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A STUDY OF THE ACTION OF ANTIMONIAL COMPOUNDS ON THE LIVER FLUKE (*FASCIOLA HEPATICA*) *IN VITRO*

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Chance and Mansour (1949) showed that the liver fluke (*Fasciola hepatica*) could be used *in vitro* as a preparation for testing the action of anthelmintics. This made it possible to compare the direct action of established anthelmintics on a representative of the platyhelminths as well as on a nematode, since a similar study has been made by Baldwin (1943) on *Ascaris in vitro*. This comparison is an essential part of studies of the mode of action of anthelmintics, and has revealed important differences between those representatives of the two phyla in their response to the same drugs. With a few notable exceptions anthelmintics have a relatively low activity on the parasites themselves ; e.g., they do not compare in potency with antibacterials. Moreover, it has been found that anthelmintic activity does not always depend upon a lethal action, but may depend upon an interference with the movements of the parasite. This can happen in more than one way. Studies on the parasite *in vitro* are therefore valuable ways of increasing our knowledge of the action of these drugs. Chance and Mansour (1949) tested anthelmintic drugs mainly used in therapeutics against intestinal parasites ; the present report is concerned with the lethal action of the chief drugs used against blood fluke infestations, namely organic antimonials. The results reveal that the preparation is sensitive to a wider range of drugs than had previously been suspected ; they also reveal some facts about the mode of action of the substances which act on blood flukes as well as on those active in the alimentary tract. Since the somatic helminths are bathed in blood or lymph during life, a comparison has been made between the action of these drugs with and without serum added to the saline medium used in the earlier study.

MATERIALS AND METHODS

Numerous organic antimony preparations are used as therapeutic agents against somatic helminths. The following compounds were therefore tested:

(*a*) Potassium antimonyl tartrate (tartar emetic).

(*b*) Antimony pyrocatechol sodium disulphonate (fouadin).

(*c*) *p*-Aminophenyl stibinate of diethylamine (neostibosan).

Kymographic records of the *in vitro* movements of the whole fluke were obtained by the method described in the earlier publication (Chance and Mansour, 1949).

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The preparations from bovine flukes were found to give rhythmical movements either in Ringer solution alone or 50 per cent (v/v) bovine serum-Ringer solution at a *p*H of 7.4 [tartar emetic is not stable in more alkaline solutions (Oelkers, 1937)] for a period of at least two hours, and frequently as long as six hours. The method of dosage employed for the therapeutic use of these drugs against schistosomiasis suggests that the rate of action is slow. Therefore, in testing these anthelmintic drugs the period of application to the preparation was extended up to 90 minutes. After that time the degree of interference with rhythmical movement of the preparation was tested by the addition of 1 : 5,000 solution of amphetamine sulphate, which has a stimulant effect on normal flukes. At least four flukes were used in each test.

RESULTS

Responses in saline.—Tartar emetic, fouadin, and neostibosan were tested at 1 : 1,000 concentration for as long as 90 minutes. The three compounds failed to cause paralysis of rhythmical activity or to prevent the response to amphetamine (Figs. 1 and 2).

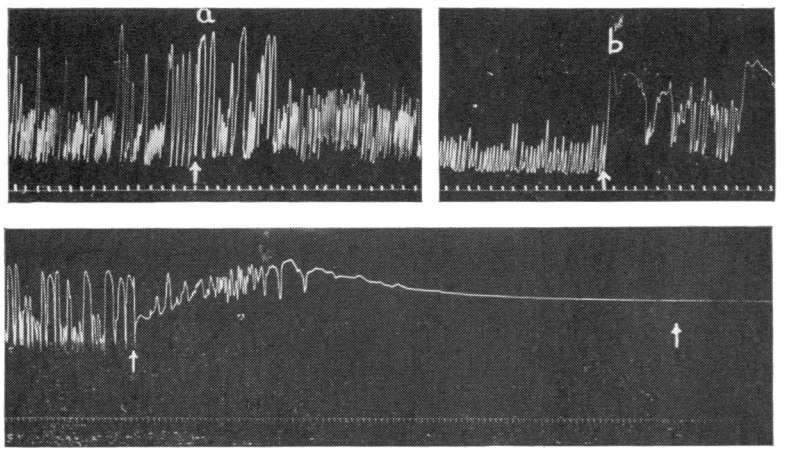

FIG. 1.—Responses to tartar emetic 1:1,000:—*Top tracing:* In saline solution at (*a*) followed by response to amphetamine after 90 minutes at (*b*). *Bottom tracing:* In serum saline medium followed by absence of response to amphetamine after 90 minutes. (In all tracings upward stroke represents contraction. Time markings in minutes.)

Responses in serum-saline.—In this medium, which was innocuous to the rhythmical activity of the preparation for as long as two hours, tartar emetic at a concentration of 1 : 1,000 caused a slight rise of tone in rhythmical movement which continued with smaller amplitude for 30 minutes (Fig. 1). Complete paralysis in the contracted form then occurred. No response to amphetamine was noticed after 90 minutes. The minimum amount of serum necessary to produce this potentiating effect was found to be 50 per cent in the saline medium. The minimal effective concentration for tartar emetic was 1 : 1,000. On the other hand, the presence of serum in the medium did not initiate any toxic response either to fouadin (Fig. 2) or to neostibosan up to a concentration of 1 : 1,000.

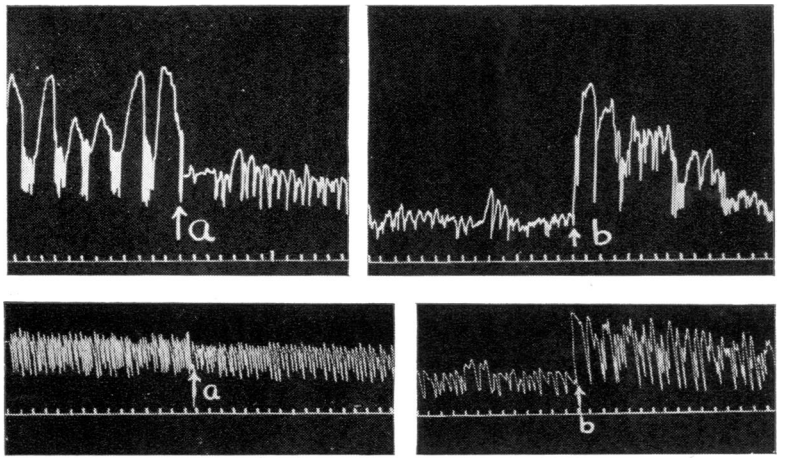

FIG. 2.—Responses to fouadin 1 : 1,000:—*Top tracing:* In saline solution at (*a*) followed by response to amphetamine after 90 minutes at (*b*). *Bottom tracing:* In serum saline medium at (*a*) followed by response to amphetamine after 90 minutes at (*b*).

Investigation of serum effect.—The potentiating effect of serum on tartar emetic drew attention to the fact that serum may play an important part in its parasiticidal action *in vivo* ; thus serum might stimulate the ingestion mechanism of the parasite *in vitro,* so that some of the drug would gain access through the gut, since serum is part of the main food for flukes (Stephenson, 1947). However, flukes with ligatured oral openings showed the same response as normal flukes to the toxicity of tartar emetic in serum.

Another factor which had to be considered was the presence of complement in the serum, but heating the serum for 30 minutes at 55° C. did not abolish its potentiating effect on the toxic action of tartar emetic.

The effect of dividing the serum into dialysable and non-dialysable fractions was also investigated.

Bovine serum (200 ml.) were dialysed in a cellophane sac against an equal volume of distilled water at 4° C. The distilled water was changed every 48 hours. Ten days' dialysate was collected, which was normally less than a litre in volume as the distilled water passed through the sac. On the other hand, the amount of serum was more than its original volume after this period of dialysis.

For kymographic tests, the amount of diluted serum, which originally contained 50 ml., was made up to 100 ml. with distilled water. Mineral content for 50 ml. of the saline medium was added to this amount ; 90 ml. of the dialysate was made up to 100 ml. with distilled water, with the addition of the mineral contents for 100 ml. of the original medium.

Neither dialysate nor serum affected the normal behaviour of movement when tested alone. Serum after dialysis was found to have lost the property of potentiating the activity of 1 : 1,000 tartar emetic (Fig. 3), whereas the dialysate possessed a partial potentiating action. When serum was dialysed against saline for a week, with a control dialysing against distilled water, it was found that the saline dialysate did not potentiate the effect of 1 : 1,000 tartar emetic, whereas the distilled water control was again active.

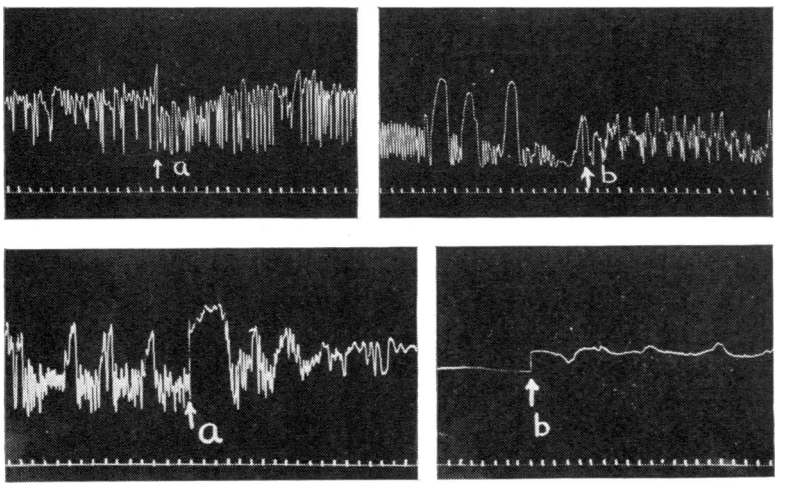

FIG. 3.—Effect of dialysis on potentiating action of serum on tartar emetic 1 : 1,000:—*Top tracing :* Effect of serum dialysed against distilled water for ten days on the action of tartar emetic at (*a*) followed by response to amphetamine after 90 minutes at (*b*). *Bottom tracing :* Effect of the freeze-dried dialysate on the action of tartar emetic at (*a*) followed by absence of full response to amphetamine after 90 minutes at (*b*).

Part of the distilled water dialysate from the previous experiment was freeze dried in order to test it in a more concentrated form. The amount of the freeze-dried powder from about 200 ml. dialysate corresponded to the total dialysate from 50 ml. bovine serum ; it was dissolved in 50 ml. distilled water and made up to 100 ml. with Ringer solution, the *p*H being adjusted to 7.4. This solution was found to potentiate the lethal action of 1 : 1,000 tartar emetic on the preparation (Fig. 3).

A petroleum-ether-soluble fraction of the freeze-dried dialysate had no potentiating effect on the toxicity of tartar emetic. On the other hand, the residual water-soluble fraction was found to potentiate the effect of tartar emetic to some extent.

DISCUSSION

The results reported here were obtained by experiments which lasted for 90 minutes. The compounds tested did not show any anthelmintic activity in the saline medium. On the other hand, a definite lethal action was demonstrated to tartar emetic in the presence of serum. This fact indicates the possible importance of the environment of the parasite on the effect of anthelmintic drugs. Lièvre (1934) reported an unsuccessful attempt to demonstrate the lethal action of tartar emetic on the liver fluke. It seems likely that this was due to the fact that a saline medium was used.

Although the antimony in fouadin is in the trivalent state, as in tartar emetic, fouadin failed to paralyse the preparations in serum saline. This might be due to its low antimony content. However, neostibosan was inactive in spite of its high antimony content. The low lethal activity of the pentavalent antimony compound, neostibosan, *in vitro* was demonstrated by Lee and Chung (1935), who found that *Schistosoma japonicum* in serum cultures was not killed at a concentration of 1 : 6,000, except after eight days.

The potentiating action of serum on tartar emetic is not due to complement and it is retained in the dialysable fraction.

Summary

1. The effects of antimonial compounds on preparations of the liver fluke (*Fasciola hepatica*) have been recorded kymographically. Both Ringer's solution and a mixture of equal volumes of bovine serum and Ringer's solution were used as media for these *in vitro* tests for a maximum period of 90 minutes.

2. Tartar emetic, fouadin, and neostibosan all failed to cause paralysis of rhythmical movement in a saline medium in a concentration of 1 : 1,000.

3. Serum was found to initiate a lethal response to tartar emetic, but not to fouadin or neostibosan. The fraction of serum responsible for this action was found to be dialysable through cellophane membrane against distilled water, but not against saline.

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