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# THE EFFECT OF 2:4-DINITROPHENOL (DNP) ON THE SMOOTH MUSCLE OF THE GUINEA-PIG'S TAENIA COLI

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The effect of DNP in increasing the oxygen consumption of whole animals and of isolated tissues is well known. Recently, Barnes & Duff (1954) described the action of DNP on mammalian striated muscle. They found that a contracture developed at a rate which was proportional to the concentration of DNP. This contracture was followed by relaxation while the DNP was still present. During the contracture the muscle became inexcitable to indirect electrical stimulation. Barnes & Duff (personal communication) also found that in some types of smooth muscle DNP produced contracture followed by relaxation, but that other types reacted differently. Generally, smooth muscle was less sensitive to DNP than striated muscle.

In the work to be described the effect of DNP on smooth muscle was studied, using methods with which it was possible to observe simultaneously the mechanical activity, the rate of oxygen consumption and the concentration of adenosine triphosphate (ATP).

## METHODS

Guinea-pigs were used. They were killed by a blow on the neck and bled out. The whole of the anterior and posterior taenia coli was dissected. Their length *in situ* was 30–35 cm and their weight varied from 277 to 458 mg. One piece of taenia was used immediately for the estimation of ATP. A second piece of about 2 cm was used to determine oxygen consumption. The remaining taenia was divided into several pieces of similar size. They were suspended together in an isolated organ bath and attached to an isometric lever. They were treated, as far as possible, in the same way as the sample used for the determination of the oxygen consumption. At different times during the experiment they were, one by one, removed to determine the ATP concentration. The experiments were carried out in the following way: the preparation was exposed to increasing concentrations of DNP for periods of 40 min, after which the bathing solution was replaced by normal solution and 40 min was allowed for recovery.

*The bathing solution* was of the following composition. To 1 l. distilled $H_2O$ were added 7·8 g NaCl, 0·35 g KCl, 0·165 g $NaH_2PO_4$, and 1·37 g $NaHCO_3$. When this solution had been saturated with a gas mixture of 95% $O_2$ and 5% $CO_2$, 1·4 ml. of a 20% $CaCl_2$ solution, 1·0 ml. 1% $MgCl_2$ solution and 1·4 g dextrose were added. The pH was 7·4.

*Recording of mechanical activity.* Tension was recorded with an isometric lever on a smoked drum. In order to estimate changes in 'active tension' the residual tension which remained during the greatest depression of the muscle in the presence of the highest concentration of DNP was subtracted from the total tension recorded at the beginning of the experiment. By taking this value = 100 the changes observed were calculated as percentages, as recorded in Fig. 7.

*Measurement of oxygen consumption.* A piece of taenia, varying in weight from 15 to 30 mg and in length from 2·0 to 4·0 cm, was inserted in a respirometer as described in detail by Bülbring (1953). The oxygen tension in the solution bathing the muscle was measured with the polarographic method devised by Davies & Brink (1942) using an oxygen cathode which was inserted into the respirometer alongside the muscle. The rate of oxygen uptake was determined with the intermittent flow method (Larrabee, Ramos & Bülbring, 1952). In this method the flow of fully oxygenated solution bathing the muscle is stopped at regular time intervals for constant periods. During these periods the tissue consumes oxygen from the solution and, consequently, a drop of current flowing between the oxygen cathode and an indifferent anode is recorded. The rate of this drop is proportional to the rate of decline in oxygen concentration in the solution. Thus in Figs. 4–6 the 'relative oxygen consumption' is expressed as the drop in current per unit of time.

*Estimation of ATP.* The method was adapted from that described by Strehler & Totter (1954). The piece of taenia was rapidly blotted, weighed and dropped into a small centrifuge tube containing 1 ml. of boiling 0·1 M-sodium arsenate buffer pH 7·5. After boiling for 5 min the taenia was homogenized in the tube in which it had been boiled. The homogenate was boiled for 2 min more, cooled and frozen at − 10° C until all the samples had been brought to this stage. Then the homogenates were thawed and centrifuged. The concentrations of ATP in the clear supernatants were determined by comparing their effects in eliciting luminescence in firefly extracts with the luminescence produced by known concentrations of ATP. Luminescence was measured by means of a photo-multiplier tube (RCA 913 A) with a current amplifier ( × 10) and a galvanometer (200 mm/$\mu$A). Control experiments showed that ATP was not significantly broken down under the conditions used for extracting it from the taenia.

The ATP used as standard was a commercial sample prepared by the Zellstofffabrik Waldhof, Wiesbaden, Germany. It was standardized by using the hexokinase reaction.

In the earlier experiments, the concentrations of inorganic phosphate and of easily hydrolysable phosphate (7 min at 100° C) were determined in trichloroacetic acid extracts of the taenia by the method of Berenblum & Chain (1938).

## RESULTS

*Mechanical activity.* The taenia coli usually displayed a spontaneous rhythm of contractions and relaxations. The lowest concentration of DNP used was $3 \times 10^{-6}$ M. It had no visible effect on spontaneous activity. However, the mean tension was slightly raised. Concentrations up to $3 \times 10^{-5}$ M increased the mean tension as well as the spontaneous activity as shown in Fig. 1*a*. But, as the concentration of DNP was further increased, the tension declined (Fig. 1*b*). Complete depression was preceded by a short period of contracture which is shown in Fig. 2. This was usually seen with concentrations of $1 \times 10^{-4}$ M or $2 \times 10^{-4}$ M. However, while these concentrations were still present, after a period of complete inactivity, spontaneous contractions frequently began again. This activity was of a new type; the contractions were smaller and more frequent than before (Fig. 3*a*). Even with the highest concentrations used ($3 \times 10^{-3}$ M) the spontaneous activity was seen to persist for some time. Occasionally, as shown in Fig. 3*b*, the high frequency activity was seen superimposed on the otherwise normal rhythm.

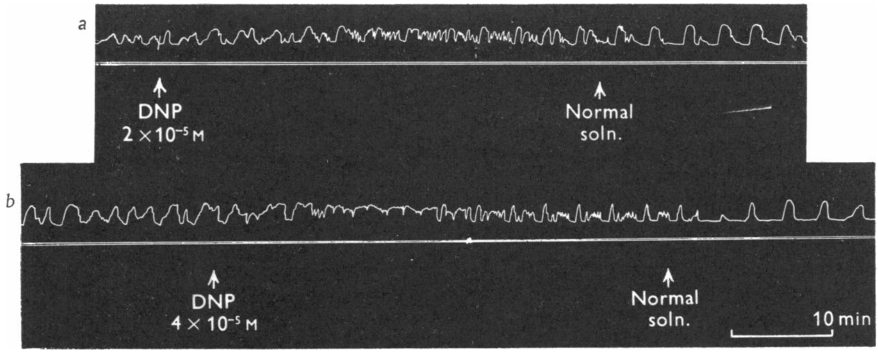

Fig. 1. Taenia coli of the guinea-pig. Record of mechanical activity increased by DNP. In (*a*) some increase of mean tension, in (*b*) a decrease is shown.

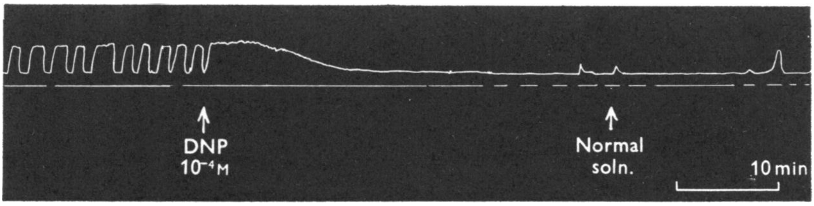

Fig. 2. Taenia coli. Contracture caused by DNP $10^{-4}$ M followed by relaxation. Note resumption of activity while DNP is still present.

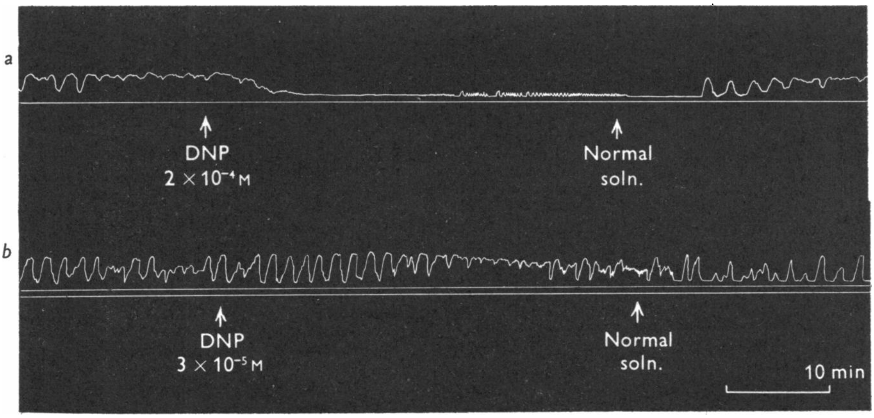

Fig. 3. Taenia coli. Small and frequent activity observed only in the presence of DNP, (*a*) after a period of inactivity, (*b*) superimposed on normal rhythm.

When the DNP solution was replaced by normal solution spontaneous activity always returned. The rate of recovery depended on the concentration of DNP which had been applied. In one experiment the taenia was exposed to $3 \times 10^{-3}$ M-DNP for 16 hr. Even after this long period it resumed spontaneous activity in normal solution.

When the average tension produced by the taenia was taken as $=100$ the change brought about by different concentrations of DNP could be calculated as a percentage. The first column of Table 1 shows the percentage changes recorded when a steady state had been reached. This was usually after 30–40 min. It will be seen that low concentrations of DNP caused some increase in mean tension, while concentrations greater than $4 \times 10^{-5}$ M caused a decrease.

*Oxygen consumption.* The mean rate of oxygen uptake by different preparations was 440 mm$^3$ $O_2$/g/hr (range 180–673). The lowest concentration of DNP used ($3 \times 10^{-6}$ M) caused a small increase in the rate of oxygen consumption of the taenia (Fig. 4). As the DNP concentration was increased to $1\times$, $2\times$ and $3 \times 10^{-5}$ M (Figs. 5, 6) the oxygen consumption increased and settled at a higher rate which was proportional to the concentration of DNP. When the DNP was removed the oxygen consumption fell again to the normal rate.

With DNP concentrations greater than $3 \times 10^{-5}$ M, the picture changed. Initially, the oxygen consumption increased, even up to 3 times the normal rate, but then it decreased and settled at a new, lower rate. The period before the decrease began and the new rate were both inversely proportional to the concentration of DNP. With the highest concentrations of DNP, the initial increases in oxygen uptake were very brief indeed, and the final rates were much smaller than the normal rate. For example, in Expt. D, in the presence of DNP $3 \times 10^{-3}$ M the decrease to a third of the normal rate was preceded by a brief period during which the normal rate was doubled. Fig. 4 illustrates the course of one experiment. The lowest concentration of DNP increased oxygen uptake only slightly; a higher concentration increased it to 3 times the normal rate, while a still higher concentration produced a brief initial increase followed by depression.

When the rate of oxygen consumption was decreased by DNP, replacement by normal solution produced an outburst of oxygen consumption before the rate returned to normal. The magnitude of this outburst was the greater the more the oxygen uptake had been depressed by DNP. An example is shown in Fig. 5. The points at which the solution of DNP was replaced by normal solution are indicated by arrows. $10^{-5}$ M-DNP raised the rate of oxygen consumption, but there was no further increase when the DNP was washed out. $2 \times 10^{-5}$ M-DNP more than doubled the oxygen uptake; yet on washing out the rate increased a little more. In the presence of $4 \times 10^{-5}$ M-DNP the oxygen consumption steadied at a lower rate and on washing out the increase was larger. With $2 \times 10^{-4}$ M-DNP the rate of oxygen uptake was further depressed and the increase on recovery was still greater.

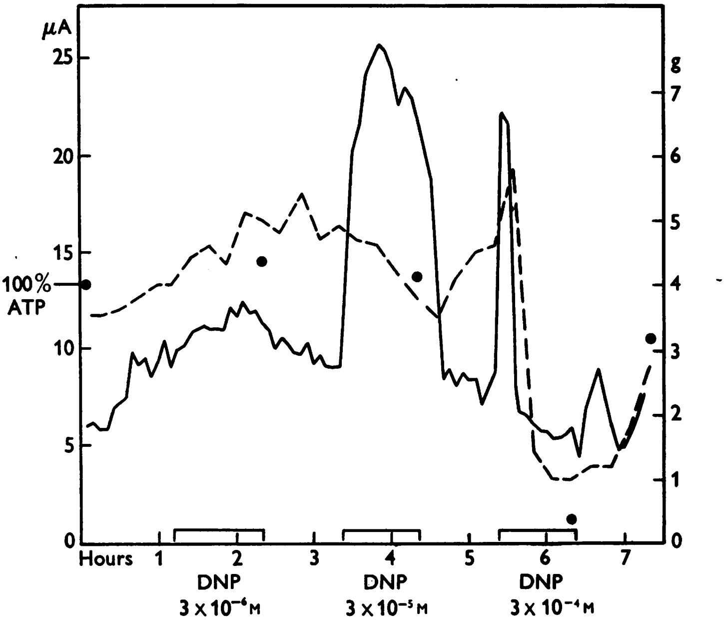

Fig. 4. Changes in tension (broken line), oxygen uptake (continuous line), and ATP content (solid circles) caused by DNP plotted against time. For description see text.

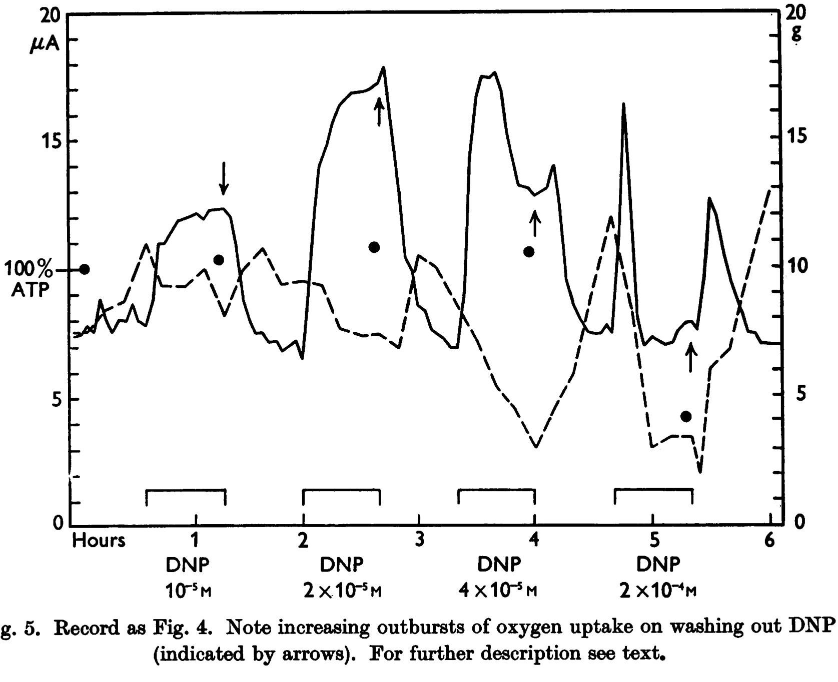

Fig. 5. Record as Fig. 4. Note increasing outbursts of oxygen uptake on washing out DNP (indicated by arrows). For further description see text.

Fig. 6 illustrates the observation that the tension rose concomitantly with the oxygen uptake as soon as DNP came in contact with the muscle. However, the tension did not rise during the outburst of oxygen consumption observed on washing out.

For the calculation of percentage changes in the rate of oxygen consumption caused by DNP the initial rise was disregarded and the final steady rate, usually attained after 30–40 min, was used. The second column of Table 1 shows that the greatest increase in oxygen consumption was produced by 3 to 4 × $10^{-5}$ M-DNP. The rate did not fall significantly below normal until the DNP concentration was greater than 3 × $10^{-4}$ M.

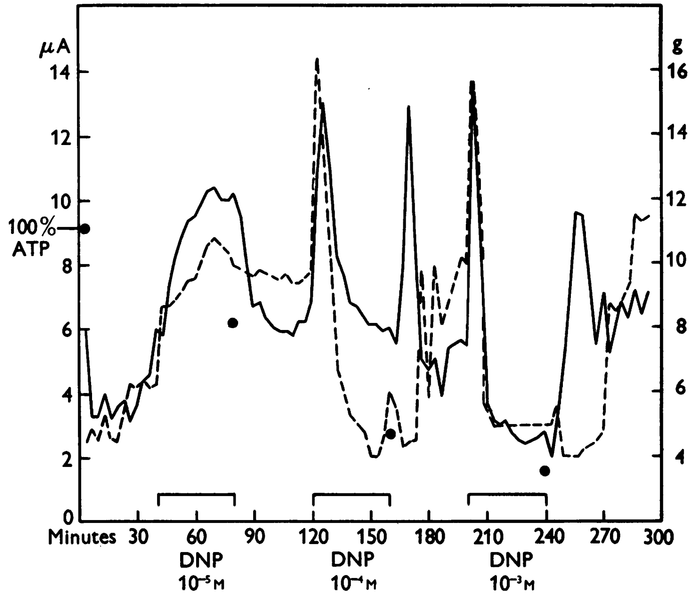

Fig. 6. Record as Fig. 4. Note concomitant changes of tension and oxygen consumption in the presence of DNP, but dissociation during recovery.

At the point where the maximum in the mean oxygen consumption was passed the tension began to fall below normal, and at the point where the oxygen consumption fell below normal, the tension was very small or zero.

*Concentration of ATP.* The concentration of ATP in taenia freshly removed from the guinea-pig varied from 900 to 1400 μg/g wet weight. The concentration of ATP in taenia which were kept in well-oxygenated Krebs's solution for several hours was not significantly different from that found in the fresh tissue.

In the third column of Table 1, the percentage changes in ATP concentration caused by DNP are recorded. The ATP content was determined after 40 min exposure to DNP and therefore corresponded to the steady states at which tension and oxygen consumption were recorded. In Figs. 4–6 the ATP concentrations are indicated as black circles.

The results of Expt. A were different from later experiments. In Expt. A the piece of taenia was at a much higher tension and it may have been overstretched. Not only its oxygen consumption but also its ATP concentration was low.

In DNP concentrations of 4 × 10$^{-5}$ M or less the ATP content of the muscle, with one exception, did not alter significantly. Higher concentrations led to a decrease. The first reduction of tension and ATP was produced by the same concentration of DNP for both. Even in the highest concentrations of DNP the ATP did not disappear altogether.

TABLE 1. Changes produced by DNP at the end of 40 min exposure (control figures = 100)

| | (1) Tension | | | | | | | (2) Oxygen uptake | | | | | | | (3) ATP content | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Expt. ... <br> DNP | A | B | C | D | E | F | G | A | B | C | D | E | F | G | A* | B | C | D | E | F | G |
| 3 × 10$^{-6}$ M | — | — | — | 125 | — | 123 | — | — | — | — | 108 | — | 125 | — | — | — | — | 103 | — | 110 | — |
| 1 × 10$^{-5}$ M | 113 | — | 160 | — | 103 | — | — | 174 | — | 196 | — | 158 | — | — | 63 | — | 67 | — | 103 | — | — |
| 2 × 10$^{-5}$ M | — | — | — | — | 77 | — | 113 | — | — | — | — | 205 | — | 215 | — | — | — | — | 109 | — | 90 |
| 3 × 10$^{-5}$ M | — | — | — | — | — | 91 | — | — | — | — | — | — | 242 | — | — | — | — | — | — | 103 | — |
| 4 × 10$^{-5}$ M | 123 | — | — | — | 25 | — | 103 | 282 | — | — | — | 158 | — | 209 | 26 | — | — | — | 107 | — | 76 |
| 5 × 10$^{-5}$ M | — | 16 | — | — | — | — | — | — | 96 | — | — | — | — | — | — | — | — | — | — | — | — |
| 8 × 10$^{-5}$ M | 60 | — | — | — | — | — | 61 | 214 | — | — | — | — | — | 147 | 42 | — | — | — | — | — | 77 |
| 1 × 10$^{-4}$ M | — | — | 18 | 26 | — | — | — | — | — | 105 | 92 | — | — | — | — | — | 29 | 46 | — | — | — |
| 2 × 10$^{-4}$ M | 37 | 10 | — | — | 20 | — | — | 130 | 60 | — | — | 95 | — | — | 17 | — | — | — | 42 | — | — |
| 3 × 10$^{-4}$ M | — | — | — | — | — | 0 | — | — | — | — | — | — | 61 | — | — | — | — | — | — | 10 | — |
| 6 × 10$^{-4}$ M | 17 | — | — | — | — | — | — | 68 | — | — | — | — | — | — | 23 | — | — | — | — | — | — |
| 8 × 10$^{-4}$ M | — | 10 | — | — | — | — | — | — | 28 | — | — | — | — | — | — | — | — | — | — | — | — |
| 1 × 10$^{-3}$ M | 0 | — | 25 | — | — | — | — | 49 | — | 43 | — | — | — | — | 17 | — | 17 | — | — | — | — |
| 3 × 10$^{-3}$ M | — | 0 | — | 0 | — | — | — | — | 12 | — | 29 | — | — | — | — | — | — | 29 | — | — | — |

* Determined by the 7 min-phosphate method.

When normal solution replaced the solution containing DNP the ATP content increased rapidly. In one experiment, after 20 min, it rose from 76 to 92 % of normal, and in two others, after 40 min, it rose from 10 to 81 and from 42 to 93 % of normal, respectively.

## DISCUSSION

The observations recorded in this paper suggest that DNP is a useful tool in the study of smooth muscle since the effect it produced, even when the highest concentrations were applied for many hours, was reversible.

Our results are summarized in Fig. 7. The averages of the percentage changes are plotted against concentrations of DNP. Below a critical concentration of DNP, 3 × 10$^{-5}$ M, there is no decrease in tension or ATP concentration although the oxygen consumption rises. At the critical concentration the oxygen consumption passes through a maximum. With higher concentrations both tension and ATP concentration fall below normal.

In the present state of knowledge it is possible to interpret the effects obtained with DNP concentrations greater than 3 × 10$^{-5}$ M as follows: first, it has been known since the work of Loomis & Lipmann (1948) that DNP interferes with the formation of ATP, and we found that the ATP concentration fell. Secondly, ATP is believed to be a key substance in the development of tension in muscle. Thus, the fall in ATP concentration may account for the observed decrease in tension. Thirdly, it has been shown (Bülbring, 1953) that the oxygen consumption of smooth muscle is proportional to the tension, and we found that the fall in tension was accompanied by a decreased rate of oxygen uptake.

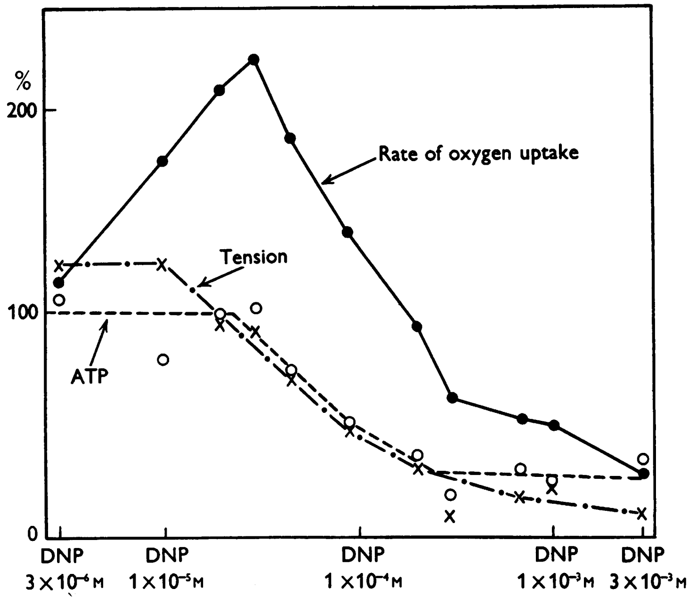

Fig. 7. Changes produced by increasing concentrations of DNP (abscissae) on tension ( × —•— × ), oxygen consumption (●——●) and ATP concentration (○---○) (ordinates) in the taenia coli. Normal = 100.

The effects obtained with DNP concentrations smaller than $3\times10^{-5}$ M may be considered in the following way: it is well known that oxygen consumption of carefully isolated surviving mitochondria can be greatly increased when phosphate-acceptor systems are added. It has been postulated that the rate of cellular respiration depends upon the relative rates of build-up and breakdown of ATP in 'phosphate cycles' and that DNP has the effect of increasing the concentration of phosphate-acceptor, thereby raising the rate of oxygen uptake (for references see Hunter, 1951; Anfinsen & Kielley, 1954).

We suggest that low concentrations of DNP lead to a faster rate of ATP turnover without net loss of ATP, and that this is the situation in smooth muscle with concentrations of DNP up to $3\times10^{-5}$ M. This concentration of DNP raises the oxygen consumption to a maximum and the turnover of ATP may be fastest while the net concentration of ATP still remains normal. With higher concentrations of DNP the rate of oxygen consumption declines and the concentration of ATP also declines, the rate of breakdown exceeding the rate of synthesis.

The tension and spontaneous activity of the taenia were actually increased by low concentrations of DNP. This suggests that the tension depends not only upon the net amount of ATP in the tissue but also upon the rate of turnover of ATP.

In this connexion it might be significant that during recovery from DNP, tone and activity return only gradually while the oxygen consumption rises rapidly above the normal rate. This increase is the greater the more the oxygen consumption had previously been depressed.

One of us (unpublished results) has found that the concentration of DNP which was critical in the case of taenia coli has an effect on the histamine contraction of the isolated ileum of the guinea-pig which higher or lower concentrations do not have. In 3–5 × $10^{-5}$ M-DNP the ileum loses the ability to respond to histamine with a *sustained* contraction. The immediate rapid component of the contraction is as great as in the absence of DNP. West, Hadden & Farah (1951) have found that the response of the isolated intestine of the rabbit to acetylcholine is affected in a similar manner by anoxia and cyanide. These findings suggest that the contractions of smooth muscle consist of two components, a rapid phase and a slow phase; and that it is only the slow component which requires a completely normal supply of metabolic energy. This will be further investigated.

## SUMMARY

1. The isolated taenia coli of the guinea-pig was used to determine the effect of DNP on tension, oxygen consumption and ATP concentration in smooth muscle. DNP was used in concentrations ranging from $3 \times 10^{-6}$ M to $3 \times 10^{-3}$ M.

2. Low concentrations of DNP increased tension and spontaneous activity. Higher concentrations at first increased and, when left in contact with the tissue, decreased tension and spontaneous movements. The highest concentrations abolished the movements at first, but after a period of inactivity smaller, more rapid movements often supervened. When the DNP was removed, even after 16 hr, the muscle resumed its tension and spontaneous activity.

3. Low concentrations of DNP increased the rate of oxygen consumption. With successively higher concentrations this increase also occurred but gave way, more and more rapidly, to a depression of oxygen uptake. This was, however, not reduced to zero but remained at a steady low rate. When, after 1 hr, the DNP was removed the oxygen consumption returned to normal. Recovery was always preceded by a period in which oxygen uptake considerably exceeded the normal rate.

4. The concentration of ATP in the taenia coli varied from 900 to 1400 $\mu$g/g wet weight. In most experiments the concentration of ATP was not significantly altered by $3 \times 10^{-5}$ M-DNP or less. In higher concentrations of DNP the ATP concentration decreased with the decrease in tension.

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