Brit. J. Pharmacol. (1952), 7, 215.

THE EFFECT OF SOME OXIDATION PRODUCTS OF PHENOTHIAZINE ON LIVER FLUKE (*FASCIOLA HEPATICA*) *IN VITRO*

BY

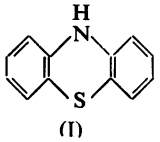
ALEXANDER MACKIE AND JOHN RAEBURN

From the Department of Chemistry, Heriot-Watt College, Edinburgh

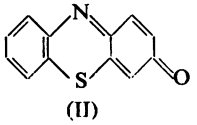
(Received November 23, 1951)

Phenothiazine (I) is used extensively as an anthelmintic and has proved to be very efficient in the removal of some intestinal worms in sheep. A considerable amount of research has been carried out in order to ascertain the fate of phenothiazine when administered to ruminants (Collier, 1940 ; Swales and Collier, 1940 ; Lipson, 1940 ; Collier, Allen, and Swales, 1942 ; Collier, Allen, and Swales, 1943 ; Clare, 1947 ; Harpur, Swales, and Dunsteht, 1950).

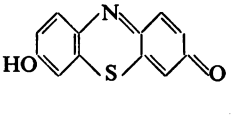
The dose of phenothiazine required appeared to be unnecessarily large when its insolubility was considered, and Taylor and Sanderson (1940) studied the possibility of the formation in the host of another substance of greater anthelmintic potency than phenothiazine.



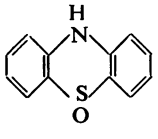
(I)



(II)



(III)



(IV)

A number of oxidation products have been found in the host dosed with phenothiazine, viz., phenothiazone (II), thionol (III), or their easily oxidizable leuco-compounds, phenothiazine sulphoxide (IV), and certain conjugates, e.g., leuco-phenothiazone conjugated with sulphuric acid.

Phenothiazone was administered to sheep by Collier, Allen, and Swales (1943), but found to have no effect on nematodes removable by phenothiazine, and both phenothiazone and thionol were tested by Taylor and Sanderson (1940) in goats heavily infected with parasitic worms, but no anthelmintic effect was observed.

As no mention was made of the liver fluke (*Fasciola hepatica*) in these experiments, the investigation described in the present paper was carried out to ascertain the action, if any, of phenothiazone, thionol, and phenothiazine sulphoxide on liver fluke *in vitro*.

METHODS

Preparative.—Phenothiazone, thionol, and phenothiazine sulphoxide were prepared by the methods of Olivier and Combé (1950), Houston, Kester, and DeEds (1949), and Barnett and Smiles (1909) respectively.

Biological testing.—The liver fluke was tested *in vitro* by the kymographic technique of Baldwin (1943) as modified by Chance and Mansour (1949). By frequent changes of the Ringer solution (1.5–2 hr.), it was possible to keep the liver fluke in a lively condition for 7 to 8 hr., after removal from the host. Aeration of the Ringer solution had no effect on prolonging the life of the parasite.

The tests were carried out at 37–38° C. with concentrations of 1 : 1,000 and less till the phenothiazine oxidation products ceased to have an effect on the helminth. As the compounds under test were very insoluble in water, emulsification by Baldwin's method was attempted. Phenothiazine sulphoxide formed only a suspension at the concentrations used.

RESULTS

It was found that phenothiazone had a lethal effect from a concentration of 1 : 1,000 to 1 : 8,000 and a paralysant effect from 1 : 8,000 to 1 : 16,000, whilst thionol and phenothiazine sulphoxide had no lethal action, and were paralysant at 1 : 1,000 and from 1 : 1,000 to 1 : 4,000 respectively.

Figs. 1–4 show the kymographic records of the three compounds at the minimum concentration required to produce a lethal or paralysant effect. The time is marked in minutes on the signal line, and the movement of the helminth in the Ringer solution is shown from the beginning of the experiment to the first long stroke, when the Ringer solution was replaced by the emulsion or suspension of the compound. At the second long stroke on the signal line, amphetamine sulphate (1 : 5,000) in Ringer's solution, as recommended by Chance and Mansour (1949), replaced the compound. Restoration of the movement of the parasite with amphetamine sulphate indicates that the compound had a paralysant effect, whilst no movement shows that the substance was lethal.

The results are summarized in Table I.

TABLE I

THE LOWEST CONCENTRATIONS AT WHICH OXIDATION PRODUCTS OF PHENOTHIAZINE EXERT A LETHAL OR PARALYSANT EFFECT ON THE LIVER FLUKE *in vitro*

Compound	Nature of preparation	Concentration	Effect
Phenothiazone	Emulsion	1 : 8,000	Lethal
	,,	1 : 16,000	Paralysant
Thionol	,,	1 : 1,000	,,
Phenothiazine sulphoxide	Suspension	1 : 4,000	,,

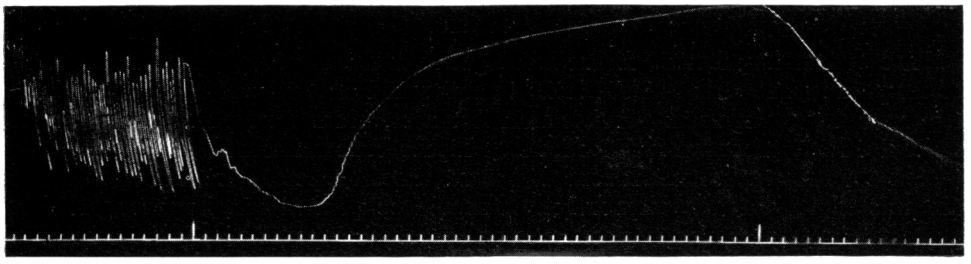


FIG. 1.—Lethal effect of phenothiazone (1 : 8,000). No response to amphetamine sulphate.

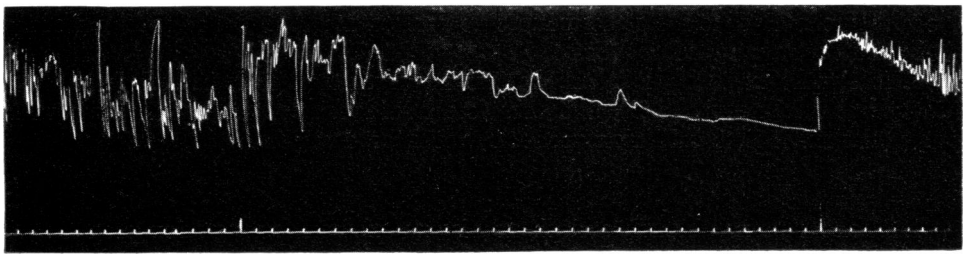


FIG. 2.—Paralysant effect of phenothiazone (1 : 16,000) after 25 min., followed by response to amphetamine sulphate.

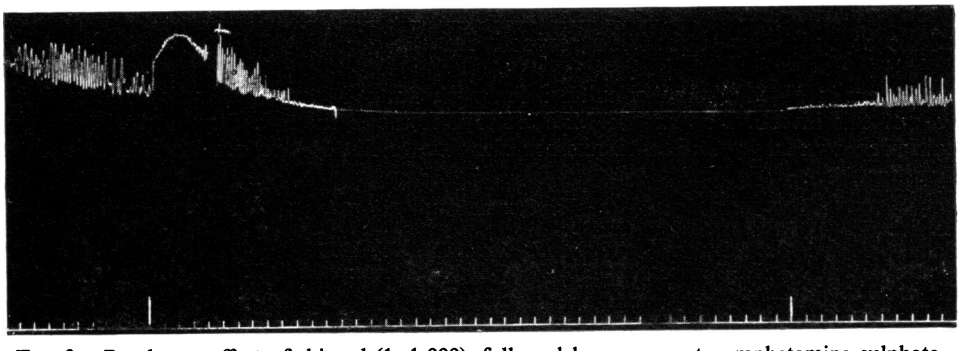


FIG. 3.—Paralysant effect of thionol (1 : 1,000), followed by response to amphetamine sulphate.

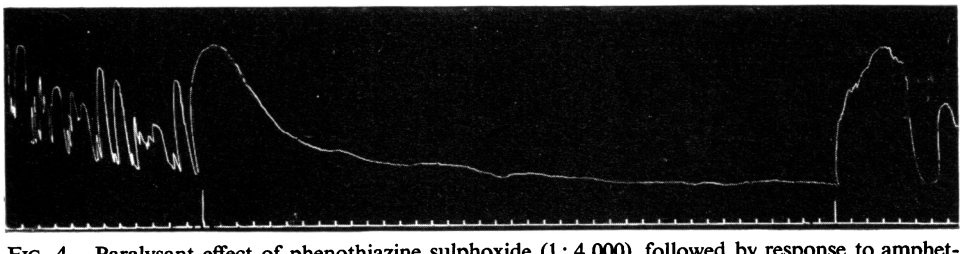


FIG. 4.—Paralysant effect of phenothiazine sulphoxide (1 : 4,000), followed by response to amphetamine sulphate.

Discussion

The outstanding feature of this investigation is the discovery of the lethal effect of the phenothiazone at 1 : 8,000 and its paralysant effect at 1 : 16,000. Chance and Mansour (1949) also found that certain substances, which were paralysants at lower concentrations, produced lethal effects at higher concentrations.

As a paralysant phenothiazone is sixteen times as potent as thionol and four times as effective as the sulphoxide. It should be pointed out, however, that the potency of phenothiazine sulphoxide is not strictly comparable with the other two compounds, since an effective concentration was only achieved by using a suspension.

It would appear, therefore, that the introduction of a hydroxyl group in position 7 of the phenothiazone molecule reduces its paralysant effect to one-sixteenth of its value and destroys its lethal effect.

It is significant that phenothiazine has no paralysant or lethal effect *in vitro* (Chance and Mansour, 1949), and therefore additional experiments *in vivo* with phenothiazine and its oxidation products would be desirable.

Summary

1. Phenothiazone, thionol, and phenothiazine sulphoxide, oxidation products of phenothiazine, have been prepared and tested at various concentrations against liver fluke (*Fasciola hepatica*) *in vitro*.

2. Phenothiazone had a lethal effect at a concentration of 1 : 8,000 and was paralysant at 1 : 16,000. Thionol and phenothiazine sulphoxide had only paralysant effects at 1 : 1,000 and 1 : 4,000 respectively.

We are indebted to the Agricultural Research Council for a grant which defrayed the cost of this investigation and to Mr. Soulsby, of the Edinburgh City Abattoir, for the collection of parasites. Our thanks are also due to Dr. Parnell for his interest in this investigation.

References

Baldwin, E. (1943). *Parasitology*, **35**, 89.
Barnett, E. de B., and Smiles, S. (1909). *J. chem. Soc.*, **95**, 1265.
Chance, M. R. A., and Mansour, T. E. (1949). *Brit. J. Pharmacol.*, **4**, 7.
Clare, N. T. (1947). *Aust. Vet. J.*, **23**, 340.
Collier, H. B. (1940). *Canad. J. Res.*, D, **18**, 272.
Collier, H. B., Allen, D. E., and Swales, W. E. (1942). *Canad. J. Res.*, B, **20**, 189.
Collier, H. B., Allen, D. E., and Swales, W. E. (1943). *Canad. J. Res.*, D, **21**, 151.
Harpur, R. P., Swales, W. E., and Dunsteht, O. F. (1950). *Canad. J. Res.*, D, **28**, 143, 162.
Houston, D. F., Kester, E. B., and DeEds, F. (1949). *J. Amer. chem. Soc.*, **71**, 3819.
Lipson, M. (1940). *Aust. J. exp. Biol. med. Sci.*, **18**, 269.
Olivier, S. C. J., and Combé, W. P. (1950). *Rec. Trav. chim. Pays-Bas*, **69**, 527.
Swales, W. E., and Collier, H. B. (1940). *Canad. J. Res.*, D, **18**, 279.
Taylor, E. L., and Sanderson, K. M. (1940). *Vet. Rec.*, **52**, 635.