A STUDY OF THE TOXICITY AND ANTHELMINTIC ACTIVITY OF *n*-BUTYLIDENE CHLORIDE

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A SERIES of chlorinated hydrocarbons were tested *in vivo* by Wright and Schaffer, who found several with promising anthelmintic activity[1]. Among the compounds tested was *n*-butylidene chloride, which they reported as 100 per cent. effective against *Ascaris*, 95 per cent. for hookworms and 28 per cent. for whipworms in dogs. They found it to have a margin of safety of 33, which, in their estimation, suggested even greater anthelmintic possibilities for the compound. In 1931, Wright *et al.*[2] reported its clinical use in the treatment of equine *Strongyloides*. Additional investigation was conducted on dogs, cats and chickens, providing further evidence of its anthelmintic activity[3].

This study was undertaken to furnish additional information concerning its toxicity and activity *in vitro*. Carbon tetrachloride and tetrachloroethylene, both similar, widely-used anthelmintics, were used for comparison.

EXPERIMENTAL

Acute Oral Toxicity in Mice. The mice were male, albino, Swiss-strain with an average weight of 24 g. A dose of 2 ml./kg. of *n*-butylidene chloride, carbon tetrachloride and tetrachloroethylene was administered by means of an oral hypodermic syringe to groups of 6 mice. The dose was then increased in 2 ml. increments to new groups until an LD100 was obtained for each compound.

Each group of animals was fasted for 24 hours prior to administration of the drug. They were observed for 36 hours following administration. Only those that died within this time were considered. All survivors were further observed for at least seven days for evidence of delayed toxicity. There was no gross evidence of delayed toxicity in any of the animals. Death appeared to be due to an overdose of a hypnotic in each instance.

The LD50 was calculated by the method of Litchfield and Wilcoxon[4]. The dose-mortality curves are shown in Figure I. The LD50s of *n*-butylidene chloride, carbon tetrachloride and tetrachloroethylene were found to be 4·5, 5·2 and 5·0 ml./kg. respectively and were within the limits previously reported[5,6,7]. The dose-mortality curves did not differ significantly when tested for parallelism and toxicity ratio[4].

Chronic Oral Toxicity in Rats. To determine the chronic toxicities, groups of 15 male Wistar strain rats averaging 240 g. were orally administered suspensions of the drugs. Aqueous suspensions were prepared by triturating 3 ml. of the drug with one gram of tragacanth per 100 ml.

of water[8]. The suspension was agitated before withdrawal of each dose to insure uniform distribution of the drug. Each animal received a dose of 0·33 ml./kg. at 4-day intervals for a total of 8 doses[9].

Liver damage was used as the criterion of chronic toxicity. This was

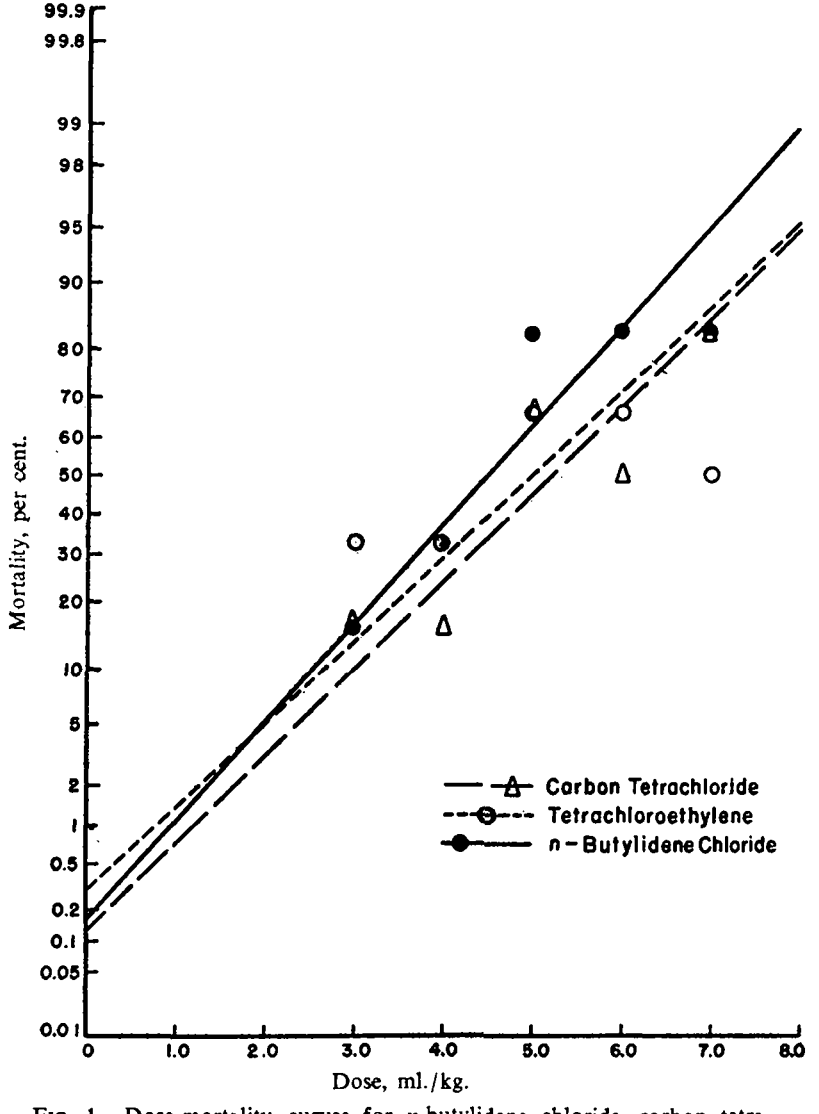

FIG. 1.—Dose-mortality curves for *n*-butylidene chloride, carbon tetrachloride and tetrachloroethylene

measured by the increase in depression time produced by a 60 ml./kg. dose of sodium hexobarbitone[10] administered intraperitoneally. Depression time of each group was taken at 4-day intervals. On the sixth day after the initial dose of the anthelmintic, the first depression time

was recorded. The last depression time was taken 3 days after the eighth dose of the drug. A normal depression time was obtained on

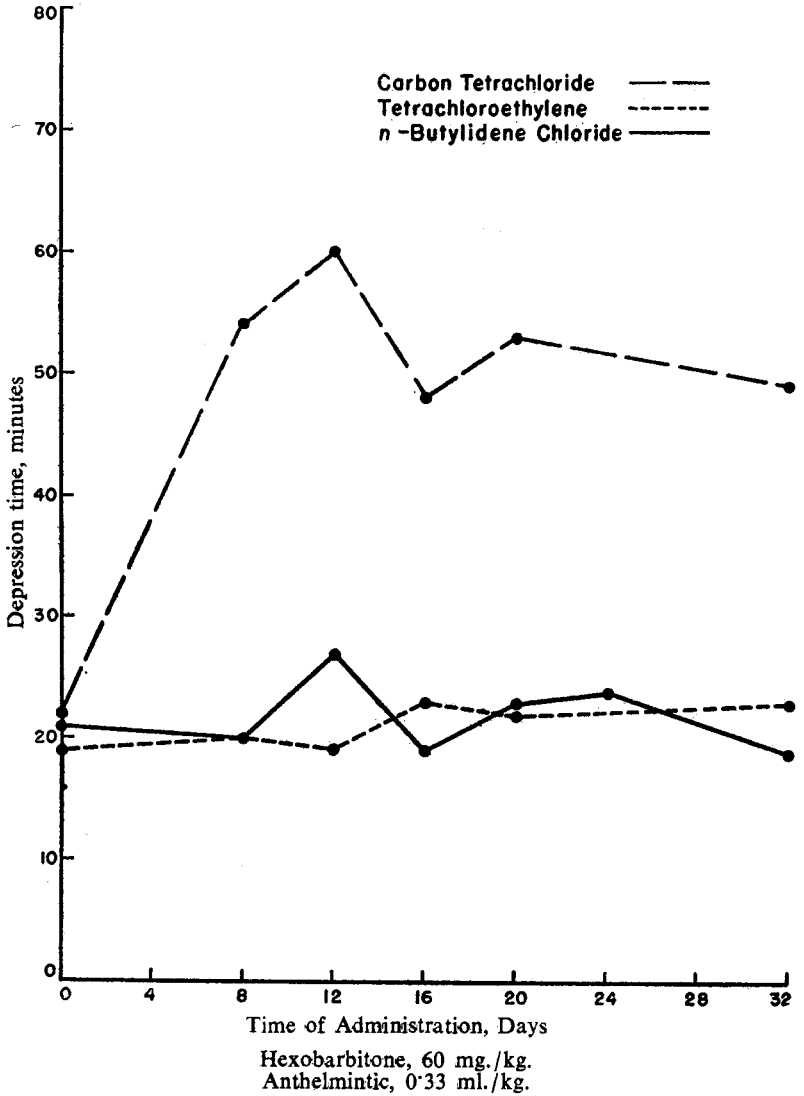

FIG. 2.—The effect of the administration of *n*-butylidene chloride, carbon tetrachloride and tetrachloroethylene on depression times

each group before administering the anthelmintics (Figure 2). The period of depression was recorded as the interval between injection and return of normal activity. The index of normal activity was opened eyes, alertness and effort to escape when held by the tail or picked up. The time was recorded to the nearest minute.

Tetrachloroethylene and *n*-butylidene chloride produced no outward

toxicological symptoms and the animals remained gentle throughout the experiment. The only apparent toxic effect produced by carbon tetrachloride was an increased irritability. The depression times of the 3 groups indicated that carbon tetrachloride produced acute liver damage, whereas the liver function of the groups receiving tetrachloroethylene and

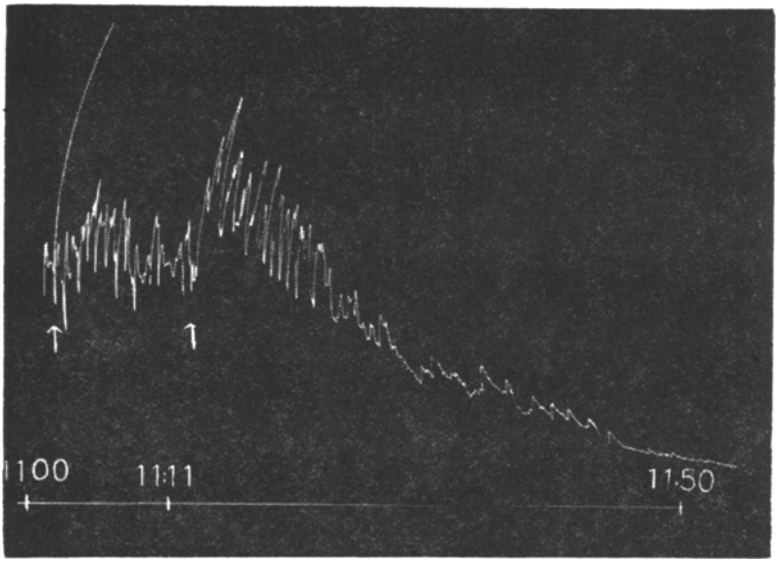

FIG. 3.—Carbon tetrachloride, 1-1000 suspension. *Ascaris lumbricoides*

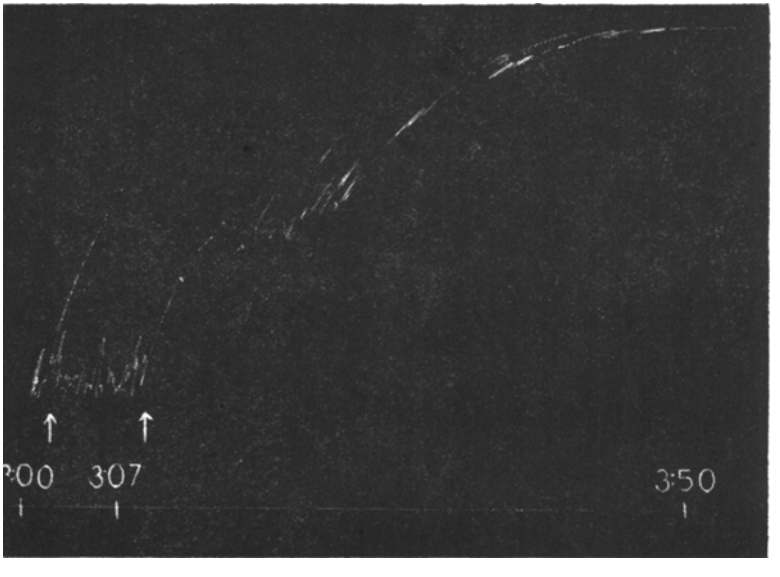

FIG. 4.—Tetrachloroethylene, 1-1000 suspension. *Ascaris lumbricoides*

n-butylidene chloride was not significantly impaired as indicated by the depression time changes in Figure 2.

The rat from each group with the greatest average depression time was sacrificed after the last dose of sodium hexobarbitone. The liver was removed and fixed in Bouin's fixative. Microscopic examination of histological sections revealed the following pathological conditions. Carbon tetrachloride produced a marked increase in the periportal and

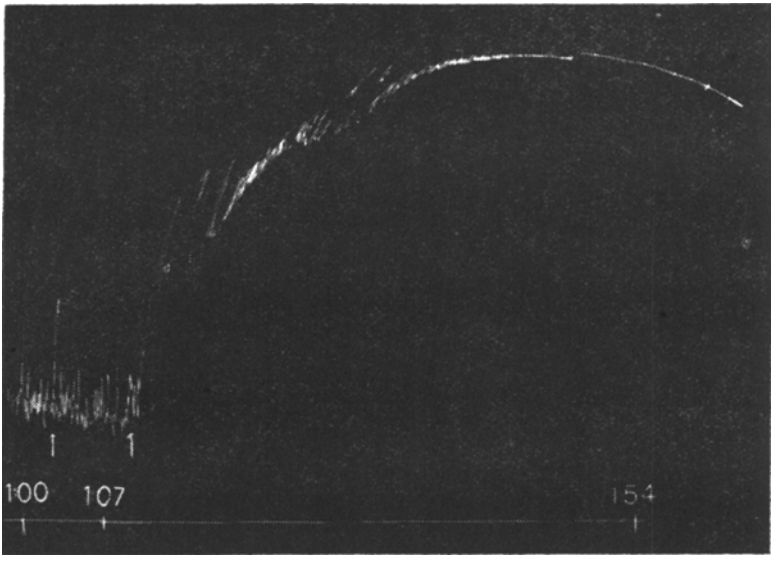

FIG. 5.—*n*-Butylidene chloride, 1-1000 suspension. *Ascaris lumbricoides*

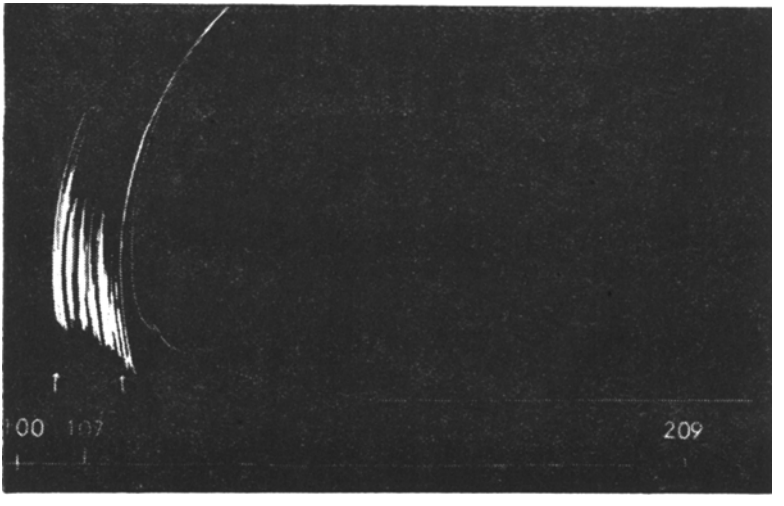

FIG. 6.—Carbon tetrachloride, 1-1000 suspension. *Lumbricus terrestris*

perilobular connective tissue. In most of these areas there were many fibroblasts, indicating young and actively growing connective tissue. In these portal areas there was an increase in bile ducts. These varied in number and structure in different areas. Some areas had 3 and 4 ducts,

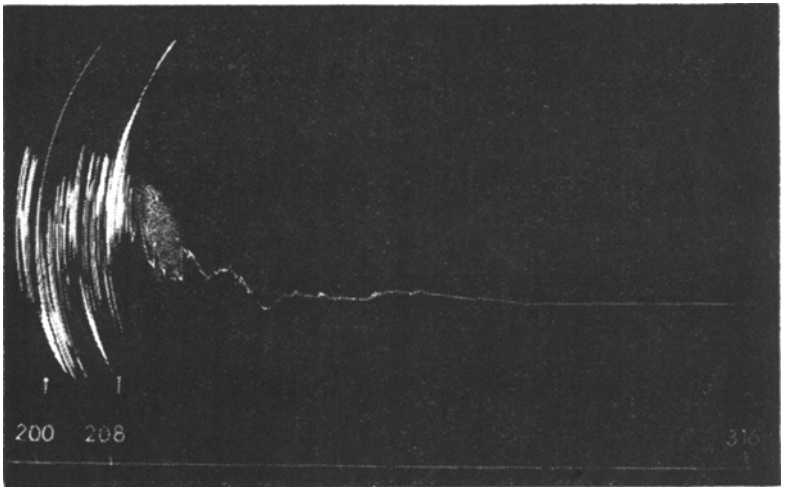

FIG. 7.—Tetrachloroethylene, 1-1000 suspension. *Lumbricus terrestris*

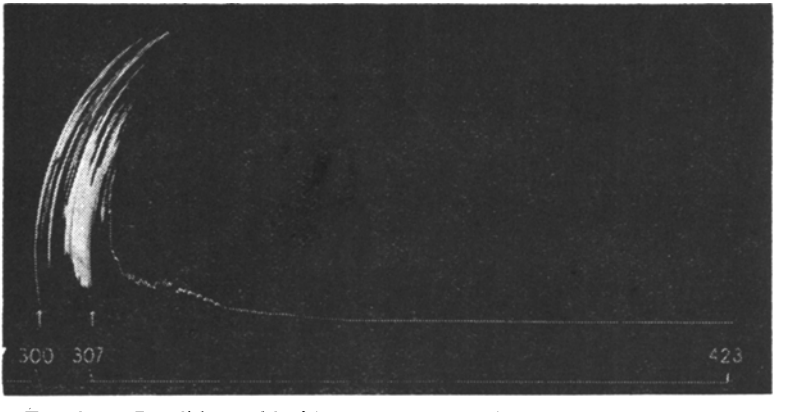

FIG. 8.—*n*-Butylidene chloride, 1-1000 suspension. *Lumbricus terrestris*

which should have had only one. The ducts were well-formed or just clumps of cells. These areas showed some leukocytic infiltration and pigment was found within the portal connective tissue. This pigment appeared to be hemosiderin rather than hematoidin, although an iron stain was not run. Necrosis of some tissues, vacuolar degeneration and generalised parenchymatous degeneration were evident. Tetrachloroethylene did not produce definite cirrhotic changes, although there were generalised parenchymatous degenerations with considerable loss of liver

substance. Foci of cellular infiltration were observed and some cells were definitely leukocytes. *n*-Butylidene chloride caused extensive degeneration of liver substance, however, no replacement with connective tissue had occurred. It was evident that parenchymatous degeneration was more extensive in the rats receiving tetrachloroethylene than in those which received *n*-butylidene chloride.

Anthelmintic Activity in Ascaris *and Earthworms.* A modified Trendelenberg recording apparatus[11] was used to record activity. The anterior end of the *Ascaris* was attached to a heart lever by means of a fine steel hook. The posterior end was secured to the bottom of a calibrated, cylindrical, pyrex container by means of another steel hook. This cylinder was filled with warmed normal saline and placed in a constant temperature bath operating at 38 to 39°C. The worm was then counterbalanced until the recording lever was in a horizontal plane. The *Ascaris* was washed with normal saline for 5 to 10 minutes, after which the normal activity was recorded on a smoked drum for 5 to 10 minutes. The kymograph drum was run at 0·308 cm./minute. At the end of the normal activity record the normal saline solution was replaced with a 1 in 1,000 solution of the drug. Movement was then recorded for 30 to 60 minutes or until the worm ceased to show any further activity (Figures 3, 4, 5).

TABLE I

VERMICIDAL ACTIVITY

Drug in 1 in 1000 concentration	*Ascaris*		Earthworms	
	Time in minutes	Killed per cent.	Time in minutes	Killed per cent.
Carbon tetrachloride	15	8	30	96
	30	48	45	100
	45	94		
	60	100		
Tetrachloroethylene	75	8	30	24
	90	50	45	100
	105	95		
	120	99		
n-Butylidene chloride	15	12	30	42
	30	34	45	100
	45	88		
	60	100		

The methods applied to the *Ascaris* were used for the earthworm, except that tap water at room temperature was used in place of normal saline solution. Tap water was substituted for the normal saline solution because of the irritating property of the latter to the earthworm[12] (Figures 6, 7, 8).

Vermicidal Activity in Ascaris *and Earthworms.* The vermicidal action of the drugs was investigated by a modification of the method of

Munch[13]. Ten 250-ml. Erlenmeyer flasks were placed in a constant temperature bath operating at 38 to 39°C. One hundred millilitres of a 1 in 1,000 suspension of the drug at the same temperature was placed in each flask and 5 active *Ascaris* were then introduced. The worms were removed at 15-minute intervals and inspected. Those which appeared dead were stimulated by a weak tetanising current from a Harvard inductorium. If no movement was evident, the worm was assumed to be dead. This procedure was continued until all were killed. The same procedure was followed for earthworms except that once again tap water at room temperature was substituted for the normal saline. The results are given in Table I.

Summary and Conclusions

1. The oral mouse LD50s of *n*-butylidene chloride, tetrachloroethylene and carbon tetrachloride are 4·5, 5·0 and 5·2 ml./kg. respectively. The differences were found to be within the range of experimental error.

2. *n*-Butylidene chloride was less hepatoxic than either carbon tetrachloride or tetrachloroethylene.

3. The value of a chronic liver toxicity determination, as indicated by an increased period of depression with sodium hexobarbitone, was corroborated by histological liver sections.

4. Carbon tetrachloride produced a flaccidity in *Ascaris*, while *n*-butylidene chloride and tetrachloroethylene caused an increased tonicity.

5. Vermicidal activity was in the descending order: Carbon tetrachloride, *n*-butylidene chloride and tetrachloroethylene.

6. Earthworms were not satisfactory test animals as they did not respond to *n*-butylidene chloride and tetrachloroethylene by increased tonicity and were markedly susceptible to all the agents tested.

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