

## The mode of action of carbon tetrachloride on *Fasciola hepatica*

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1. A biliary fistula was prepared in twelve sheep.
  2. Each animal was given 2 ml. of  $^{14}\text{C}$ -carbon tetrachloride (specific activity 15 mc/m-mole) and bile collected at 2, 4, 24 and 48 hr.
  3. Specific activity was measured by liquid scintillation counting before and after each specimen had been heated to 75° C for 10 min to drive off volatile content (carbon tetrachloride).
  4. Only traces of activity were present in the bile and heating did not alter this.
  5. There was no difference in the survival times of adult *Fasciola hepatica* incubated in Hedon Flieg medium with and without 25% bile from non-medicated sheep, or with carbon tetrachloride in a concentration of 0.03%, but 25% of bile from sheep given 2 ml. of carbon tetrachloride 24 hr before greatly increased lethality *in vitro*.
  6. The anthelmintic action of carbon tetrachloride on *F. hepatica* does not result from a direct action due to excretion in bile of unchanged carbon tetrachloride but of an unidentified toxic element not necessarily derived from the drug but which arises as a result of its administration.
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Carbon tetrachloride is widely used in veterinary practice for the treatment of liver fluke (*Fasciola hepatica*) infestation and has been used for hookworm infestation in man. Robbins (1929) was of the opinion that all of an orally administered dose in dogs was excreted by the lungs. McCollister, Beamer, Atchinson & Spencer (1951) gave  $^{14}\text{C}$ -labelled carbon tetrachloride by inhalation to monkeys and concluded that the major portion was destroyed in the body and simple degradation products were excreted in the urine and by the lungs (approximately 51% by this route). Nevertheless, Daykin (1960) states that carbon tetrachloride is excreted in the bile and that it exerts its therapeutic action by destroying mature *F. hepatica* as a result. Khalidi & Frankul (1965) collected specimens of bile from fistulae for 2-48 hr after oral dosing of sheep with 1 ml. of carbon tetrachloride and were unable to detect the drug by Fugiuware's chemical test (Gonzales, Vance & Umberger,

1954). It may be said justifiably that this test is not sensitive enough to detect small quantities of carbon tetrachloride. The opportunity has therefore been taken to repeat and extend the work with  $^{14}\text{C}$ -labelled carbon tetrachloride.

## Methods

In twelve adult sheep a permanent bile-duct fistula was made as described previously (Khalidi & Frankul, 1965) and bile specimens were collected in polythene tubes attached to the shaven skin of the animal's flank. The sheep were starved for 24 hr pre-operatively and dosed orally by syringe via a soft rubber tube into the oesophagus with 2 ml. pure carbon tetrachloride labelled with  $^{14}\text{C}$  having a specific activity of 15 mc/m-mole, obtained from Amersham, England. The sheep were maintained on water only by mouth. They drank regularly and copiously and all the bile secreted was collected at 2 hr, 4 hr, 24 hr and 48 hr after dosing.

(A) One ml. aliquots of bile were added to 16 ml. dioxane scintillator fluid and counted in triplicate in a Packard Tri-Carb automatic spectrometer before and after heating to  $75^\circ\text{C}$  for 10 min to expel any carbon tetrachloride present. Control specimens of bile from the pre-dosing period were also examined.

(B) Mature *F. hepatica* were collected from the livers of freshly slaughtered infected sheep. Eight flukes were placed in each of a number of bottles which were arranged in groups of five or six and kept at  $37^\circ\text{C}$ . The bottles contained (1) 500 ml. of Hedon Flieg (HF) medium, as a control, (2) HF medium containing 25% v/v of bile from an unmedicated sheep (n.m.b.), (3) HF with 5%, 10% or 25% of bile from a sheep premedicated 24 hr previously with 2 ml. of carbon tetrachloride (p.m.b.), (4) HF medium to which was added  $\text{CCl}_4$  to a final concentration of 0.03%. The bottles were examined daily, dead flukes removed and cumulative mortality expressed as percentage deaths.

TABLE 1. Specific activity in counts/ml. bile per min.  $\pm$  S.E.M. before and after heating for 10 min at  $75^\circ\text{C}$ .

Control	Before 0	After 0	<i>n</i> =12
2 hr	$15.8 \pm 3.1$	$15.1 \pm 2.9$	<i>P</i> >0.4
4 hr	$11.7 \pm 5.5$	$8.7 \pm 4.3$	<i>P</i> >0.2
24 hr	$6.7 \pm 4.0$	$4.5 \pm 2.8$	
48 hr	$2.8 \pm 3.0$	$3.1 \pm 3.2$	

TABLE 2. Effect of bile from non-medicated sheep (n.m.b.) and from sheep premedicated with 2 ml. of carbon tetrachloride by mouth 24 hr before (p.m.b.) and of carbon tetrachloride on the survival of *F. hepatica* in Hedon Flieg medium, expressed as cumulative daily deaths as a percentage of the initial culture number.

Medium	Mortality of flukes (%)				
	1	2	3	4	5 days
H.F.	0	0	12	56	78
+25% n.m.b.	0	2.5	12	42	75
+5% p.m.b.	0	0	6	56	67
+10% p.m.b.	0	0	6	62	68
+25% p.m.b.	0	97	100	—	—
+0.03% $\text{CCl}_4$	0	4	8	53	74

## Results

The volumes of bile passed by individual sheep in the 48 hr period were remarkably constant, namely,  $81.5 \pm 0.3$  ml. ( $\bar{x} \pm$  S.E. of mean).

(A) Table 1 shows the results of the examination of bile specimens from sheep dosed with  $^{14}\text{C}$ -labelled  $\text{CCl}_4$ . It can be seen that the level of specific activity detected is low and declines with time and that no statistically significant difference was produced by heating. It is concluded that the activity detected is not due to a volatile component (carbon tetrachloride), but to water-soluble labelled substances.

(B) *Fasciola hepatica* did not survive well beyond 3 days in HF medium, 56% being dead on the fourth day and 78% on the fifth. Addition of bile from a non-medicated non-infected sheep (n.m.b.) did not appreciably alter this pattern or affect mortality. The addition of bile (p.m.b.) from a sheep pre-treated by mouth with carbon tetrachloride increased the mortality of the cultured flukes and there appeared to be a close-dependent relationship. The addition of 5 or 10% of p.m.b. caused no increase in mortality over the controls by day 3 whereas 25% p.m.b. caused 97% mortality by day 2. On day 3 all the flukes in 25% p.m.b. were dead. The trial with HF medium containing 25% v/v of bile from a premedicated sheep was repeated on forty flukes in five flasks and gave substantially the same result—97% lethality on day 2 and 100% on day 3. Carbon tetrachloride was not by itself lethal to *F. hepatica* when added to HF medium in a concentration of 0.03% v/v. It is concluded that carbon tetrachloride has no direct toxic action on *F. hepatica* *in vitro*.

## Discussion

The findings do not support the idea that *F. hepatica* is affected by unchanged carbon tetrachloride excreted by the liver in the bile. Nevertheless, carbon tetrachloride is an effective anthelmintic when given orally. It may be postulated that carbon tetrachloride is excreted in small amounts and is really of high potency, but the absence of *in vitro* activity of carbon tetrachloride added to the medium does not support this. There may be an extremely active metabolite of carbon tetrachloride excreted in bile; if so, it is not volatile and is present in trace amounts only (see Table 1). Alternatively, the anthelmintic action may be indirect. As to the possible nature of this, one can only hazard a guess. Local anoxaemia and increased intra-biliary pressure consequent on hepato-parenchymal oedema may kill flukes directly or from lack of oxygen or other nutrients. The damaged liver cell may perhaps release a product which does not derive directly from the catabolism of the labelled carbon tetrachloride but is lethal to *F. hepatica*. The latter theory may be thought to fit the findings best and if proved correct is a novel mode of action of a chemotherapeutic agent.

We are grateful to the Radioisotope Centre, Amersham, for providing the labelled  $^{14}\text{C}$ -carbon tetrachloride and to the M.E. Regional Radioisotope Centre, Cairo, for performing the scintillation counts, and to the authorities of the College of Veterinary Medicine, Baghdad, for providing and maintaining the sheep.

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(Received November 19, 1968)