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Some hepatotoxic actions of hexachloroethane and its metabolites in sheep

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1. Pentachloroethane and tetrachloroethylene were major metabolites of hexachloroethane in sheep.

2. Concentrations of hexachloroethane, pentachloroethane and tetrachloroethylene were determined by gas-liquid chromatography in blood, bile, faeces, urine and tissues after oral administration of hexachloroethane emulsions to sheep.

3. Increased blood concentrations of sorbitol dehydrogenase, glutamate dehydrogenase, and ornithine carbamoyl transferase were found to follow oral administration of hexachloroethane or pentachloroethane.

4. The rate of bromsulphthalein transfer from liver cells to bile was found to decrease after oral administration of hexachloroethane.

Hexachloroethane has been widely used in cattle and sheep as an anthelmintic (Thienal, 1926; Olsen, 1944; Olsen, 1946). It is effective against mature liver flukes *Fasciola hepatica* which are found in the bile ducts of sheep and cattle, but has little activity against immature flukes migrating through the liver parenchyma.

Hexachloroethane is a volatile, fat soluble, solid. It is given orally in the form of a suspension or bolus (Olsen, 1946; Olsen, 1947); parenteral administration has not been favoured because it leads to carcase taint in treated animals.

Large numbers of animals were treated without reports of marked toxicity until Olsen published an account of poisoning in sheep (Olsen, 1946). Adverse reactions have included intoxication, inco-ordination, muscle tremors and death (Olsen, 1947; Southcott, 1951; and Byewater, 1955). As with carbon tetrachloride, the toxicity of hexachloroethane is enhanced by a high protein diet and withdrawal of such feed-stuffs at the time of dosage has been advocated (Gibson, 1962). The present work on hexachloroethane comprised an investigation of the metabolism and excretion of the drug, and its hepatotoxicity. Previously hexachloroethane metabolism has been studied by ^{14}C-labelling techniques (Jondorf, Parke & Williams, 1957) and *in vitro*, by the action of tissue homogenates with particular reference to sulphydryl group conjugation (Bray, Thorpe & Vallance, 1952).

Methods

Experimental animals

Scottish Blackface (1, 2, 3, 4, 27, 28) or Cheviot cross (5, 6, 7, 8, 9, 10, 11, 12, 17, 18, 19, 20, 25, 26) male (6, 8) or castrated male (others) sheep weighing 15–23 kg each were used. Animals were housed with hay and water available *ad lib.*

Administration of drugs

Hexachloroethane was dissolved in olive oil (15% w/v) then emulsified with water using gum acacia (12.5 %w/v) and gum tragacanth (0.7% w/v) as emulsifying agents to produce a final volume of about 300 ml. This was orally administered by drenching bottle.

Pentachloroethane and tetrachloroethylene were dissolved in olive oil (33% v/v) and administered orally by a syringe with a four inch tubular extension.

Dosage rates: Hexachloroethane, 0.5 g/kg, sheep 1, 2, 3, 4, 5, 6, 11, 12, 27, 28. Hexachloroethane, 0.75 g/kg, sheep 7. Hexachloroethane, 1.0 g/kg, sheep 8. Pentachloroethane, 0.3 ml/kg, sheep 19, 25. Tetrachloroethylene, 0.3 ml/kg, sheep 20, 26. Olive oil (control), 0.6 ml/kg, sheep 9, 10, 17, 18.

Sample collection

1. Blood samples

Blood samples (5 ml.) were taken from the jugular vein with evacuated glass tubes containing sodium heparin as anticoagulant. Plasma and erythrocytes were separated by centrifugation for 20 min at RCF 1600. Plasma was used immediately for the various analyses, or stored frozen at $-20°$ C for subsequent use. Erythrocytes were washed three times with 0.15 M sodium chloride solution before use or storage.

2. Urine and faeces samples

Urine and faeces samples were collected from sheep 11 and 12 while they were confined in metabolism cages. Hay and water were available *ad lib.* throughout the collection period of 4 days. Faeces were collected on polyethylene sheeting and recovered at 24, 48, 72 and 96 hr for examination. Urine was collected by the method of Warwick (1966) into a receiver maintained at low temperature by ice/water jacket.

3. Bile and tissue samples

Sheep 27 and 28 were fasted for 24 hr and then anaesthetized with pentobarbitone sodium. The hepatic duct was cannulated with 4 mm (outside diameter) polyvinyl tubing and the cystic duct ligated. The common duct was also ligated and the duodenum occluded above and below the sphincter of Oddi with bowel clamps to avoid any chance of samples becoming directly contaminated with hexachloroethane. After collection of a sample of bile, hexachloroethane solution (15% w/v in olive oil) was injected at a dose of 0.5 g/kg directly into the rumen and lower duodenum (divided dose). Bile was collected continuously and 2 ml. was retained each 30 min for analysis ; any remaining bile was returned to the lower duodenum after recording its volume.

The preparation was artificially respired with a mixture of 95% oxygen and 5% carbon dioxide for 1 min every 30 min to supplement normal respiration and to reduce any tendency to hypoxia. Anaesthesia was maintained by intravenous infusion of pentobarbitone sodium and the experiments were terminated 8.5 hr after administration of hexachloroethane.

Samples (3–4 g) of the following tissues were taken within 10 min of death and stored frozen at −20° C until required: brain; liver (diaphragmatic and visceral surfaces); kidney (cortex and pelvis); muscle (masseter, peroneus tertius, infraspinatus); perinephric, subcutaneous and inguinal fat.

4. Storage of biological samples

Urine and faeces samples were used as procured. Aliquots of plasma, erythrocytes, bile or tissues were stored frozen in glass tubes closely covered with metal foil secured by an elastic ring. Before use, bile, blood and erythrocyte samples were stood at room temperature until liquid; tissue samples were ground from the frozen state.

Study of the metabolism of hexachloroethane

1. Hexane extraction of biological samples

Samples (1 ml.) of bile or plasma, or weighed samples of erythrocytes, were extracted in closed tubes with hexane (2 ml.) containing hexachlorobut-1, 3-diene as internal standard. 2.5 M ammonium sulphate solution (1 ml.) was included to reduce the extent of emulsion formation, and saponin (2 ml. 0.01% w/v solution) was added to erythrocyte extraction tubes to aid haemolysis. The tubes were tightly covered with metal foil, shaken for 40 min and centrifuged to break down any emulsion. Rapid cooling to −20° C followed, which enabled the hexane to be decanted from the frozen aqueous portion. The hexane extracts were examined within 24 hr by gas-liquid chromatography. When prolonged storage was required, samples were sealed into ampoules.

Urine samples were extracted by a similar technique. 100 ml. of urine was extracted by a single partition with 20 ml. of hexane which was then washed to remove interfering substances. (Successive washings with equal volumes of water, 1 N sodium hydroxide, 1 N hydrochloric acid and finally water, followed by drying with anhydrous sodium sulphate.) Faecal samples were macerated under warm hexane, and washed in a similar manner.

These methods reduced losses by volatilization and recovery rates (determined by addition of standard solutions to control samples) were greater than 90%.

Tissue samples were weighed and ground with silver sand using a glass pestle-and-mortar. Extraction was by three partitions with hexane containing internal standard. In order to avoid contamination of the columns used in the gas-liquid chromatographic analysis, the fat content of samples was reduced by chilling (3 hr at −20° C) and a Pyrex glass trap was incorporated in the injector port of the gas chromatograph.

2. Analysis of samples by gas-liquid chromatography

A concentric tube electron capture detection system was used on the Aerograph Hi-Fi 600-C model gas chromatograph and aliquots of hexane extracts were injected with a Hamilton syringe.

Retention times of extracted materials were compared on three columns with those due to reference solutions of hexachloroethane, pentachloroethane and tetrachloroethylene. Retention times for 1,2-dichloroethane and 1,1,2,2-tetrachloroethane were also determined. Aliquots of compounds administered to sheep, of extracts of samples from sheep and of solvents used in making extractions were analysed on the following columns:

Column 1. 2.12m × 3 mm stainless steel tubing packed with acid-washed Celite 60/72 mesh, coated with 5% (w/w) SE-30 grease.

Column 2. 2.00 m × 3 mm stainless steel tubing packed with firebrick, coated with 1.5% (w/w) SE-30 grease and 2% (w/w) polyethylene glycol 20 M.

Column 3. 1.25 m × 3 mm stainless steel tubing packed with Chromosorb 'G' 60/80 mesh, coated with 3% (w/w) dinonylphthalate.

3. *Interpretation of gas-liquid chromatographic results*

In the gas-liquid chromatographic analysis of extracts of samples from sheep which received hexachloroethane 0.5 g/kg. two unidentified peaks (referred to as "X" and "Y") were detected in hexane extracts of blood, bile, urine and faeces. The "X" and "Y" peaks were identical on three columns and in all conditions tested with peaks produced by standard solutions of tetrachloroethylene and pentachloroethane in hexane. The evidence afforded by the retention times of the unknown compounds "X" and "Y" present in the extracts was taken as circumstantial evidence for identifying these as the same as the reference compounds tetrachloroethylene and pentachloroethane. This is in accordance with present criteria of peak identification in gas-liquid chromatography (Perry, 1967).

The areas of peaks "X" and "Y" were calculated by triangulation and their ratio to the internal standard peak area computed. The concentrations of "X" and "Y" were estimated by comparison of these peak ratios with ratios due to several concentrations of tetrachloroethylene and pentachloroethane dissolved in hexane containing hexachlorobut-1, 3-diene as internal standard.

Samples were diluted where necessary to obtain a linear response from the electron capture detector (utilizing less than 30% of the available standing current). Standard mixtures containing reference compounds in similar concentrations to the compounds in extracts were analysed frequently during analysis of samples from sheep experiments.

4. In vitro *metabolism of hexachloroethane*

(a) 1–1.5 mm slices of fresh liver in olive oil emulsion (10 ml.); (b) the same as (a) with hexachloroethane (18 mg or 52 mg/l.); (c) as (b) but liver slices previously boiled (5 min at 100° C); (d) as (b) but liver slices previously heated (5 min at 70° C); (e) olive oil emulsion (10 ml.) with hexachloroethane, tissue omitted. The emulsion contained olive oil (3 ml.); powdered acacia (1 g) per l. of Hedon-Fleig solution (0.1% glucose). Hexachloroethane was dissolved in the olive oil before emulsification.

The liver slices were incubated at 37° C for 4 hr in a water bath with continuous agitation, before hexane extraction. Hexane extracts were examined by gas-liquid chromatography.

Reagents

Analytical grade reagents were used where available. Commercial hexachloroethane was purified by sublimation; technical grade pentachloroethane and tetrachloroethylene by two fractional glass distillations.

Fractions distilling at 158° C (uncorr.) (pentachloroethane) and 121° C (uncorr.) (tetrachloroethylene) were collected. After purification, hexachloroethane and tetrachloroethylene each produced a single response from the gas chromatograph; however, pentachloroethane was still contaminated with tetrachloroethylene at a level of 7.6 p.p.m.

Hexane fraction of petroleum b.p. 67°–70° C was purified in 200 ml. aliquots as follows: successive washes with concentrated sulphuric acid (20 ml.) (specific gravity 1.84); water (100 ml., twice) sodium bicarbonate solution (100 ml.) (1.1 M); water (100 ml., twice), and finally dried with anhydrous sodium sulphate (50 g).

Olive oil was B.P. grade, hexane extracts of which gave no response when analysed by gas-liquid chromatography.

Study of the hepatotoxicity of hexachloroethane

1. Plasma enzymes

Glutamate dehydrogenase E.C.1.4.1.3 (GD) was determined by the method of Ford & Boyd (1962); sorbitol dehydrogenase E.C.1.1.1.14 (SD) by the method of Ford (1967) and ornithine carbamoyl transferase E.C.2.1.3.3. (OCT) by the micromethod of Moore (1967).

Aspartate aminotransferase E.C.2.6.1.1 (GOT) was determined colorimetrically by the method of Reitman & Frankel (1957), but with modifications as suggested by Wootton (1964).

Plasma enzyme concentrations were expressed in International Units (i.u.) or, in the case of OCT, in m-i.u.

Fresh or recently unfrozen plasma was used throughout for enzyme determinations; activities of the above enzymes were stable for at least a week at −20° C (storage temperature).

2. Bromsulphthalein dye clearance tests

Bromsulphthalein (BSP) dye clearance tests were carried out on sheep 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. BSP sodium solution (50 mg/ml. distilled water) was injected intravenously at a dose rate of 5 mg/kg. Six blood samples were collected at accurately timed intervals between 2 and 30 min after injection of BSP. Optical density (O.D.) was determined at 575 nm after dilution of 1 ml. aliquots of plasma. The control reading was obtained by dilution of plasma with 2 ml. of 0.05 N hydrochloric acid; the test reading by dilution of plasma with 2 ml. of 0.5 N ammonium hydroxide. The concentration of BSP in each sample of plasma was calculated by reference to a standard curve relating O.D. at 575 nm to concentration of several standard solutions of BSP. A water blank and a standard solution of BSP were read with each diluted plasma sample.

In the sheep a dose of BSP 5 mg/kg disappears in a biphasic manner as described by Cornelius, Holm & Jasper (1958); this allows calculation to be made of two

transfer rates for BSP as described by Richards, Tindall & Young (1959). Resolution of the plasma disappearance curve into two components was by the graphical " trial-and-error " method described by Clarkson & Richards (1967).

3. Assessment of hepatotoxicity

Plasma enzyme concentrations were determined daily until stable. After drug administration, plasma enzymes were estimated at least daily until predosage concentrations were present ; this was usually for 7 days. A BSP dye clearance test was conducted 1 hr before and 72 hr after drug administration. BSP transfer rates were calculated and results were expressed as " percentage change in BSP transfer rate from liver to bile," any reduction at 72 hr being attributed to hepatic dysfunction. BSP clearance tests were discontinued when it became apparent that simultaneous increase of concentrations of OCT, GD, SD, and GOT accompany hepatic damage in the sheep, thus confirming the findings of Ford (1967).

Results

1. Clinical response to drug administration

Hexachloroethane (0.5–1.0 g/kg), was well tolerated, animals continuing to eat after administration. Slight tremors of the facial muscles occurred in three sheep between 1 and 4 hr after dosage.

Pentachloroethane (0.3 ml./kg) produced narcosis ; after 30 min sheep 19 became recumbent and only regained normal posture 9 hr after dosage. During this period, the limbs were flaccid and normal reflexes depressed ; defaecation and urination did not occur but labial tremors were noticed. The heart rate was slightly increased. Twenty-four hours after dosage sheep 19 was taking some hay and 72 hr after dosage appeared normal. Sheep 25 became recumbent 20 min after dosage and remained so for 40 min. Labial tremors were noted during this period. Feeding recommenced 1.5 hr after pentachloroethane and the animal appeared normal.

Tetrachloroethylene (0.3 ml./kg) and olive oil (0.6 ml./kg) produced no adverse reaction.

2. Metabolism and excretion of hexachloroethane

Blood concentrations of hexachloroethane. Blood concentrations in unanaesthetized sheep were in every case maximal at the 24 hr sample (Fig. 1) and approximately 100 times greater than in anaesthetized sheep, where the maximum concentrations occurred 6 hr after administration.

Concentrations of hexachloroethane, pentachloroethane and tetrachloroethylene in the plasma of sheep 5 and 6 24 hr after hexachloroethane administration were 2.3–2.6 times greater than the corresponding concentrations in erythrocytes.

Urine and faeces concentrations of hexachloroethane. More than 80% of the total faecal hexachloroethane (1–2 mg) was excreted in the 24 hr following drug administration and little hexachloroethane was detected in the urine (Table 2).

Bile and tissue concentrations of hexachloroethane. Hexachloroethane was first detected in bile 15 min after administration, but was not detected in venous blood

until after the 27th minute (sheep 28). The maximum concentrations were 8–10 times greater in bile than in blood (Table 3). Fat concentrations were highest and muscle lowest (sheep 27, Table 3). Muscle and fat from different sites did not show significant variation in hexachloroethane concentrations.

Metabolites of hexachloroethane. Two metabolites were detected in sheep which had received hexachloroethane and shown to be tetrachloroethylene and pentachloroethane.

Pentachloroethane was detected in venous blood 24 hr after hexachloroethane administration and was still present after 96 hr (Table 1, Fig. 1). The urine content was relatively high (Table 2); however, in anaesthetized sheep, blood, bile and tissue levels were very low (Table 3).

TABLE 1. *Blood concentrations of hexachloroethane (HCE) pentachloroethane (PCE) and tetrachloroethylene (TCE) as μg/ml. whole blood and change in the excretory capacity of the liver following administration of hexachloroethane*

Sheep No.	HCE dose g/kg	24 hr blood conc. HCE	TCE	PCE	72 hr BSP transfer rate (as % of 0 hr. transfer rate)
1	0·5	27	0·6	0·15	25
2	0·5	—	—	—	12
3	0·5	28	0·7	0·30	66
4	0·5	10	0·8	0·06	47
5	0·5	27	1·0	0·50	79
6	0·5	23	1·1	0·15	35
7	0·75	—	—	—	8
8	1·0	—	—	—	36
9	Control	—	—	—	94
10	Control	Nil	Nil	Nil	105

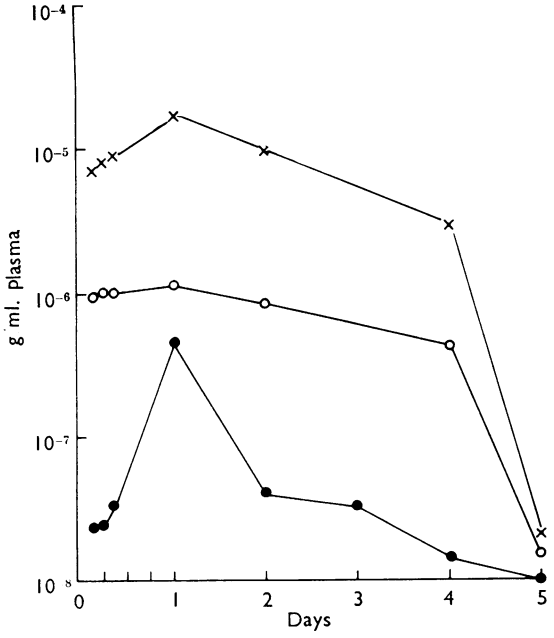

FIG. 1. Concentrations of hexachloroethane (×----×), pentachloroethane (●----●) and tetrachloroethylene (○----○) in venous blood samples of sheep 5 which received an oral dose of hexachloroethane 0.5 g/kg at day 0.

Sheep 19 and 25, which received pentachloroethane 0.3 ml./kg showed blood concentrations of pentachloroethane of the same order as the concentrations of hexachloroethane following administration of hexachloroethane. The second metabolite of hexachloroethane, tetrachloroethylene, was also present. This was the only metabolite of pentachloroethane detected (Fig. 2).

Tetrachloroethylene was always found in the blood of sheep given hexachloroethane and the maximum concentration was usually reached at 24 hr (Table 1, Fig. 1). After pentachloroethane, blood concentrations of tetrachloroethylene reached the maximum at 3 hr and remained at similar concentrations to pentachloroethane for at least 3 days (Fig. 2).

TABLE 2. *Total (μg) hexachloroethane (HCE), pentachloroethane (PCE) and tetrachloroethylene (TCE) in the urine and faeces of two sheep that received hexachloroethane*

	Sheep 11			Sheep 12		
Faeces	HCE	TCE	PCE	HCE	TCE	TCE
0–24 hr	780	854	Trace	1260	1300	468
24–48 hr	Trace	22	Trace	280	440	Trace
48–72 hr	Trace	Trace	Trace	Trace	15	Trace
72–96 hr	Nil	Trace	Nil	Trace	Trace	Trace
	Sheep 11			Sheep 12		
Urine	HCE	TCE	PCE	HCE	TCE	PCE
0–24 hr	50	25	20	70	29	25
24–48 hr	4·4	8·8	1·3	10·8	8·9	1·0
48–72 hr	0·4	6·5	0·4	Trace	5·4	0·5
72–96 hr	Nil	Nil	Nil	Trace	5·4	Trace

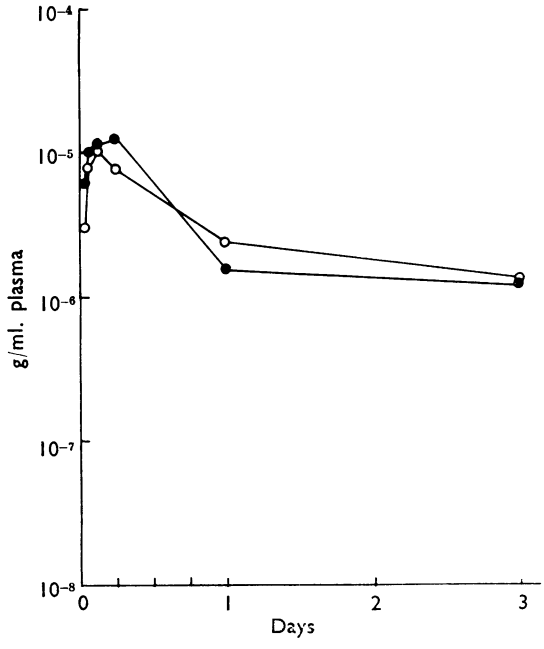

FIG. 2. Concentrations of pentachloroethane (●- - -●) and tetrachloroethylene (○- - -○) in venous blood samples of sheep 19 which received an oral dose of pentachloroethane 0.3 ml./kg at day 0.

Concentrations of tetrachloroethylene were high in the urine and faeces and could be detected when hexachloroethane was absent (Table 2); they were also high in the anaesthetized sheep whilst hexachloroethane concentrations were low, indicating that although absorption was slower, metabolism was not as markedly affected by anaesthetics (Table 3). Brain and muscle contained least of all the tissues, whereas liver showed the highest content in sheep 28 and fat in sheep 27.

Blood from sheep which had received tetrachloroethylene 0.3 ml./kg showed only one peak on gas-liquid chromatography, that due to tetrachloroethylene.

Other metabolites of hexachloroethane. These are presumed to be water soluble, and were not detected in hexane extracts. There were no unidentified peaks, and none corresponding to those produced by reference solutions of 1,2-dichloroethane or 1,1,2,2-tetrachloroethane, the most toxic of the series of chlorinated ethanes (Williams, 1959).

Controls. Blood samples from sheep which received olive oil 0.6 ml./kg produced no response on gas-liquid chromatography.

In vitro *experiments.* Fresh liver slices liberated pentachloroethane and tetrachloroethylene from hexachloroethane emulsions over a period of 4 hr at 37° C (Table 4).

Boiled liver slices took up hexachloroethane but pentachloroethane and tetrachloroethylene were not detected (Table 4).

TABLE 3. *Concentrations (μg/g) of hexachloroethane (HCE), pentachloroethane (PCE) and tetrachloroethylene (TCE) in two anaesthetized sheep which received hexachloroethane. Post-mortem tissue samples (8·5 hr)*

	Sheep 27			Sheep 28		
Tissue	HCE	TCE	PCE	HCE	TCE	PCE
Bile (4 hr)	1·7	0·3	Trace	2·2	0·5	Nil
Blood (6 hr)	0·2	0·4	Trace	0·2	0·2	Nil
Brain	0·2	0·9	0·02	Trace	Trace	Trace
Fat	1·1	2·1	0·02	Trace	0·6	Nil
Kidney	0·1	1·2	Trace	Trace	0·6	Trace
Liver	0·2	0·9	0·01	Trace	2·8	Trace
Muscle	0·04	0·5	0·01	Trace	Trace	Trace

TABLE 4. *Liberation of tetrachloroethylene (TCE) and pentachloroethane (PCE) from hexachloroethane (HCE) by sheep liver as fresh, heated (70° C for 5 min) or boiled (100° C for 5 min) slices incubated at 37° C for 4 hr*

Tissue	No. of expts	HCE μg/g (±S.D.)	TCE μg/g (±S.D.)	PCE μg/g (±S.D.)
(1) Olive oil emulsified in Hedon–Fleig solution (0·1% glucose)				
Fresh	10	Nil	Nil	Nil
Boiled	10	Nil	Nil	Nil
(2) Emulsion+hexachloroethane, 18 μg/ml.				
Fresh	10	13·3±0·5	9·1±0·1	0·76±0·01
Heated	10	50·8±0·1	2·4±0·8	1·74±0·66
Boiled	10	58·4±0·9	Nil	Nil
None	4	15·9±0·8	Nil	Nil
(3) Emulsion+hexachloroethane, 54 μg/g				
Fresh	10	56·4±3·0	56·4±8·6	0·95±0·35
Heated	16	20·2±1·5	0·36±0·01	0·12±0·01
None	4	50·3±1·5	Nil	Nil

Heated liver slices liberated pentachloroethane and tetrachloroethylene from hexachloroethane but considerably less tetrachloroethylene was detected (Table 4).

3. *Hepatotoxicity of hexachloroethane and its metabolites*

Plasma enzyme determinations. Hexachloroethane administration was followed by simultaneous increases in the concentrations of GD, SD and OCT, reaching a maximum at 48 hr and returning to normal 4–5 days later (Fig. 3). GOT concentrations increased slightly and were maximal at 24–48 hr (sheep 2, 4 and 5) or 48–72 hr (sheep 3, 6, 7 and 8); no increase occurred in sheep 1.

GD was increased fifty-five times in sheep 1, but a typical increase was three to six times (sheep 3, 5, 6, 7 and 8). SD always increased by three to six times. OCT increased from two to ten times after hexachloroethane. Administration of pentachloroethane resulted in large increases in the plasma concentrations of GD, SD and OCT; GD increased 200 times in both sheep; SD increased 30 fold (sheep 19) and 20 fold (sheep 25). OCT showed a 15-fold increase in sheep 19 and a 19-fold increase in sheep 25.

Tetrachloroethylene administration (two sheep) caused some plasma enzyme increases. OCT did not change in either sheep; GD increased eight-times in one and three times in the other (48 hr maxima); SD increased two fold (sheep 20, 24 hr maximum; sheep 26, 48 hr maximum).

BSP dye clearance tests. Plasma disappearance of BSP (5 mg/kg) occurred in two phases as described by Cornelius *et al.* (1958). Transfer rates for BSP showed that hexachloroethane did not affect uptake from plasma by liver cells, but that

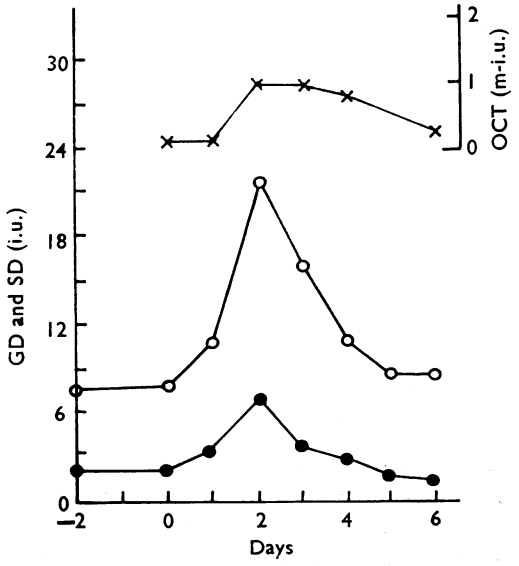

FIG. 3. Concentrations of GD (●----●) and SD (○----○) expressed in i.u. and of OCT (×----×) m-i.u. in plasma of sheep 6, which received an oral dose of hexachloroethane 0.5 g/kg at day 0.

72 hr after administration a marked reduction of transfer from liver cells to bile occurred. BSP dye clearance tests conducted on sheep 9 and 10, which received olive oil, showed little variation within the 72 hr test period (Table 1).

Discussion

Metabolism of hexachloroethane

The metabolism of hexachloroethane in the rabbit was studied by Jondorf *et al.*, (1957), using ^{14}C-labelled drug together with isotopic dilution techniques. Gas-liquid chromatography with electron capture detection provided a sensitive and easily quantitated method for separation and detection of hexachloroethane and its hexane soluble metabolites in sheep.

The absorption of orally administered hexachloroethane as reflected by jugular venous blood concentrations of the drug was slow, especially in anaesthetized sheep ; pentobarbitone seemed to depress intestinal motility ; also release of bile and pancreatic lipase were interrupted by the duct cannulation and duodenal clamping. Despite these limitations, three important points were demonstrated by the anaesthetized preparations: firstly, hexachloroethane excretion in the bile was established ; secondly, biliary concentrations may markedly exceed blood concentrations and, finally, hexachloroethane is widely distributed.

Excretion of hexachloroethane in bile allows direct contact with parasites such as the mature liver fluke. Moreover, contact time may be increased by enterohepatic circulation which, if concentrations in bile are greater than those in systemic blood, may maintain portal venous concentrations after systemic venous concentrations have fallen.

The metabolism of various chlorinated hydrocarbons by an enzyme present in the liver, kidney and spleen, was demonstrated by Heppel & Porterfield (1948). Bray *et al.* (1952), however, questioned these findings and described the reactions of certain aliphatic chloro' compounds with the sulphydryl groups of amino-acids, liberating chloride. Boiled tissue extracts were capable of liberating chloride from several compounds including hexachloroethane and pentachloroethane, so enzymatic systems were discounted, in favour of $-SH$ conjugation systems (Bray *et al.*, 1952).

The *in vitro* experiments with liver slices did not confirm the findings of Bray *et al.* (1952) ; although fresh liver slices produced pentachloroethane and tetrachloroethylene from hexachloroethane, boiled liver slices did not, which suggested that an enzymatic process might be involved in the metabolism of the drug. Further support for this was afforded by the partial inactivation which occurred at 70° C ; this affected metabolism of pentachloroethane more markedly than metabolism of hexachloroethane and indicated that probably at least two enzymes were involved in the degradation of hexachloroethane, both of which were present in liver.

The rapid appearance of tetrachloroethylene in the systemic circulation may be contrasted with the apparent lag in the appearance of pentachloroethane, possibly the metabolism of pentachloroethane was rapid in sheep. The removal of H–Cl and introduction of the double bond of tetrachloroethylene is analogous to the metabolism of DDT which yields DDE ; however, this reaction appears to proceed slowly in mammals.

Toxicity of hexachloroethane

It is interesting to note that the metabolism of hexachloroethane, which probably occurred to a large extent in the liver, was closely linked with hepatotoxicity. This is similar to the situation with carbon tetrachloride (Slater, 1966).

The results of plasma enzyme determinations and BSP dye clearance tests indicated that a degree of hepatic dysfunction followed the oral administration of hexachloroethane.

BSP dye clearance tests and plasma enzyme concentrations have been widely used as indices of hepatic function in sheep (Cornelius *et al.*, 1958 ; Alexander & MacDonald, 1960 ; Ford & Boyd, 1962 ; Ford & Lawrence, 1965 ; Ford, 1967). The elevations of plasma concentrations of GD, SD, OCT and GOT which followed hexachloroethane administration were greatest at 48 hr ; this compares with 24 hr for carbon tetrachloride and 144 hr for sporidesmin (Ford, 1967).

Elevation of plasma enzyme concentrations after drug administration suggested that cell membrane permeability changes occurred and because GD is a mitochondrial enzyme (de Duve, Wattiaux & Baudhuin, 1962), permeability changes probably occurred in intracellular membranes. The decreased BSP dye clearance 72 hr after hexachloroethane administration and unchanged hepatic uptake rates for BSP implicated a decreased excretory capacity of the liver, not merely an increased albumin-BSP binding effect (Crawford & Hooi, 1968), even though it was shown that plasma albumin concentrations fell in sheep which received chlorinated hydrocarbons (Alexander & MacDonald, 1960).

The causes of hepatic dysfunction following hexachloroethane administration are not known, but Alexander & MacDonald (1960) suggested that the anthelmintic actions of carbon tetrachloride might depend on the release of products of liver damage rather than direct action on intrabiliary flukes.

The excretion of hexachloroethane in the bile allows direct contact of the drug with adult liver flukes in addition to contact with products of liver damage. Hexachloroethane and/or pentachloroethane may have a direct action on the liver and liver fluke, or their metabolism may yield active radicals as suspected with carbon tetrachloride (Slater, 1966). The presence of dechlorinated radicals with a high electron affinity such as the CCl_3, which may result from carbon tetrachloride metabolism, would be expected to produce severe, if only very localized damage to the endoplasmic reticulum and possibly to the mitochondria (Albert, 1968). Assuming that the liver fluke also metabolizes hexachloroethane and pentachloroethane, this provides a basis for an hypothesis on the nature of the hepatotoxic and anthelmintic action of hexachloroethane.

Apart from metabolism of these drugs which probably occurs in liver cells, the nervous symptoms and narcosis which followed the administration of hexachloroethane and pentachloroethane indicated that the drugs also entered the cells of the central nervous system. It is not known if metabolism occurred in this tissue ; if the dechlorination of these drugs is related to their toxicity, damage will occur in tissues which have enzyme systems available for the metabolism of hexachloro- and pentachloroethane.

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