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THE CHARACTERISTICS OF SPONTANEOUS AND EVOKED ACTION POTENTIALS RECORDED FROM THE RABBIT'S UTERINE NERVES

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SUMMARY

1. A method is described for recording *in vivo* the action potentials of afferent and efferent fibres in whole nerves supplying the rabbit's uterus.

2. Examination of these nerves under the light microscope and the electron microscope showed them to be composed almost entirely of non-myelinated fibres.

3. Two types of spontaneous action potential were observed; one travelled at about 4 m/sec and probably came from the myelinated fibres, the other travelled at 0·4–1·4 m/sec and certainly came from non-myelinated fibres.

4. The efferent fibre spikes were shown to be faster and higher than the spikes from uterine afferent fibres, but slower and smaller than spikes from broad ligament afferent fibres.

5. Apart from differences in conduction velocity and height, all spikes were basically similar, lasting about 1·5 msec. The height was related to the square of the velocity. Some more complex spikes were also observed.

6. The compound action potential evoked by stimulation of the uterine nerve had three peaks, conducted at 1·3, 0·8 and 0·6 m/sec, respectively, and thought to correspond to the fast afferent fibres, the efferent fibres and the slow afferent fibres, respectively. There were also some late peaks due to reflexion of the antidromic action potentials from the ganglion cells.

7. Stimulation of the hypogastric nerve also evoked a compound action potential in the uterine nerves. Stimulation of the pelvic nerves had no effect.

8. By means of ganglion blocking agents, the uterine ganglia were shown to lie in the pelvic plexus, peripheral to the hypogastric nerve, but central to the uterine nerves.

9. It is argued that the spontaneous action potentials came from individual fibres rather than Remak bundles, and that the recording technique used detected the activity of all but the smallest fibres.

INTRODUCTION

An electrophysiological investigation of the rabbit's uterine nerves was undertaken in the hope of overcoming some of the limitations imposed on indirect methods by the sensitivity of the uterine muscle to non-nervous factors. Previous experiments on the uterine innervation have been reviewed by Reynolds (1949) and Marshall & Chassar Moir (1952). The nerves contain myomotor and vasomotor efferent fibres which leave the spinal cord in the lumbar roots and travel to the pelvic plexus in the hypogastric nerve (Langley & Anderson, 1895*a*; Schofield, 1952). They also contain afferent fibres from mechano-receptors in the uterus and broad ligament (Bower, 1959). It is not certain whether there is a sacral contribution to the uterine nerves, nor is the site of the uterine ganglia definitely established (Langley & Anderson, 1895*a*, *b*; Schofield, 1952; Varagić, 1956). Histological investigations have shown there to be no intramural plexuses analogous to the intestinal nerve plexuses, though there may be a few scattered cells (Pallie, Corner & Weddell, 1954). Present information about the structure of the nerve endings gives little indication of their functions. The efferent nerves appear to be adrenergic; stimulation of the hypogastric nerve causes the release of adrenaline and noradrenaline into a uterine perfusate (Mann & West, 1951), and Schofield (1952) and Varagić (1956) have reported further evidence which supports this.

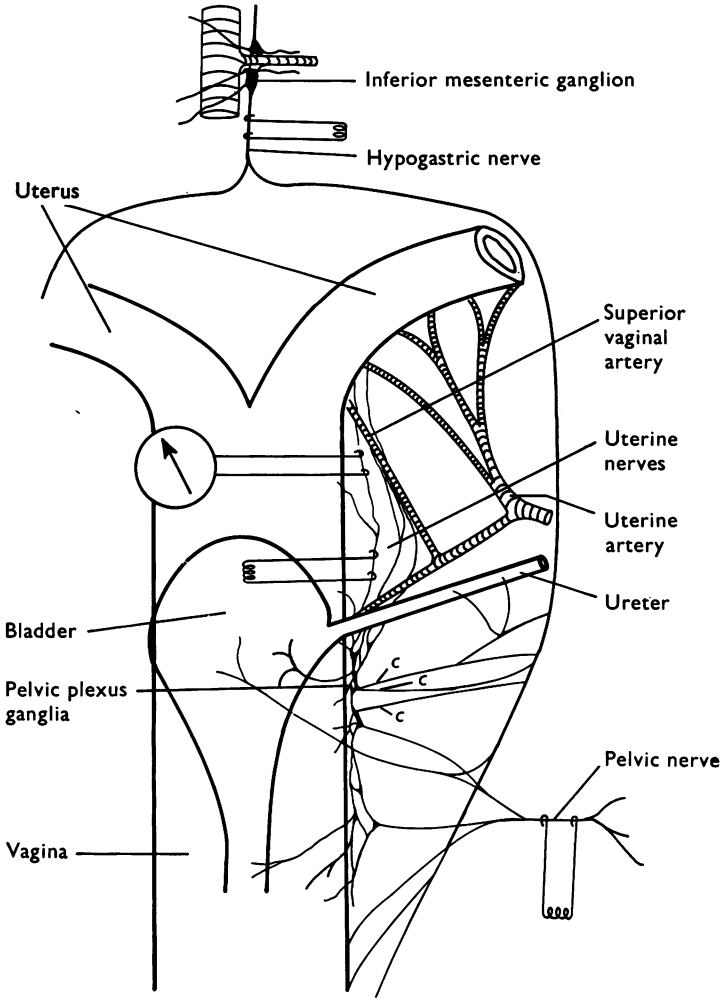
In this paper the experimental technique is described and the action potentials recorded are shown to have come from non-myelinated fibres and to have velocities which place them in group C. An interpretation of the compound action potentials evoked in the uterine nerves by stimulation is suggested, and it is shown that the uterine ganglia must lie within the pelvic plexus. Preliminary accounts of some of the results have been published elsewhere (Bower, 1959, 1962).

METHODS

The experiments were carried out on nerves supplying the rabbit's uterus *in vivo*. Control of the reproductive state was not attempted, but the condition of each animal was noted during the experiment.

The anatomy of the rabbit's pelvic plexus. This has been described by Langley & Anderson (1896), and the relevant details are shown diagrammatically in Text-fig. 1. The uterine nerves arise from a cluster of small ganglia in the pelvic plexus on the lateral wall of the vagina, and pass to the uterine horns in the broad ligament close to the superior vaginal artery. Reynolds & Kaminester (1935) have shown by physiological techniques that there are no intramural nerve tracts. There were usually about a dozen of these fine branching nerves, each between 50 and 200 μ in diameter, in many instances accompanied by small blood vessels, with some of the branches anastomosing to form a network. Peripherally they plunged into the uterine fat, which precluded tracing them up to the uterus; their ultimate terminations were inferred from the distribution of their sensory receptors. Occasionally a

nerve was found next to the uterine vein, containing afferent fibres from the Fallopian tube and bursa. The pelvic plexus is supplied by the pelvic nerve, from spinal roots S II, III and IV, and by the hypogastric nerve, which is a single nerve in the rabbit, lying just ventral to the aorta. The hypogastric nerve arises from the inferior mesenteric ganglion and divides



Text-fig. 1. Diagram of innervation of the rabbit's uterus showing the positions of the recording and stimulating electrodes. Adapted from Langley & Anderson (1896) Fig. 7.

distally to supply the left and right pelvic plexuses. Several fine nerves branch off during its course and rejoin it distally, forming loops in the mesocolon. The inferior mesenteric ganglion is connected with the sympathetic chain by three inferior splanchnic nerves on each side; a fourth nerve emerges between the bifurcation of the aorta to join the hypogastric nerve directly. There are thus three possible sites for the uterine ganglionic synapses: sympathetic chain ganglia; the inferior mesenteric ganglia; the pelvic plexus ganglia.

The experimental procedure. The rabbits were anaesthetized with urethane solution (25 g/100 ml. in NaCl solution 0·9 g/100 ml.) injected into an ear vein. The animal was secured on its back on a warm table and the trachea was cannulated. Cannulae were also placed in the left jugular vein for the intravenous injection of drugs and in the right carotid artery for recording the arterial blood pressure.

The abdomen was opened by a mid-line incision from the pelvic brim to the umbilicus. The nerves to the left uterine horn were used for most experiments. A slit was made in the left broad ligament, either medial or lateral to the uterine vein according to size, through which a dissecting platform could be passed. The platform was of Perspex, with a black background and a narrow cork rim to which the tissues could be pinned; its total area was 1·5 cm square. The broad ligament was tented out from the vagina and pinned down on the platform to display the superior vaginal artery. The abdomen was then closed as far as possible and any exposed tissues were packed with moist swabs.

After the top layer of the broad ligament had been pulled away, the nerves could be seen through a binocular dissecting microscope in the triangle formed by the superior vaginal artery, the vesical artery and the vagina. One nerve was freed, cut and stripped away from its blood vessel if possible. The cut end, peripheral or central according to the experiment planned, was lifted onto a fragment of non-wettable glass coverslip. The glass had been made water-repellent by treatment with a solution of 2 % (v/v) dimethyldichlorosilane in CCl_4 ('Repelcote'; Hopkin and Williams Ltd). The pair of recording electrodes 1 mm apart were placed on the nerve on the glass, and the preparation was covered by a drop of silicone oil at 37° C (silicone fluid MS 200/1000; Midland Silicones Ltd.). Silicone was preferred to liquid paraffin as it was more viscous at body temperature, and the nerves survived better and kept their normal appearance. The impedance of the 1 mm length of nerve, judged by the noise level, was at least 100 kΩ, and was sometimes nearly 1 MΩ. It will be shown that this electrode separation was adequate for recording the non-myelinated fibre spikes without serious distortion (see p. 737).

To compare spikes from afferent and efferent fibres, it was necessary to record them under identical conditions; that is, from the same nerve at the same time. A nerve was dissected free without being cut, a fragment of siliconed coverslip was eased under it, and the preparation was set up as before. A considerable length of nerve had to be freed to minimize short-circuiting of the electrodes by inactive tissue, which made this a difficult preparation to use.

The nerve was rinsed with silicone oil at 37° C before each series of records taken for measurement; no attempt was made to measure the temperature of the nerve, and it is likely that this was actually less than 37° C.

The fine dissection was facilitated by an injection of heparin (10 mg in 1 ml. saline i.v.) originally given to keep the cannulae free from clots. It prevented clotting of the tissue fluid and blood at the dissection site; this was an advantage which outweighed the disadvantage of increased bleeding.

The stimulating electrodes. Before the recording apparatus was set up, the hypogastric nerve was usually exposed and lifted on to a pair of silver wire stimulating electrodes. In some experiments the hypogastric nerve and its colonic loops were cut to eliminate central connexions and touched with 3 % procaine solution, but when the normal efferent discharge was to be observed these nerves were left intact; this made no difference to the results of stimulation by the short low-frequency trains of stimuli used in these experiments. After the dissection was finished, the abdominal contents were replaced over the electrodes.

In a few rabbits the left pelvic nerve was approached by dissecting down next to the vagina at the level of the pelvic brim, and a pair of silver wire stimulating electrodes enclosed in a Perspex shield were slipped under the nerve. This dissection was not carried out as a routine procedure as it endangered the pelvic plexus.

After the recording electrodes had been set up, a pair of silver wire stimulating electrodes

were rested on the same nerve without further dissection 0·5–1·5 cm central to the recording electrodes.

Apparatus. The recording electrodes were a pair of 46 s.w.g. bright silver wires about 5 mm long mounted on a pair of steel needles. The latter were mounted on a manipulator consisting of a ball and socket joint giving horizontal movement, carried on a rack and pinion for vertical movement. The flexibility of these fine wires allowed some movement of the preparation without disturbance to the recording (e.g. during a vaginal contraction). An earth connexion was made through a silver plate placed on the bladder.

The action potentials were amplified by an a.c. differential amplifier and displayed on a Cossor split-beam oscilloscope; the maximum deflexion sensitivity was 17 μV/cm, and time constants of 10 or 500 msec could be selected. The spikes were also monitored by means of a loudspeaker. The high-frequency limit of the amplifier was 5 kc/s; this was usually reduced to 1 kc/s to improve the signal-to-noise ratio, but it was increased to its maximum when spikes were recorded for measurement. The oscilloscope display was generally photographed on stationary paper using a sweeping spot; the records were measured with grids prepared photographically using the oscilloscope to allow for the distortion introduced by this instrument.

Single and repeated stimuli were supplied by a single channel square-wave stimulator which could also trigger the oscilloscope.

Time marks at 1 msec and 10 msec were provided by a Furzehill tuning fork time marker.

Blockade of ganglionic transmission. This was induced by an injection of hexamethonium bromide (15 mg/ml.) or, in a few experiments, of nicotine hydrogen tartrate (0·3 mg/ml.), pentamethonium bromide (15 mg/ml.) or tetraethyl ammonium bromide (T.E.A.) (5 mg/ml.) All salts were dissolved in NaCl solution (0·9 g/100 ml.). All weights refer to the active principle.

Histological techniques. To demonstrate the presence or absence of myelinated fibres, short lengths of nerves from which records had been obtained were stained overnight in osmium tetroxide solution (0·75 g/100 ml.). They were then dehydrated, cleared in xylol and mounted whole in canada balsam.

For preparation of electron micrographs, short lengths of uterine nerve were gently stretched by tying them round coverslips, and fixed by Palade's standard method. They were then embedded in methacrylate, sectioned at 60 mμ and mounted on grids between formvar and carbon. The electron micrographs were prepared by Professor A. F. Huxley.

RESULTS

The structure of the uterine nerves

Altogether, twenty-six nerves from ten rabbits were examined by means of the osmium technique described, after records had been obtained from their afferent fibres. Electron micrographs were prepared from adjacent lengths of two of these nerves as well, to confirm the results of the osmium staining. One osmium-stained nerve is shown in Pl. 1, fig. 1, with the spikes recorded from it in Pl. 1, fig. 2, and a field from the electron micrograph of the same nerve is shown in Pl. 2. The nerves were sufficiently fine for any myelinated fibres present in the whole mount to show clearly as grey strands against a brown background; the non-myelinated fibres were faintly outlined in grey. It was evident from the electron micrographs that these were true non-myelinated fibres, and not an intermediate type; e.g. lightly myelinated fibres or fibres with a long mesaxon. The electron micro-

graphs also confirmed that the osmium had stained all the myelinated fibres present.

None of the nerves examined contained more than five myelinated fibres; most contained none at all. The diameters of the myelinated fibres ranged from 2 to 5 μ. A broad ligament from one rabbit was rolled up, stained in osmium, embedded in paraffin and sectioned serially at 3 μ. There were nine nerves in the bundle, from 30 to 100 μ in diameter; the smallest contained one myelinated fibre, and in the rest there were none at all. This 30 μ nerve also contained at least 130 non-myelinated fibres and two Schwann cell nuclei.

The range of non-myelinated fibre sizes could not be judged from the electron micrographs available. Many of the fibres appeared to occupy individual Schwann sheaths, and few sheaths contained more than three fibres. The sheaths were separated by unusually large amounts of collagen. As serial sections were not prepared, it is not known whether the sheaths formed a syncytium similar to that described by Gasser (1955).

The spontaneous action potentials

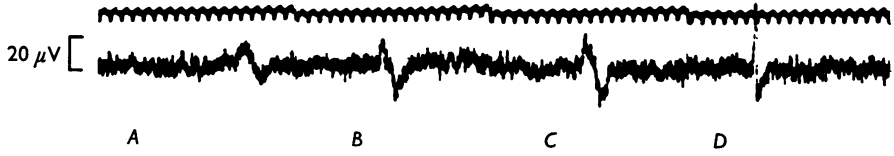
When the electrical activity of the uterine nerves was compared with their structure, as in Pl. 1, it was immediately apparent that most of the spikes recorded must have come from non-myelinated fibres.

Conduction velocities were calculated from the distance between the recording electrodes (about 1 mm) and the times between the peaks of the diphasic action potentials. Measurements were only taken from spikes in which the two phases were similar, indicating that the spikes had been conducted past both electrodes; i.e. that the fibres were not damaged at the recording site. In view of the poor signal-to-noise ratio and the uncertainty about the temperature, any figures given must be regarded as orders of magnitude rather than absolute values. They are nevertheless useful for comparisons between spikes recorded under identical conditions.

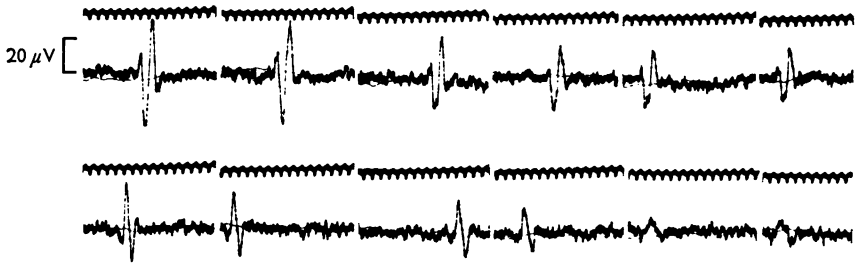
Types of action potentials. Two distinct types were recorded; the first probably came from the myelinated fibres, whereas the second certainly came from the non-myelinated fibres as it was yielded by nerves containing no myelinated fibres at all.

An example of the first type of spike is shown in Text-fig. 2, together with three of the second type from the same nerve for comparison; another is shown in Pl. 1, fig. 2. These spikes were rarely observed, and have been recorded from afferent fibres only. Their velocities could not be measured accurately, but were of the order of 4 m/sec, which places them in group A. The spikes were characterized by a large first phase and a small second phase. The short interval between the two peaks of the diphasic spike caused a sharp crack on the loud-speaker, clearly different from the dull

pop given by the C spikes. The A spikes were usually larger than the C spikes, though not very much so; indeed, the A spike in Pl. 1, fig. 2*B*, is smaller than the exceptionally large C spike in Pl. 1, fig. 2*A*, recorded from the same nerve.



Text-fig. 2. Four action potentials recorded from afferent fibres in the peripheral end of a cut uterine nerve. *A*, *B*, *C*, typical action potentials from non-myelinated fibres; *D*, an action potential probably from a myelinated fibre. Time marks: 1 msec.



Text-fig. 3. Action potentials recorded concurrently from an uncut uterine nerve. Upper row: potentials from efferent fibres. Lower row: potentials from afferent fibres. The tetraphasic form of the spikes is probably due to an artifact of the type described by Donaldson & Robson (1965). It can be seen that the efferent and afferent fibre spikes are essentially similar. Time marks: 1 msec.

Spikes of the second type were recorded in all experiments from both afferent and efferent fibres; they travelled at 0·4–1·5 m/sec, which places them in group C. Text-figure 3 shows a series of such afferent and efferent fibre spikes recorded concurrently from an uncut nerve. It can be seen that all the spikes are similar in appearance, differing only in height and interphase spacing, except for the very smallest ones which were more rounded and elongated; this may have been due to distortion by the nerve sheath, which probably had a space constant comparable to the lengths of the shortest action potentials. The spikes in Text-fig. 3 are tetraphasic; the first and last phases are artifacts associated with recording from an uncut nerve earthed at both ends, as they were not seen in spikes recorded from cut nerves. They are probably similar in nature to the artifact described by Donaldson & Robson (1964).

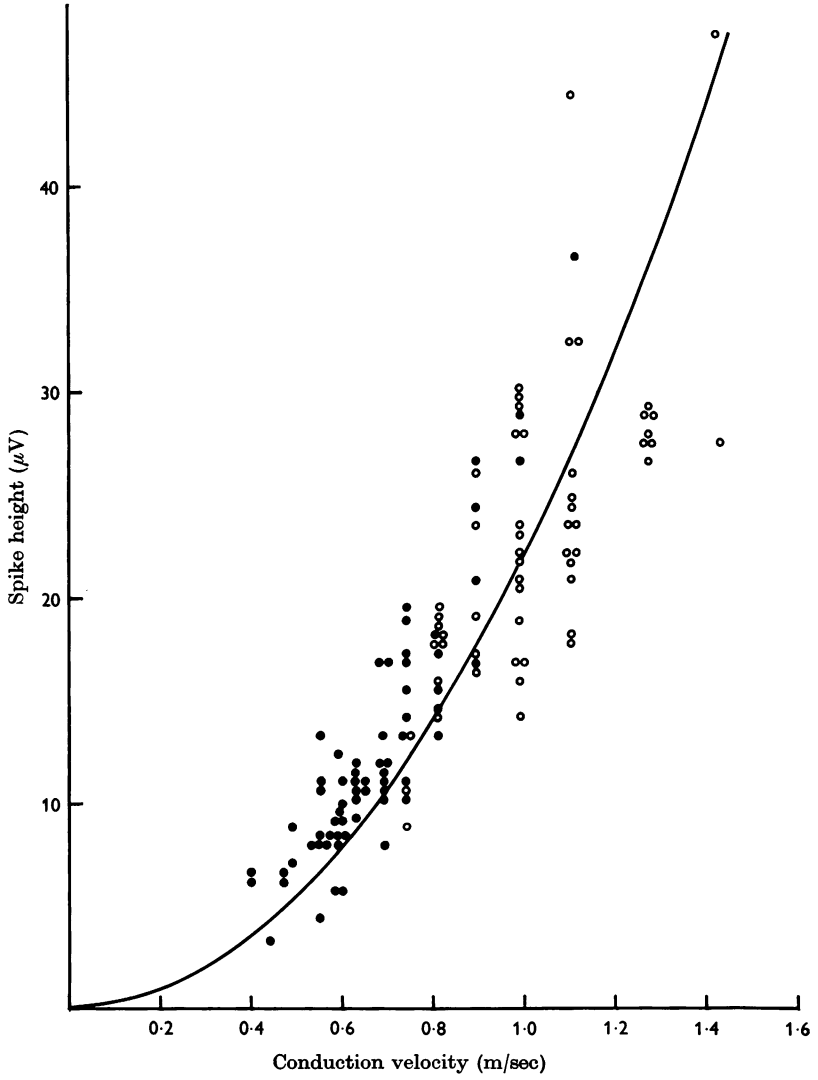
The duration of the action potentials. Monophasic action potentials have not been recorded successfully, but their durations can be estimated by

subtracting the time between the two peaks from the total duration of the diphasic potential. The duration of all except the smallest potentials measured, both A and C, was about 1·5 msec, regardless of conduction velocity. The smallest potentials were somewhat longer, lasting up to 2 msec. The rise time of all spikes was about 0·25 msec, but the true value may have been shorter, as this was near the high-frequency limit of the amplifier. From these figures, it appears that an electrode spacing of 1 mm could accept the rising phase of a spike travelling at up to 4 m/sec, but would attenuate the falling phase of any spike faster than about 1 m/sec. In the case of an impulse conducted at about 4 m/sec or above, the recorded spike would approximate to the first differential of the action potential; this could account for the distinctive shape of the A spikes, which have the long second phase much smaller than the short first phase. Experimentally, increasing the distance between the electrodes to 2 or 3 mm did not alter the shapes of the C spikes, apart from increasing the inter-phase spacing, whereas reducing the distance to 0·25 mm attenuated the second phase, making the C spikes resemble the A spikes recorded in other experiments; no A spikes were observed in this experiment.

The relation between size and velocity. The sizes of the spikes depended on the recording conditions; typically they were about 40 μV, but 150 μV spikes have been recorded from efferent C fibres under favourable conditions. As described before (Bower, 1959), two classes of afferent spikes were observed: large C and A spikes from receptors in the broad ligament, and small C spikes from receptors in the uterus. The efferent fibre spikes were intermediate in size. To confirm this observation, and to establish that there were corresponding differences in conduction velocity, the spikes from the experiment illustrated in Text-fig. 3 were measured from the base line to peak of the first phase, and the heights so obtained were plotted against the conduction velocities (Text-fig. 4). Two other experiments on uncut nerves and two on cut nerves gave similar results but fewer points. Although there is considerable overlap, the spikes from the afferent fibres are clearly smaller and slower than those from the efferent fibres. Most of the afferent fibre spikes had velocities in the range 0·4–0·9 m/sec whereas most of the efferent fibre spikes were in the range 0·8–1·3 m/sec. No large afferent fibre C or A spikes were noticed during this experiment. Despite the scatter of points due to the poor signal-to-noise ratio, the spike height is clearly related to the conduction velocity; a second-power curve through the origin fits the points reasonably well.

Complex spikes. In addition to the simple spikes described so far, complex spikes were seen, especially in the more crowded records. Some examples are shown in Text-fig. 5. Most were like those in Text-fig. 5*C*, and were probably due to coincidental superposition of spikes. A very few

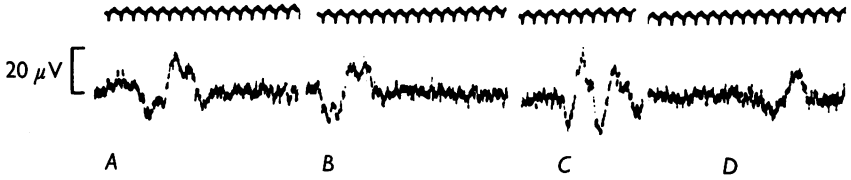
(Text-fig. 5*A*, *B*) resembled the interacting spikes shown by Katz & Schmitt (1940), and could have been caused by interaction between adjacent fibres.



Text-fig. 4. The relation between the heights and conduction velocities of spikes recorded concurrently from afferent and efferent fibres in an uncut uterine nerve. ●, afferent fibre spikes; ○, efferent fibre spikes. The continuous line is a second power curve fitted by eye.

The evoked action potentials

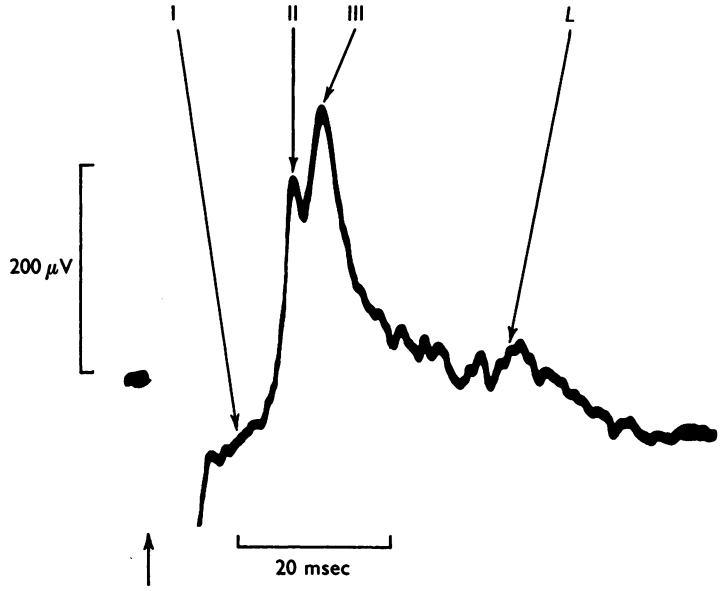
Compound action potentials were recorded from a uterine nerve when the hypogastric nerve or the same uterine nerve were stimulated. Stimulation of other uterine nerves, or of the pelvic nerve, had no effect. The pelvic nerve has been stimulated in only five rabbits, so the last result is not conclusive in view of Schofield's (1952) observation of uterine contractions in four out of twenty rabbits after pelvic nerve stimulation.



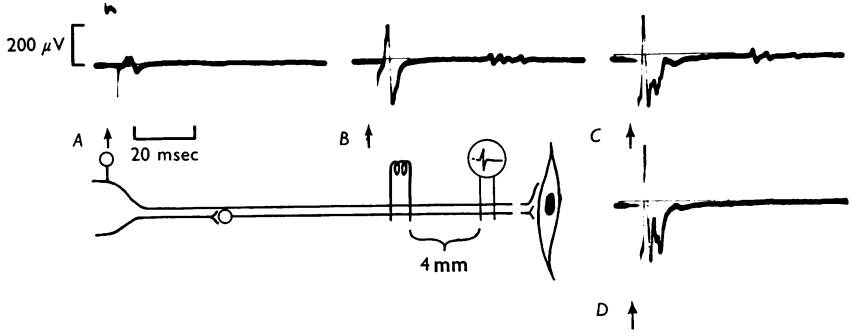
Text-fig. 5. *A*, *B*, *C*, complex spikes recorded from efferent fibres in a uterine nerve; *D*, a simple spike from the same nerve for comparison. Time marks: 1 msec.

The potential evoked by uterine nerve stimulation. The wave form and conduction velocity of this potential were examined. The difficulties of recording monophasically from such short fine nerves are considerable, and few acceptable records were obtained; one is shown in Text-fig. 6. These were supplemented by measurements from diphasic potentials such as those in Text-fig. 7, in which the peaks of the waves could be distinguished, although the waveform was much distorted. Conduction distances had to be measured on the nerves *in situ*, as the nerves were too easily stretched by dissection; for this reason, and because of the uncertainty about the temperature, figures given for conduction velocities must again be regarded as orders of magnitude rather than absolute values, and are probably lower than the true values.

The compound action potential recorded from a uterine nerve after stimulation 0·5–1·5 cm central to the recording electrodes had three distinct peaks, each with successively higher thresholds for electrical stimulation. The first wave was small and was not noticed in every nerve; it is shown in Text-fig. 7 as a double-peaked wave with a distinctly lower threshold than the second wave. Its velocity was 1–1·3 m/sec. The second and third waves were large and present in all the nerves examined; their peaks travelled at 0·8 and 0·5–0·6 m/sec, respectively. The slowest units in the third wave conducted at about 0·35 m/sec. All these velocities are definitely within the group C range, even if allowances are made for the low temperature; no group A waves have been observed, but this is not surprising in view of the scarcity of myelinated fibres in these nerves. Comparison of the compound action potential with Text-fig. 4 suggests that the second and third waves correspond to the efferent and afferent



Text-fig. 6. A compound action potential recorded monophasically from a uterine nerve still connected to its ganglion. The stimulating cathode was 15 mm central to the recording electrode. I, II, III are the three fibre groups referred to in the text; *L* is the late wave reflected from the ganglion. ↑, point at which the stimulus was applied.



Text-fig. 7. The origin of the late waves of the uterine nerve compound action potential. Conduction through the ganglia had been blocked by an injection of hexamethonium (63·5 mg/kg I.V. divided into four doses). *A*, *B*, *C*, compound action potentials recorded following stimulation of the uterine nerve at increasing stimulus strengths, recruiting waves I, II and III, respectively; *D*, the stimulus strength is the same as in *C*, but the nerve has been cut just central to the stimulating electrodes. The diagram indicates the relative positions of the stimulating and recording electrodes and the ganglia. The late waves had the same threshold and maxima as wave II, and were abolished by cutting the nerve between the stimulating electrode and the ganglion. ↑, point at which the stimulus was applied.

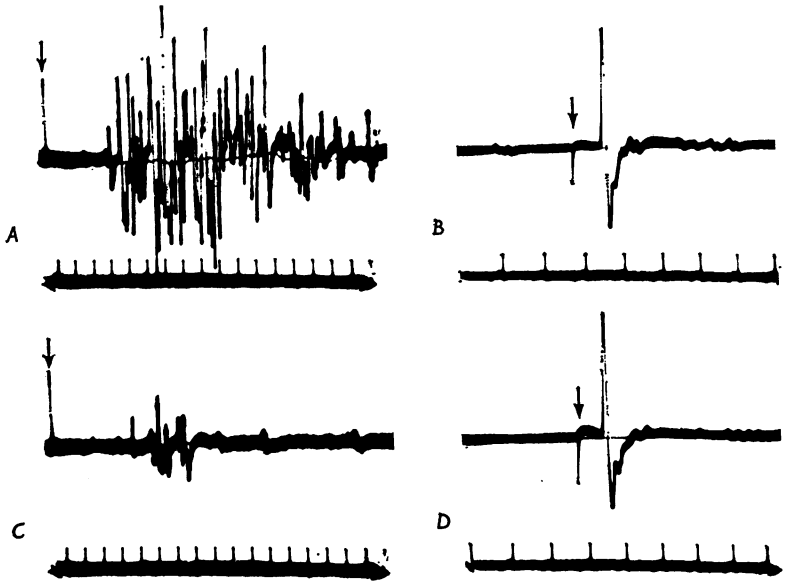
fibres, respectively; the first wave probably corresponds to the fast afferent fibres which are absent from Text-fig. 4.

Provided the nerve was still attached to the pelvic plexus, some additional late waves were recorded which followed those already described and which are shown in Text-fig. 7. They had the same threshold and maximum stimulus strengths as the second wave of the compound action potential (i.e. the efferent fibres), and appeared to have been reflected back from the uterine ganglion cells, since they were abolished by cutting the nerve between the stimulating electrodes and the pelvic plexus, but were not affected by doses of hexamethonium greatly in excess of that required to block conduction through the ganglia completely (20 mg/kg I.V.). In the case of the experiment illustrated in Text-fig. 7, the pelvic plexus ganglia lay 1·5–2·5 cm central to the stimulating electrodes and the latency of the late waves was 35–60 msec; this is what would be expected if the fibres had a conduction velocity of about 1 m/sec, and if the spikes were reflected from the ganglia with little or no delay.

The site of the uterine ganglia. More information on the site of the uterine ganglia has been obtained from the action of ganglion blocking agents on the compound action potential conducted from the hypogastric nerve. This was greatly reduced, though not abolished, by an intravenous injection of hexamethonium (20 mg/kg); the potential from stimulation of the uterine nerve was not affected (Text-fig. 8). Repetition of the injection did not cause any further change in either potential. The same result was obtained by injecting nicotine (0·1 mg/kg). Pentamethonium (20 mg/kg I.V.) and T.E.A. (5 mg/kg I.V.) have been shown to abolish the post-ganglionic after-discharge which follows stimulation (Bower, 1966), but the compound action potentials have not been observed in these cases. That part of the potential conducted from the hypogastric nerve insensitive to blocking agents can be accounted for by the uterine afferent fibres, which must travel to the spinal cord through the hypogastric nerve, there being no response to stimulation of the pelvic nerve. Therefore all, or nearly all, of the ganglion cells supplying the rabbit's uterus must lie peripheral to the hypogastric nerve but central to the uterine nerves.

The ganglia were more precisely identified in one pregnant rabbit with a suitably large pelvis and broad ligament. After the ganglia had been blocked with hexamethonium, the pelvic plexus was exposed, the fine nerves shown in Text-fig. 1 were stimulated and the resulting compound action potentials in the uterine nerves were observed. Stimulation of the uterine nerve at any point back to the ganglia on the vagina elicited a full-sized action potential similar to that in Text-fig. 8*b*. Stimulation of the hypogastric nerve or of the fine strands marked '*c*' in Text-fig. 1 gave a reduced action potential similar to that in Text-fig. 8*C*. Stimulation of

the pelvic nerve or of any other nerves in the pelvic plexus had no effect. The transition from the small to the large action potential at the ganglia was fairly abrupt. In another rabbit these ganglia were dissected out, stained in methylene blue and examined as a whole mount. The ganglia were very diffuse, having several large groups of cells resembling sympathetic neurones but with numerous scattered cells extending up the roots of the uterine nerves.



Text-fig. 8. The action of hexamethonium on compound action potentials recorded from the uterine nerves. *A*, a compound action potential recorded from a uterine nerve following stimulation of the hypogastric nerve; *B*, a compound action potential from the same nerve following stimulation of the nerve 5 mm central to the recording electrodes; *C*, *D*. As for *A* and *B*, respectively, after an injection of hexamethonium (22·6 mg/kg i.v.). Time marks: 10 msec. ↓, points of application of stimuli. The amplification in *A* and *C* is 10× that in *B* and *D*.

The potential evoked by hypogastric nerve stimulation. It can be seen from Text-fig. 8 that the afferent fibre spikes were among the earliest to reach the uterine nerve from the hypogastric nerve, although in the uterine nerves they have been shown to travel slower than the efferent fibre spikes (Text-fig. 4) and to have higher electrical thresholds (Text-fig. 7). In the few monophasic potentials recorded, the wave conducted fastest from the hypogastric nerve was seen to have an appreciably higher threshold than the other waves, implying that the afferent fibres were among the slowest in the hypogastric nerve as in the uterine nerves. The late arrival of many of the efferent fibre spikes from the hypogastric nerve must have been due to delays in the ganglia rather than slow conduction in the fibres.

DISCUSSION

Although there have been several recent reports of activity recorded from mammalian nerve fibres with conduction velocities in group C (e.g. Iggo, 1958), no description of their action potentials has been published since that by Adrian, Bronk & Phillips (1932). Indeed, no description appears to exist of action potentials which are known to have come from mammalian non-myelinated fibres.

Adrian *et al.* (1932) considered that the action potentials which they recorded from the cat's hypogastric nerve were so large that they represented the synchronized activity of groups of fibres rather than individual units. As the potentials in their illustrations were some 20 msec long, their suggestion was probably correct, though the synchronization could not have been close and the number of units contributing to each wave must have been large. The grouping of non-myelinated fibres into communal Schwann sheaths revealed by the electron microscope lent further support for the idea of interaction between the action potentials, and Katz & Schmitt (1940) showed that action potentials in two adjacent crab nerve fibres can interact and keep in step, but only if their conduction velocities do not differ by more than 10%. Gasser (1955) has argued that there is normally little or no interaction between non-myelinated fibres in mammalian nerves, mainly because of the repeated transition of fibres from one bundle to another.

There are several reasons for believing that the simple spontaneous spikes described in this paper represent the activity of individual non-myelinated fibres rather than of synchronized groups. First, the time relations of the spikes were constant under constant conditions, were the same for all C spikes over a wide range of conduction velocities and were the same as or similar to those of the few A spikes observed. This constancy of the time relations agrees with the predictions of the cable theory (Rushton, 1951). Secondly, there is a clear relation between the spike height and the conduction velocity which fits both afferent and efferent fibres (Text-fig. 4); this would not be expected if the spike height depended on a varying number of interacting fibres. Thirdly, the single units encountered, both afferent and efferent, gave spikes of constant height and shape which were entirely comparable to other spikes observed. The largest spikes came from afferent fibres supplying the most localized receptors: those in the broad ligament. Fourthly, the structure of the uterine nerves, with few fibres per sheath, must restrict the amount of interaction possible.

The spikes in Text-fig. 5 suggest that interaction between fibres can occur, but spikes like this were rarely seen, and may have been due to

coincidence. Efferent discharges have been observed to increase from a single unit to an intense discharge without any significant increase in the sizes of the spikes (e.g. Bower, 1966, Fig. 9), implying that interaction plays little if any part at least in the uterine nerves.

The relation between the height and the conduction velocities of the spikes does not fit the predictions from the cable theory. From the cable theory it would be expected that, for non-myelinated fibres, spike height would vary with diameter^2 (Hodgkin & Rushton, 1946) and conduction velocity with $\sqrt{}$ diameter (Rushton, 1951); hence spike height should vary with velocity^4. But no fourth-power curve passing through the origin will fit the points in Text-fig. 4, whereas a second-power curve fits quite well. Gasser (1950) assumed a linear relation between spike height, conduction velocity and fibre size for his reconstructions of dorsal root group C compound action potentials; a straight line can be drawn through the points in Text-fig. 4, but not through the origin. In the case of myelinated fibres it is known that the spike height varies directly with the diameter of the fibres, instead of with the diameter^2; it is not yet known whether this also applies to the uterine nerve C fibres.

The points in Text-fig. 4 and the uterine nerve compound action potentials both cover approximately a fourfold range of conduction velocities. The lowest velocity of the compound action potential was 0·35 m/sec, whereas the slowest spikes in Text-fig. 4, which were at the limit of certain identification, travelled at 0·4 m/sec. It is evident that the technique used was detecting all but the very slowest action potentials in the nerve, even though the recording conditions in this experiment were not unusually favourable; there certainly appears to be no distinct group of fibres in the uterine nerves with potentials too small to be detected by the method described in this paper.

The waves reflected from the uterine ganglia on antidromic excitation of the uterine nerve resemble those reflected from ventral horn cells described by Renshaw (1941), and have not been previously reported from autonomic ganglia. Their insensitivity to ganglion blocking agents differentiates them from similar phenomena described by Brown & Pascoe (1952).

The evidence given here locating the uterine ganglia in the pelvic plexus agrees substantially with the conclusions of Langley & Anderson (1895*b*) and Cushny (1906), who examined the actions of nicotine. Other evidence supports this conclusion. Asphyxia caused a marked increase in the discharge in the uterine efferent fibres, with a respiratory grouping of the spikes (Bower, 1966); this was abolished by any of the blocking agents used, implying that there were no preganglionic fibres at the position of the recording electrodes. Antidromic or orthodromic stimulation of the post-ganglionic neurones by a single shock was followed by a characteristic

after-discharge (Bower, 1966); after the ganglia had been blocked orthodromic stimulation through the hypogastric nerve gave no such response, although antidromic stimulation was still effective, implying that the hypogastric nerve contained no post-ganglionic fibres of this type. However, Schofield (1952) found that although nicotine reduced the uterine response to hypogastric nerve stimulation, pentamethonium in doses of up to 18 mg/kg I.V. had no effect; she therefore concluded that the uterine ganglia were central to the hypogastric nerve. Varagić (1956), using an excised hypogastric nerve–uterus preparation from the rabbit, found that the contractions following nerve stimulation could be abolished by hexamethonium but the dose needed was sometimes very large. He therefore agreed with Schofield, attributing the action of hexamethonium to some unidentified toxic effect.

The evidence presented above leaves little doubt that the ganglia are in the pelvic plexus, and that they can be blocked by both hexamethonium and pentamethonium. The doses required for a complete block are undoubtedly large, and both have the undesirable side-effect of stimulating the ganglia (Bower, 1966), but transmission through the ganglia can be substantially reduced by more moderate doses (e.g. 7 mg/kg), so it is surprising that Schofield (1962) and Varagić (1956) ran into difficulties with these drugs. A possible explanation lies in the syncytial behaviour of the oestrogen-dominated uterus (Bozler, 1937; Kuriyama & Csapo, 1961); this would presumably be capable of giving a full-sized contraction in response to local stimulation by only a few fibres, and thus may be little affected by ganglion blocking agents until the ganglia are completely blocked.

This tendency for a contraction to spread over an oestrogen-treated uterus may also explain why stimulation of the pelvic nerve causes uterine contractions in some rabbits (Schofield, 1952) although no evidence for a pelvic contribution to the uterine nerves could be found in the present experiments. Such contractions may be due to spread of activity from some other parasympathetically innervated part of the genital tract, e.g. the vagina; this interpretation is supported by the long latency of the uterine contraction following pelvic nerve stimulation (Schofield, 1952, Fig. 8).

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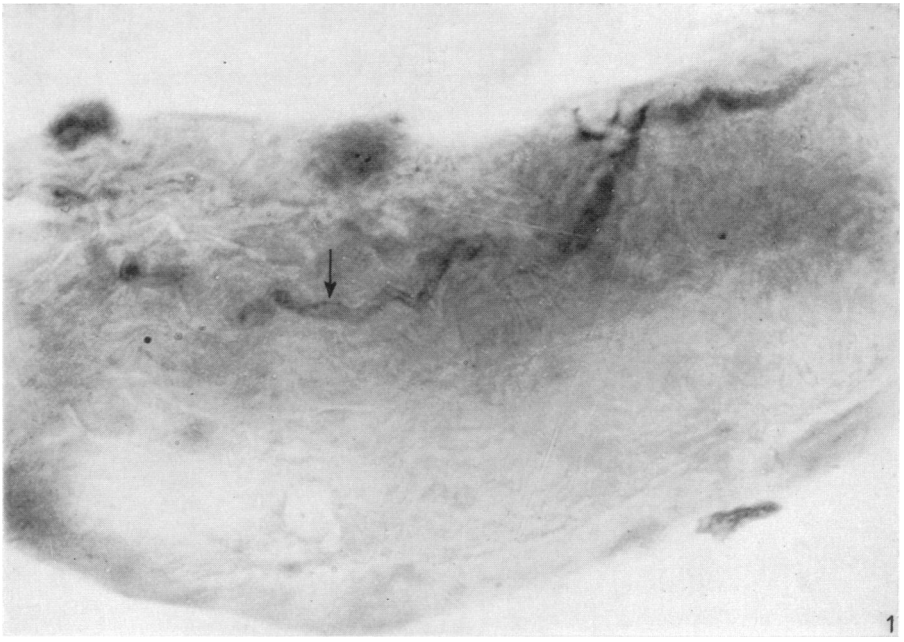

Fig. 1

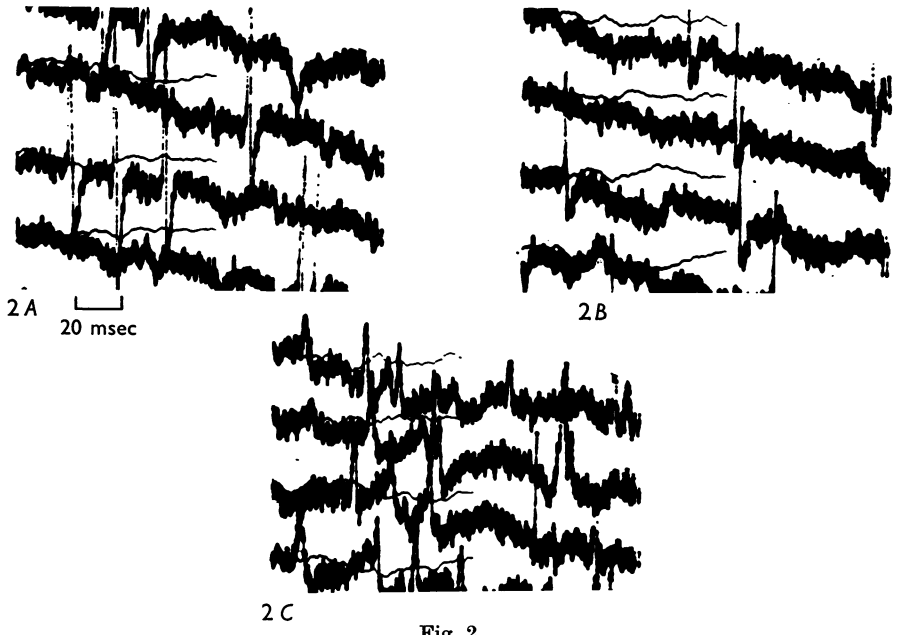

Fig. 2

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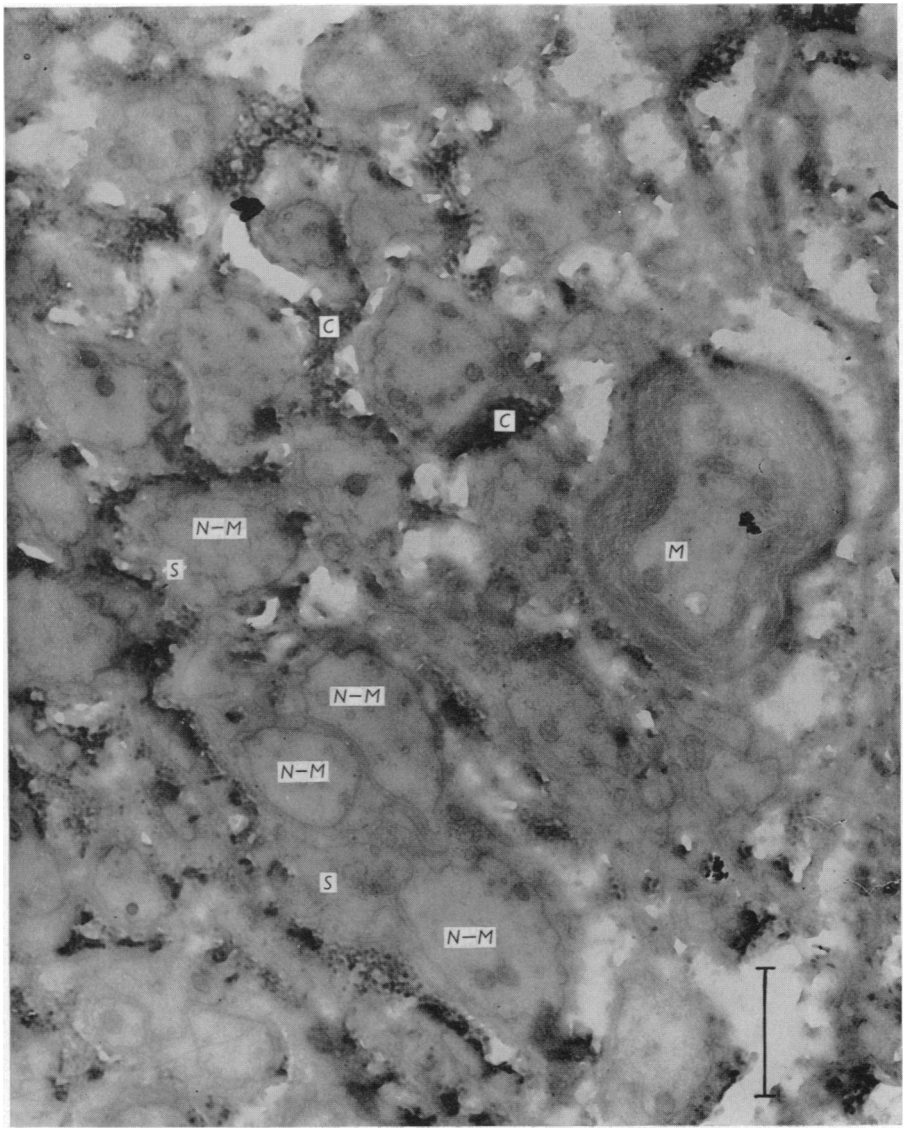
(*Facing p.* 746)



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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Whole mount of a uterine nerve fixed in OsO_4. The only myelinated fibre in the nerve is indicated by an arrow.

Fig. 2. Action potentials recorded from the nerve shown in Fig. 1. *A*, potentials evoked by pressure on a discrete point on the broad ligament; *B*, potentials from pressure on another point on the broad ligament; these were probably conducted in the myelinated fibre; *C*, potentials evoked by pressure on the uterus.

PLATE 2

Electron micrograph of transverse section of the nerve shown in Pl. 1, fig. 1, showing the single myelinated fibre, with adjacent non-myelinated fibres. The specimen was fixed by Palade's standard method; there is much crushing and damage due to the unexpectedly large collagen content of the nerve. The calibration is 1 μ. *M*, myelinated fibre; *N–M*, non-myelinated fibres; *S*, Schwann cells; *C*, collagen bundles.