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PROBLEMS IN AERIAL APPLICATION:

A COMPARISON OF THE EFFECTS OF DIELDRIN POISONING IN COLD-ADAPTED AND ROOM-TEMPERATURE MAMMALS

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Comparison of the Effects of Dieldrin Poisoning in Cold-Adapted and Room-Temperature Mammals

I. Introduction.

The delivery of insecticides by air (crop dusting) continues to increase. These insecticides are poisonous to humans and constitute a hazard to those engaged in this work. Many of these poisons are halogenated hydrocarbons. Although there has been a great amount of research on the pathological effects of carbon tetrachloride poisoning and some on DDT poisoning, there is virtually nothing on the pathology resulting from poisoning by such halogenated hydrocarbons as endrin, dieldrin, etc., except that from an overwhelming dose. In such cases, the widespread effects of the poison make it difficult to determine which of the effects are directly due to the poison and which are secondary. It is also difficult to extrapolate from these widespread effects to what might be expected from the smaller dosages that would be more likely to occur in handling these chemicals. It would also be impossible to extrapolate from these acute effects to the possibility of residual damage. In spite of the paucity of information, accounts of the toxicology of the halogenated hydrocarbons stress liver damage. With carbon tetrachloride or trichlorethylene poisioning,[1] there can be no question of widespread liver necrosis, but with other halogenated hydrocarbons this may not be the case.[2] In a preliminary study in rats to which near-fatal levels of endrin or dieldrin were given, there was found evidence of minor liver alterations and little else.[3] In this study, it was found that the liver damage was not only relatively light but was also quite different from the damage produced by carbon tetrachloride administration. Some of these changes were indicative of altered lipid metabolism. Since there are marked changes in lipid metabolism in cold adaptation, it was thought that the combination of cold adaptation and dieldrin poisoning might serve to elucidate the resulting liver alterations.

II. Materials and Methods.

Forty-nine male rats of the Charles River strain were used. Twenty-five of these (164 to 247 grams) were placed in a room at 5°C for 4 weeks. Purina checkers and water were available as desired. On removal from the cold, all the rats had varying, but mild, frostbitten ears. Two animals were removed from the group; one had bloody urine and the other a badly frostbitten tail. The remaining 23 rats all increased in weight during their stay in the cold. While the others were kept in the cold, the remaining 24 male rats were kept at room temperature with water and food as desired.

Treatment with the poison was instituted 4 days after removal from the cold. Twelve each of the cold-adapted and room-temperature rats were given 15 mg of dieldrin dissolved in mineral oil intraperitoneally daily for 3 days. There were thus four groups of animals: cold-adapted and treated, cold-adapted controls, room-temperature treated, and room-temperature controls.

In the earlier study,[3] it was found that food consumption of poisoned animals dropped precipitously. Because of this drop in food consumption, one-half of the controls were given only the average food consumption of the treated animals. At this time it was also decided that, if any animals in the treated groups died, a corresponding number would be dropped from each of the other groups. With the treated animals the ones with the largest numbers would be dropped while with the control groups the final (size of number of rat) two of the rats on full diet would be dropped and then the final ones of the animals on limited diet. One of the treated cold-adapted rats died within 48 hours and two more within 72 hours. Ninety-six hours after treatment began, the treated animals were sacrificed; the controls were sacrificed on the following day. The animals were killed by the intraperitoneal injection of

25 mg of nembutal and were perfused through the heart with acacia saline followed by acacia formol saline.[4] Then the livers were excised, and a thin slice was placed in 80% ethyl alcohol and a much larger slice in 10% formalin.

The following routine stains were used: Sudan Black B, Nile Blue Sulfate, periodic acid schiff (PAS), and hematoxylin and eosin(H and E). In addition three stains that are routine for nervous tissue were used. These are Cresyl Violet Acetate, which is specific for nucleic acids, Swank Davenport Marchi, which, in the central nervous system, is specific for unsaturated fatty acids, and a modified Weil stain, which in nervous tissue is specific for the secondary and tertiary amines found in myelin.

A. *Nile Blue Sulfate.*—
   1. Wash frozen sections (10 m) in distilled water.
   2. Place in 0.5 ml aqueous 1% Nile Blue Sulfate in a small vial for 20 minutes in an oven maintained at 60°C.
   3. Add 10 ml of 1% aqueous acid and leave in oven for 20 minutes.
   4. Wash in distilled water.
   5. Mount in glycerine, wipe slide dry, and ring cover slip with Permount.

B. *Cresyl Violet Acetate.*—As described previously.[5]

C. *Sudan Black B.*—As described previously.[6]

D. *H and E.*—As described previously.[6]

E. *Swank Davenport Marchi.*—Using diluted Poirer solution.[6]

The routine method, impregnation of a 1-mm block, was used but better results were obtained with frozen sections.[7]

F. *Modified Weil Stain.*—
   1. Decerate and hydrate mounted paraffin sections.
   2. Place filled staining rack in a dry staining dish.
   3. Pour on slide 2.5 ml of a 10% alcoholic hematoxylin diluted to 100 ml and 100 ml of 1% ferric ammonium sulfate.
   4. Thoroughly mix solutions by raising and lowering staining rack several times. Leave in stain for 30 minutes.
   5. Wash in tap water and then in three changes of distilled water.
   6. Place in 1% ferric ammonium sulfate solution for 1 minute.
   7. Wash in three changes of distilled water.
   8. Place in 0.5 M sodium oxalate solution overnight.
   9. Dehydrate, clear, and mount with Permount.

III. Results.

Table 1 presents a summary of the effects seen on the livers of these rats. The only stain that showed a difference between the control rats on limited diet and those on full diet was the PAS. This was expected due to the known loss of liver glycoyen that occurs in inanition. Unlike carbon tetrachloride, whose effects are primarily centrolobular, dieldrin poisoning results in changes located around the periportal canals. The extent of damage (whether this represents actual damage or is a reflection of altered function elicited by the poison remains a subject for investigation) is indicated on a scale of 4 with 0 indicating no change from control and 3 indicating the maximal changes seen. Thus, in Figure 1 is shown the Swank Davenport Marchi section, which was assigned a grade of 3. The periportal area where the fat is localized is to the

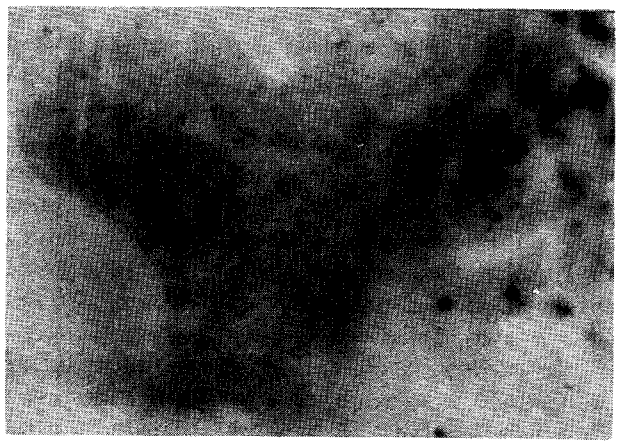

FIGURE 1. Marchi Swank Davenport-stain single cell to left with black globules lies at edge of central vein. Numerous cells at right are in periportal region. Area between comprises a large portion of lobule. Dieldrin-poisoned rat.

right and the single fat-containing cell to the left lies adjacent to the central vein of the lobule. The intervening area comprising over one-half of the lobule is free from fat. It is evident that the number of cells containing appreciable amounts of fat is quite small. With the four fat stains there is general agreement, although there are few cases where the same value was judged

TABLE 1. Summary of effects on liver as seen with various stains. The ratings are on a scale of 4 with 0 no effect and 3 maximal. With PAS, the scale used was of 5 with 4 maximal and 0 least. S = scattered, MZ = midzonal, PP = periportal.

Rat No.	Swank Davenport Marchi	Modified Swank Davenport Marchi	Sudan Black B	Nile Blue Sulfate	Cresyl Violet	H and E	PVS
Room-temperature and poisoned							
1 W	3 PP	3 PP	3 PP	1 PP	3	1	2
2 W	2 PP	1 PP	2 PP	1 PP	1	0	2
3 W	2 PP	3 PP	3 PP	1 PP	3	0	0
4 W	2 PP	0	2 PP	2 PP	2	1	2
5 W	2 PP	2 PP	2 PP	2 PP	2	1	1
6 W	3 PP	3 PP	3 PP	3 PP	3	1	1
7 W	1 PP–S	1 PP	2 S	1 PP	1	1	2
8 W	2 PP	3 PP	2 S–PP	2 S	1	1	2
9 W	2 PP	1 S–PP	1 S	1 S	3	1	2
Room-temperature controls							
13 W	1 S	0	2 S	1 S	0	0	3
14 W	1 S	2 PP	3 PP	2 PP	1	0	3
15 W	1 S	0	0	0	0	0	3
16 W	3 PP	1. PP	2 PP	1 PP	1	1	3
19 W	0	0	0	1 S	0	0	0
20 W	0	1 PP	2 PP	1 S	0	0	0
21 W	1 S	0	0	0	2	0	0
22 W	2 PP	0	0	0	0	0	3
23 W	2 PP	0	0		1	0	0
Cold-adapted and poisoned							
1 C	2 PP	0	0	0	1	1	0
2 C	2 PP	2 PP	2 PP	1 S	2	1	1
3 C	1 PP	1 PP	1 PP	1 S	1	0	2
4 C	2 PP	3 PP	3 PP	2 PP	1	1	1
5 C	1 S	1 PP	1 PP	1 S	0	0	2
6 C	2 S	3 MZ	1 MZ	2 S	2	2	1
7 C	2 PP	2 PP	1 S	1 S	2	1	2
9 C	2 PP	3 PP	3 PP	1 S	2	1	1
14C	2 PP	3. PP	3 PP	2 S	0	0	1
Cold-adapted controls							
15 C	1 PP	0	0	1 S	0	0	0
16 C	1 PP	1 PP	1 PP	0	1	0	4
17 C	1 PP	0	1 PP	0	0	0	4
18 C	1 PP	0	0	0	0	0	3
21 C	0	0	0	0	0	0	4
22 C	1 S	0	0	0	0	0	0
23 C	1 PP	1 PP	2 PP	0	1	1	0
24 C	0	0	1 S	0	1	1	0
25 C	2 PP	0	1 PP	0	0	0	3

for each strain. The differences between groups are evident with the cold-adapted nontreated animals showing much less evidence of fatty deposits and the room-temperature treated rats the greatest amount. A measure of the relative amount of fat can be made by adding the estimates of fat for the groups. These values are 59 (warm treated), 40 (cold-adapted treated), 27 (warm control), and 17 (cold-adapted control). Thus, in order of decreasing amount of fat, the groups are warm poisoned, cold-adapted poisoned, warm controls, and cold-adapted controls least. It should be stressed that the livers of the poisoned animals cannot be considered fatty livers since the number of fat-containing cells is only a small percentage of the total number of cells. The effects seen with Cresyl Violet Acetate and with H and E are similar. In the most marked

TABLE 2

Cold-adapted and treated		Room-temperature and treated	
Rat No.	Average absorbance	Rat No.	Average Absorbance
1C	0.2874	1W	0.2890
2C	0.1129	2W	0.3130
3C	0.1781	3W	0.3036
4C	0.1739	4W	0.3559
5C	0.2964	5W	0.2816
6C	0.1973	6W	0.3156
7C	0.2604	7W	0.3017
9C	0.2240	8W	0.2961
14C	0.2351	9W	0.3511
	x_1=0.2184		x_2=0.3119
Cold-adapted controls		Room-temperature controls	
15C	0.1823	13W	0.3556
16C	0.2571	14W	0.2502
17C	0.2258	15W	0.1905
18C	0.1063	16W	0.1714
21C	0.0470	19W	0.0888
22C	0.1292	20W	0.3540
23C	0.1604	21W	0.1549
24C	0.2616	22W	0.2829
25C	0.1471	23W	0.4494
	x_3=0.1685		x_4=0.2553

Analysis of variance of absorbance of Weil-stained rat liver:

Source	df	SS	MS	F	P
Total	3S	0.2802			
Treatment	3	0.0988			
Temp	1	0.07319	0.0732	12.9	PC 0.005
Drug	1	0.02555	0.0256	4.49	PC 0.05
Temp & Drug	1	0.00007	0.00007	<1	n. s.
Error	32	0.18138	0.005668		

examples, the difference between the periportal and centrolobular areas is clearly demonstrated. With Cresyl Violet Acetate, the cytoplasmic RNA in the periportal areas is almost granular (Figure 2), while in the centrolobular areas, the RNA is found in distinct clumps (Figure 3). In slides marked 0, this clumping of the RNA extends throughout the lobule. With H and E, differences are not as great but involve both the hematoxylin and the eosin. The cytoplasmic material stained with hematoxylin in the centrolobular area is clumped, while in the periportal areas, it is more granular in those marked 1. In addition, the periportal cells are redder than the centrolobular.

A modification of the Weil stain for myelin sheaths was also used on formalin-fixed paraffin liver sections. As modified,[8] the differentiation of this stain is self-limiting since, while differentiation requires about 8 hours, an eightfold increase in differentiation time produces no further loss in stain. The slides were read with a Photovolt electronic photometer. Ten readings were taken on each section with the locations random except that fields containing blood vessels were not used. The averages of these readings are found in Table 2. A factorial 2×2 method was used with the results found as shown in the table legend. It is evident that all four distributions differ significantly from one another. The staining density is increased in the poisoned animals and decreased in the cold-adapted animals with no interaction between the two conditions. The meaning of this is not known at present. By

FIGURE 2. Cresyl Violet Acetate. Note small size of cytoplasmic granules in dark area adjacent to portal canal.

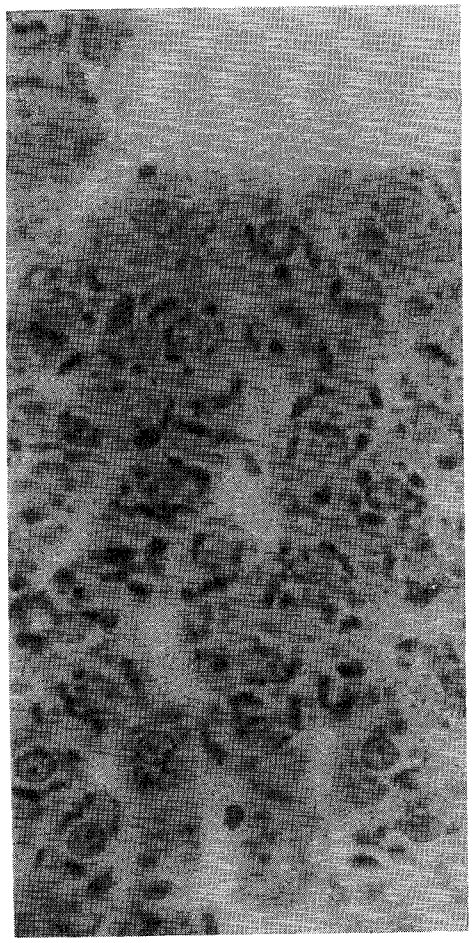

FIGURE 3. Cresyl Violet Acetate. Note large size of cytoplasmic granules.

analogy with the use of this stain for myelin sheaths,[9] the functional group to which the stain attaches is a secondary or tertiary amine and the compound is a phospholipid. Other inferences could be made by analogy with the results obtained in the study on myelin sheaths, but these should be verified on the liver before conclusions are drawn. It is interesting to speculate on the possible function of the material stained. The amount is increased by poisoning but decreased by cold adaptation. Is this material a necessary substrate for detoxification of the halogenated hydrocarbon, a "side-product" of the detoxification, or an artifact? If it is any of these, why should it be decreased in cold adaptation? Answers to these questions may give a rationale for treatment of sublethal dosage with dieldrin and possibly other halogenated hydrocarbons.

IV. General Discussion.

Although it should also be stressed that rank orders of all the measures (weight loss and the various stains) showed little correlation with one another, some generalizations are warranted. The cold-adapted rats were more severely affected by the poison than were the room-temperature rats. Thus, three of the treated cold-adapted rats died, while none of the treated room-temperature rats died. The food consumption of the treated cold-adapted rats was less and they lost more weight than the treated room-temperature rates. The reverse was true with the livers. There was a greater amount of fat in the livers of the warm treated rats; however, the cytoplasmic RNA showed greater alterations in the treated cold-adapted animals than in the room-temperature rats, and, finally, the material stained by the Weil stain was considerably less in the cold-adapted rats. Is all this a reflection of the altered lipid metabolism of the cold-adapted rats or are other factors involved? The answer to this question would be of interest from many viewpoints beyond that of poisoning by an insecticide.

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