AN ISOLATED NERVE-MUSCLE PREPARATION FROM *ASCARIS LUMBRICOIDES*

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(With Five Text-figures)

INTRODUCTION

In an earlier publication (Baldwin, 1943) we described a method for the detection and approximate measurement of anthelminthic potency. The method consisted essentially in recording by a kymographic technique the movements executed by short, sausage-like fragments of *Ascaris lumbricoides* (from the pig), prepared by tying off portions of the worm between tightly drawn silk ligatures. It was shown that there exists a high degree of correlation between anthelminthic potency in a given drug and positive reactions to it (i.e. eventual contracture or paralysis) on the part of our preparations. On the other hand, numerous drugs devoid of anthelminthic efficacy elicited no response, and among these were acetylcholine, adrenaline and a number of other sympathetico- and parasympathetico-mimetics.

In the past it has been almost standard practice in the chemotherapeutic assay of anthelminthic potency to carry out *in vitro* tests on tissues prepared from the body wall of earthworms and leeches (e.g. Trendelenburg, 1916; Lautenschläger, 1921; von Oettingen, 1929; Rosenmund & Schapiro, 1934; Oelkers & Rathje, 1941). These annelid materials are notoriously sensitive to the action of drugs of many different kinds. Earthworm muscle, taken from the body wall, responds to acetylcholine, eserine, choline, pilocarpine, to adrenaline, to ephedrine, and to cocaine, strychnine, caffeine and many others, none of which evoked any response from the *Ascaris* material used in our earlier experiments. The musculature of the alimentary tract of the earthworm likewise responds to many of these compounds (Wu, 1939). These facts suggest that either (*a*) the nematode cuticle, which was present in our preparations, is selectively permeable to some drugs and impermeable to others, or else (*b*) that the neuro-muscular apparatus of the nematode differs, perhaps fundamentally, from that of annelids. In either case the use of annelid material in the study of anthelminthics is not only illogical (cf. Lamson & Ward, 1936) but fundamentally unsound, and should be abandoned forthwith. In the hope of gaining more precise information we attempted to prepare from *Ascaris* functional fragments of the body-wall musculature of a kind in which there should be no cuticular barrier, so that a comparative study of the physiology and pharmacology of nematode material might be attempted.

The need for such a preparation has long been felt, not only for studies of anthelminthics, but also for the sake of the light that might be thrown with its aid upon

the neuro-muscular physiology of this phylum. Very little is known about the physiology of the Nematoda as a whole (see Lapage, 1937). Morphologically, as is well known, its members are very peculiar indeed and, from the biochemical point of view also, numerous peculiarities have been noted, such, for example, as the presence in the tissues of remarkably high concentrations of volatile fatty acids (see p. 284) and the occurrence of the apparently unique compound, ascaryl alcohol (Flury, 1912; Schulz & Becker, 1933). It is therefore to be anticipated that the physiology of the nematodes might also present some unusual features.

A few experiments on isolated *Ascaris* muscle were carried out by Trendelenburg (1916) in the course of his classical researches on the pharmacology of santonin and its derivatives, but the only record published in his paper does little to support his claim 'Das die Regenwürmer in derselben Weise wie die Spülwürmer auf Santonin mit Erregung reagieren würden, schien höchst wahrscheinlich'. Trendelenburg's authority appears to have been largely responsible for the introduction and subsequent wide employment of annelid tissues in anthelminthic studies: indeed, he himself soon abandoned nematode in favour of earthworm muscle because of the extreme difficulty of removing the cuticle of *Ascaris* from the muscular layer without damaging the latter. Our own experience has amply confirmed this difficulty.

We have now devised a simple preparation by which the musculature can be exposed directly to the action of drugs without previous removal of the cuticle. Observations on the physiology and pharmacology of preparations of this kind will be reported in further publications: for the present we propose to describe only the operative procedure, the general treatment and the normal behaviour of the new preparations.

MATERIAL AND METHODS

The collection of our animals, the general conditions of their maintenance in the laboratory and the experimental procedure were substantially the same as in our previous work (Baldwin, 1943). We have, however, adopted a new 'keeping medium'. We were fortunate in having at our disposal the results of a number of analyses, each obtained from a number of pooled samples, of the body-fluid of *Ascaris* and of the (centrifuged) contents of the small intestine of pigs, and are very much indebted to Prof. A. D. Hobson, Dr W. Stephenson and Dr A. Eden for permission to make use of these data. The variations between one mixed sample and the next were considerable, and large enough, in our opinion, to justify the use of rounded average figures: these are set out in Table 1.

Table 1. *Composition of* Ascaris *body fluid, contents of small intestine of pig, and old 'keeping medium'*

	mM total					Equiv. mM NaCl	
	Na	K	Ca	Mg	Cl	Conduct.	Osm. press.
Ascaris, body fluid	130	25	6	5	53	143	198
Pig, gut contents	124	27	14	6	61	174	257
Old 'keeping medium' (Baldwin, 1943)	136	2·7	1·8	0·4	—	—	—

A striking feature of these new data is the great disparity between the amounts of chloride and total base. If the figures given by McCance (1936) for the human subject are in any way applicable to the pig, it is very probable that the deficit of anions in the pig-gut contents must be largely made up by bicarbonate. In view of the mutual replaceability of chloride and bicarbonate ions in most biological systems we carried out some survival experiments on intact worms in media made up from the chlorides Na, K, Ca and Mg in the proportions indicated by the analytical data for the pig-gut contents, but most of the specimens died within 2 or 3 days, i.e. much earlier than in the very dissimilar medium employed in our earlier work. This, we suspect, is largely due to the high [Ca^{++}] of the mixture; the data of Table 1 correspond to total and not to ionic concentrations.

Much better results were obtained with media patterned on the body fluid, buffered to *p*H 6·7 with phosphate and having a total salinity equivalent to 170 m*M* NaCl, a concentration very similar to that of the electrolytic components of the pig-gut contents (174 m*M*). In relative composition this saline closely resembles the natural external medium of the worm (see Table 3) except in so far as the Ca content and total osmotic pressure are concerned. Worms kept in this medium appeared to be more active than in our older 'keeping medium', individual specimens surviving as a rule for 4–12 days, but not longer than in the 'old keeping medium'. We adopted the newer medium on account of its closer resemblance to the natural environment of the parasites. Experience showed that useful results are only occasionally to be obtained with worms that have been in the laboratory for more than 24 hr., and we did not therefore go any further into the question of survival.

The new 'keeping medium' is prepared as follows. We keep a stock of concentrated medium containing 75·5 g. NaCl, 14·2 g. KCl, 13·1 g. $CaCl_2.6H_2O$ and 10·2 g. $MgCl_2.6H_2O$ per litre. A stock phosphate buffer is prepared containing 250 ml. 0·2 *M* KH_2PO_4 and 21 ml. *N* NaOH made up to 1 l. One volume of the concentrated stock receives 1 vol. of the stock buffer and is diluted to 10 vol. with distilled water and warmed to 38° C. for use. The product has the following properties:

	m*M*
Na^+	130
K^+	24
Ca^{++}	6
Mg^{++}	5
Total phosphate	5
Total salinity	*c.* 170 (as NaCl)

*p*H = 6·7

With this modification our worms are kept under the conditions previously described (Baldwin, 1943).

Preparation of muscle strips

When our work began there was nothing to indicate what kind of saline bathing medium might be suitable for maintaining the physiological condition of fragments

of isolated nematode tissue, and it was accordingly difficult to be sure whether, when a given preparation failed to show any lasting activity, the saline or the preparation itself was at fault. Occasionally, however, individual fragments of the body wall showed a more or less rhythmic activity that enabled us in time to develop the following operative technique. Large, active female worms were used throughout.

A suitable specimen is dropped from a height of about 12 in. on to the bench. This treatment leads to a sharp contraction of the musculature, immobilizes the animal for the time being, and considerably increases the firmness of the whole body, thus facilitating the procedure. It is essential as a preliminary measure since, if it is omitted, the final muscle strip usually contracts to a length shorter than the minimum which, in our experience, is necessary for satisfactory performance.

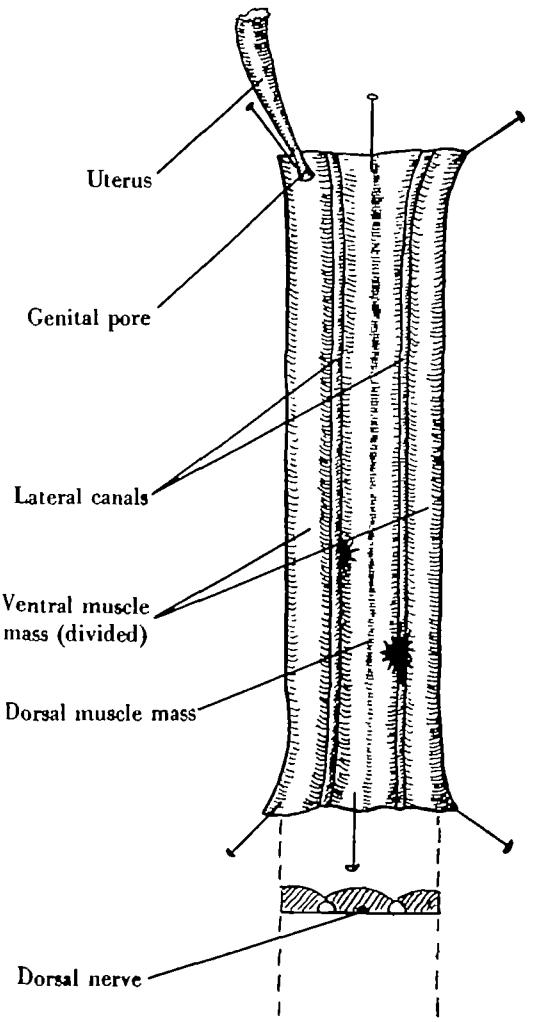

Fig. 1. See text for explanation.

The 'stunned' worm is pinned down to a cork mat, ventral surface upwards, by passing a pin vertically through the body about 5 mm. behind the genital pore. A second pin is passed through about 4 cm. in front of the pore and the external parts are cut away. The upper (ventral) muscle is divided by a longitudinal incision with a very sharp scalpel, the divided portions are deflected and pinned down, and the gut carefully removed with fine forceps. The preparation now has the appearance illustrated in Fig. 1. Further cuts are made along the lateral canals, great care being taken to avoid injury to the dorsal muscle. Silk ligatures are loosely applied to the strip of dorsal muscle thus isolated, the first being applied about 2–3 mm. in front of the level of the genital pore and the second 2·5 cm. farther forward. Variations of ± 1–2 mm. are immaterial, but the optimal length appears to be 2·5 cm. After tying the ligatures the remainder of the tissue is removed. Better results were obtained with loose than with tight ligatures, and it has been found necessary to leave rather long 'tails' (4–5 mm.) beyond the ligatures in order to avoid slipping.

Each muscle strip thus prepared consists essentially of a ribbon-like piece of dorsal muscle together with the corresponding dorsal and sublateral nerves. While the external surface of the strip retains its cuticle, the inner side is exposed. Strips of ventral muscle can be similarly prepared by starting with a dorsal incision. The region used corresponds to the 'intermediate preparations' of our earlier work; we have not so far attempted to obtain exposed fragments comparable with our 'anterior preparations'.

It is best to prepare several strips at a time. Each in turn is transferred to a bath at 38° C. containing the special buffered experimental medium described below (p. 286), a load of 20–40 mg. is applied to each and the whole batch is kept under observation. The immediate response to being placed in the warm bath consists in a powerful, long-lasting contraction, followed by gradual relaxation. After a period of relative quiescence, a more or less rhythmic activity sets in in about 60% of all strips prepared from a good batch of material, and full activity is usually established in 30–60 min.

It may be pointed out that different batches of worms yield preparations of very variable degrees of usefulness. This variability, we believe, is due to circumstances beyond our control, i.e. to the treatment accorded to the animals during collection and transport. It is particularly noticeable that very few successful preparations can be obtained if the temperature of the medium in which the worms are transported has fallen below 30° C. before reaching the laboratory.

Loading and recording

The movements executed by these preparations vary widely. They consist essentially of alternating longitudinal contractions and relaxations, but on account of the closeness of attachment of the muscle to the cuticular layer and the considerable rigidity of the latter, the strips often assume bizarre shapes in the contracted condition. The best preparations are selected for attachment to the recording apparatus.

A very light isotonic lever with a Gimbal-mounted writing point was used in all our later experiments, but in some of the early work we used simple levers with adjustable loads. It was found in many cases that, if the load is gradually increased, a critical point is reached at which the strip suddenly relaxes very sharply indeed and at once contracts again, leading in some specimens to the onset of vigorous activity. In some experiments we have seen these small strips of muscle working actively under loads of as much as 50 g. Later, however, we abandoned heavy loads entirely in favour of weights of only 20–40 mg.

A number of representative tracings are shown in Fig. 2. Individual strips give records that vary markedly in amplitude and frequency, but the behaviour of a given specimen is usually very consistent. Strips showing aberrant behaviour or any serious irregularity are usually rejected if they fail to settle down after 60–90 min. Particularly noteworthy is the typical difference between dorsal strips and those prepared from ventral muscle. This difference is remarkably consistent and must presumably correspond to some difference in physiological constitution, the precise

nature of which remains to be investigated. In the meantime the bulk of our experiments have been carried out on dorsal preparations, since a larger proportion of these gives useful behaviour on the kymograph.

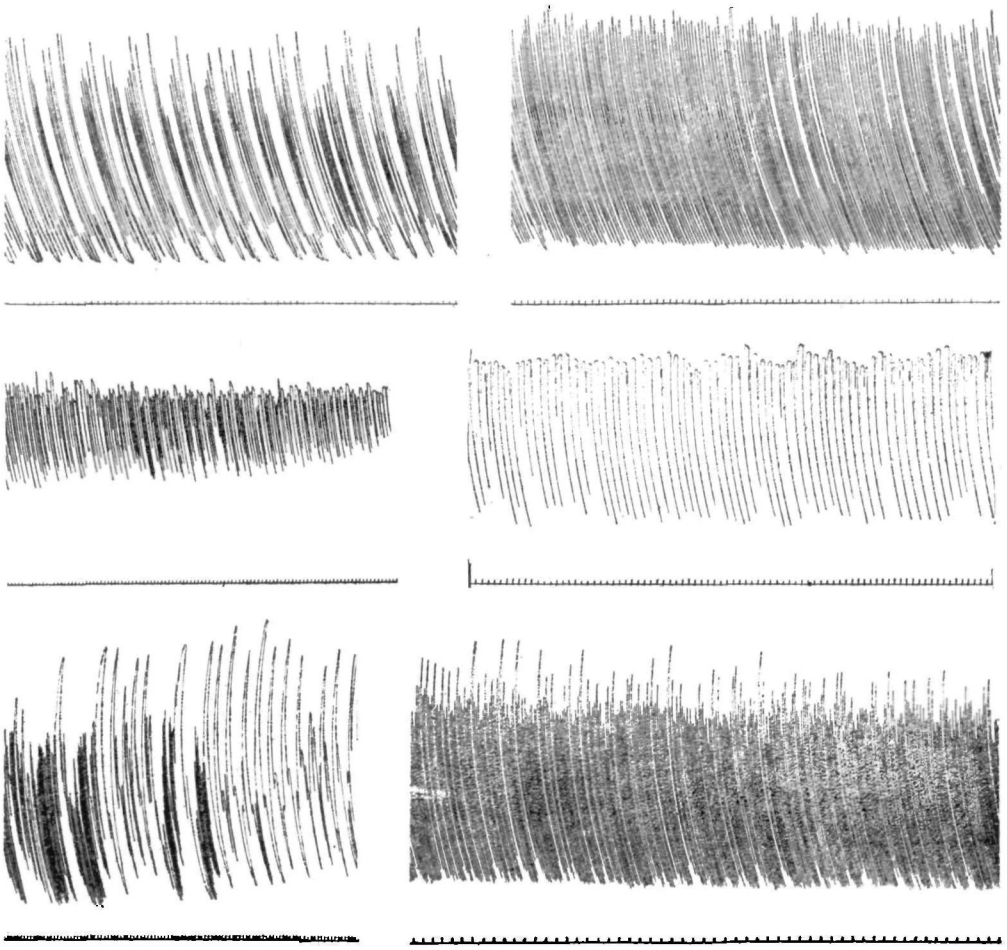

Fig. 2*a*. Typical tracings of behaviour of dorsal muscle strips. Upward stroke of lever corresponds to contraction; time-marker intervals, minutes in all cases. Read from left to right. Records taken in experimental medium (p. 286) unless otherwise stated.

Environmental conditions

(i) *General.* In the absence of much specific information about the general physico-chemical conditions prevailing in the normal internal and external environments of *Ascaris in situ* in the gut of the host, we had of necessity to make numerous empirical trials to find conditions under which the physiological activity of our preparations might be maintained. The conditions to be taken into account, apart from the relative ionic composition of the medium and the possibility of specific peculiarities, included temperature, *p*H, osmotic pressure, oxygen tension,

carbon dioxide tension and so on. A temperature of 38° C. was used throughout, this being the one factor of which the suitability seemed to be logically assured.

Bunge (1883, 1890) and Weinland (1901) are among those who have claimed that *Ascaris* can live under strictly anaerobic conditions. Slater (1925) severely criticized their conclusions on the grounds that the precautions taken to ensure complete anaerobiosis in their experiments were quite inadequate. That the tissues of *Ascaris* can utilize oxygen is certain, and there are in the literature many records of measurements of the Q_{O_2} of intact worms and of their several tissues under a variety of experimental conditions (e.g. Harnisch, 1933, 1935; Krüger, 1936). According to

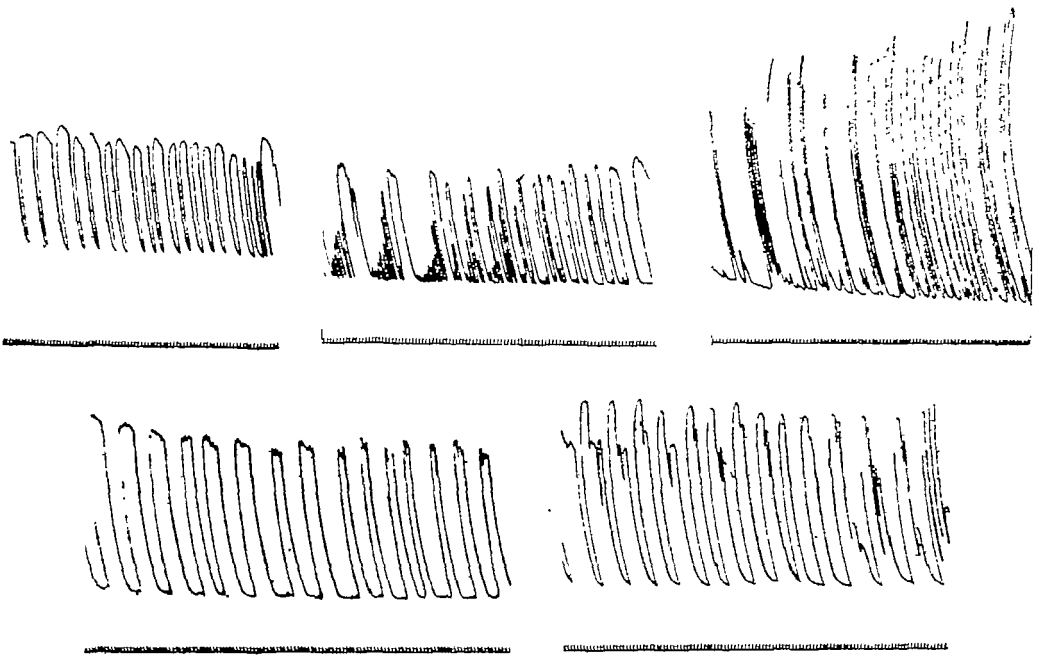

Fig. 2*b*. Typical tracings of behaviour of ventral muscle strips. Upward stroke of lever corresponds to contraction; time-marker intervals, minutes in all cases. Read from left to right. Records taken in experimental medium (p. 286) unless otherwise stated.

Laser (1944), the rate of oxygen consumption of the intact worm is not much smaller than that of intact mammalian organisms kept under comparable conditions of temperature and oxygen tension, though high oxygen tensions were found to have definitely toxic effects.

There is little reason to think that much oxygen is normally present in the habitual environment of *Ascaris*. Long & Fenger (1917), whose results have been substantially confirmed by von Brand & Weise (1932), reported that the intestinal gases of pigs contain variable amounts of oxygen, averaging about 5 %. The experiments of Bunge, Weinland, Slater and many others show, however, that this parasite can live for long periods at very low oxygen tensions and, in our earlier work, we have ourselves seen tubular fragments of *Ascaris* actively recording on the kymograph

after 24 hr. or more under experimental conditions in which no provision whatever was made for oxygenation of the medium.

We decided for our present experiments, therefore, to use cylinder nitrogen to provide the atmosphere in our media. Ordinary commercial nitrogen usually contains a few per cent of oxygen, and this, it was hoped, would suffice to meet the oxygen requirements of the tissues without exposing them to the danger of oxygen poisoning (see Laser, 1944). Our media were 'gassed' at the beginning of each experiment: maintenance of gassing throughout the experiments resulted in no improvement in the behaviour of our preparations, nor did frequent replacement of the medium by freshly gassed samples. Indeed, it has been our experience that, once a muscle strip has settled down to a steady pattern of behaviour, the less it is disturbed the better.

(ii) *Body fluid as experimental medium.* We anticipated that the properties and composition of the body fluid of *Ascaris* (Table 1) would provide a useful guide to the conditions required for maintaining the activity of our muscle strips. Several features of this body fluid call for special comment however.

The large disparity between total chlorides (53 m*M*) and total base (166 m*M*) is largely due to the presence of volatile fatty acids. Bunge (1890) long ago noticed the peculiar smell of media in which *Ascaris* have been kept, and attributed it to the presence of a volatile fatty acid. Weinland (1901, 1904) believed that a valeric and a caproic acid are concerned, and that these are essentially excretory products. Although these acids have been studied by a number of workers (e.g. Schulte, 1917; Flury, 1912; Krüger, 1936; von Brand, 1934), they have still not been satisfactorily identified. Much discussion has centred round the possibility that they might be formed, not by the worms themselves, but by bacteria, a possibility that could not easily be eliminated because of the virtual impossibility of sterilizing *Ascaris* itself. Most of the work hitherto carried out on these acids has been done on material collected, usually by steam distillation, from media in which *Ascaris* has been housed and in which, therefore, bacterial activity has probably or even certainly been considerable. Schimmelpfennig (1902), however, claimed that the same fatty acids are present in ethereal extracts of the whole worm, an observation later confirmed by Flury (1912).

We ourselves carried out a number of estimations on freshly collected body fluid previously deproteinized with tungstic acid. Aliquot portions of the filtrates were steam-distilled in a Markham (1942) apparatus, and the acids estimated in the distillates by titration with CO_2-free NaOH in a CO_2-free atmosphere. Added valeric acid was recoverable to the extent of 96–98 % under the conditions employed. The results obtained are presented in Table 2 and indicate the presence in the body fluid of some 50 m*M* steam-volatile fatty acids.

The fact that these acids are present in such large quantity in the perienteric fluid itself would seem to militate against the supposition that they owe their origin to bacterial activity and argue for them a biological role of considerable importance, though what this role may be is at present uncertain. It has usually been supposed that they are excretory products, but they may conceivably discharge an osmotic

role comparable with that of the urea which is so characteristic a feature of the blood and tissues of the Elasmobranchii (Smith, 1936) or with that of the glycine, taurine and other extractives which appear here and there in the animal kingdom, often in remarkably large concentrations (cf. Krogh, 1939, p. 196).

Together with chloride, these acids are equivalent to about two-thirds of the total base found by analysis. To what extent they are ionized is uncertain, the *p*H of the body fluid being somewhat unsure. The remaining anions have not been identified, but in view of the close resemblance between the compositions of the body fluid and the gut contents of the host, and of the probably high bicarbonate content of the latter, it seems likely that they must consist largely of bicarbonate.

Table 2. *Steam-volatile fatty acid content of body fluid of* Ascaris

Exp. no.	Days in laboratory	m*M* volatile acids
1	1	53
2	2	64
3	1	52
4	1	58
5	Fresh	50
6	,,	51
7	,,	35
8	,,	34
9	,,	57
10	,,	60
	Average	51

Table 3. *Relative composition of some biological and experimental fluids*

	Na	K	Ca	Mg	m*M* total base
Ascaris, body fluid	100	19	5	4	166
Pig-gut contents	100	21	11	4·8	171
Ringer's solution	100	1·7	1	0	116
Tyrode's solution	100	1·8	1·2	0·7	155
New 'keeping medium'	100	19	5	4	166
Experimental medium (p. 286)	100	3	1·5	3	120

Anions apart, the relative composition of the perienteric fluid resembles that of the external environment (i.e. the pig-gut contents) rather closely (Table 3) and differs markedly from that of the internal media of animals in general. Table 3 includes the relative ionic composition of Ringer's and Tyrode's solutions, which may be taken as roughly representative of the normal internal environment of animals as a whole. Because of the numerous known peculiarities of the Nematoda we were tempted to believe that the perienteric fluid of *Ascaris* might, in spite of its somewhat peculiar composition, represent the true *milieu intérieur* of that animal, and carried out many experiments in solutions made up to resemble this fluid in inorganic composition. We did not, however, attempt to imitate the fatty acid composition, the acids being still unidentified, but replaced them by chloride in preference to running the risk of introducing foreign anions of a possibly toxic nature.

Numerous strips became active in the 'synthetic body fluid', but in no case was the activity long maintained, and we finally came to the conclusion that the body fluid does not in reality correspond to the true *milieu intérieur*, using that term in its classical sense. Its true biological status awaits elucidation: conceivably it is, in essence, an excretory product.

(iii) *Experimental medium.* Media of the Ringer and Tyrode types seemed to offer the best prospects of success, and some hundreds of experiments in all were carried out in a search for the most satisfactory conditions. Magnesium was found to be a necessary constituent as, indeed, is usually the case for invertebrate tissues. For buffering purposes we decided on a mixture of carbon dioxide and bicarbonate, together with phosphate, since the combination gave more satisfactory results than either alone.

We are indebted to Prof. Hobson for informing us that 'the *p*H of the body fluid is rather uncertain owing to the fact that it changes very rapidly on exposure to air. When taken with a glass electrode as quickly as possible after "bleeding" it seems to average about 6·8, but I have known it as low as 6·5 and as high as 7·0' (personal communication). Probably the drift observed in these determinations must have been due to the loss of volatile fatty acids when the fluid was exposed, and we believe that the *p*H of the body fluid *in situ* must probably lie nearer to 6·5 than to 7·0. Our muscle strips, however, seem to be little affected by changes of *p*H between 6·5 and 7·5, and we settled finally on a *p*H of 7·1 as a matter of experimental convenience.

The osmotic pressure of the body fluid likewise proved no guide to the total salinity desirable in an experimental saline. Muscle strips prepared in the usual manner were suspended in a series of media having the same relative ionic composition but differing in total salinity, and were weighed at intervals. At 120 m*M* the changes of weight were usually within 5% of the initial value over periods of 3 hr., larger changes being observed at higher and at lower osmotic pressure. We therefore determined to use a total salinity of 120 m*M*.

It is not necessary here to give a detailed account of the experiments carried out with media of various compositions. Our aim was to find, if possible, an experimental medium in which rhythmic activity could be developed and subsequently maintained on a steady base-line, and eventually we adopted a medium, the preparation of which is described below, having the following properties:

$Na^+ : K^+ : Ca^{++} : Mg^{++} = 100 : 3 : 1{\cdot}5 : 3$,
$HCO_3^- = 6$ m*M*,
Total phosphate = 3 m*M*,
Total salinity = 120 m*M* (as NaCl),
*p*H = 7·1,
Thiamine hydrochloride = 1 : 10,000.

In this medium we were able to maintain the activity of our strips for periods up to 9 hr., with a usual survival of 5–8 hr. Longer survivals were not obtained under any of the numerous conditions tested, but, having due regard to the nature of the

muscle strip which, unlike the isolated frog heart for example, is in no sense an intact physiological unit, we feel that these results may be regarded as satisfactory.

The incorporation of thiamine into the medium followed an observation that this vitamin encourages relaxation of the muscle strips. Many preparations show a marked tendency to remain contracted for relatively long periods, relaxing only infrequently and partially. The general impression gained by careful observation of preparations of this kind was that some sort of nervous dysfunction was involved and, recalling the polyneuritis associated with vitamin B_1 deficiency in other animals, we tried the effect of adding small amounts of thiamine to the medium. A typical record is shown in Fig. 3. The type of behaviour in the early part of the record is far from rare in these preparations. The dramatic effect of adding thiamine is well shown in the figure: almost immediately the strip begins to relax more fully and more frequently and after a brief interval a smooth, long-lasting activity is attained.

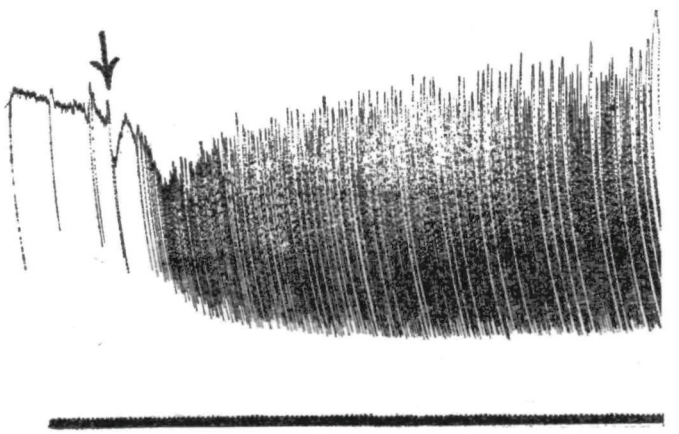

Fig. 3. Effect of thiamine on muscle strip. Thiamine added at arrow.

After careful consideration we have come to the conclusion that the routine addition of a small amount of thiamine to our experimental saline is justified, and that the activity elicited in this way must be regarded as physiological. Other experiments have been carried out with the addition, together and separately, of members of the vitamin B_2 complex, including riboflavine, nicotinic amide, pantothenic acid, pyridoxin and *p*-aminobenzoic acid, but in none of these was there any change in the behaviour of the muscle.

We also tried the effect of adding small amounts of thiamine to our 'keeping medium' in the hope that the worms might be maintained in better functional condition. Their general appearance and activity seemed to be appreciably improved, but a series of survival experiments showed that the average life period is not increased by thiamine. The cause of death of *Ascaris* in laboratory media is not known: starvation alone seems unlikely to be the cause, for the tissues are still rich in glycogen at death. By analogy with what is known about the culture of parasitic micro-organisms it seems reasonable to assume that the isolated nematode dies in

culture because it depends upon its host, in the ordinary way, for day-to-day supplies of some essential nutrient or nutrients, but our results with *Ascaris* seem clearly to indicate that, whatever this material may be, it does not consist of thiamine alone.

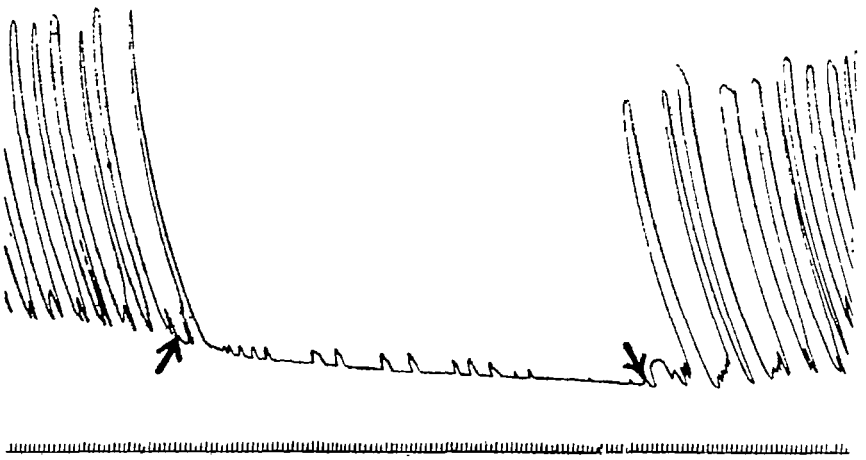

Fig. 4*a*

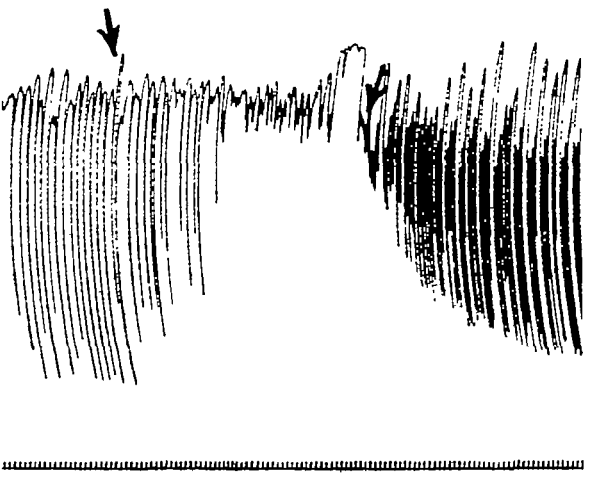

Fig. 4*b*

Fig. 4. Influence of changes in osmotic pressure of medium. Both records start at 120 m*M*, (*a*) decreased to 80 m*M* at first arrow, returned to 120 m*M* at second arrow; (*b*) increased to 150 m*M* at first arrow, returned to 120 m*M* at second arrow.

Preparation of the experimental medium

(i) *Stock solutions.* Molar solutions of NaCl, KCl, $CaCl_2.6H_2O$ and $MgCl_2.6H_2O$ are kept as stocks, together with *M* $NaHCO_3$ and 120 m*M* $NaH_2PO_4.H_2O$ and $Na_2HPO_4.2H_2O$. All these are prepared from A.R. reagents and made up in glass-distilled water. All subsequent dilutions are carried out with glass-distilled water.

(ii) *Working solutions*. The working solution of Na^+ is prepared by taking 117 ml. *M* NaCl, adding 7·5 ml. 120 m*M* NaH_2PO_4 and 17·5 ml. 120 m*M* Na_2HPO_4 and making up to 1 l. 50 ml. of the product are replaced by 50 ml. freshly diluted 120 m*M* $NaHCO_3$. This forms a buffered working solution containing 120 m*M* Na^+.

The remaining solutions are freshly prepared by dilution of the molar stocks to 120 m*M*. 30 ml. KCl, 15 ml. $CaCl_2$ and 30 ml. $MgCl_2$ (120 m*M* in each case) are added to each litre of the solution of Na^+, followed by the addition of 100 mg.

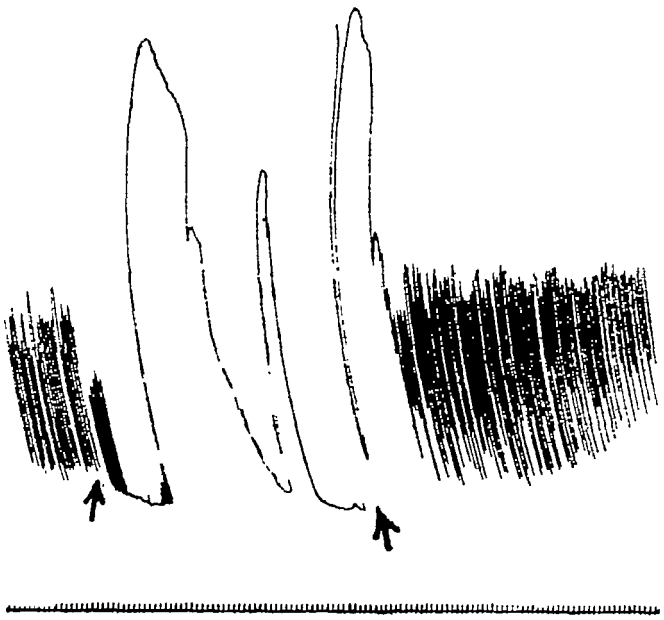

Fig. 5. Influence of changing from normal experimental medium to 'synthetic body fluid' (first arrow) and return to normal (second arrow).

thiamine hydrochloride. Finally, the mixture is gassed with 5 % carbon dioxide in 95 % nitrogen from a cylinder, no steps being taken to remove traces of oxygen. The gas mixture is bubbled through in the form of a very fine spray until the solution reaches a *p*H of 7·1 as judged by testing a sample against bromthymol blue (*p*H = 7·1). The gas stream may then be reduced but should not be cut off completely, since the dissolved gases tend to escape slowly from the solution.

Variations on this standard composition can readily be made by adding different amounts of KCl, $CaCl_2$, etc., while the *p*H can be modified by adding less or more of the $NaHCO_3$ or by varying the proportions of the two phosphates.

DISCUSSION

Many experiments have now been performed on muscle strips prepared in the manner and under the conditions described here. Experiments on the pharmacology of the muscle strips will be presented in a later paper: for the present it may be stated that these preparations are capable of giving useful indications of the influence of various drugs with anthelminthic and other properties and that the results obtained are, in the main, freely repeatable.

As illustrations of the reactions of the strips to unfavourable conditions in their immediate external environment, reference may be made to Fig. 4, which shows the effect of changes in osmotic pressure, and to Fig. 5, which shows the effect of changing from the standard experimental medium to 'synthetic body fluid' and back again.

SUMMARY

1. A technique is described for the preparation from the body wall of *Ascaris* of semi-isolated strips of muscle. These strips are exposed on one side to the surrounding medium and are suitable for studies of the action of anthelminthic and other drugs upon the exposed musculature.

2. A medium suitable for use in such experiments has been devised and its preparation is described.

3. Media made up to represent the body fluid of *Ascaris* fail to support physiological activity in the exposed muscle strips, and it seems that this perienteric fluid does not correspond to the true *milieu intérieur* of this nematode.

4. Some new observations on the nature and composition of the perienteric fluid are presented incidentally in the text.

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