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TRANSMISSION FROM INTRAMURAL INHIBITORY NERVES TO THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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SUMMARY

1. Membrane potential changes of smooth muscle cells were recorded during stimulation of the intramural inhibitory nerves to the taenia coli.

2. Stimulation across the taenia coli with single pulses of 200 μsec duration excites the intramural nerves and not the muscle directly.

3. The membrane potential changes due to stimulation of the intramural inhibitory nerves were different from those produced by perivascular inhibitory nerve stimulation in the following ways: hyperpolarizations (i.j.p.'s) of up to 25 mV were produced in response to *single* pulses; the latency, i.e. the time taken for the membrane to hyperpolarize after a stimulus of maximal strength, was as short as 80 msec; when the nerves were stimulated repetitively the membrane was hyperpolarized by up to 35 mV and all spontaneous activity was abolished; the mean hyperpolarization due to repetitive stimulation increased with the frequency of stimulation up to 10 pulses/sec and then remained constant; the hyperpolarization due to stimulation at frequencies greater than 5 pulses/sec was not maintained but decreased after 3–5 sec of stimulation; and finally when stimulation had ceased action potentials commenced firing at frequencies greater than normal.

4. The amplitude and rate of hyperpolarization of the i.j.p. increased with increasing strength of stimulation until a maximum amplitude and rate of hyperpolarization was reached. The recovery or depolarizing phase of the i.j.p. was exponential with a time constant which varied from about 250 msec to 500 msec and could not therefore be due to the discharge of the membrane capacitance. In some cases there was an inflexion on this depolarizing phase and in these cases recovery led directly into an action potential.

5. Spontaneous hyperpolarizations of the membrane were seen in some cells, and these hyperpolarizations were similar to those recorded on submaximal stimulation of the intramural nerves.

6. There were no changes in the characteristics of the i.j.p. in the presence of guanethidine or bretylium.

INTRODUCTION

Only two inhibitory junctions with smooth muscle have been examined electrophysiologically so far, that of the sympathetic nerves to the distal colon by Gillespie (1962*a*) and the perivascular inhibitory nerves to the taenia coli by Bennett, Burnstock & Holman (1966). Both these studies showed that there was no hyperpolarization of the smooth muscle cells until the inhibitory nerves were stimulated above 5 to 10 pulses/sec. This is in contrast to transmission of excitation from autonomic nerve to smooth muscle in which a depolarization of the smooth muscle membrane occurs when the excitatory nerves are stimulated with single pulses (Burnstock & Holman, 1961; Ursillo, 1961; Gillespie, 1962*b*; Orlov, 1962; Speden, 1964).

Pharmacological studies of the innervation of the guinea-pig taenia coli have shown that the smooth muscle is supplied with two types of inhibitory nerves; perivascular inhibitory nerves and intramural inhibitory nerves (Burnstock, Campbell & Rand, 1966). This paper describes the electrophysiological characteristics of the transmission of inhibition from intramural nerves to the smooth muscle cells of the taenia coli. In particular, we have attempted to find an explanation for two of the observations described by Burnstock *et al.* (1966). One of these is the relaxation of the taenia coli caused by low frequencies (less than 5 pulses/sec) of stimulation and the other is the inability of sympathetic blocking drugs to block the relaxation due to intramural nerve stimulation.

Preliminary reports of this work have already been published (Burnstock, Campbell, Bennett & Holman, 1963*a*, *b*, 1964).

METHODS

Guinea-pigs of either sex, weighing between 200 and 500 g, were used. The animals were stunned and bled to death. Strips of taenia coli together with the entire underlying caecal wall were cut away from the caecum and divided into lengths of about 2 cm.

These strips were mounted on a Perspex block 3 cm long and 1 cm wide, with the taenia uppermost. The block was then secured in a 10 ml. rectangular bath containing modified Kreb's solution (Bülbring, 1953). Both ends of the strip were tied with cotton, one piece of which was passed through a pulley to a Grass tension transducer. The other piece of cotton was used to draw the strip through two 1·5 mm diameter loops of platinum wire which were separated by 2 mm and partly embedded in Araldite. About 2 mm of the strip protruded from one side of these loops and about 1·5 cm from the other side.

The method of recording the electrical activity of the smooth muscle cells of the taenia was the same as that described in a previous paper by Bennett *et al.* (1966).

The solution in the bath always contained atropine sulphate (10^{-7} g/ml.) so as to eliminate the effects of stimulating cholinergic excitatory nerves.

RESULTS

In the presence of atropine, stimulation across the taenia coli with single pulses of 200 μsec duration caused a transient hyperpolarization of the smooth muscle cell membrane. This response was observed in all cells, the amplitude varying up to 25 mV. Some examples of this hyperpolarization are shown in Fig. 1.

A check was made to determine if this hyperpolarization could have been propagated through the smooth muscle cells from the stimulating electrode. If this was the case, the time from the moment of stimulation to the beginning of hyperpolarization should have increased with an increase in the distance from the stimulating electrode, that is, the latency should have increased with distance. Figure 1 shows the hyperpolarizations recorded at 8, 5 and 2 mm from the stimulating electrode. The latency for the hyperpolarization at these distances was 90 ± 10 msec. Since the latency for the hyperpolarization was approximately constant it seems unlikely that the hyperpolarization could have propagated through the smooth muscle cells.

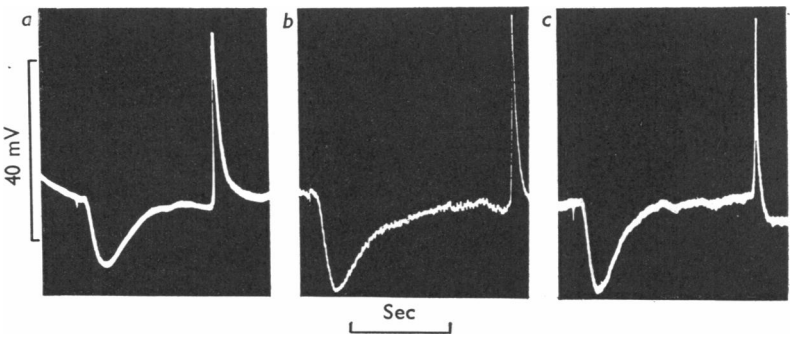

Fig. 1. Hyperpolarizations recorded in cells at different distances from the stimulating electrode on stimulating across the taenia coli with single pulses of 200 μsec duration. Distances from the stimulating electrode were 8, 5 and 2 mm for *a*, *b* and *c*, respectively. The time between the moment of stimulation and the beginning of hyperpolarization was 90 ± 10 msec in each case. Records in *a*, *b* and *c* taken from cells 83, 32 and 78, respectively. Records *a* and *c* retouched.

Stimulation across the taenia coli did not initiate any action potentials. The possibility existed that action potentials were initiated at the stimulating electrode but that the hyperpolarization due to stimulation interfered with their propagation. However, the latency for hyperpolarizations recorded 2 mm from the stimulating electrode was about 80 msec, whereas the time for an action potential to propagate this far would only be about 25 msec (Bülbring, Burnstock & Holman, 1958). Hence if any action

potentials had been initiated they would have reached the point of recording some 50 msec before hyperpolarization occurred and would not have been blocked. It seems likely then that stimulation with pulses of 200 μsec duration does not excite the muscle cells directly.

Burnstock *et al.* (1966) have presented evidence that stimulation across the taenia coli stimulates intramural inhibitory nerves. This evidence and that produced in this paper make it likely that the hyperpolarization in response

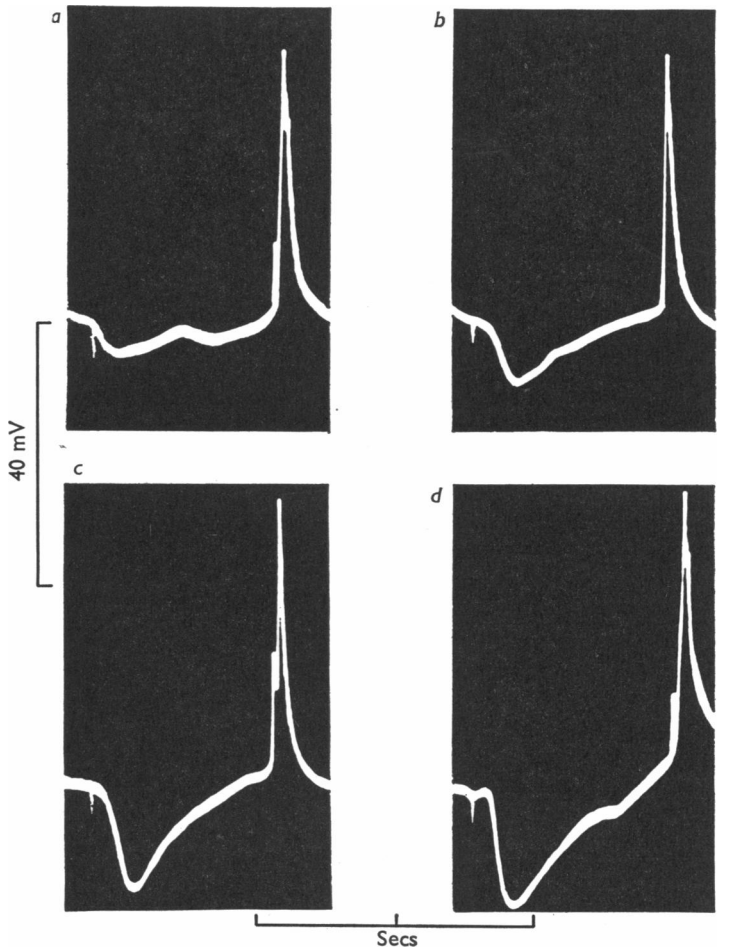

Fig. 2. The effects of increasing the strength of stimulation across the taenia coli on the i.j.p. recorded in cell 84. The pulse strengths used in each case, when expressed as a fraction of the pulse strength which produced the maximum i.j.p. were 0·37, 0·47, 0·73 and 1·0 for *a*, *b*, *c* and *d*, respectively. The duration of the stimulation pulse was 200 μsec. Note the inflexion on the recovery phases of the i.j.p.'s in *b* and *d*. Records retouched.

to stimulation across the taenia is due to the stimulation of intramural inhibitory nerves. This hyperpolarization will be called an inhibitory junction potential (i.j.p.).

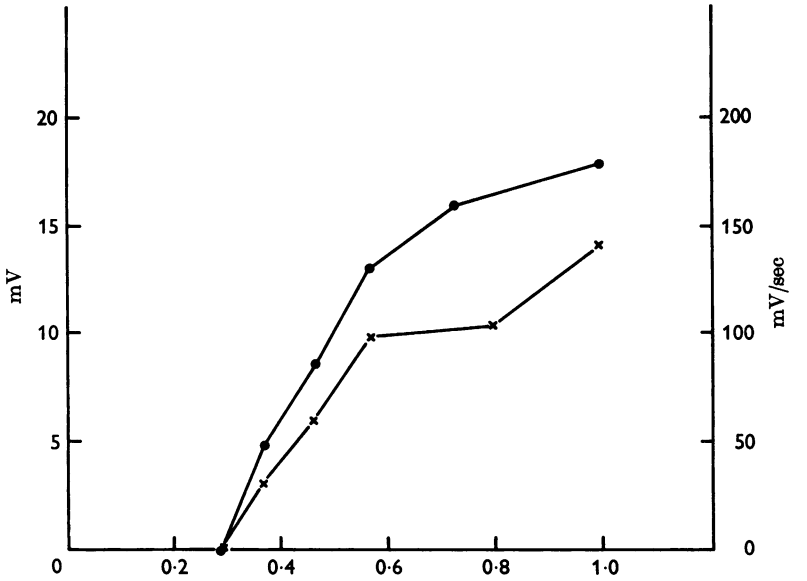

Fig. 3. Changes in the characteristics of the i.j.p. of cell 84 due to varying the strength of stimulation across the taenia coli. Abscissa, stimulating pulse strength expressed as a fraction of the pulse strength which produced the maximum i.j.p. Ordinate, closed circles give the amplitude of the i.j.p. and crosses the maximum rate of rise of the i.j.p.

Characteristics of the hyperpolarization due to intramural nerve stimulation

Many of the characteristics of the hyperpolarization due to intramural nerve stimulation depended on the strength and frequency of stimulation. The following sections of this paper describe the characteristics of the hyperpolarization when either the strength or frequency of stimulation was kept constant.

Stimulation with single pulses of submaximal strength. The size and rate of hyperpolarization of the i.j.p. increased with increasing strength of stimulation. Figure 2 shows the effects of increasing the strength of stimulation on the i.j.p. recorded in one cell. Figure 3 shows how the amplitude and rate of hyperpolarization of the i.j.p. in this cell increased with the strength of stimulation, until the maximum values of 18 mV and 140 mV/sec, respectively, were reached. In this cell, a pulse strength 0·3 of that which produced the maximum i.j.p. did not cause any membrane potential changes.

Stimulation with single pulses of maximal strength. The time course of the i.j.p. in response to single pulses of maximal strength varied from cell to cell and their latencies varied from 80 to 120 msec. The i.j.p. decayed approximately exponentially. In Fig. 4 the ratio of the amplitude of an i.j.p. (V) to its maximum amplitude (V_o) was plotted against the time after it had reached its maximum amplitude. This graph shows that the i.j.p. shown in Fig. 5*a* decayed with a time constant of 250 msec, and that the recovery phase of another i.j.p. had a time constant of 470 msec. Six others analysed in this way also decayed exponentially with time constants between 250 and 500 msec. The latency therefore remained fairly constant but there was great variation in the time course of the i.j.p.'s.

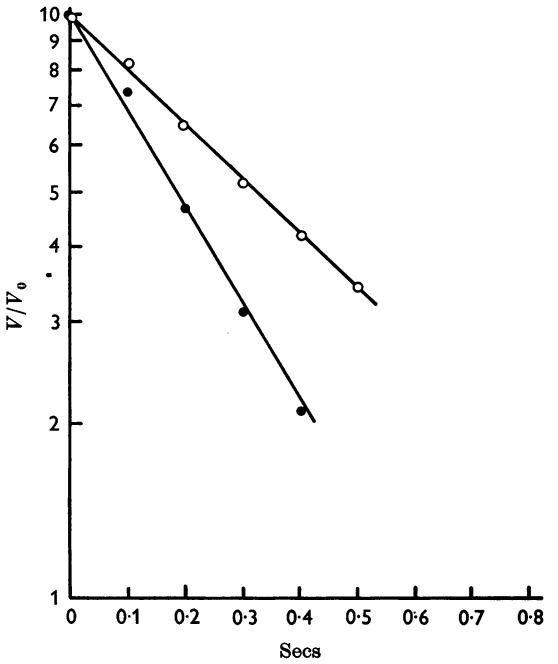

Fig. 4. Time course of the recovery phase of two i.j.p.'s. Abscissa, time after the i.j.p. had reached its maximum amplitude. Ordinate, ratio of the amplitude of the i.j.p. (V) to the maximum amplitude of the i.j.p. (V_o). Log scale. Closed circles are for an i.j.p. recorded in cell 79 and illustrated in Fig. 5*a*. Time constant 250 msec. Open circles are for an i.j.p. in cell 39. Time constant 470 msec.

Some i.j.p.'s had inflexions on their recovery phase which consisted of a momentary slowing down of the recovery. Such an inflexion occurred on the i.j.p. shown in Fig. 5*b* and is indicated by an arrow. The time course of the decay before and after the inflexion on this i.j.p. was exponential as shown in the graph of Fig. 6. The time constant for recovery up to the

inflexions was 760 msec, while the time constant for recovery after the inflexion was 280 msec. This fast depolarization after the inflexion led almost immediately into the rising phase of an action potential. It was often noticed that when an i.j.p. had an inflexion it led directly into an action potential. When there was no inflexion the depolarizing phase brought the membrane potential back to its resting value where it re-

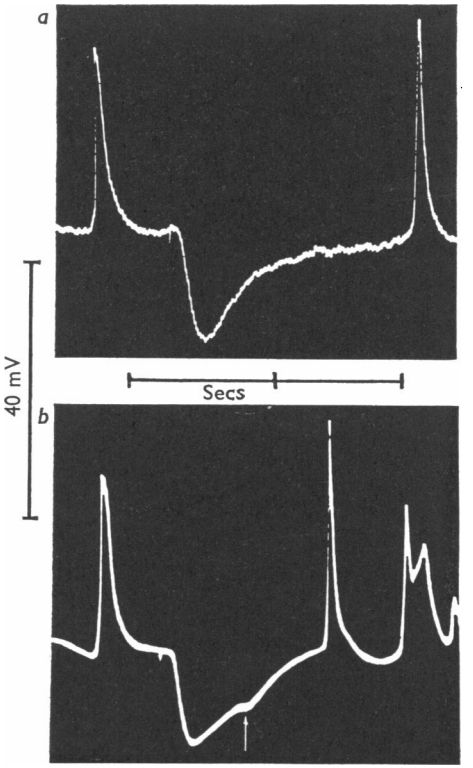

Fig. 5. I.j.p.'s recorded in two cells in response to stimulation with single pulses of maximal strength. (*a*) I.j.p. recorded in cell 39 which shows no inflexion on the recovery phase. (*b*) I.j.p. recorded in cell 83 which shows an inflexion on the recovery phase marked by an arrow. Note the increase in amplitude of the action potential immediately following the i.j.p. in both cases. Pulse duration 200 μsec. Record *b* retouched.

mained for about 1 sec before any action potentials occurred. This effect is similar to that observed by Katz & Miledi (1963) in the motoneurone where sometimes the depolarizing phase of an i.p.s.p. leads directly into the rising phase of an action potential.

Stimulation with repetitive pulses of submaximal strength. Repetitive stimulation across the taenia coli gave hyperpolarizations whose amplitude depended on the frequency of stimulation as well as the pulse strength.

At a pulse strength 0·4 of that which produced the maximum i.j.p., stimulation at 2 pulses/sec and at 20 pulses/sec hyperpolarized the membrane of one cell by 3 and 10 mV respectively, as is shown in Fig. 7. During stimulation the intervals between action potentials increased at 2 pulses/sec, whereas all spontaneous activity ceased at 20 pulses/sec in this cell. Thus the effect of intramural nerve stimulation on spontaneous activity depends on the frequency of stimulation.

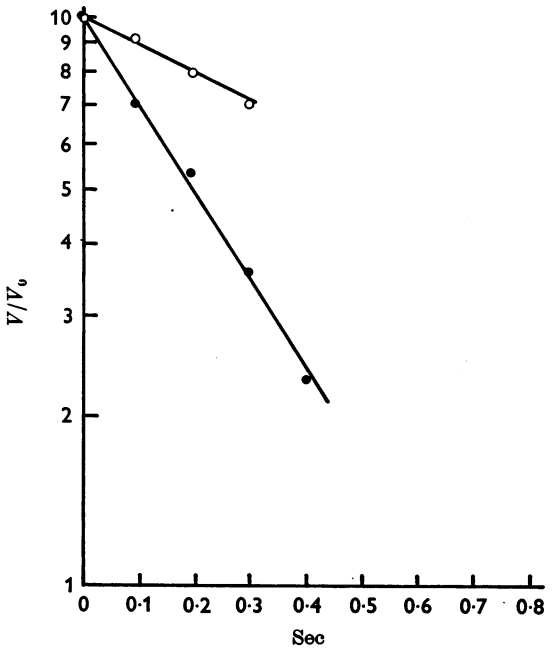

Fig. 6. Time course of the recovery phase of an i.j.p. before and after an inflexion. *Open circles.* Abscissa, time after the i.j.p. had reached its maximum amplitude. Ordinate, ratio of the amplitude of the i.j.p. to its maximum amplitude. Log. scale. Time constant for this phase, 760 msec. *Closed circles.* Abscissa, time after the inflexion occurred on the recovery phase of the i.j.p. Ordinate, ratio of the amplitude of the i.j.p. to its amplitude at the inflexion. Log scale. Time constant for this phase, 280 msec. Cell 83 shown in Fig. 5*b*.

Stimulation with repetitive pulses of maximal strength. When the intramural inhibitory nerves were stimulated repetitively with pulses of maximal strength, successive i.j.p.'s summed with each other to hyperpolarize the membrane further than the hyperpolarization due to a single i.j.p. The records taken from three cells during this summation process are illustrated in Fig. 8. For frequencies of stimulation less than 5 pulses/sec, as in Fig. 8*b*, *c*, and *d*, the second i.j.p. commenced before the first i.j.p. had depolarized back to the resting potential and therefore hyperpolarized the

membrane by a greater amount than did the first i.j.p. Subsequent i.j.p.'s did not hyperpolarize the membrane much beyond the value reached by this second i.j.p. When the nerves were stimulated at frequencies greater than 5 pulses/sec no individual i.j.p.'s could be distinguished and the membrane was hyperpolarized to a maximum value of between 30 and 35 mV as shown in the record taken from one cell in Fig. 8*a*. This process of summation of successive i.j.p.'s is very similar to that recorded by Kuffler & Eyzaguirre (1955) in the crustacean stretch receptor on stimulating its inhibitory nerves.

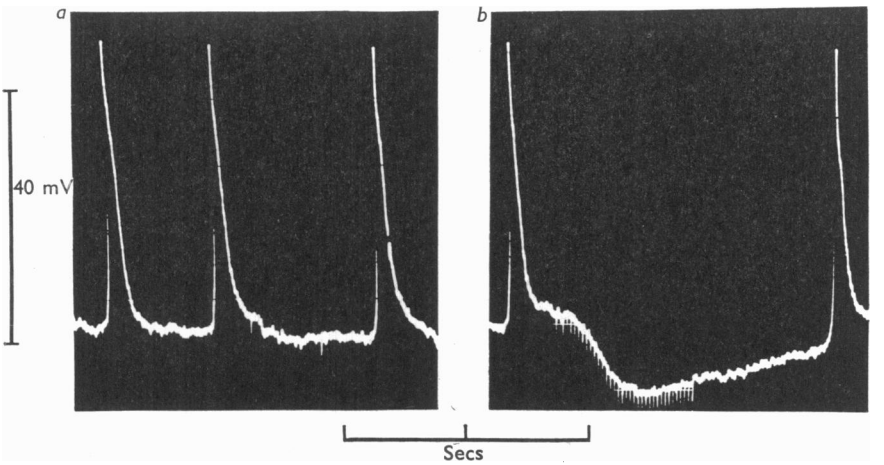

Fig. 7. Effect of stimulating across the taenia coli with repetitive pulses of strength 0·4 of that which produced the maximum sized i.j.p. Stimulation at 2 and 20 pulses/sec in *a* and *b*, respectively. Pulse duration 200 μsec. *a*, cell 38; *b*, cell 33.

The amplitude of the mean hyperpolarization due to intramural nerve stimulation increased with an increase of the frequency of stimulation. The mean hyperpolarization was studied in preference to the maximum hyperpolarization because of the large fluctuations of the membrane potential which occurred during stimulation at frequencies less than 5 pulses/sec. In one preparation this mean hyperpolarization increased with frequency in the manner shown in Fig. 9. The amplitude of the mean hyperpolarization in this case increased up to 10 pulses/sec and then remained approximately constant up to 60 pulses/sec.

In other preparations the amplitude of the mean hyperpolarization also increased with frequency up to 10 pulses/sec. At higher frequencies, the amplitude of the hyperpolarization remained approximately the same though it differed considerably between preparations at any one frequency of stimulation.

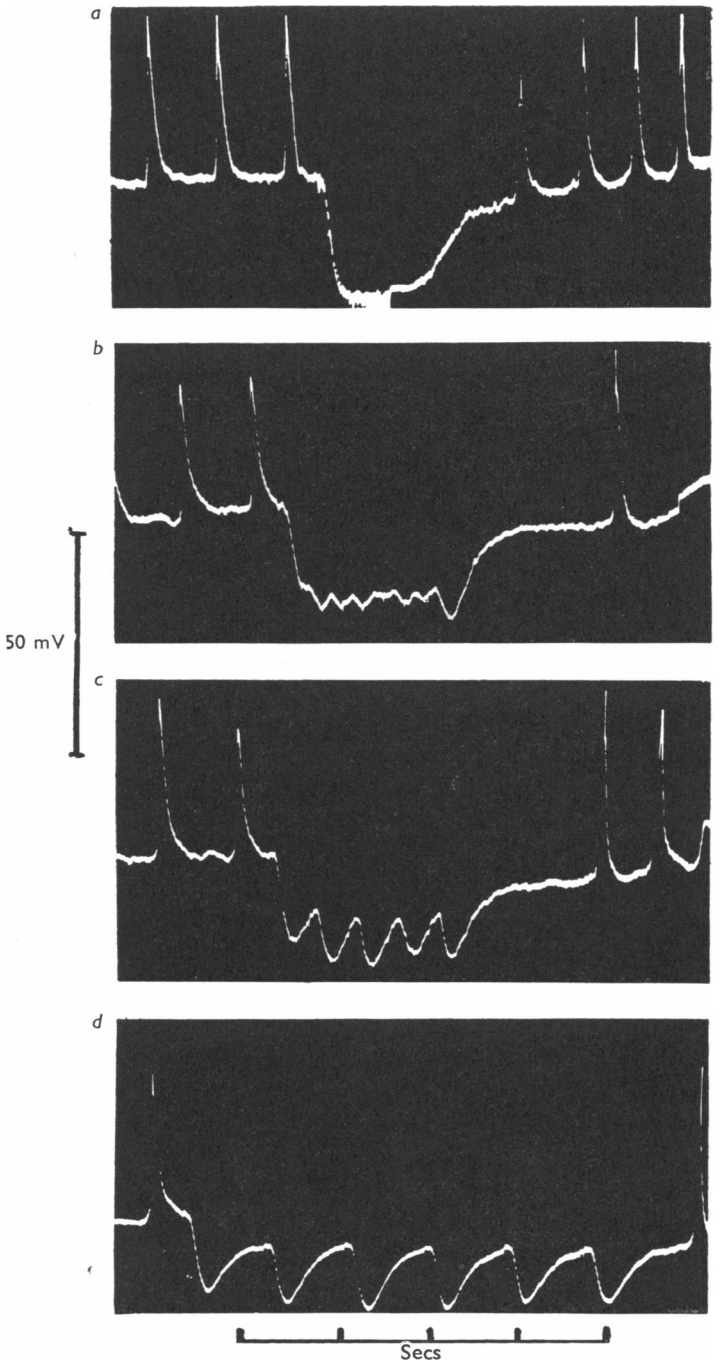

Fig 8. For legend see opposite page.

The latency between the beginning of stimulation and the beginning of hyperpolarization did not change with the frequency of stimulation, but remained constant at about 100 msec. The rate of hyperpolarization, however, increased for frequencies above about 10 pulses/sec. For example, the rate of hyperpolarizations illustrated in Fig. 8 remained the same at 120 mV/sec for frequencies below 10 pulses/sec but increased to a maximum of 180 mV/sec for frequencies above 10 pulses/sec.

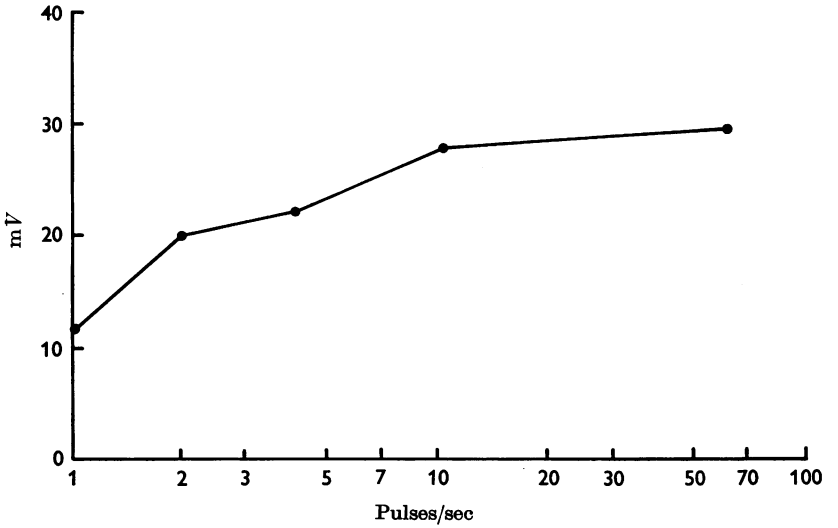

Fig. 9. Change in amplitude of the i.j.p. in different cells of one taenia coli preparation with change of the frequency of stimulation. Abscissa, frequency of stimulation on log scale. Ordinate amplitude of the mean hyperpolarization during the i.j.p. The cells recorded from, for stimulation at 1, 2, 4, 10 and 60 pulses/sec were 38, 37, 37, 40, and 35, respectively.

When the intramural nerves were stimulated above 5 pulses/sec, the membrane potential did not remain at its maximum hyperpolarized value during the period of stimulation but slowly depolarized towards the resting level. A record taken from one cell showing this effect is shown in Fig. 10; the membrane depolarized 8 mV from its maximum hyperpolarized value of 19 mV after 3 sec of stimulation at 10 pulses/sec. We

Legend to Fig. 8.

Fig. 8. Effect of stimulating across the taenia coli with repetitive pulses of maximal strength at different frequencies. Frequencies of stimulation 60, 4, 2 and 1 pulses/sec in *a*, *b*, *c* and *d*, respectively. Pulse duration 200 μsec. Records taken from cells 35, 36, 37 and 38 in *a*, *b*, *c* and *d*, respectively. Note the increase in amplitude of the action potential after the i.j.p. in *b*, *c* and *d*, and the increased rate of firing after the i.j.p. in *a* and *c*. Electrode was dislodged from the cell in the record at the end of *b*.

have not yet studied this recovery for durations of stimulation greater than 5 sec.

When repetitive stimulation was stopped, the membrane potential returned to its resting value, and action potentials resumed firing at a frequency which was generally much greater than that seen before stimulation. Such an increased rate of firing is shown in Fig. 8*a*, after stimulation at 60 pulses/sec. This increase in the frequency of firing of action potentials immediately after stimulation is probably responsible for the increased tone observed by Burnstock *et al.* (1966) after intramural nerve stimulation.

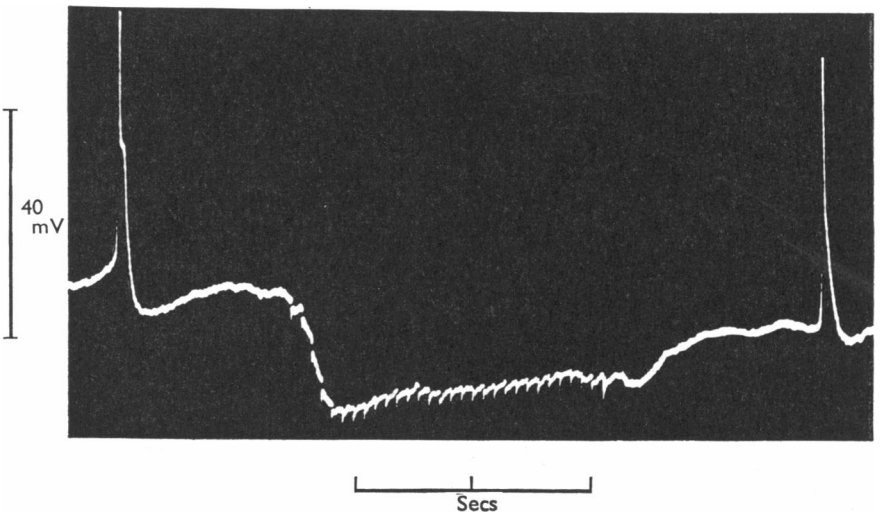

Fig. 10. Recovery of the hyperpolarization during repetitive stimulation across the taenia coli at 10 pulses/sec for 3 sec. Cell 82.

No action potentials or small depolarizing potential changes were observed during stimulation with pulses of maximal strength. This is in contrast to the small depolarizing potentials and action potentials observed by Bennett *et al.* (1966) during perivascular inhibitory nerve stimulation. This difference is probably due to the large hyperpolarizations of the membrane when the intramural nerves were stimulated compared with those which occurred when the perivascular nerves were stimulated. These large hyperpolarizations are probably sufficient to block all action potential activity whereas the small hyperpolarizations due to perivascular nerves are not.

Spontaneous hyperpolarizations of the smooth muscle membrane. In some preparations hyperpolarizations of the smooth muscle membrane occurred without stimulation of the nerves, and these hyperpolarizations had similar time courses to those i.j.p.'s caused by pulses of submaximal strength.

Two examples of these spontaneous hyperpolarizations are shown in Fig. 11*a* and *b* where they are indicated by arrows. Figure 11*c* shows the effect of stimulation at submaximal strength in the same cell as that illustrated in Fig. 11*b*. It seems likely that these hyperpolarizations are due to the spontaneous release of transmitter from inhibitory intramural nerve fibres. Alternatively, these nerve fibres may have been excited during impalement of the smooth muscle cell by the micro-electrode.

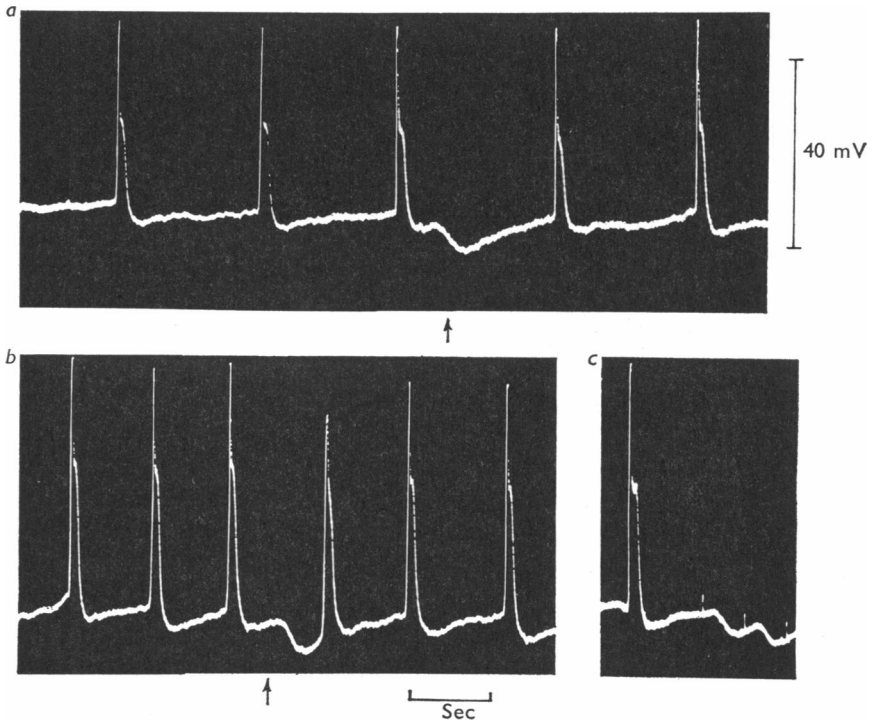

Fig. 11. (*a*) and (*b*). At the arrows, spontaneous hyperpolarizations of the membranes of two smooth muscle cells. (*c*) I.j.p.'s in the same cell as in *b* due to stimulation with pulses of sub-maximal strength at 2 pulses/sec. Pulse strength 200 μsec in (*c*).

The effect of sympathetic blocking drugs on intramural inhibitory nerve transmission. Sympathetic blocking drugs had no effect on the characteristics of the i.j.p. Neither guanethidine in a concentration of 10^{-6} g/ml. or bretylium in a concentration of 5×10^{-6} g/ml. affected the transmission from the intramural inhibitory nerves. For example, Fig. 12*a* shows an i.j.p. recorded in a preparation before guanethidine was added; the characteristics of this i.j.p. were unchanged 50 min after the addition of the guanethidine, as shown in Fig. 12*b*. These results are in agreement

with those obtained by Burnstock *et al.* (1966) who showed that the relaxation due to intramural inhibitory nerve stimulation was not blocked by guanethidine or bretylium.

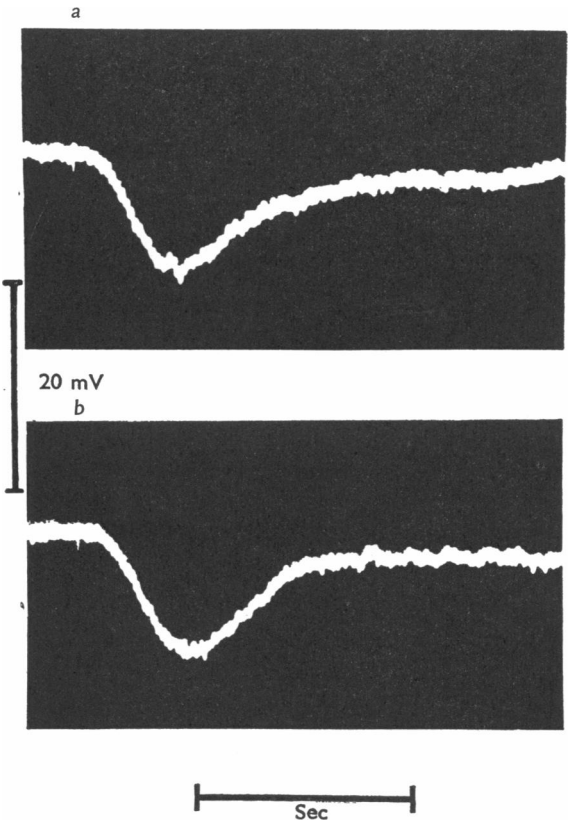

Fig. 12. Effect of guanethidine (10^{-6} gm/ml.) on the i.j.p. in response to single pulses 200 μsec duration. (*a*) I.j.p. recorded before guanethidine added (*b*) I.j.p. recorded 50 min after the addition of guanethidine.

DISCUSSION

Similar i.j.p.'s to those described in this paper were observed in response to stimulation of the taenia coli with brief pulses of less than 500 μsec duration while recording from this smooth muscle in the sucrose gap (Burnstock *et al.* 1963*b*). The large hyperpolarizations in response to single stimuli recorded in these experiments suggested that the taenia coli might be innervated by inhibitory nerve fibres which were very different from sympathetic inhibitory nerves of the type described by Gillespie (1962*a*) for the distal colon of the guinea-pig. Burnstock *et al.* (1964) have given further evidence for the existence of inhibitory nerves in the taenia coli which are distinct from the perivascular nerves. The principal evidence

which they presented was: the existence of a guanethidine resistant relaxation of the taenia coli when the caecal wall was electrically stimulated; a relaxation due to the ganglion-stimulating action of DMPP after this drug had produced a guanethidine-like blockade of the perivascular nerves and the inability of DMPP to inhibit strips of the taenia cut in such a manner as to exclude neurones of Auerbach's plexus.

In this paper further evidence has been presented that stimulation across the taenia with pulses of low duration excites nerves and not the muscle directly. First, since there was no detectable change in the latency of the i.j.p. with a fourfold increase in distance from the stimulating electrode, it is unlikely that the i.j.p. could have been propagated through the smooth muscle cells. Secondly, stimulation with pulses of 200 μsec duration did not elicit any action potentials.

The principal evidence presented in this paper for the existence of inhibitory nerves distinct from the perivascular inhibitory nerves is that the characteristics of the i.j.p. are not changed by sympathetic blocking drugs and that the characteristics of the hyperpolarization produced by intramural stimulation are entirely different to those of the hyperpolarization produced by perivascular stimulation. For example, stimulation of the intramural nerves with single pulses of maximal strength gave i.j.p.'s of up to 25 mV while stimulation of the perivascular nerves with single pulses did not change the membrane potential.

The latency of most i.j.p.'s was about 100 msec and most of this time was probably due to the time for transmission from intramural inhibitory nerve to the smooth muscle cell. Gasser (1950) has estimated the slowest conduction velocity of a C fibre as 0·7 m/sec. In our experiments the furthest distance between an impaled cell and the stimulating electrode was 1·5 cm. Hence the longest conduction time for C fibres over this distance would be 20 msec. The inhibitory nerves are probably C fibres and therefore most of the latent period would be accounted for by the time for transmission.

The depolarizing phase of the i.j.p. was exponential with a time constant as long as 500 msec. Kuriyama & Tomita (1964) have recently shown that the membrane time constant of the smooth muscle cells of the taenia coli is only about 3 msec. Hence the long time for recovery of the i.j.p. cannot be due to the passive discharge of the membrane capacitance (Eccles, 1961).

It is unlikely that the long time course of the i.j.p. can be due to an asynchronous release of transmitter. The I.P.S.P. recorded in pyramidal cells of the hippocampus by Anderson, Eccles & Løyning (1963) has a very long time course of from 200 to 500 msec. They claimed that this long time course was due to an asynchronous inhibitory bombardment from basket

cells, the asynchronism showing itself as a high frequency ripple on the I.P.S.P. No ripples were seen on the i.j.p. of the taenia coli therefore it is unlikely that the long time course in this case was due to asynchronous release of transmitter.

The long time course of the i.j.p. is probably due to the transmitter action maintaining the junctional currents for times which are long compared with the membrane time constant. The long time course of some postsynaptic potentials have been explained in this way. For example: the I.P.S.P. of the lobster cardiac ganglion (Hagiwara, Watanabe & Saito, 1959); the E.P.S.P. of the motoneurone (Araki & Terzuolo, 1962) and the E.P.S.P. of frog sympathetic neurones (Nishi & Koketsu, 1960).

There was a summation of the i.j.p.'s when the nerves were repetitively stimulated, and the maximum hyperpolarization of about 30 mV was reached at 10 pulses/sec. There was also an increase in the rate of hyperpolarization for frequencies above about 10 pulses/sec. This increase is probably due to an increase in the amount of transmitter released in a short time at high frequencies, thus causing a faster increase in membrane permeability and therefore an increase in the rate of hyperpolarization.

Burnstock *et al.* (1965) observed very large relaxations of the taenia coli for low frequencies of stimulation of the intramural nerves, an effect which may be explained by the frequency versus mean hyperpolarization curve for intramural stimulation. Bennett *et al.* (1966) have shown that the depression of spontaneous electrical activity was greater the larger the hyperpolarization of the smooth muscle membrane, and Bülbring (1954) and Bülbring & Kuriyama (1963*a*) have shown that the tension developed by the taenia coli is related to the frequency of firing of the action potentials. Hence the larger the hyperpolarization, the lower the frequency of firing of action potentials and the smaller the tension. Since even single pulses can hyperpolarize the membrane up to 25 mV it is not surprising that the low frequencies of stimulation can cause a large relaxation of the taenia coli.

After stimulation of the intramural nerves had ceased, action potentials began firing at frequencies greater than normal. This may explain why Burnstock *et al.* (1965) observed that the tone of the taenia was greater than normal after intramural nerve stimulation.

Spontaneous hyperpolarizations of the membrane were seen in some smooth muscle cells. These hyperpolarizations have also been observed by Bülbring & Kuriyama (1963*b*) and are similar to the hyperpolarizations recorded on stimulating the intramural inhibitory nerves with a pulse of submaximal strength. It may be that these hyperpolarizations are due to the release of transmitter from intramural inhibitory nerves.

The transmitter which is involved in the generation of i.j.p.'s in the

taenia coli remains to be identified. Preliminary experiments have suggested that its action is to cause a specific increase in conductance for K ions (Bennett, Burnstock & Holman, 1963).

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REFERENCES

ANDERSON, P., ECCLES, J. C. & LØYNING, Y. (1963). Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses. *Nature, Lond.*, **198**, 541–542.

ARAKI, T. & TERZUOLO, C. A. (1962). Membrane currents in spinal motoneurones associated with the action potential and synaptic activity. *J. Neurophysiol.* **23**, 772–789.

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1963). *J. Physiol.* **69**, 33–34*P*.

BENNETT, M. R., BURNSTOCK, G. & HOLMAN, M. E. (1966). Transmission from perivascular inhibitory nerves to the smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **182**, 527–540.

BÜLBRING, E. (1953). Measurements of oxygen consumption in smooth muscle. *J. Physiol.* **122**, 111–134.

BÜLBRING, E. (1954). Membrane potentials of smooth muscle fibres of the taenia coli of the guinea-pig. *J. Physiol.* **125**, 302–315.

BÜLBRING, E., BURNSTOCK, G. & HOLMAN, M. E. (1958). Excitation and conduction in the smooth muscle of the isolated taenia coli of the guinea-pig. *J. Physiol.* **142**, 420–437.

BÜLBRING, E. & KURIYAMA, H. (1963*a*). The effect of adrenaline on the smooth muscle of guinea-pig taenia coli in relation to the degree of stretch. *J. Physiol.* **169**, 198–212.

BÜLBRING, E. & KURIYAMA, H. (1963*b*). Effects of changes in the external sodium and calcium concentrations on spontaneous electrical activity in smooth muscle of the guinea-pig taenia coli. *J. Physiol.* **166**, 29–58.

BURNSTOCK, G. & HOLMAN, M. E. (1961). The transmission of excitation from autonomic nerve to smooth muscle. *J. Physiol.* **155**, 115–133.

BURNSTOCK, G., CAMPBELL, G., BENNETT, M. R. & HOLMAN, M. E. (1963*a*). The effect of drugs on the transmission of inhibition from autonomic nerves to the smooth muscle of the guinea-pig taenia coli. *Biochem. Pharmac.* (Suppl.) **134**.

BURNSTOCK, G., CAMPBELL, G., BENNETT, M. R. & HOLMAN, M. E. (1963*b*). Inhibition of the smooth muscle of the taenia coli. *Nature, Lond.*, **200**, 581–582.

BURNSTOCK, G., CAMPBELL, G., BENNETT, M. R. & HOLMAN, M. E. (1964). Innervation of the guinea-pig taenia coli. Are there intrinsic inhibitory nerves which are distinct from sympathetic nerves? *Int. J. Neuropharmac.* **3**, 163–166.

BURNSTOCK, G., CAMPBELL, G. & RAND, M. (1966). The inhibitory innervation of the taenia of the guinea-pig caecum. *J. Physiol.* **182**, 504–526.

ECCLES, J. C. (1961). Membrane time constants of cat motoneurones and time courses of synaptic action. *Exp. Neurol.* **4**, 1–22.

GASSER, H. S. (1950). Unmedullated fibres originating in dorsal root ganglia. *J. gen. Physiol.* **33**, 651–690.

GILLESPIE, J. S. (1962*a*). Spontaneous mechanical and electrical activity of stretched and unstretched intestinal smooth muscle cells and their response to sympathetic nerve stimulation. *J. Physiol.* **162**, 54–75.

GILLESPIE, J. S. (1962*b*). The electrical and mechanical responses of intestinal smooth muscle cells to stimulation of their extrinsic parasympathetic nerves. *J. Physiol.* **162**, 76–92.

HAGIWARA, S., WATANABE, A. & SAITO, N. (1959). Potential changes in syncytial neurons of lobster cardiac ganglion. *J. Neurophysiol.* **22**, 554–572.

KATZ, B. & MILEDI, R. (1963). A study of spontaneous miniature potentials in spinal motoneurons. *J. Physiol.* **168**, 389–422.

KUFFLER, S. W. & EYZAGUIRRE, C. (1955). Synaptic inhibition in an isolated nerve cell. *J. gen. Physiol.* **39**, 155–184.

KURIYAMA, H. & TOMITA, T. (1964). Effect of polarizing currents on the membrane activity of single smooth muscle cells. *J. Physiol* **173**, 10–11*P*.

NISHI, S. & KOKETSU, K. (1960). Electrical properties and activities of single sympathetic neurons in frogs. *J. cell. comp. Physiol.* **55**, 15–30.

ORLOV, R. S. (1962). On impulse transmission from motor sympathetic nerve to smooth muscle. *Sechenov Physiol. J. U.S.S.R.* **48**, 342–349.

SPEDEN, R. N. (1964). Electrical activity of single smooth muscle cells of the mesenteric artery produced by splanchnic nerve stimulation in the guinea-pig. *Nature, Lond.*, **202**, 193–194.

URSILLO, R. C. (1961). Electrical activity of the isolated nerve-urinary bladder strip preparation of the rabbit. *Am. J. Physiol.* **201**, 408–412.