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

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

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the volume of hydropericardium (HP) was determined according to the procedure outlined by Douglass and Flick (1961). While the heart was still beating, blood was obtained by cardiac puncture of the right ventricle with freshly heparinized needles and syringes. Hematocrits were determined by the semi-micro procedure described by Natelson (1961), using heparinized capillary tubes which were hand-sealed and centrifuged[2] for fifteen minutes. Hemoglobin was determined by the cyanmethemoglobin method.[3] Whole blood glucose was determined by an enzymatic method.[4]

The animals were then autopsied, and gross pathological changes were observed and recorded.

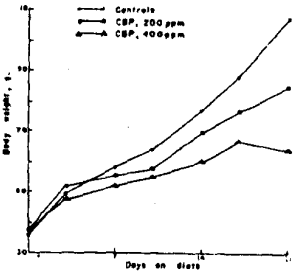

FIG. 1. Twenty-one day growth curves of day-old S. C. White Leghorn cockerels fed chlorinated biphenyl (CBP).

RESULTS AND DISCUSSION

Plots of body weights of chicks fed 0, 200 or 400 p.p.m. CBP are shown in Figure 1. For the first week all chicks grew at approximately the same rate, but within the second week differences in weight gains became apparent, and these differences were more pronounced by the end of the third week. The growth depression obtained was directly related to level of CBP fed. The most adverse effect on growth was found among the chicks fed 400 p.p.m. CBP; they actually lost weight during the last four days of the feeding regimen. Our observations of the adverse effects of CBP on growth confirm and extend the studies reported by McCune *et al.* (1962) who used a heavy breed of chick.

At the end of three weeks, surviving chicks were autopsied. Observations are summarized in Table 1. The three deaths which occurred were among the birds fed 400 p.p.m. of CBP. Edema formation was virtually absent from the group fed 200 p.p.m. of CBP, except for one bird with elevated HP. However, this level of CBP elicited marked organ and skin changes, including paleness of kidney and pancreas, enlargement of adrenal, and small spleen. Two birds had dermatitis and one had a loss of feathers on the dorsum of the neck. Frank edema occurred among birds fed 400 p.p.m. CBP. Three of seven chicks had slight subcutaneous edema, and four had slight to moderate edema in the abdominal area. This group had a high incidence of HP; 5 of 7 birds had more than 0.21 ml. fluid in the pericardial sac. The organ and tissue changes of chicks fed 400 p.p.m. of CBP, compared to those fed 200 p.p.m., were marked by both degree of change and incidence. Most prominent of the gross changes was that every bird fed 400 p.p.m.

[2] Capillaries were sealed with Seal-Ease, No. A-2080, and centrifuged with the Adams Micro-Hematocrit Centrifuge No. CT-2900, products of Clay-Adams, Inc., New York 10, New York.

[3] The cyanmethemoglobin kit containing diluent (Aculute) and standard (Acuglobin) was purchased from Ortho Pharmaceuticals Corp., Raritan, New Jersey.

[4] Glucose was determined by using the Glucostat kit, a prepared enzymatic glucose reagent, and following Method R as outlined by the Worthington Biochemical Corp., Freehold, New Jersey.

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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.

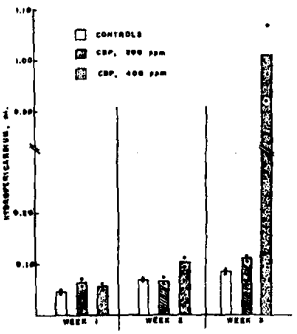

FIG. 2. Effect of chlorinated biphenyl (CBP) on hydropericardial response (ml. ± S. E. of the mean).

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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week among these chicks may have been due to compensation of hematopoiesis.

Blood glucose levels (Table 2) among control chicks apparently increased with time. It was found after the first week, however, that CBP at both 200 and 400 p.p.m. levels was accompanied by a moderate hyperglycemia. Blood glucose levels among chicks fed 400 p.p.m. CBP were below normal at three weeks. This hypoglycemic response, though not so marked, is similar to that observed among chicks with the edema disease following intake of crude toxic fat.

TABLE 2.—*Hemoglobin, hematocrit and whole blood glucose levels of chicks fed chlorinated biphenyl (CBP)*

CBP (p.p.m.)	Week 1	Week 2	Week 3
	Hemoglobin, g %		
0	9.16±0.30[1]	8.33±0.35	7.08±0.44
200	7.77±0.13	7.50±0.23	6.93±0.32
400	6.92±0.36	5.97±0.23	6.24±0.29
	Hematocrit, % cells		
0	35.0±0.56	31.8±0.80	31.5±1.28
200	30.4±0.73	29.5±1.01	28.4±0.53
400	28.4±2.20	22.3±1.05	29.2±2.02
	Whole blood glucose, mg. %		
0	186.8±4.1	—	205.6±6.9
200	203.3±6.1	161.5±5.2	196.5±3.1
400	208.8±6.4	183.3±6.4	171.1±7.3

[1] Values are means of 6-7 determinations (±S.E. of the mean).

From the studies in this report, it appears that the symptoms elicited by CBP are in part similar to the chick edema disease, particularly in gross appearance of the living chick, observations at necropsy, and ability of CBP to depress growth. Certain details, however, are different. One important difference is that, until recently, the chick edema factor concentrate was about 200 times more potent than CBP in the production of comparable symptoms. In more recent studies, Wootton *et al.* (1962) and Wootton and Courchene (1964) postulated that 5 μg. of one fraction, designated as α-3.02, of their purified chick edema factor could kill one chick. Flick *et al.* (unpublished observations) determined under controlled feeding conditions that about 16 μg. of their preparation led to 50% mortality and, further, that less than 6 μg. of the chick edema factor led to development of edema.

A possibility to be considered (McCune *et al.*, 1962) is that the chick edema factor may be present in the CBP preparation. This possibility, however, is not supported by recent analyses of CBP and CEF by the microcoulometric gas chromatograph (Firestone, personal communication). With this technique a number of peaks were found in CBP, but none had relative retention times near that of a highly purified fraction of the CEF under similar experimental conditions. Also to be considered is the possibility that we may be dealing with a specific molecular configuration or an active nucleus of a molecule which, either alone or as a metabolite, is responsible for the chick edema disease.

There are many reports on toxicological aspects of chlorinated aromatic compounds for the chick, as well as other animals, according to Negherbon (1959). For example, chlorinated naphthalenes were reported by Pudelkiewicz *et al.* (1958) to inhibit growth of Broad Breasted Bronze poults fed at 5 p.p.m. or higher in a practical type of ration. In a later study, Pudelkiewicz *et al.* (1959) found that not only did chlorinated naphthalenes depress growth but when it was fed at 100 p.p.m. and higher there were decreases in plasma and liver vitamin A levels, serum cholesterol, and hematocrit values. The studies by Pudelkiewicz *et al.* suggest that the potency of naphthalenes containing 5-8 chlorine atoms per molecule may be equal to or higher than chlorinated biphenyl. It is felt, however, that the toxicity of the chick edema factor is many times more potent for the chick than any chlorinated aromatic compound reported to date.

It is thus obvious that more studies are needed not only to identify the specific

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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.

REFERENCES

Brew, W. B., J. B. Dore, J. H. Benedict, G. C. Potter and E. Sipos, 1959. Characterization of a type of unidentified compound producing edema in chicks. J. Assoc. Offic. Agr. Chemists, 42: 120–128.

Douglass, C. D., and D. F. Flick, 1961. Collaborative bioassay for chick edema factor. J. Assoc. Offic. Agr. Chemists, 44: 449–456.

Firestone, D., W. Ibrahim and W. Horwitz, 1963. Chick edema factor III. Application of microcoulometric gas chromatography to detection of chick edema factor in fats or fatty acids. J. Assoc. Offic. Agr. Chemists, 46: 384–396.

Firestone, D., 1964, Division of Food Chemistry, Food and Drug Administration, Washington, D. C. 20204, personal communication.

Flick, D. F., C. D. Douglass and L. Gallo, 1963. Studies of the chick edema disease. 1. Body water distribution and effect of diet. Poultry Sci. 42: 855–862.

Flick, D. F., D. Firestone and J. P. Marliac, 1965. Studies of the chick edema disease 2. Purification and biological effects of the chick edema factor. Poultry Sci. 44: 1214–1222.

Flick, D. F., V. A. Childs and R. G. O'Dell, unpublished observations.

Friedman, L., D. Firestone, W. Horwitz, D. Banes, M. Anstead and G. Shue, 1959. Studies of the chick edema disease factor. J. Assoc. Offic. Agr. Chemists, 42: 129–140.

Friedman, L., 1962. Progress in the chick edema problem. Feedstuffs, 34 (11): 18.

Harman, R. E., G. E. Davis, W. H. Ott, N. G. Brink and F. A. Kuehl, Jr., 1960. The isolation and characterization of the chick edema factor J. Am. Chem. Soc. 82: 2078–2079.

McCune, E. L., J. E. Savage and B. L. O'Dell. 1962. Hydropericardium and ascites in chicks fed a chlorinated hydrocarbon. Poultry Sci. 41: 295–299.

Natelson, S., 1961. Microtechniques of Clinical Chemistry, 2nd Ed. Charles C. Thomas, Springfield, Ill., 74–79.

Negherbon, W. O., 1959. Handbook of Toxicology. III. Insecticides, a Compendium, Aero Medical Laboratory, Wright Air Development Center, Air Research and Development Command, United States Air Force, Wright-Patterson Air Force Base, Ohio.

Pudelkiewicz, W. J., R. V. Boucher, E. W. Callenbach and R. C. Miller, 1958. Some physiological responses of Broad Breasted Bronze poults to chlorinated naphthalene. Poultry Sci. 37: 185–187.

Pudelkiewicz, W. J., R. V. Boucher, E. W. Callenbach and R. C. Miller, 1959. Some physiological responses of New Hampshire chickens to a mixture of penta- and hexachloronaphthalenes. Poultry Sci. 38: 424–430.

Wootton, J. C., and J. C. Alexander, 1959. Some chemical characteristics of the chicken edema disease factor. J. Assoc. Offic. Agr. Chemists, 42: 141–148.

Wootton, J. C., N. R. Artman and J. C. Alexander, 1962. Isolation of three hydropericardium-producing factors from a toxic fat. J. Assoc. Offic. Agr. Chemists, 45: 739–740.

Wootton, J. C., and W. L. Courchene, 1964. A contribution to the knowledge of the structure

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of two hydropericardium-producing factors from a toxic fat. Agr. Food Chem. 12: 94–98.
Yartzoff, A., D. Firestone, D. Banes, W. Horwitz, L. Friedman and S. Nesheim, 1961. Studies of the chick edema factor. II Isolation of a toxic substance. J. Am. Oil Chemists Soc. 38: 60–62.

Avian Tracheal Rings*

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IN A recent publication, Mennega (1964) shows the supposed structure of the rings of the trachea of the chicken, the turkey and the duck. Mennega depicts these structures in sketches. It seems apparent that the sketch of the tracheal rings of the chicken is actually based on the description by Bradley and Grahame (1950) which follows: "A relatively long trachea, composed of complete rings of hyaline cartilage united by narrow membranous ligaments, connects the cranial larynx with the caudal larynx or *syrinx*. The cartilaginous rings are of alternate sizes, so that a large ring half overlaps a small ring, and nearly touches the end of the previous small ring. The rings have thick middle portions and thin edges. The ends of the rings are joined by a band of connective tissue. Commonly, ossification takes place in the ventral part of the tracheal rings."

McLeod *et al.* (1964) say: "The *trachea* is a long tube connecting the cranial and caudal larynges. It has a skeleton of complete cartilaginous rings which have a tendency toward progressive ossification with age. Ossification occurs earlier in the goose and the duck than in the chicken and turkey. The rings do not have a uniform diameter. Some are larger at the caudal end; others are beveled on the outside at both ends. This arrangement allows a certain amount of telescoping of the rings and modification of the length of the trachea with flexing and extending of the neck. The adjacent rings are connected by ligaments which are physically elastic in structure. The total length of the trachea is subject to wide functional variation, but is always shorter than the corresponding part of the vertebral column. The trachea of the leghorn hen averages about six inches in length."

Sisson and Grossman (1953) say: "The trachea is a long tube, whose cartilaginous rings are complete."

Salt and Zeuthen (1960) say: "The trachea is held open by hyaline cartilaginous rings, partly ossified on the ventral side. The rings are narrower dorsally there so the tracheal wall is more flexible."

Romanoff (1960) says: "The rings are of alternating sizes, so that a large ring half overlaps a small ring and nearly touches the free edge of the large ring."

TECHNIQUES

The use of magnifier, preferably a dissecting microscope, is helpful.

Digestion of the trachea in 1% trypsin for an hour or more makes the rings stand out. Further help in observation is afforded by slitting the trachea lengthwise along either the left or the right side and spreading it flat.

Staining with aqueous malachite green (5%) followed by a tap-water rinse and counter-staining with Wright's stain makes

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