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A METHOD FOR RECORDING THE EFFECTS OF ANTHELMINTICS ON THE MOVEMENTS OF *ASCARIS LUMBRICOIDES*

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Adult roundworms (*Ascaris lumbricoides*) obtained from pigs were suspended in modified Tyrode solution at 37° in fine nylon stockings, and the movements of the stockings were recorded. This method of suspension avoided damage to the cuticle or occlusion of the alimentary tract of the worm.

Piperazine salts caused gradual narcosis, the time of onset of which was related linearly to the logarithm of the concentration of drug in the bath. The method is suitable for the assay of preparations of piperazine. Tetrachlorethylene, hexylresorcinol, and oil of chenopodium all caused stimulation of movement before the worm was immobilized. Santonin caused incoordination of the movements of the anterior end of the worm; the movements of the body were apparently unaffected. Piperazine is the safest preparation available for the treatment of ascariasis.

Baldwin (1943) studied the effect of anthelmintics on the movements of isolated portions of *Ascaris lumbricoides* prepared from the anterior and intermediate parts of the worm and suspended in nutrient fluid in an isolated organ bath. Baldwin and Moyle (1949) investigated the action of drugs on portions of worm from which the cuticle had been removed and the muscle exposed. More recently, Norton and de Beer (1957) have used similar muscle preparations for the investigation of the action of piperazine. Preparations of this kind do not survive long *in vitro* and they give little indication of what may happen to a worm when the anthelmintic reaches it through the alimentary canal of the host in which it lives. Some drugs, such as piperazine, act slowly (Standen, 1955; Brown, Chan and Hussey, 1956) and are probably ingested by the worm. Baldwin (1943) recognized these difficulties.

The main problem of recording the movements of whole worms is that of harnessing them to the recording lever in such a way that the cuticle is not damaged by stitches, the alimentary canal is not constricted by ligatures and the movements of the worm are unimpeded. A suitable method has been devised in which the worm is introduced into a fine nylon stocking and the movements of the stocking are recorded.

METHODS

Ascaris lumbricoides. — Worms were obtained from pigs at the slaughterhouse and transported to the laboratory in a vacuum flask containing modified Tyrode solution at 37° (NaCl 0.8%, KCl 0.02%, $CaCl_2$ 0.02%, $MgCl_2$ 0.01%, $NaHCO_3$ 0.015%, Na_2HPO_4 0.05%, glucose 0.5%). They were stored in an incubator in covered glass pans of the solution, which was changed daily.

Stockings.—Tubes about 16 in. long and $\frac{3}{16}$ to $\frac{1}{4}$ in. in diameter were prepared from discarded nylon stockings (12 denier, 60 gauge); the seam was made in fine cotton with a sewing machine. One end of the stocking was left open, the other was tapered to the shape of the anterior end of the worm and was closed. Stockings of this size are suitable for adult female worms; male worms, which are smaller, require narrower and shorter stockings.

The worm was introduced into the stocking in a dish of warm Tyrode solution. The open end was closed behind the worm with a rubber band; the band was attached to a sinker made from a sealed glass ampoule (5 ml.) filled with lead shot. The closed (anterior) end of the stocking was attached to a length of fine cotton which was used to lower the worm into a bath 16 in. long and $2\frac{1}{2}$ in. in diameter (capacity about 400 ml.) containing Tyrode solution maintained at 37°. A small cap made from black glass tubing was threaded over the cotton to shade the light-sensitive anterior tip of the worm. The cotton was

attached to a frontal writing lever with sufficient counterweight to keep the worm upright in the bath. A diagram of the apparatus is shown in Fig. 1. Movements were recorded on a slow drum without magnification. No oxygen was bubbled through the nutrient solution; *Ascaris* is an anaerobe.

In some experiments a bath was used which restricted the movements of all but the anterior ¼ in. of the worm. This was made from a length of ¼ in. bore glass tubing fused to a wide tube above. The body of the worm in its stocking was imprisoned in the narrow tube; the anterior tip was free to move and the movements were recorded with a light lever magnifying ten times.

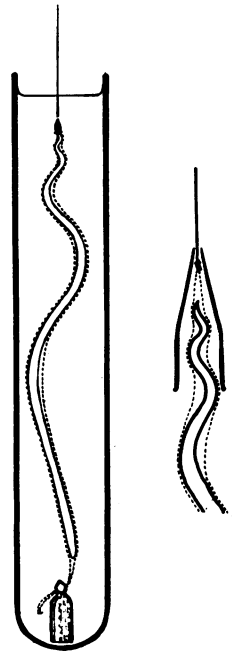

FIG. 1.—Worm in stocking suspended in a bath of Tyrode solution. The lower end of the stocking is anchored by an ampoule full of lead shot and the upper end is shaded by a small cap of black glass.

Drugs.—Water-soluble anthelmintics were dissolved in nutrient medium and added to the bath. Those which were insoluble were emulsified or brought into solution with medium containing 0.5% sodium tauroglycocholate.

At least ½ hr. was allowed for a worm to settle down before adding a drug. A continuous record was taken for 1 hr. after adding the drug; with slow-acting substances, tracings were recorded for ¼ hr. periods once every hour for the following 24 hr. This was arranged by interposing a Sangamo-Weston interval-timer in the mains supply to the kymograph motor. Drugs were left in contact with the worms for the whole period of observation.

RESULTS

Normal Movements.—The worm moved by arching the body in the dorsiventral plane, first in one direction and then in the other. Movements of the adult female were most vigorous in the anterior ⅓ to ½ of the body; the posterior part, occupied by the gravid uterus, was relatively quiescent unless stimulated by drugs. The arching of the body took place fairly regularly at intervals of 2 to 3 min. A sinuous wave appeared to run forwards along the anterior part of the worm, decreasing in both amplitude and wavelength as it approached the anterior end. The extreme tip of the worm was very active and carried out independent searching movements. The normal tracing (Fig. 2) shows the arching movements as large excursions, with the more rapid movements of the tip superimposed. The function of the searching movements of the anterior end was sometimes demonstrated in a stocking with a small hole in it. If the hole was large enough to admit the extreme tip of the worm, vigorous sinuous movements of the body thrust it through; the constriction formed around the worm by the edges of the hole stimulated it to even greater activity and it soon enlarged the hole sufficiently for the whole body to pass through. Observation of these powerful movements makes clear the method by which the worm can maintain its position in face of the peristaltic waves in the small intestine of the host, and the reason why displaced worms are occasionally found in the appendix, the bile duct and the nose. In early experiments, before a black glass cap was used to cover the anterior end of the worm, the creature sometimes doubled back in the stocking, often making two or three folds of its body in a part of the stocking which would stretch enough to hold them; sometimes it reversed its position completely. These manœuvres were always accompanied by very vigorous contractions. If the anterior end was shaded the worm always remained in its original position.

FIG. 2.—Normal tracing. The large excursions correspond to arching movements of the body, the small excursions to searching movements of the anterior end. Time, min.

The use of a sinker to anchor the stocking to the bottom of the tube was a more satisfactory method than attachment to the lower end of a

hooked glass rod. Worms sometimes wound themselves around the rod, used it as a support and became relatively quiescent.

Piperazine.—The effect of a concentration of 1:250 of piperazine citrate in the bath is shown in Fig. 3. The worm was slowly narcotized and after about 6 hr. its movements ceased. Lower concentrations of drug also produced narcosis but took longer to do so. Fig. 4 shows that the mean time taken to immobilize the worms was linearly related to the logarithm of the concentration of drug in the bath. The activity of piperazine citrate was equal to that of other salts, such as the adipate and phosphate, in amounts containing an equivalent quantity of base. Piperazine adipate is completely soluble at 1:250. Pharmaceutical preparations of piperazine in syrup also had activity proportional to their content of piperazine base; syrup alone had no significant effect. No significant stimulation of movement by piperazine was observed in experiments with several hundred worms. Worms narcotized with piperazine began to move again when immersed in fresh medium but never recovered their original vigour. Male worms were 10 times as sensitive to piperazine as female worms.

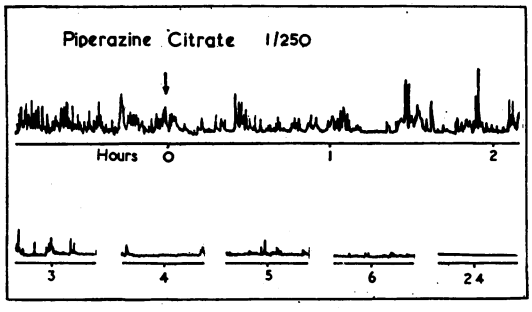

FIG. 3.—Effect of piperazine citrate 1:250. The movements gradually diminished and the worm became quiescent in about 6 hr. There was no stimulation.

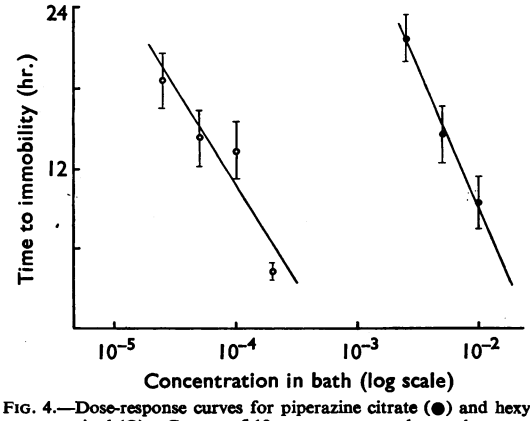

FIG. 4.—Dose-response curves for piperazine citrate (●) and hexylresorcinol (O). Groups of 10 worms were used at each concentration of drugs and the mean time to immobility (max. 24 hr.) was plotted against log concentration. The vertical lines indicate the standard errors of the means. Calculation of relative potency by the method of Specification 911 of the British Standards Institute (1940) shows hexylresorcinol to be 98.8 times as active as piperazine citrate. Tests for linearity and parallelism were satisfactory and the true fiducial limits of the activity ratio were 66.0 to 158.5% ($P=0.95$).

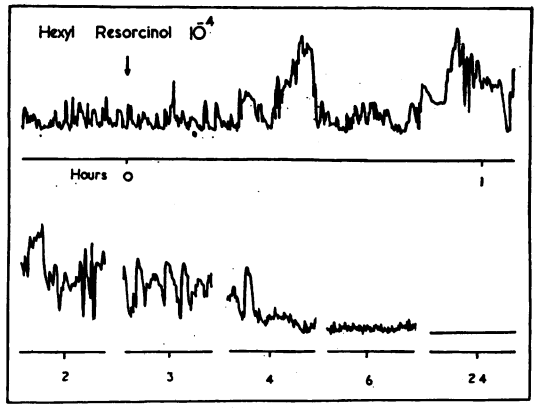

FIG. 5.—Effect of hexylresorcinol 1:10,000. The worm was immobilized, but only after several hours of increased activity.

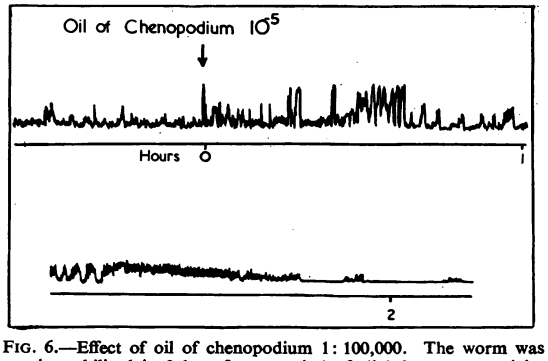

FIG. 6.—Effect of oil of chenopodium 1:100,000. The worm was immobilized in 2 hr. after a period of slightly greater activity than normal.

Hexylresorcinol.—This drug was about 100 times as active as piperazine (Fig. 4) but caused marked stimulation before the worm became immobile (Fig. 5). Worms which had been immobilized with hexylresorcinol did not recover in fresh medium. Thymol had the same type of action as hexylresorcinol but was much less potent.

Oil of Chenopodium.—A concentration of 10^{-4} of oil of chenopodium, emulsified with bile salts, brought the movements of the worm to a standstill in a few minutes. Lower concentrations took longer and sometimes caused preliminary stimulation (Fig. 6).

Santonin.—Santonin had no detectable effect on the arching movements of *Ascaris*; 24-hr. tracings made by worms in a concentration of 10^{-3} of santonin solubilized with bile salts were similar to those made by normal worms. On the other hand, the searching activities of the anterior end of the worm were impaired very soon after the drug was added to the bath. The movements became feeble and incoordinated. When the arching movements were suppressed by enclosing the worm in a narrow tube, only the movements of the anterior end were recorded on the drum; on the addition of santonin these movements became too feeble to move the light lever (Fig. 7).

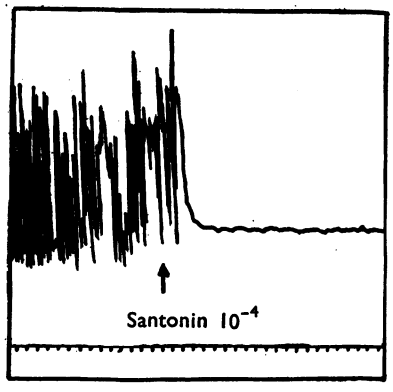

FIG. 7.—Effect of santonin 1: 10,000 on the movements of the anterior ¼ in. of the worm. The movements of the body of the worm were suppressed by enclosure in a narrow tube; only the tip was free to move. Time, min.

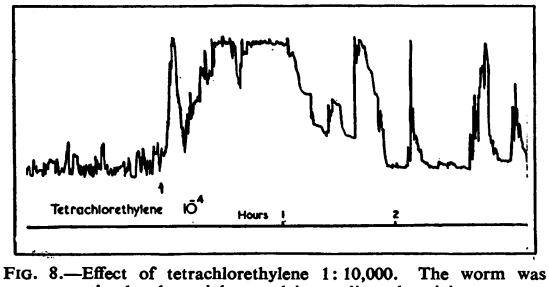

FIG. 8.—Effect of tetrachlorethylene 1: 10,000. The worm was stimulated to violent and incoordinated activity.

Tetrachlorethylene.—An emulsion of tetrachlorethylene stimulated the worm to violent, convulsive activity (Fig. 8). The posterior part of the female worm, usually quiescent, also convulsed and often remained for many minutes in a state of contracture in bizarre looped or angled attitudes. The worms did not recover in fresh medium.

DISCUSSION

These observations show that although piperazine is less potent, weight for weight, than the other anthelmintics tested, it has more desirable properties than the other drugs at present available for the treatment of ascariasis. It takes several hours to exert its action, and produces gradual narcotization without any preliminary phase of stimulation; it has a wide margin of safety in man. The effectiveness of a single large dose of piperazine as an ascarifuge has been shown by Goodwin and Standen (1954, 1958).

Small children in the tropics frequently carry a burden of 30 or 40 adult roundworms, and some have several hundreds. An adult female is 12 to 14 in. in length and ¼ in. in diameter; a group of 50 females occupies a volume of about 200 ml. The worms are strong and muscular, and it is not surprising that drugs such as tetrachlorethylene and hexylresorcinol which stimulate them to violent activity sometimes cause obstruction or perforation of the intestine when given to patients with heavy worm burdens. Sappenfield, Swartzwelder, and Miller (1957) have reported the successful use of piperazine citrate given by mouth or by intestinal tube to children less than 4½ years of age suffering from partial intestinal obstruction caused by roundworms. Surgical intervention was unnecessary. Pena-Chavarria, Lizano, and Xirinachs (1957) have used the drug to obviate the dangers of perforation by roundworms in patients with typhoid fever.

Norton and de Beer (1957) showed that 1:20,000 of piperazine partially inhibits the contraction produced by acetylcholine in preparations of *Ascaris* in which the muscle is exposed to the action of the drug. Electrically-stimulated contractions of the muscle were unaffected by piperazine; the drug appeared to have an action on neuromuscular transmission in the worm resembling that of tubocurarine in the mammal.

The higher sensitivity of male worms to the action of piperazine explains some observations made in the course of field trials in the Gambia (Goodwin and Standen, 1958). A single dose containing the equivalent of 4 g. of piperazine hexahydrate removed all the ascarides from more than 80% of patients. The remaining patients were freed from most of their worms, but a few eggs were still present in the faeces a week after treatment. Most of these eggs were infertile, showing that the worms that remained were mostly unmated females.

Oil of chenopodium, although a very potent ascaricide, is toxic to the liver of the host and is

dangerous to use in patients with obstruction caused by roundworms.

The action of santonin was studied by Baldwin (1943) on fragments of *Ascaris*. He observed that the drug affected preparations of the anterior end, but had no action on preparations from the intermediate part of the worm. He concluded that santonin exerted its effect on the cephalic ganglia. These observations have been confirmed on whole undamaged worms, and it is clear that the action of santonin as a vermifuge must depend upon this incoordinating action. It is likely that, unless the searching activity of the anterior end can find and penetrate the central canal of an advancing peristaltic wave, the arching movements of the body which brace it against the walls of the gut are not sufficiently well-directed for it to maintain its position. A worm which has been affected by santonin therefore, like one narcotized by piperazine, becomes swept along the gut by the peristaltic contractions and is expelled.

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