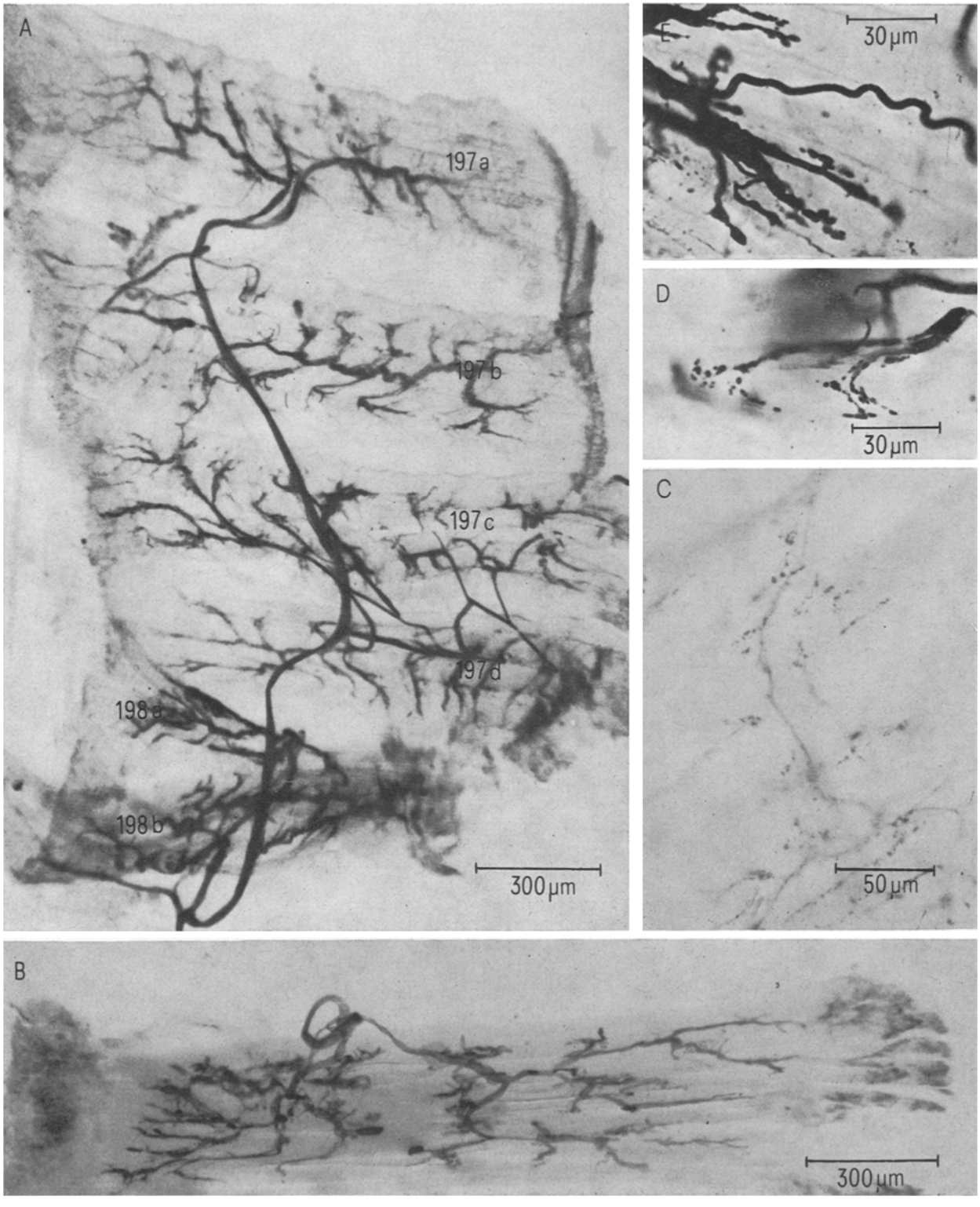


Fig. 2. Innervation of abdominal muscles. A) M197 and 198, showing regularly spaced multiterminal innervation. B) M199; innervation in two distinct fields. C) Nerve endings in M202. D) Beaded endings in M202 (Timm's method). E) Compact endings in M198 (Timm's method).

cannot be excluded. Sometimes, very thin (0.4 μm diameter) beaded arborizations of the nerve fibres were seen (Figure 2c), resembling, for instance the endings in the depressor tibiae muscle of the locust, stained with methylene blue by AUBER[5].

Better resolution was obtained by applying Timm's intensification method after cobalt-staining[6]. This revealed two different types of nerve-endings: a compact type in muscles M197 and M198 (Figure 2e) and a more dispersed one (Figures 2c and d) in M202 and M203 (nomenclature after[7]). Electrophysiological and ultrastructural investigations have shown that the abdominal intersegmental muscles of locusts each receive nerve endings from several axons[8–10]. However, with cobalt-staining multiple innervation could not be demonstrated as yet, although in some cases, two fibres could be seen ramifying side by side. An interesting feature is the innervation of some muscles in two distinct fields which are similar in different individuals (Figure 2b). Whether this finding has also physiological implications concerning, for instance innervation by two functionally different nerve fibres, remains to be investigated.

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## The Origin and Propagation of Upper Urinary Tract Contraction Waves. A New in vitro Methodology

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*Summary.* A new in vitro experimental method which has enabled direct visualization of the upper urinary tract with minimal damage to the wall of the region is described. Evidence is presented which supports the concept of an autonomous pacemaker system located proximal to the pelviureteric junction.

The present paper describes an experimental method which enables direct visualization of the region in which contraction waves originate in the upper urinary tract, namely, that part proximal to the pelviureteric junction. New data are provided about the origin and progagation characteristics of contraction waves in unicaliceal and multicaliceal systems demonstrating their species differences in pacemaker activity.

*Materials and methods.* The kidneys and ureters were removed in toto and the cortex was incised along its lateral border in a plane midway between the anterior and posterior surfaces from upper to lower poles, through the cortex into the outer medulla. In the dog, the medulla formed a single ridge projecting into the lumen of the ureter and the plane of the incision divided this ridge longitudinally. In the pig, each minor calix lying in the plane of the incision was identified by blunt dissection of renal medullary tissue. The interior was then partially exposed by an incision minimizing interference with the renal attachments of the minor calyces. Specimens were transferred into a tissue bath containing Krebs solution. The anterior and posterior surfaces of each kidney were retracted along the line of the original incision to allow visualization as far distally as the pelviureteric junction (Figure). The ureter was placed so that ureteric contraction waves could be easily visualized. Observations and filming were begun after a tissue stabilization.

*Results and discussion.* Regular spontaneous contraction waves were readily observed crossing the exposed inner surface of the upper urinary tract as soon as the preparations were examined. These rhythmical events continued throughout the period of observation (up to 3 h). Whilst the exact sites of origin and the rates and form of propagation of each wave varied according to the species, every contraction began proximal to, and always moved initially towards, the pelviureteric junction. The fact that spontaneous activity can be directly observed is evidence against those who believe that contraction waves are initiated solely by the stretching forces[1]. In the present study, distention forces were reduced since the renal pelvis was converted into an open system thereby decreasing the resting tension on the renal attachments. Thus, the present findings support the concept of spontaneously active pacemaker cells responsible for the initiation of contraction waves[2–4]. It is argued that the active pacemaker site is located at the pelviureteric junction[5]. The present observations, however, clearly indicate that in both unicaliceal and multicaliceal systems, contractions always develop proximal to the pelviureteric junction and propagate towards this region.

In the dog, regular contraction waves occurred at a rate of 13–17/min, and were seen to arise on the wall of the renal pelvis a few mm distal to the septa which attached the pelvis to the renal parenchyma (Figure). Each wave moved distally along a concentric front and approached the pelviureteric junction at 1–2 cm/sec but waves failed to propagate along the ureter. In the pig, contraction waves usually originated from circumscribed regions in the junctional area between one minor calix and the renal pelvis. Although one such region acted as the source for several contractions, this activity frequently changed to a similar region in another part of the system. Contraction waves were observed 5–9/min and each moved from its origin across the adjacent part of the renal pelvis towards the pelviureteric junction forming a crescentic wave front (1–2.5 cm/sec). At the pelviureteric junction, however, the wave frequently continued across the pelvis in a direction away from the ureter moving towards that pole of the kidney opposite to the one in which the contraction had originated. As in the dog, peristaltic waves are not observed along the pig ureter.

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