J. Physiol. (1957) 136, 310–323

# THE EFFECT OF METABOLIC INHIBITORS ON THE ELECTRICAL AND MECHANICAL ACTIVITY OF THE SMOOTH MUSCLE OF THE GUINEA-PIG'S TAENIA COLI

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(*Received* 4 *December* 1956)

The rate of oxygen consumption by smooth muscle is normally proportional, over a wide range, to the tension produced in isometric conditions (Bülbring, 1953). Under the influence of 2:4-dinitrophenol (DNP) in concentrations from $3 \times 10^{-6}$ to $3 \times 10^{-4}$ M the oxygen consumption is increased (Born & Bülbring, 1955). In the lower part of this range of DNP concentrations there is also some slight, though not proportional, increase in tension and spontaneous activity. But with higher concentrations of DNP the oxygen consumption may rise to more than twice the normal rate, while the muscle tension remains unchanged or declines. At the same time the adenosine triphosphate (ATP) content in the muscle remains unchanged until, during exposure to high DNP concentrations, it decreases in parallel with the declining muscle tension. Born (1956) found a close parallelism between smooth muscle tension and creatine phosphate content in the absence of glucose or of oxygen.

In normal conditions any change in tension which the smooth muscle of the taenia coli produces is associated with a change in membrane potential, in the frequency of spike discharge (Bülbring, 1955) and also with a change in spike configuration (Bülbring, 1956, 1957). We have now studied the effect of metabolic inhibitors on the electrical activity in relation to muscle tension. We have found a dissociation of the electrical from the mechanical manifestations which may in part account for the disproportion between the rate of metabolism and the mechanical activity.

## METHODS

The smooth muscle preparation was the isolated taenia coli of the guinea-pig. A piece measuring *in situ* 3 mm in length was excised. It was held flat by fixing the underlying circular muscle layer to a small Perspex disk (2 × 3 mm: described in detail by Bülbring, 1954, though at that time

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a larger frame was used). The piece was then stretched to 4 or 5 mm length for further immobilization, thereby producing an initial tension which varied in different experiments. The tension was recorded with a mechano-electronic transducer valve (R.C.A. 5734) (Talbot, Lilienthal, Beser & Reynolds, 1951).

Neutralized solutions of various substances were either injected, prewarmed, through a coil of fine polythene tubing or added to the continuously flowing bathing solution. The solution contained (mM): 133 NaCl, 4·7 KCl, 1·38 $NaH_2PO_4$, 16·3 $NaHCO_3$, 2·5 $CaCl_2$, 0·105 $MgCl_2$, 7·8 glucose, and was equilibrated with 95% $O_2$ + 5% $CO_2$; the pH was 7·4. The temperature in all experiments was 35° C.

Intracellular electrical records were taken with glass micro-electrodes (Ling & Gerard, 1949) filled with 3 M-KCl. The outer tip diameter was less than 0·5 $\mu$, the resistance varied from 15 to 25 M$\Omega$. A conventional cathode follower input stage was used (input valve M.E. 1400). Further details of the method are described in earlier papers by Bülbring & Hooton (1954) and Bülbring (1954, 1955).

## RESULTS

### *The normal behaviour*

The normal spontaneous mechanical activity of the taenia coli showed two patterns: (i) pendular movements consisting of fast changes in muscle tension of the order of several grams and covering a range between 3·5 and 7·0 g (Figs. 3, 7), and (ii) a maintained tension of about 5·0 g with small undulations of the order of fractions of 1 g (Figs. 2, 8). In preparations which showed large excursions of tension, the mechano-electronic transducer could only be used at low sensitivity. In those which maintained a fairly uniform tension it was possible to increase the sensitivity and then small increments of tension could be observed which occurred at the same frequency at which spike potentials were discharged.

The frequency of spike discharge was very similar in different preparations, usually about 12–13 in 10 sec. Higher frequencies (up to 20 in 10 sec) occurred at the peaks of tension during pendular activity. Some fibres were silent; if they fired, the frequency was never less than 5 in 10 sec. Good correlation existed between spike frequency and muscle tension.

The spike height varied mostly between 3 and 15 mV; occasionally larger spike potentials were seen, the highest value was 40 mV. Their duration and configuration was equally variable, not only in different preparations but also from one impalement to another, and from one spike potential to the next, recorded in the same fibre (see also Bülbring, 1957). The membrane potential measurements showed the same scatter (20–80 mV) as previously reported (Bülbring, 1954); the mean was 38·8 mV S.E.M. ± 0·56 (394 observations).

### *The action of* 2:4-*dinitrophenol* (*DNP*)

DNP was administered for periods from 10 to 60 min in concentrations from $10^{-5}$ M to $5 \times 10^{-4}$ M in the bathing solution; or it was given as an injection of 0·2–0·5 ml. of DNP $10^{-3}$ to $10^{-2}$ M into the 2·0 ml. bath.

*Tension.* The effect of DNP started immediately on contact with the tissue; the muscle tension decreased during the first minute (see Figs. 1, 2 and 4). In

some preparations a short rise preceded the fall in tension (see Fig. 3), which then continued to decline until the muscle was fully relaxed. With low concentrations of DNP complete relaxation was not always obtained, but sporadic developments of tension recurred. The sensitivity of different taenia coli preparations varied; some muscles relaxed completely with $2 \times 10^{-5}$ M DNP, others required ten times this concentration.

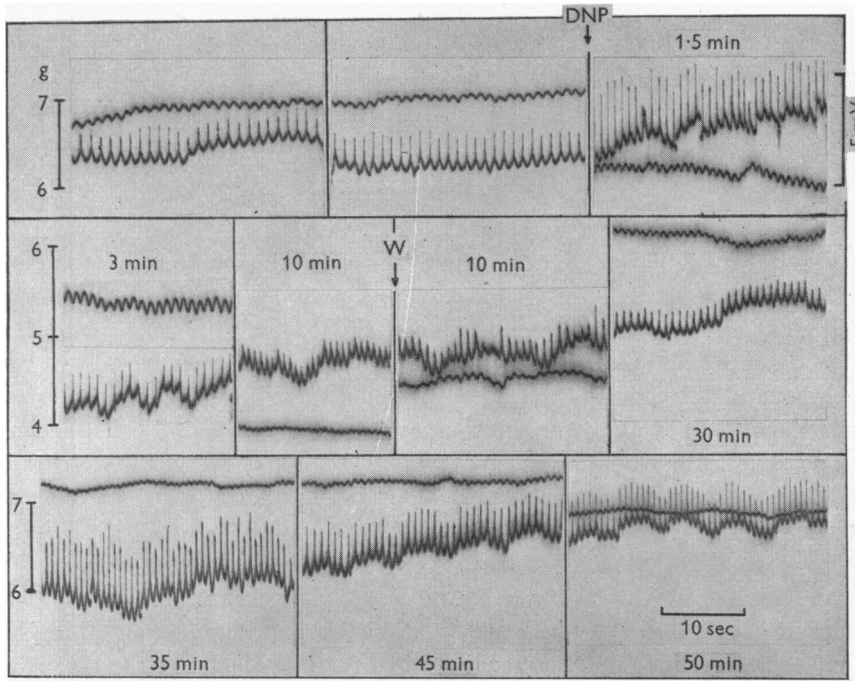


Fig. 1. Effect of DNP. Records of tension and membrane potential: the time after exposure to DNP $2 \times 10^{-4}$ M and after return to normal solution (W) is given with each record.

*Spike frequency.* The first effect of DNP on the discharge of spike potentials was to increase the frequency. This occurred without exception in all experiments and with all concentrations used. It was more pronounced if the DNP reached the muscle quickly. This increased spike frequency was seen irrespective of whether there was a short transitory rise in tension (Fig. 3) or whether there was an immediate fall in tension, which was usual (Figs. 1, 2 and 4). In Fig. 1 records are shown from a muscle preparation which maintained a fairly steady tension between 6·7 and 7·0 g. After 90 sec exposure to DNP $2 \times 10^{-4}$ M the tension had fallen to 6 g in spite of an increase in spike frequency from 8·4 to 11·4/10 sec. After 10 min exposure to DNP the spike frequency was 13·9/10 sec, but the tension had declined to 4 g. After the

removal of the DNP the tension returned gradually to 7 g, but the increased rate of spike discharge was still evident 45 min later. It is interesting to note that throughout the exposure to DNP, as during the control periods, each spike was followed by a small increment in muscle tension. However, whereas in normal solution the tension was maintained, in the presence of DNP the individual small tension changes did not summate but, in spite of the fast rate at which they followed each other, the total tension declined. This observation contrasted with the normal sequence of events in which any increased rate of spike discharge was accompanied by an increase in tension due to summation of the small increments following each spike (Bülbring, 1957).

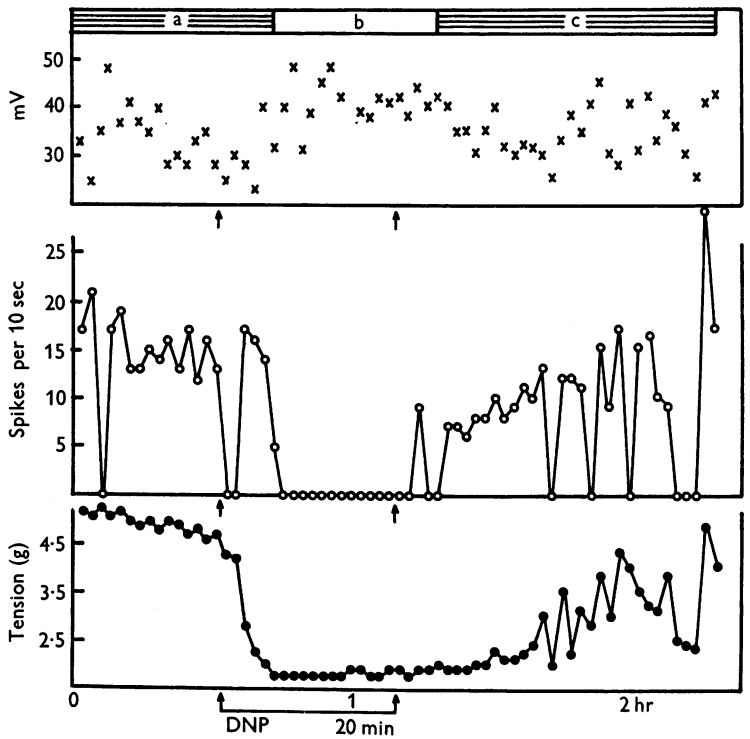


Fig. 2. Effect of 20 min exposure to DNP $5 \times 10^{-4}$ M on membrane potential (*A*), spike frequency (*B*), and tension (*C*): (a, b, c indicate groups of observations used for Table 1).

In Fig. 4 is shown, for comparison, the response of a muscle preparation to (*a*) a dose of histamine, and (*b*) a dose of DNP. While in (*a*) spike frequency and tension rose in parallel, the tension in (*b*) fell as the rate of spike discharge increased. The dissociation is also shown in Fig. 2. Though in Fig. 3 a transitory rise in tension accompanied the acceleration of spike discharge, the muscle relaxed while the spike frequency was still high.

The initial increase of electrical activity by DNP was followed by depression. The rate of discharge gradually slowed and rates of less than 5/10 sec, which were not seen normally, were now observed. With high concentrations of DNP, or long exposure to lower concentrations, spike potentials disappeared completely. After washing out they reappeared slowly, returned to the normal rate, often after a period of acceleration (Figs. 1, 2). Muscle tension also recovered, but usually more slowly than the spike frequency.

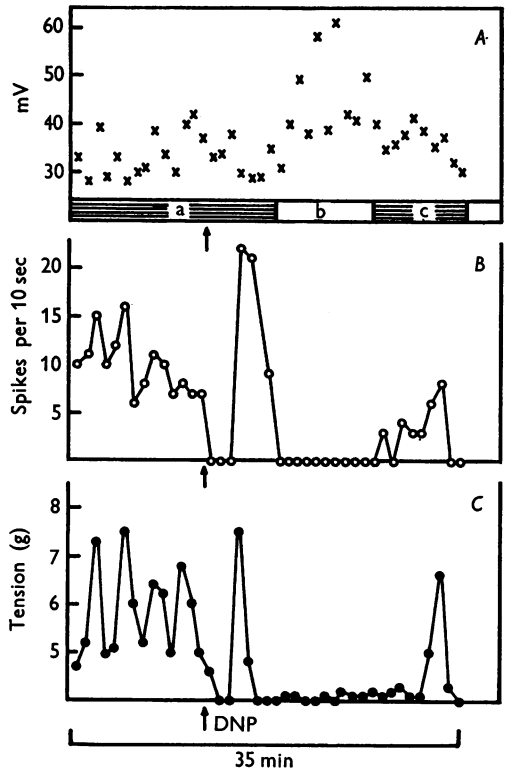


Fig. 3. Effect of an injection of DNP (at arrow) producing an initial concentration of $5 \times 10^{-3}$ M in the bath, on membrane potential (*A*), spike frequency (*B*), and tension (*C*). (a, b, c indicate groups of observations used for Table 1.)

*Spike configuration.* Recently (Bülbring, 1957) it was found that a correlation existed between the configuration of spike potentials and muscle tension. Thus stimulating substances (e.g. histamine), or direct current depolarization which increased the spike frequency, caused a delay of repolarization of the individual spike potentials producing spikes of long duration; this was associated with a rise in muscle tension. On the other hand, a low spike frequency and fast repolarization, i.e. spikes of short duration, were associated with relaxation of the muscle. In the present experiments it was found that DNP,

while increasing the rate of spike discharge, also prolonged their duration. In Fig. 5 are shown, for comparison, records obtained after an injection (*a*) of a dose of histamine and (*b*) of a dose of DNP. Both substances increased the spike frequency, both increased the duration of the spike potentials and produced double spikes. The difference between the two substances (as shown in Fig. 4) was, however, that histamine caused a rise but DNP a fall in tension.

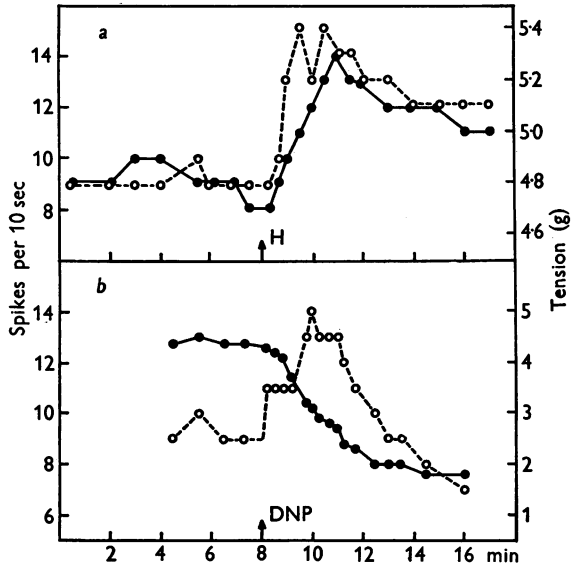


Fig. 4. Ordinates: spike frequency (○) and tension (●). (*a*) effect of an injection of histamine (initial concentration $1 \times 10^{-6}$); (*b*) effect of an injection of DNP (initial concentration $5 \times 10^{-3}$ M).

When the first phase of stimulation by DNP gave way to the second phase of depression spikes were discharged less frequently and the slow depolarization leading up to each spike became more and more prolonged. Finally, the 'prepotentials' proceeded for as long as 1 sec before spikes were discharged. These were of long duration, deformed to plateaus carrying irregular small deflexions. Slow waves of about 2 sec duration carrying such abnormal spikes sometimes continued for a long time before activity ultimately ceased. Fig. 6 shows this gradual change in spike configuration progressing during one hour exposure to DNP $10^{-4}$ M. After this time activity ceased altogether except for a short burst at 72 min. When the DNP was washed out the effects described were reversed and the shape of the spike potentials slowly reverted to normal.

*Membrane potential.* The membrane potential varied widely during control periods, and there was a similar scatter in the short initial period of excitation. No significant change of membrane potential could be detected during the first minutes of the action of DNP. Unlike the initial phase, however, the

subsequent phase of depression could be prolonged at will in order to obtain sufficient measurements. The readings obtained during this period, in which there was a much reduced or no spike discharge as a result of the action of DNP, were compared with the measurements obtained before and after, as indicated in Figs. 2, 3 and 7. The results are given in Table 1, which shows a significantly higher membrane potential at the height of the DNP action in the three experiments in which the measurements were made with the same microelectrode.

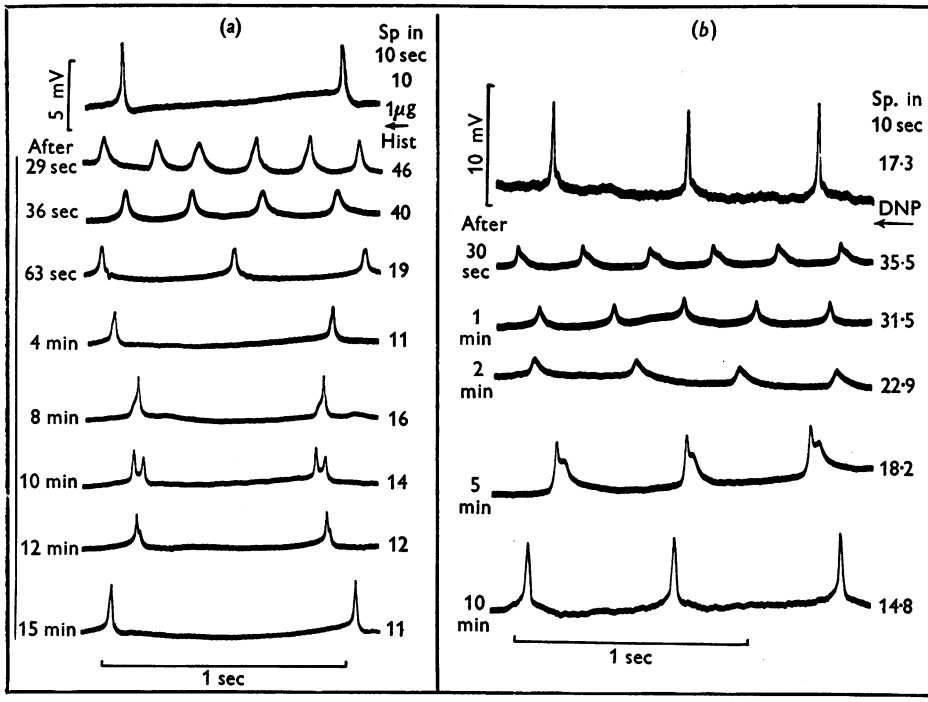


Fig. 5. The effect on spike configuration produced by (*a*) a dose of histamine (initial concentration $5 \times 10^{-7}$); and (*b*) a dose of DNP (initial concentration $10^{-5}$ M).

The action of DNP in the concentration used in these experiments was completely reversible. The membrane potential, the shape and frequency of spike potentials and the tension returned to control values, though often only after a considerable time. However, a second dose of DNP had always a stronger effect than the first.

*The action of sodium azide* ($NaN_3$)

The effect produced by $NaN_3$ was similar to that of DNP, but less striking. The concentrations investigated ranged from $10^{-3}$ M in the perfusion fluid to an initial concentration in the bath of $5 \times 10^{-2}$ M produced by injection. The lower concentrations caused some fall in tension and a decrease of pendular activity.

Higher concentrations diminished the tension considerably and stopped spontaneous activity (see Fig. 7). In the majority of preparations $NaN_3$ produced an increase in the spike frequency and prolonged the duration of the individual spike potentials within the first minute of contact with the tissue (see Fig. 8*a*).

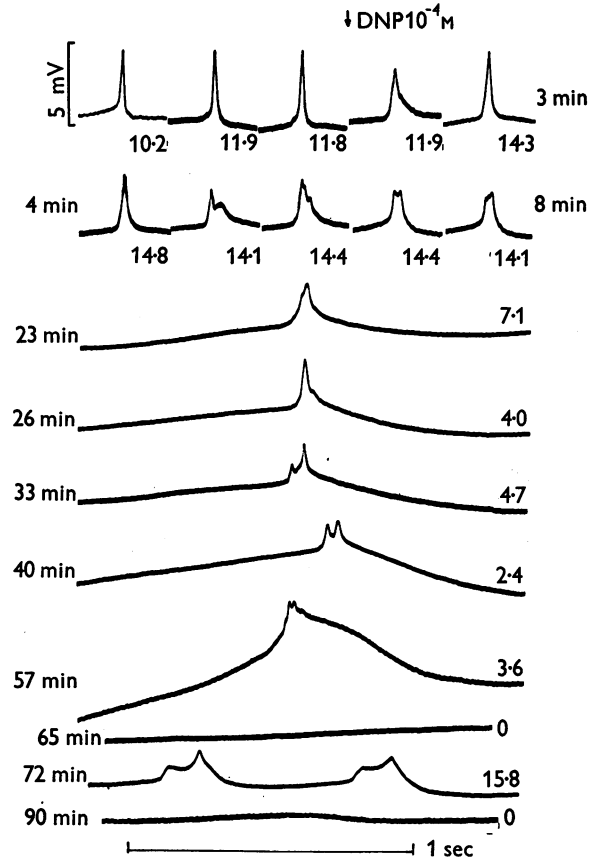


Fig. 6. The effect of prolonged exposure to DNP $10^{-4}$M on spike configuration. Top row, three controls and two records after 1 and 3 min in DNP $10^{-4}$M; second row, record obtained every min from 4th to 8th min: subsequent times on the left of each record; spike frequency on the right of each record.

Stronger concentrations then stopped spike discharge. During this period the mean membrane potential was higher than during control periods before or after (Fig. 7, Table 1). The $NaN_3$ effect was completely reversible.

### *The action of mono-iodoacetic acid (MIAA)*

The onset of the action of MIAA, unlike that of DNP and $NaN_3$, was slow. A typical experiment is shown in Fig. 9. This preparation maintained, in

normal solution, a fairly uniform tension. During the exposure to MIAA $10^{-3}$ M there was at first only a slight, gradual fall in tension. Then periods of relaxation intervened and the muscle became less able to resume its previous tension and to maintain it. After about one hour the muscle relaxed fully.

MIAA caused no dissociation between electrical activity and tension; on the contrary, a good correlation existed throughout between the spike frequency

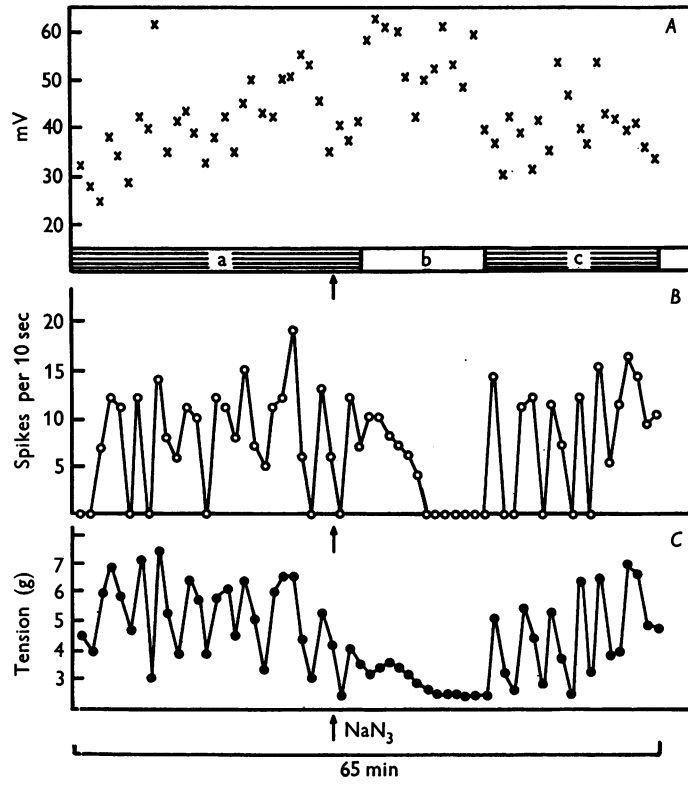


Fig. 7. Effect of an injection of $NaN_3$ (at arrow) producing an initial concentration of $5 \times 10^{-2}$ M in the bath, on membrane potential (*A*), spike frequency (*B*) and tension (*C*). (a, b, c indicate groups of observations used for Table 1.)

TABLE 1

| Substance and concentration used | Mean membrane potential (mV) ±S.E. (a) Before | (b) During | (c) After |
|---|---|---|---|
| DNP $5 \times 10^{-4}$ M in bathing solution | 33·0±1·43 ($n$=20) | 40·5±1·07 ($n$=18) | 34·5±0·99 ($n$=29) |
| DNP $5 \times 10^{-3}$ M by injection | 33·4±0·99 ($n$=20) | 44·9±2·97 ($n$=10) | 36·3±3·47 ($n$=10) |
| $NaN_3$ $5 \times 10^{-2}$ M | 40·7±1·56 ($n$=29) | 53·5±2·12 ($n$=13) | 39·5±1·55 ($n$=18) |

(For all differences $P<0·01$.)

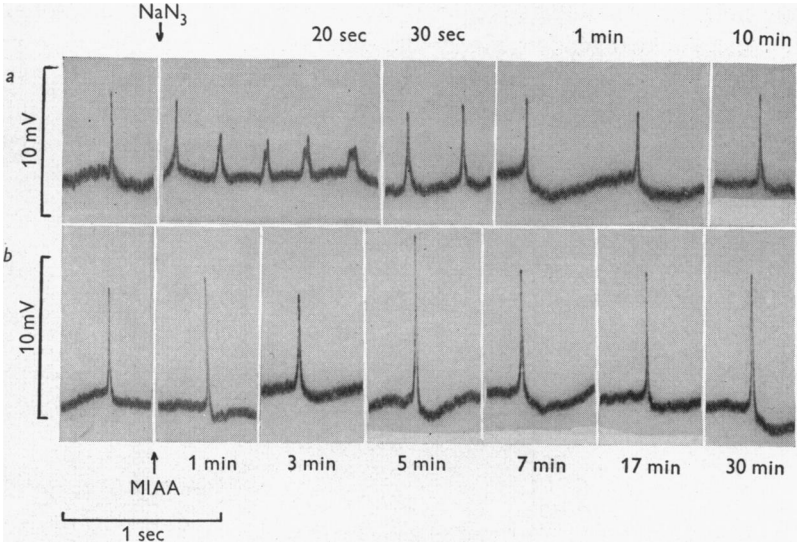


Fig. 8. (*a*) Effect of an injection of $NaN_3$ (initial concentration $10^{-2}$ M). (*b*) Effect of an injection of MIAA (initial concentration $5 \times 10^{-3}$ M). First record in both rows before, subsequent records at specified times after the injection (at arrow). (Activity stopped 40 min after the application of MIAA.)

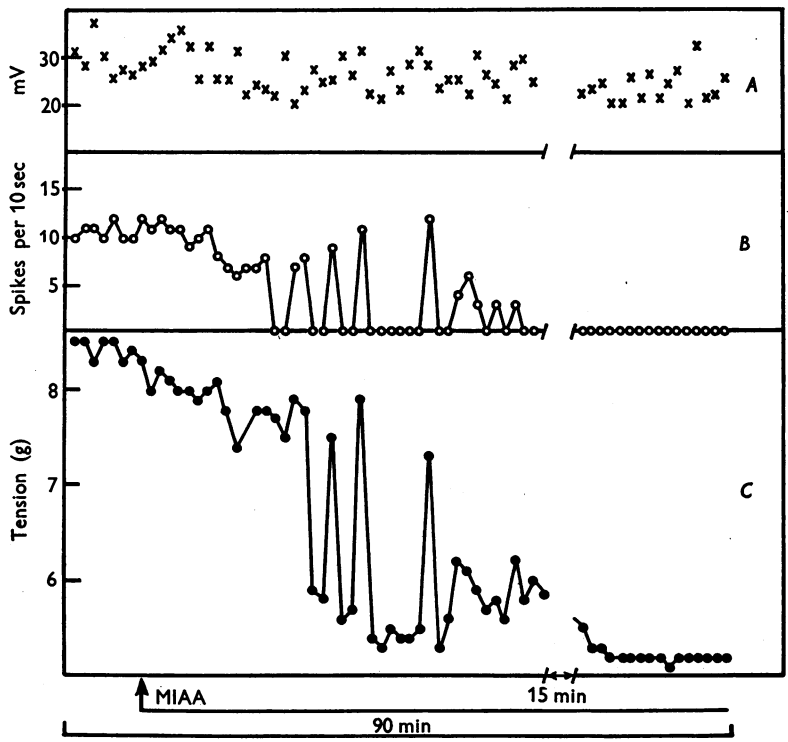


Fig. 9. Effect of MIAA $10^{-3}$ M on membrane potential (*A*), spike frequency (*B*), and tension (*C*).

and the muscle tension. The configuration of the individual spike potentials also remained unchanged (see Fig. 8*b*) though more and more silent fibres were encountered. There appeared to be a slight fall in membrane potential. However, during 90 min exposure to MIAA $10^{-3}$ M this fall was not significant, though spike discharge had ceased and the muscle was fully relaxed. The action of MIAA was irreversible.

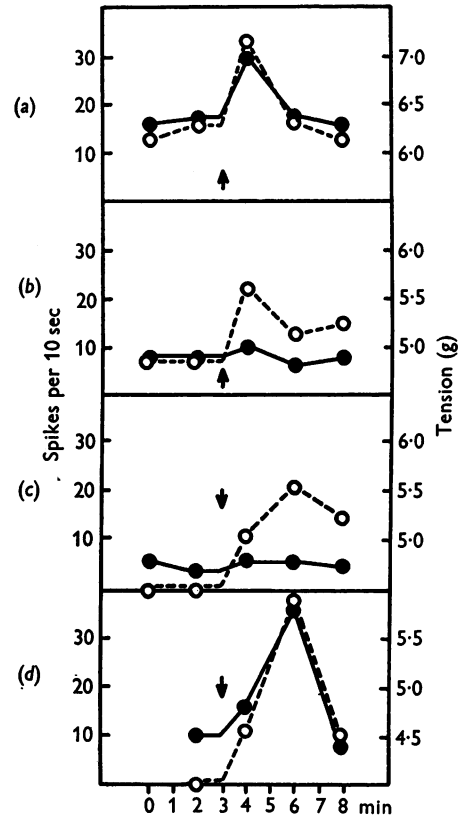


Fig. 10. Effect of injecting (at arrow) acetylcholine (initial concentration $10^{-5}$) on spike frequency (○) and tension (●) (*a*) before, (*b*) during exposure to DNP $10^{-4}$ M, 20 min from start, (*c*) 45 min from start, (*d*) 22 min after returning to normal solution.

*The influence of DNP and* $NaN_3$ *on the effect of acetylcholine and histamine*

Fig. 10 shows the effect of the injection of 20 $\mu$g ACh before, during and after exposure to DNP. The normal response to acetylcholine (*a*) was a rise in spike frequency and tension. After 20 min exposure to DNP $10^{-4}$ M, (*b*), both tension and spike frequency were lower than before; acetylcholine still caused an increased rate of discharge (from 8 to 22/10 sec), but failed to increase the tension. After 45 min exposure to DNP, (*c*), the spike discharge was zero.

Nevertheless, acetylcholine was still able to stimulate, causing a discharge of 20 spikes/10 sec but no change in tension. When DNP was washed out, (*d*), the injection of acetylcholine produced its normal effect, in spite of the fact that neither the rate of spike discharge nor the tension had yet completely recovered.

Similar experiments were done with histamine and the same dissociation of electrical activity and tension was observed. In the presence of DNP the muscle still responded to histamine with an increase in spike frequency but not with an increase in tension. Acetylcholine and histamine were both able to increase the rate of spike discharge in the presence of $NaN_3$, but the accompanying production of tension was either reduced or abolished.

## DISCUSSION

Excitation and inhibition of the smooth muscle of the taenia coli have previously been shown to be associated with characteristic electrical changes, the tension which the muscle produces being inversely related to the membrane potential but directly related to the rate of spike discharge and to the spike duration (Bülbring, 1955, 1957). The experiments now described have shown that during the exposure to DNP this correlation was abolished. When DNP came in contact with the tissue, the spike frequency rose immediately. At the same time DNP slowed the rate of repolarization of individual spike potentials, thereby prolonging their duration. In spite of this excitation at the membrane, the muscle relaxed.

When histamine or acetylcholine were administered in the presence of DNP they were still able to increase the rate of spike discharge, but were unable to increase the muscle tension. In low concentrations of DNP they occasionally caused an initial rise which soon gave way to relaxation (see Born, 1955) and this occurred while the rate of spike discharge was accelerated. Thus DNP prevented the translation of membrane excitation into the production of tension. Born (1955) found that at the time when the tension had fallen to about 25% of the initial maximum (produced in response to histamine) the concentration of ATP had only fallen to 75% of the controls, thus not to the same extent as the tension. It may be that a small reduction of ATP is sufficient for the disruption of a chain of reactions which couples the events at the membrane to the contractile mechanism, and that only a further depletion in ATP leads to complete cessation of membrane activity (Born & Bülbring, 1955).

It is known that the effect of DNP on skeletal muscle is to cause a contracture (Cori & Cori, 1936). However, Barnes, Duff & Threlfall (1955) describe an experiment (Fig. 3, p. 589) in which DNP $10^{-4}$M was applied to the rat diaphragm during tetanic stimulation. They found that in the stimulated muscle DNP caused relaxation within less than one minute. The taenia coli

which is normally continuously active is probably more comparable to the activated skeletal muscle than to resting skeletal muscle, and the relaxation observed in the rat diaphragm during a tetanus may be analogous to the relaxation of the taenia coli during its spontaneously maintained tension or 'tone'.

During the second phase of the action of DNP, when the frequency of spike discharge was diminished, the membrane potential rose. As an increased membrane potential is normally associated with a decreased spike frequency, it may well be that the inhibition of the spontaneous spike discharge during the second phase of the DNP effect was the cause for the rise in membrane potential, i.e. that the rise was the consequence of inactivity.

The effect of DNP in first accelerating and then diminishing spike discharge was parallel to that on the rate of oxygen consumption described in a previous paper (Born & Bülbring, 1955); both these changes were entirely unrelated to the changes in tension. This suggests that the initial changes in oxygen consumption produced by DNP are connected with the energy supply required for the ion transport at the membrane rather than with the energy supply to the contractile system.

SUMMARY

1. The isolated smooth muscle of the guinea-pig's taenia coli was used to study the effect of metabolic inhibitors on membrane potential, spike discharge and tension.

2. The action of 2:4-dinitrophenol was biphasic. Its immediate effect was to increase spontaneous electrical activity, accelerating the spike discharge and prolonging the duration of individual spike potentials. During the second phase of its action DNP depressed spontaneous electrical activity until it ultimately ceased.

3. During the initial phase of membrane excitation by DNP there was a fall in muscle tension. Only sometimes, with low concentrations of DNP, there was a transient rise in muscle tension which soon gave way to relaxation at a time when the spike frequency was still raised. Thus during the first phase of its action DNP abolished the normal relation between the events taking place at the membrane and the contractile system.

4. During the second phase of the action of DNP, when electrical activity had ceased, the muscle remained relaxed. The effect of DNP was entirely reversible.

5. The action of sodium azide ($NaN_3$) was similar to that of DNP but weaker; it was completely reversible.

6. The effect of mono-iodoacetic acid (MIAA) was different. It caused no increase of spontaneous electrical activity and no change in spike configuration. There was no change in the relation between spike frequency and tension; both gradually declined and were ultimately abolished irreversibly.

7. In the presence of DNP and of $NaN_3$, acetylcholine and histamine still caused an increase in spike frequency, which was comparable to their effect in normal solution, but they failed to produce an increase in tension.

8. The biphasic effect of DNP on the membrane is discussed. It is parallel to and follows the same time course as the biphasic effect on the rate of oxygen uptake in smooth muscle.

We wish to thank Mr O. B. Saxby and Mr D. Groves for their unfailing technical assistance.

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