Brit. J. Pharmacol. (1949), **4**, 145.

A CONTRIBUTION TO THE PHYSIOLOGY AND PHARMACOLOGY OF *ASCARIS LUMBRICOIDES* FROM THE PIG

BY

ERNEST BALDWIN AND VIVIEN MOYLE

From the Biochemical Laboratory, Cambridge

(Received December 17, 1948)

The observations reported in this paper arose indirectly out of an attempt to devise a logical and satisfactory method for the detection of anthelminthic potency *in vitro*. In the past, and mainly on the authority of Trendelenberg (1916), *in vitro* tests for anthelminthics have usually been carried out on earthworm muscle (e.g., by Lautenschläger, 1921; Oelkers and Rathje, 1941; von Oettingen, 1929; Rosenmund and Schapiro, 1934), some workers supplementing this by leech preparations. Since, however, the Nematoda show no trace of the metameric segmentation which characterizes the Annelida and, indeed, differ from them in almost every respect (Chitwood and Chitwood, 1937–8; Lapage, 1937), this seems an entirely unjustifiable procedure unless it can first be demonstrated that members of the two phyla do in fact react in substantially the same manner to a diversity of physiological and pharmacological agents. But Lamson and Ward (1936) carried out comparative tests of no less than 121 different chemical substances on earthworms and on pig *Ascaris*; they could find no correlation between the respective responses, and they condemned the use of earthworm material in anthelminthic studies as "irrational."

In our own work we have been concerned only with nematodes, and the use of the term "anthelminthic" in the present paper is accordingly restricted to substances which are anthelminthically active towards nematodes; the observations have been made exclusively with *Ascaris lumbricoides* obtained from the pig. Our earlier *in vitro* method (Baldwin, 1943) made use of tied-off, sausage-like pieces of *Ascaris*, these "sausages" being surrounded by the cuticle which is such a prominent and important feature of the Nematoda. Two kinds of "sausages" were used—viz., "anterior" preparations each comprising a terminal anterior portion about 2 cm. in length, and "intermediate" preparations taken from the region immediately in front of the genital pore and about 2.5 cm. in length.

A method based on the use of intact specimens of pig *Ascaris* had previously been introduced and extensively used by Lamson and his colleagues (1935, 1936). We, however, doubted the reliability of this procedure because of the well-known resistance, first recorded by von Schroeder (1885), of intact *Ascaris* to a number of anthelminthics, including even nicotine and santonin. In our anterior "sausage" preparations, on the other hand, santonin produces complete paralysis within a few minutes at concentrations of 10^{-5}; this effect seems to be due to a specific action of santonin upon the central nervous system, the "nerve ring," which is situated very close to the anterior extremity (Rauther, 1925).

With these "sausages" a strong correlation was established between positive reactions in the tests and acknowledged anthelminthic potency in the substances tested. At the same time, however, the "sausage" preparations showed not the slightest response to a wide range of pharmacologically active compounds such as acetylcholine and adrenaline and drugs which potentiate, imitate, or antagonize them. It seemed desirable to discover why these drugs should be inert. Two possibilities suggested themselves: (*a*) these drugs might fail to penetrate the cuticle, which is known to show highly selective permeability (Trim, 1949), or (*b*) nematode tissues might be insensitive to these substances. In either case we should have powerful arguments against the use of annelid preparations for anthelminthic testing, since neither the earthworm nor the leech possesses anything comparable

with the nematode cuticle, while both are notoriously sensitive to many compounds which possess no known anthelminthic activity.

Our first problem consisted in devising a nematode preparation in which the substances to be investigated could be directly applied to the muscle. The preparation devised, and a saline medium suitable for its maintenance, have already been described (Baldwin and Moyle, 1947).

The present paper reports the results of a study of the reactions of such "exposed" preparations towards a number of compounds of physiological and pharmacological interest, most of which are devoid of action upon the earlier "sausage" preparations (Baldwin, 1943). The observations are offered as a contribution to the at present scanty knowledge of the physiology and pharmacology of the Nematoda. They provide at the same time fresh factual evidence of the complete unsuitability of annelid material for tests of anthelminthic activity.

Results

1. *Perfusion experiments.*—Before we succeeded in obtaining active preparations of exposed *Ascaris* muscle we carried out a number of experiments in which short pieces of the worm were taken from the "intermediate" region—i.e., immediately in front of the genital pore. Fine cannulae were tied between the gut and the muscle masses of these preparations, which were then perfused with physiological saline, with and without the addition of various drugs. Perfusion was carried out with a mechanically operated syringe at a rate of about 6 ml. per hour and the movements executed by the muscles were kymographically recorded in the same manner and with the same apparatus as was used with the original "sausages" (Baldwin, 1943). Thymol and hexylresorcinol (Fig. 1*a*) were tested at concentrations of 5×10^{-4}, and, as was to be anticipated from their action upon the "sausage" preparations, they produced rapid paralysis and subsequent contracture of the muscle. Santonin (Fig. 1*b*) at 2×10^{-4} led to a near-paralysis, while acetylcholine (10^{-6}) now produced marked stimulation of the muscle. Pilocarpine and adrenaline (10^{-3}) were quite inert, and *p*-benzylphenyl carbamate, which produces paralysis in 20–30 min. when applied at 2×10^{-4} to the "sausages," was quite without action when applied by perfusion. These results seemed to justify a renewal of our efforts to obtain active preparations of the exposed muscle, especially since santonin, which paralysed our perfused preparations, is known to produce violent stimulation of denervated earthworm muscle (Trendelenberg, 1916). Furthermore, we now had evidence of sensitivity of the *Ascaris* tissue towards acetylcholine. At about this time we began to get more promising results with exposed preparations, and the perfusion technique, which proved tedious and not a little difficult, was abandoned soon after the pilot experiments just mentioned had been completed.

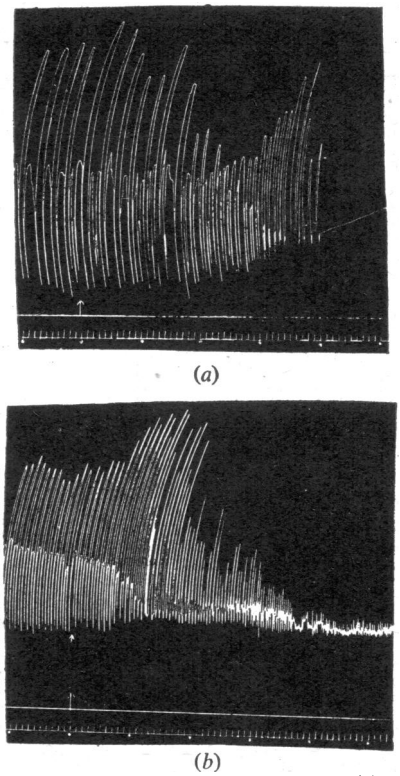


FIG. 1.—Perfused preparation of *Ascaris*. (*a*) 5×10^{-4} hexylresorcinol at signal; (*b*) 2×10^{-4} santonin at signal.

2. *Experiments with exposed preparations.*—The technique has already been described (Baldwin and Moyle, 1947). Each preparation consists of a ribbon-like strip of dorsal musculature about 2.5 cm. in length taken from the "intermediate" region. The cuticle is still present on the external face of the strip, but the other side is exposed directly to the bathing medium. These preparations are sometimes exceedingly sensitive to mechanical stimulation so that, when the bathing medium is changed, the greatest care must be taken to avoid spoiling the tracings.

The Table summarizes the effects of a number of drugs when applied to the exposed preparations: their effects upon the "sausages" (Baldwin, 1943) are given side by side for comparison.

TABLE

THE CONCENTRATIONS ARE, WITH INERT COMPOUNDS, THE HIGHEST AT WHICH THEY WERE TESTED AND, WITH ACTIVE COMPOUNDS, THE LOWEST AT WHICH ACTIVITY WAS STILL DETECTABLE

s and *p* indicate that stimulant or paralysant effects respectively were observed within 30 min. at the concentrations indicated. ± indicates a weak or dubious response and – that no effect could be detected

Compound	Exposed preparation		"Sausage" preparation	
	Concentration	Effect	Concentration	Effect
A				
Acetarsone	10^{-4}	—	2×10^{-3}	—
Acetylcholine	10^{-6}	*s*	2×10^{-4}	—
Adrenaline	10^{-4}	—	2×10^{-4}	—
Arcain	10^{-4}	—	10^{-3}	—
Atropine	10^{-3}	—	10^{-3}	—
Caffeine	10^{-3}	—	10^{-3}	—
Chloral hydrate	10^{-3}	±	10^{-3}	—
Choline	10^{-4}	*s*	10^{-3}	—
Cocaine	10^{-4}	*p*	10^{-3}	—
Ephedrine	10^{-4}	—	10^{-3}	—
Histamine	10^{-4}	—	5×10^{-4}	—
Morphine	10^{-4}	—	10^{-3}	—
Pelletierine	10^{-4}	*s*	10^{-3}	—
Phenothiazine	sat. soln.	—	10^{-3}	—
Phenylurea	10^{-3}	—	10^{-3}	—
Pilocarpine	10^{-4}	—	10^{-3}	—
*Pseudo*aconitine	10^{-3}	—	10^{-3}	—
Quinine	10^{-3}	±	10^{-3}	—
Strychnine	10^{-3}	—	10^{-3}	—
Sulphanilamide	10^{-4}	—	10^{-2}	—
Sulphapyridine	10^{-4}	±	10^{-3}	—
Sulphathiazole	10^{-4}	—	5×10^{-5}	—
Tyramine	10^{-4}	—	10^{-3}	—
B				
Arecoline	10^{-4}	*p*	10^{-3}	±
,,	10^{-3}	*s*	10^{-3}	±
Coumarine	10^{-3}	*p*	10^{-3}	—
Santonin	10^{-5}	*p*	2×10^{-4}	±
C				
p-Benzylphenyl-carbamate	10^{-4}	*p*	2×10^{-4}	*p*
Hexylresorcinol	10^{-4}	*s*	10^{-4}	*p*
β-Naphthol	10^{-4}	*p*	2×10^{-4}	*p*
Nicotine	10^{-7}	*s*	5×10^{-4}	*p*

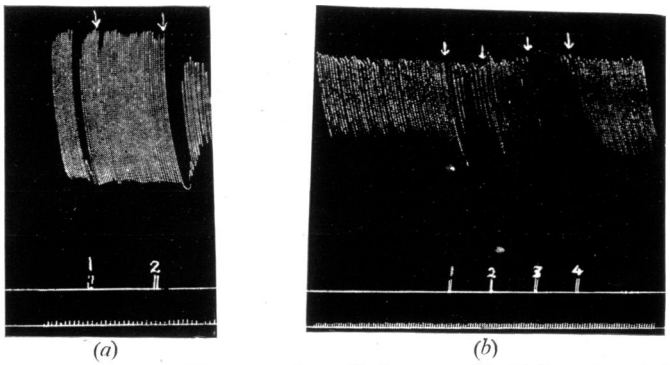
Within Group A (drugs devoid of action upon "sausages") there are four compounds that produce definite effects when applied directly to the muscle, indicating that their failure to act upon the "sausages" must be attributed to inability to penetrate the cuticular layer. In Group B (drugs which paralyse "anterior sausages" but have little action upon "intermediate sausages") all three compounds tested showed considerably greater activity and acted at lower concentrations upon the "exposed" than upon the "sausage" preparations, again indicating slowness or failure of penetration of the cuticle. In Group C (compounds which act both upon "anterior" and "intermediate sausages") the four compounds examined again showed activity, but, whereas the two phenolic substances and the phenol carbamate showed about the same activity whether applied directly or by way of the cuticle, which they may therefore be presumed to penetrate rapidly, nicotine was far more active when directly applied. This presumably indicates that nicotine can pass only relatively slowly through the cuticle.

These preliminary experiments showed that the resistance of the "sausages" to pharmacological agents is at least sometimes due to the protective action of the cuticle. The fact nevertheless remains that *Ascaris* muscle, even when directly exposed, fails to react to such compounds as adrenaline, histamine, pilocarpine, strychnine, and many others.

This made it desirable to study more closely its general pharmacological behaviour. We accordingly carried out further experiments on the influence of a number of substances including adrenaline, acetylcholine, and drugs which simulate, potentiate, or antagonize their usual effects.

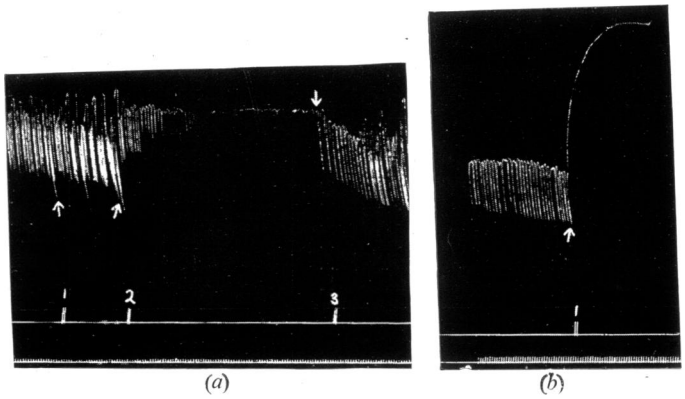
Adrenaline, ephedrine, ergotoxine.—Adrenaline was without detectable influence at concentrations up to 10^{-4}, whether applied alone or in the presence of ephedrine (10^{-4}), which itself was likewise inert. Ergotoxine, which antagonizes the augmentor effects of adrenaline, was also inert at 10^{-4}, whether applied before or after the preparations had been treated with adrenaline.

Acetylcholine, eserine, choline.—At concentrations below 10^{-6} acetylcholine is inert, even after previous treatment with eserine (10^{-5}). Eserine alone (10^{-5}) is also without action (Fig. 2*a*). At 10^{-6} acetylcholine (Fig. 2*b*) produces a typical stimulation marked by a sharp rise of tone and corresponding diminution of amplitude. Higher concentrations gave only an exaggeration of this response; at 10^{-5}—10^{-4} the rise of tone is so great that relaxation often fails altogether. These effects are imitated by choline, which, at 10^{-4}, produces responses that are qualitatively and quantitatively similar to those of acetylcholine at 10^{-6}—10^{-5}. The effects of acetylcholine, which are reversed by washing, are quite uninfluenced by previous treatment of the preparation with eserine and do not fall off with time. It is difficult therefore to escape the conclusion that cholinesterases are totally absent from our preparations.



(*a*) (*b*)

FIG. 2.—"Exposed" preparation of *Ascaris*. (*a*) 10^{-5} eserine at 1; 10^{-6} acetylcholine at 2. (*b*) Acetylcholine, 10^{-6} at 1, 10^{-5} at 3; wash at 2 and 4.



(*a*) (*b*)

FIG. 3.—"Exposed" preparation of *Ascaris*. (*a*) Nicotine, 10^{-8} at 1, 10^{-7} at 2; wash at 3. (*b*) 10^{-3} nicotine at signal.

Nicotinic effects: nicotine, tubocurarine, strychnine, cocaine.—Nicotine at 10^{-7} (Fig. 3*a*) exactly reproduces the effects of acetylcholine at 10^{-6}—10^{-5}. Higher concentrations (10^{-5}) evoke responses of the same qualitative kind but of greater intensity; even at 10^{-3} there is no indication of any reversal of the stimulatory effects (Fig. 3*b*). Thus nicotine does not have the paralysant action which it so often displays at high concentrations in other tissues.

The effects of nicotine at 10^{-7} are abolished by tubocurarine (Fig. 4*a*) at 10^{-4}, a concentration which, in the absence of nicotine, leads to complete paralysis after a short time. Tubocurarine at 10^{-4} also abolishes the effect of acetylcholine at 10^{-5} (Fig. 4*b*). Strychnine (10^{-3}) resembles tubocurarine in abolishing the action of acetylcholine (10^{-5}) (Fig. 4*c*) but differs from it in having no obvious or reproducible effect when applied by itself (10^{-3}). Cocaine at 10^{-4}, like tubocurarine, also leads to complete paralysis, presumably by narcosis, but full activity can be restored by nicotine at 10^{-7}. At higher concentrations (10^{-3}), however, the action of cocaine is no longer abolished by nicotine (10^{-5}).

These results show clearly that nicotine can imitate the effects of acetylcholine upon the *Ascaris* tissues. The influence of nicotine is also imitated by Ba^{++}, which at 10^{-4} has effects exactly comparable with those of nicotine at 10^{-7} or acetylcholine at 10^{-6}—10^{-5} (Fig. 4*d*). In addition, arecoline, which is reputed to have nicotine-like effects in high concentrations (Feldberg and Vartiainen,

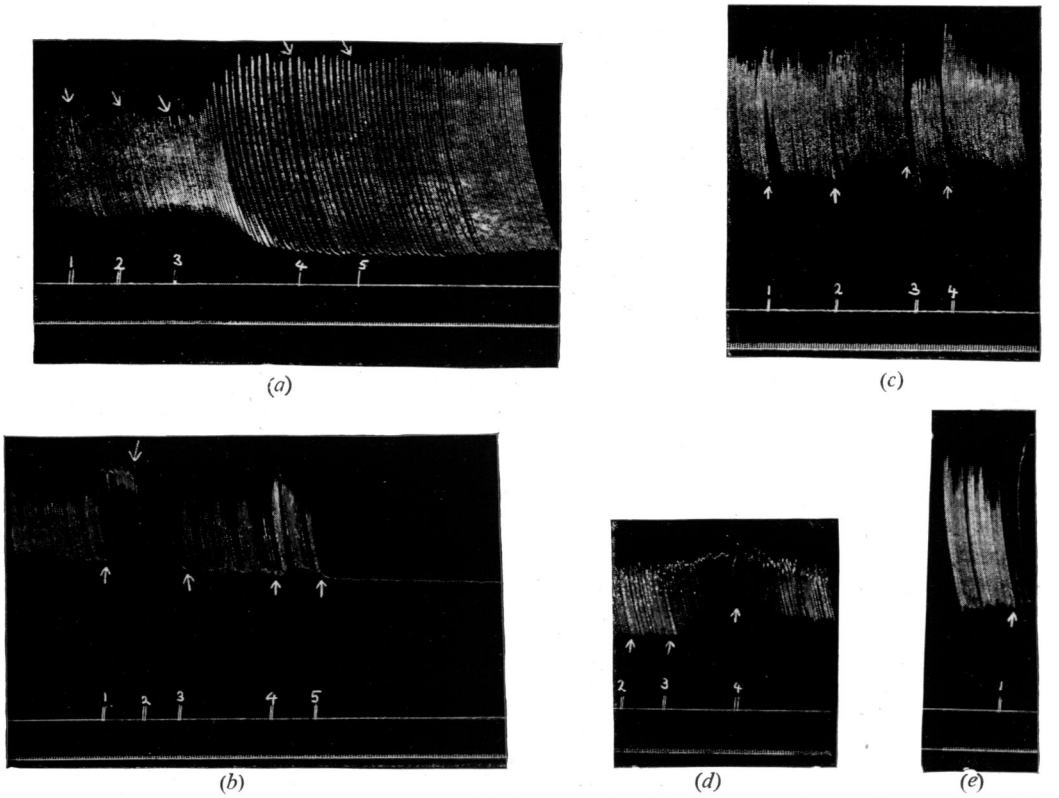


FIG. 4.—"Exposed" preparation of *Ascaris*. (*a*) Tubocurarine-nicotine antagonism: tubocurarine, 10^{-6} at 1, 10^{-5} at 2, 10^{-4} at 3; 10^{-4} tubocurarine + 10^{-7} nicotine at 4; 10^{-4} tubocurarine + 2×10^{-7} nicotine at 5. (*b*) Tubocurarine-acetylcholine antagonism: 10^{-5} acetylcholine at 1; wash at 2 and 3; 10^{-4} tubocurarine at 4; 10^{-4} tubocurarine + 10^{-5} acetylcholine at 5. (*c*) Effect of strychnine: 10^{-4} at 1, 10^{-3} at 2; 10^{-3} strychnine + 10^{-5} acetylcholine at 3; wash at 4. (*d*) Effect of barium: $BaCl_2$, 10^{-5} at 2, 10^{-4} at 3; wash at 4. (*e*) Effect of arecoline (high concentration): 10^{-3} at signal.

1935), produces at 10^{-3} a stimulant effect quantitatively comparable with that of nicotine at 10^{-6}—10^{-5} or acetylcholine at 10^{-5}—10^{-4} (Fig. 4*e*).

While nicotine faithfully imitates the action of acetylcholine at low or moderate concentrations it cannot be concluded that acetylcholine has a typical "nicotine-action" upon *Ascaris* tissue, since, according to the classical definition of Dale (1914), such effects are characteristically abolished by high concentrations of nicotine.

Muscarinic effects: pilocarpine, atropine, 2,268 F.—The effects of acetylcholine are not influenced in any way by atropine in concentrations as high as 10^{-4}, nor is atropine alone active at this concentration (Fig. 5*a*). This appears to show that the action of acetylcholine in this tissue does not include any "muscarine-action" (cf. Dale, 1914). Pilocarpine, which ordinarily reproduces the muscarinic effects of acetylcholine and of muscarine itself, is quite inert at 10^{-4}. Arecoline, however, which is reputed to simulate the muscarinic effects of acetylcholine when applied at low concentrations, produces paralysis at 10^{-4} (Fig. 5*b*), though it is inert at lower concentrations and has at 10^{-3} a stimulant action, resembling that of acetylcholine and nicotine (Fig. 4*e*); this is the only indication so far of any muscarine-like response by the *Ascaris* tissue.

Muscarine itself was not procurable and we were therefore obliged to have recourse to 2,268 F (ethylal-trimethylammonium-propanediol), a compound which is reputed to imitate faithfully the pharmacological effects of natural muscarine (Fourneau, Bovet, Bovet, and Montezin, 1944). This substance proved inert at 10^{-6} but at 10^{-5} produced an effect closely resembling that of nicotine at 10^{-7} or acetylcholine at 10^{-6}—10^{-5} (Fig. 5*c*). Its effect was not antagonized by atropine at 10^{-4} (Fig. 5*d*).

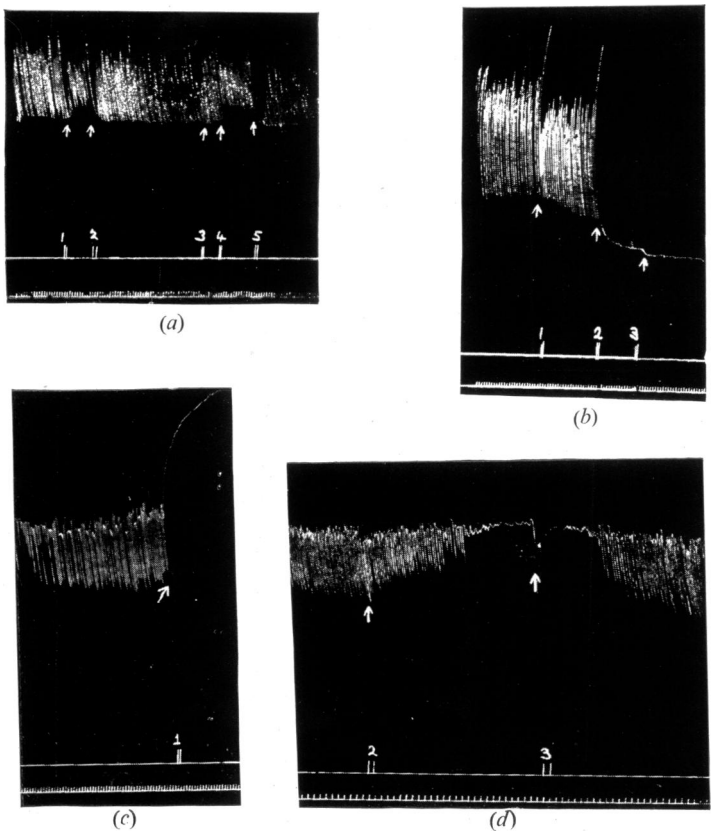


FIG. 5.—"Exposed" preparation of *Ascaris*. (*a*) Effect of atropine: 10^{-6} acetylcholine at 1; wash at 2; 10^{-4} atropine at 3; 10^{-4} atropine + 10^{-6} acetylcholine at 4; wash at 5. (*b*) Effect of arecoline (moderate concentration): 10^{-5} at 1; 10^{-4} at 2; wash at 3. (*c*) Effect of 2,268 F: 10^{-4} at signal. (*d*) Effect of 2,268 F + atropine: 10^{-4} atropine at start; 10^{-4} atropine + 10^{-5} 2,268 F at 2; wash at 3.

Since the effect of 2,268 F is not antagonized by atropine it cannot be regarded as typically muscarine-like (cf. Dale, 1914) any more than that of acetylcholine. The response to arecoline at 10^{-4} appears to contradict this conclusion, but it should be remembered that the pharmacology of arecoline itself is not very clearly understood and that its paralysant action in this particular tissue may perhaps be only a specific narcotic effect.

Other inactive compounds included caffeine, histamine, morphine, quinine, and tyramine, all at 10^{-4}—10^{-3}.

DISCUSSION

The use of annelid muscle as a means of testing anthelminthic potency was originally adopted by Trendelenberg (1916) for two reasons; first, the technical difficulty of exposing *Ascaris* muscle free from cuticle, and, secondly, because he believed that earthworms probably respond to santonin, with which he was primarily concerned, in much the same manner as *Ascaris* itself does. In our experiments, however, we find that santonin, which violently stimulates the denervated body-wall muscle of the earthworm, actually paralyses *Ascaris* muscle, usually after transient preliminary stimulation. This apart, the substitution of annelid for nematode material could hardly be justified in the absence of positive proof that members of the two phyla were similarly affected by a wide range of diverse chemical substances. No such evidence has been forthcoming, and, indeed, the extensive experiments of Lamson and Ward (1936) and the present work point to a wholly contrary conclusion. It is, of course, true that a number of phenolic anthelminthics of high reputation act similarly upon earthworms and roundworms, but these belong to the group of "general protoplasmic poisons" and have toxic actions upon most, if not all, kinds of animal tissues.

Even among parasitic helminths as a whole, differences between species in respect of response to different drugs are well known. It is therefore not surprising that such differences should also exist between these parasites and free-living Annelida. In point of fact, the responses of exposed *Ascaris* muscle differ in many ways from those observed in studies of annelid materials—

for example, earthworm body-wall (Wu, 1939; and our own observations mentioned below), earthworm gut (Ambache, Dixon, and Wright, 1945; Millott, 1943; Wu, 1939), lugworm body-wall (Wu, 1939), lugworm gut (Wells, 1937), and leech body-wall (Fühner, 1918; Minz, 1932).

In the absence of eserine, we found that both *Ascaris* and earthworm muscle responded by mild stimulation to acetylcholine at *ca* 10^{-6}, but whereas eserine was without influence on *Ascaris* muscle it showed a weak acetylcholine-like action upon earthworm muscle at 10^{-6} and also sensitized it to the action of acetylcholine itself. Moreover, the effects of acetylcholine upon earthworm muscle diminished with time, a phenomenon not observable with *Ascaris*. These results provide suggestive evidence for the presence of cholinesterases in the earthworm and their absence from *Ascaris*. Furthermore, although small concentrations of nicotine produce acetylcholine-like effects in both tissues, higher concentrations (10^{-4}; Wu, 1939) antagonize the effect of acetylcholine in the earthworm but not in *Ascaris* (even at 10^{-3}). Pilocarpine, while provoking no response in *Ascaris* muscle, produces a slight stimulation in earthworm muscle at 10^{-6}—10^{-5}, changing to a depressant effect at 10^{-3}, which suggests that this tissue, again unlike that of *Ascaris*, is susceptible to the action of muscarine-like drugs. Further, although atropine (10^{-5}) has very little action upon earthworm muscle by itself, it does in part abolish the action of acetylcholine, which again indicates that the action of the latter on earthworm muscle, unlike that on *Ascaris* muscle, includes muscarine-like as well as nicotine-like components. Adrenaline at 10^{-5} had a weak and transient stimulant effect upon our earthworm preparations but was inert at 10^{-6}. Ephedrine (10^{-4}) produced an effect closely resembling that of adrenaline, but neither drug showed activity towards *Ascaris* muscle. These differences, added to numerous differences previously noted (Baldwin, 1943, 1948) between "sausage" preparations of *Ascaris* and earthworm muscle, and between intact specimens of the two species (Lamson and Ward, 1936), establish real and far-reaching differences between the two organisms.

Quite apart from the physiological and pharmacological differences noted here, the properties of the nematode cuticle have to be reckoned with. As our preliminary experiments showed, acetylcholine, arecoline, choline, cocaine, nicotine, pelletierine, and santonin pass slowly or not at all through the cuticle of *Ascaris*. Among these only arecoline, nicotine, and santonin had any action upon our original "sausages," and it is worthy of note that their action was far more rapid and powerful upon "anterior sausages" than upon those from the region immediately in front of the genital pore. This, no doubt, is because the cuticle is much thinner in the anterior region. But acetylcholine, choline, cocaine, and pelletierine have no action at all upon "sausages" even when applied at high concentrations to the anterior region, despite the thinness of the cuticle, and it is only when the muscle is directly exposed to the action of the drugs in question that any response can be observed. It may be presumed, therefore, that these compounds fail entirely to penetrate the cuticle.

Every one of these substances evokes more or less profound responses in earthworm muscle, and there can be no doubt that the cuticular layer of the nematodes constitutes a real and formidable barrier to the penetration of many substances, among them a number of compounds of established anthelminthic efficacy. Since there is nothing in the ordinary preparations of earthworm body-wall to offer a similar barrier to penetration, the use of earthworm body-wall can yield no information about the ability of a compound to penetrate the nematode cuticle; and such ability is necessary for anthelminthic activity.

The use of earthworm muscle (and probably other annelid material as well) in anthelminthic studies is therefore to be condemned on two main grounds: these are, first, that important physiological and pharmacological differences exist between annelid and nematode muscles, and, secondly, that earthworm preparations fail to reproduce any of the important effects attributable to the nematode cuticle, which plays an important part in determining the resistance or the susceptibility of parasitic nematodes to drugs of physiological or pharmacological interest.

Summary

1. The reactions of exposed *Ascaris* muscle to a number of compounds of physiological and pharmacological interest have been studied.

2. The muscle is stimulated by acetylcholine but appears to contain no cholinesterases.

3. The effects of acetylcholine are imitated by choline, nicotine, and barium ions, and are antagonized by tubocurarine, strychnine, and cocaine, but not by excess of nicotine.

4. The effects of acetylcholine are also imitated by 2,268 F, but atropine does not antagonize the effects of either.

5. Adrenaline, atropine, caffeine, ephedrine, ergotoxin, eserine, histamine, morphine, pilocar-

pine, quinine, and strychnine have no direct action on *Ascaris* muscle.

6. The cuticle acts as a barrier to the penetration of many compounds of physiological and pharmacological interest, including some substances possessing anthelminthic activity.

7. There are thus many important differences between *Ascaris* muscle and the muscles of annelids, and the use of the latter as test materials for the detection of anthelminthic potency is to be condemned.

One of us (V. M.) is indebted to the Agricultural Research Council for a grant, during the tenure of which the greater part of this work was carried out. We are also indebted to Dr. W. Feldberg for gifts of tubocurarine chloride and 2,268 F as well as for much valuable discussion. Our thanks are also due to Sir Henry Dale for a gift of 2,268 F, and to Professor E. Verney and Dr. C. F. A. Pantin, who read and criticized the manuscript. We also wish to thank the manager of the St. Edmundsbury Co-operative Bacon Factory, who kindly made the arrangements for our regular supplies of freshly collected *Ascaris*.

References

Ambache, N., Dixon, A. St. J., and Wright, E. A. (1945). *J. exp. Biol.*, **21**, 46.
Baldwin, E. (1943). *Parasitology*, **35**, 89.
Baldwin, E. (1948). *Brit. J. Pharmacol.*, **3**, 91.
Baldwin, E., and Moyle, V. (1947). *J. exp. Biol.*, **23**, 277.
Chitwood, B. G., and Chitwood, M. B. (Ed.) (1937–8). *An Introduction to Nematology*. Baltimore.
Dale, H. H. (1914). *J. Pharmacol.*, **6**, 147.
Feldberg, W., and Vartiainen, A. (1935). *J. Physiol.*, **83**, 123.
Fourneau, E., Bovet, D., Bovet, F., and Montezin, G. (1944). *Bull. Soc. Chim. biol., Paris*, **26**, 516.
Führer, H. (1918). *Arch. exp. Path. Pharmak.*, **82**, 51.
Lamson, P. D., and Brown, H. W. (1935). *J. Pharmacol.*, **53**, 227.
Lamson, P. D., and Brown, H. W. (1936). *Amer. J. Hyg.*, **23**, 85.
Lamson, P. D., and Ward, C. B. (1936). *Science*, **84**, 293.
Lamson, P. D., Brown, H. W., and Ward, C. B. (1935). *J. Pharmacol.*, **53**, 198.
Lamson, P. D., Brown, H. W., Stoughton, R. W., Harwood, P. D., Baltzly, R., and Bass, A. (1935). *J. Pharmacol.*, **53**, 218, 234, 239.
Lamson, P. D., Stoughton, R. W., and Bass, A. D. (1936). *J. Pharmacol.*, **56**, 50, 60, 63.
Lapage, G. (1937). *Nematodes Parasitic in Animals*. London.
Lautenschläger, L. (1921). *Ber. dtsch. Pharmak. Ges.*, **31**, 279.
Millott, N. (1943). *Proc. Roy. Soc.*, **131B**, 271, 362.
Minz, B. (1932). *Arch. exp. Path. Pharmak.*, **168**, 292.
Oelkers, H. A., and Rathje, W. (1941). *Arch. exp. Path. Pharmak.*, **198**, 317.
Oettingen, W. F. von (1929). *J. Pharmacol.*, **36**, 335.
Rauther, W. (1925). In Kukenthal's *Handbuch der Zoologie*, **2**, 249. Berlin.
Rosenmund, K. W., and Schapiro, D. (1934). *Arch. Pharmak. Berlin.*, **272**, 313.
Schroeder, W. von (1885). *Arch. exp. Path. Pharmak.*, **19**, 290.
Trendelenberg, P. (1916). *Arch. exp. Path. Pharmak.*, **79**, 190.
Trim, A. R. (1949). *Parasitology*, **38**, 281.
Wells, G. P. (1937). *J. exp. Biol.*, **14**, 117.
Wu, K. S. (1939). *J. exp. Biol.*, **16**, 184, 251.