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EXHIBIT 18

Studies of the Chick Edema Disease

3. SIMILARITY OF SYMPTOMS PRODUCED BY FEEDING CHLORINATED BIPHENYL*

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INTRODUCTION

In EARLY attempts to chemically identify the chick edema disease factor, a number of investigators (Brew *et al.*, 1959; Wootton and Alexander, 1959; Friedman *et al.*, 1959; Harman *et al.*, 1960; and Yartzoff *et al.*, 1961) reported that the factor was heavily chlorinated. More recent reports (Friedman, 1962; Wootton *et al.*, 1962; Firestone *et al.*, 1963; and Wootton and Courchene, 1964) have confirmed and extended the early studies and have shown that the factor is quite toxic for the chick. In the latter report, Wootton and Courchene (1964) "estimated that 5μg. of one of the toxic factors is enough to kill one chick."

McCune *et al.* (1962) reported that a chlorinated hydrocarbon used as a plasticizer for an epoxy-resin cage coating material elicited symptoms similar to those found when chicks were fed the chick edema factor or fats containing the factor. Using Vantress-White Rock chicks, these workers reported that hydropericardium, edema (subcutaneous and abdominal), hemorrhages of internal organs and enteritis resulted when the active material, chlorinated biphenyl, was fed at a level of 0.01% or higher.

It was, therefore, considered of interest to study in more detail the responses of chicks after they had been fed chlorinated biphenyl at various dietary levels.

EXPERIMENTAL

Day-old S. C. White Leghorn cockerels were used for these studies. On day of receipt from the hatchery, the chicks were randomized in the shipping carton, individually wing banded and weighed. They were divided into groups of twelve and placed in battery brooders with raised wire mesh bottoms. Room temperature (23.9°C.) and relative humidity (50%) were kept constant by automatic control. Incandescent lights were controlled automatically for ten hours of darkness and fourteen hours of light. Chicks were immediately placed on diets containing 0, 200 or 400 p.p.m. chlorinated biphenyl[1] (CBP). They were fed and watered *ad libitum* and were weighed twice each week.

A practical type of ration was used for these studies. The composition of the ration is reported elsewhere (Flick *et al.*, 1965). The CBP was added to cottonseed oil as carrier and thoroughly mixed, and the carrier was then mixed into the dry components of the ration.

At weekly intervals of a three-week feeding regimen six or seven chicks were randomly removed from each group, weighed, and anesthetized with ether, and

* Portions of this report were presented at the 48th Annual Meeting of the Federation of American Societies for Experimental Biology, Chicago, Illinois, April 12–17, 1964.

[1] The chlorinated biphenyl, sold as Aroclor 1242, was obtained from Monsanto Chemical Company, St. Louis, Missouri.

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TABLE 1.—*Mortality and pathological observations of chicks fed chlorinated biphenyl (CBP) for three weeks*

CBP (p.p.m.)	No. chicks on diet	Deaths	No. chicks autopsied	Edema incidence			Tissue and organ pathology[2]
				HP[1]	Abdominal	Subcutaneous	
0	21	0	6	0	0	0	None
200	24	0	10	1	0	0	Pancreas (8), kidney (5), spleen (3), skin and feathers (2) and adrenal (1).
400	24	3[3]	7	5	4	3	Pancreas (7)[4], adrenal (6), kidney (5), spleen (1), heart (1)[5], skin and feathers (1).

[1] Only HP volumes of >0.21 ml. were included.
[2] Organ changes observed included: hemorrhages, enlargement, paleness, smallness (spleen), and dermatitis and/or defeathering. Numbers in parentheses are incidence of observations/organ or tissue change.
[3] Two deaths occurred during the second week and one during the third week. No autopsies on these chicks.
[4] All animals in this group had a pale cream-colored pancreas.
[5] Pronounced heart enlargement of a bird having hemoglobin level of 4.05 g. % and hematocrit of 19.1

CBP had a pale cream-colored pancreas. The kidneys were extensively hemorrhagic and enlarged, and the adrenals (6/7) in this group were also enlarged and hemorrhagic.

The results of HP volume determination are shown in Figure 2. At the first and second weeks, the mean HP volumes of the three groups were about the same, except that the chicks fed 400 p.p.m. CBP had slightly elevated HP volumes at two weeks. At three weeks, the mean HP volume of the group fed 400 p.p.m. CBP was approximately 10-fold higher than that of the group fed 200 p.p.m., and the latter, in turn, was somewhat higher than that of the controls.

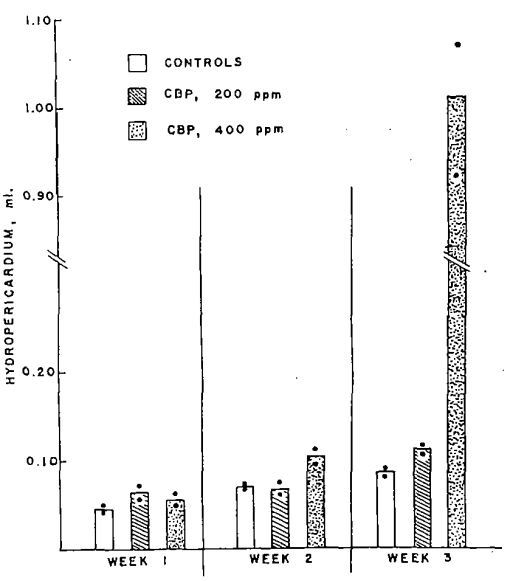

FIG. 2. Effect of chlorinated biphenyl (CBP) on hydropericardial response (ml. ± S. E. of the mean).

The results of the blood studies are shown in Table 2. Blood hemoglobin levels decreased weekly among control chicks. A comparison of levels at each week revealed that the birds fed CBP had lower hemoglobin levels than controls. The birds fed 200 p.p.m. CBP showed a slight but steady decrease in hemoglobinemia throughout the 3 week study; those fed 400 p.p.m. CBP showed a marked hypohemoglobinemia at the second week but recovered somewhat at the third week. This recovery may have been due to the development of a compensatory mechanism to overcome the inhibitory effect of CBP on hematopoiesis manifested in the earlier days of the experiment.

Hematocrit levels (Table 2) followed a pattern similar to hemoglobin levels. Maximal depression of hematocrit levels was found at the second week among the chicks fed 400 p.p.m. CBP. As with hemoglobin levels, the increased hematocrit at the third

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compound (isomer) responsible for the chick edema disease, but to elucidate the observed relationships between toxicity and potency of CEF and the toxic nature of chlorinated aromatic compounds.

SUMMARY

Chlorinated biphenyl (CBP), fed to S. C. White Leghorn cockerels at 200 or 400 p.p.m. in a practical type of ration for three weeks, produced signs which partially simulated those of the chick edema disease factor, a chlorinated aromatic hydrocarbon.

CBP feeding was accompanied by growth depression in direct proportion to the dietary level. Edema formation in the pericardial sac and in the abdominal and subcutaneous areas was pronounced only at the 400 p.p.m. level of CBP. Transient changes in blood glucose, hemoglobin and hematocrit levels accompanied CBP feeding, particularly at the 400 p.p.m. level.

Pathological changes observed were as follows in decreasing order of incidence: cream-colored pancreas, enlarged hemorrhagic kidney, enlarged adrenal, small spleen, dermatitis and defeathering.

Relationships between the toxic nature and relative potency of CBP and the chick edema factor are discussed.

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