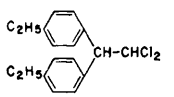
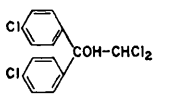
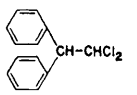
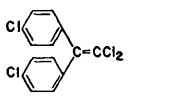
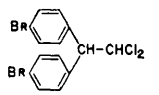
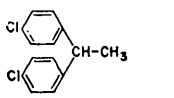
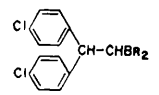
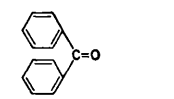
# Further Observations on Effect on Plasma 17-OH-Corticosteroids in the Dog of Derivatives of 2,2-Bis-(p-Chlorophenyl)-1,1-Dichloroethane (DDD, TDE).* (23784)

FLORAPEARL A. COBEY, ISABEL TALIAFERRO AND HARVEY B. HAAG
*Departments of Pharmacology and Medicine, Medical College of Virginia, Richmond*

Production of adrenal cortical atrophy in dogs by 2,2-bis-(p-chlorophenyl)-1,1-dichloroethane (DDD, TDE) was first observed by Nelson and Woodard(1). Larson *et al.*(2) demonstrated that while DDD and certain of its derivatives produce adrenal cortical atrophy, still other derivatives cause hyperplasia or hypertrophy. The production of adrenal cortical atrophy is dependent upon the presence of the intact 2,2-diphenyl-1,1-dichloroethane structure. Alteration of the number of chlorines on the 1-position of the ethane abolishes the ability to produce adrenal cortical atrophy. Hydroxylation at the 2-position or desaturation to the ethylene analogue forms compounds which produce adrenal cortical hypertrophy. In an investigation of the effect of DDD and compound I (Table I) on the level of plasma 17-hydroxycorticosteroids (corticoids) in dogs, Cobey, Taliaferro and Haag(3) found that adrenal cortical atrophy obtained with these compounds was accompanied by decreased response to ACTH as measured by changes of plasma corticoids. However, a similar decrease was found using compound V which has been shown(2) to produce adrenal cortical hypertrophy.

TABLE I. Structural Formulae of the 8 DDD Derivatives Studied. Compound I is referred to as Perthane® and Compound V as FW-152.

| REFERENCE NUMBER | COMPOUND | REFERENCE NUMBER | COMPOUND |
|---|---|---|---|
| I |  | V |  |
| II |  | VI |  |
| III |  | VII |  |
| IV |  | VIII |  |

In the present investigation was primarily to obtain further information on the effect on corticoid secretion of DDD derivatives, incluing several which have been previously studied and certain additional ones. In some instances, the histopathological effects on the adrenal cortex were also determined.

*Procedure.* Mongrel dogs of both sexes weighing from 15 kg to 30 kg were used. With the exception of Compound I, the DDD derivatives† were dissolved in corn oil and placed in gelatin capsules for oral administration. Compound I was used for long-term feeding studies in which it was administered thoroughly mixed with finely ground Purina Dog Chow Kibbled Meal. Routinely, 2 hours before blood samples were taken, 20 U.S.P. units of ACTH‡ was administered intravenously so as to determine the secretory responsiveness of the adrenal cortex(3). Plasma 17-hydroxycorticoids§ were determined by the method of Nelson and Samuels(4).

*Results.* The derivatives studied are shown in Table I, and, with the exception of compounds VII and VIII, the results obtained are shown in Fig. 1-3.

*Compound I* (Perthane®) was given to 2 dogs in the diet at levels of 500 and 1000 p.p.m. (parts per million) rather than in capsule form as we had done previously(3). Within 2 weeks at each level a marked diminution was noted in ability of the adrenal

* This work was supported by Grant from the Nat. Heart Inst., N.I.H., U. S. Public Health Service.

† Kindly furnished through the courtesy of Rohm and Haas Co., Philadelphia.

‡ Armour's Corticotrophin (ACTH), 25 I.U./vial.

§ Standard 17-hydroxycorticosteroid was kindly supplied by Dr. H. Molitor, Merck Institute for Therapeutic Research, Rahway, N. J.

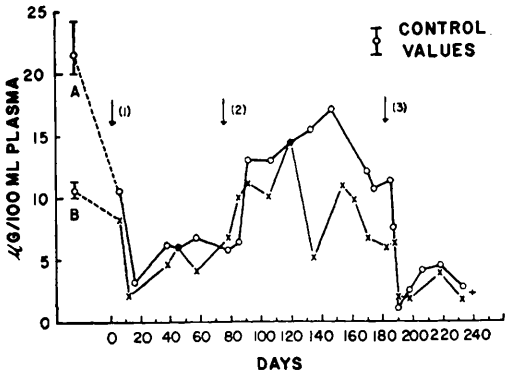



FIG. 1. Effect of Compound I in 2 dogs (A and B) on level of plasma 17-hydroxycorticosteroids 2 hr after inj. of ACTH. Arrows indicate addition of Compound I to the diet as follows: 500 p.p.m. level started at (1) and discontinued at (2); 1000 p.p.m. started at (3). Animals sacrificed at ×.

cortex to respond to ACTH as measured by levels of plasma corticoids (Fig. 1). The plasma corticoids showed some increase after the initial drop, but adrenal responsiveness was maintained at a definitely lower than normal level as long as treatment was continued. The decrease in corticoids while the dogs were on the diet of 1000 p.p.m. was essentially the same as that noted while on the diet of 500 p.p.m. In one animal (Dog A)

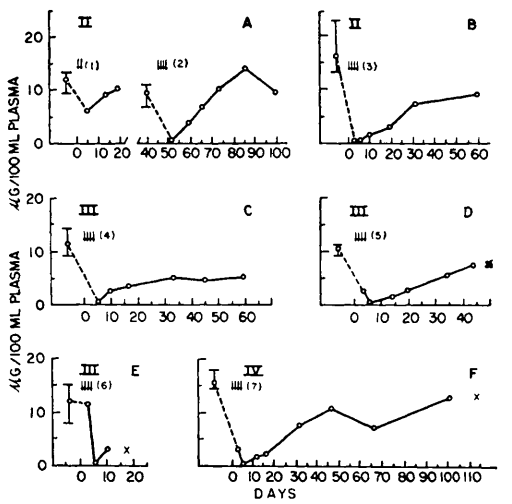



FIG. 2. Effect of Compounds II, III and IV in dogs on level of plasma 17-hydroxycorticosteroids 2 hr after inj. of ACTH. ♀ represents control values; ↓ indicates oral administration of compounds in corn oil solution as follows: (1) and (6) 100 mg/kg/day; (2), (3), (4), (5) 200 mg/kg/day. Animals sacrificed at ×.

the plasma corticoid values did not return to the normal level after compound I was discontinued. In this connection it might be pointed out that with this series of compounds it was frequently observed that after completion of treatment during which a marked decrease in plasma corticoid output was obtained, the plasma levels would not return to their former value for periods of up to several months. Larson *et al.*(2) as well as the authors(3) have previously found that this compound produces adrenal cortical atrophy in dogs. Similarly, in the present study Dog

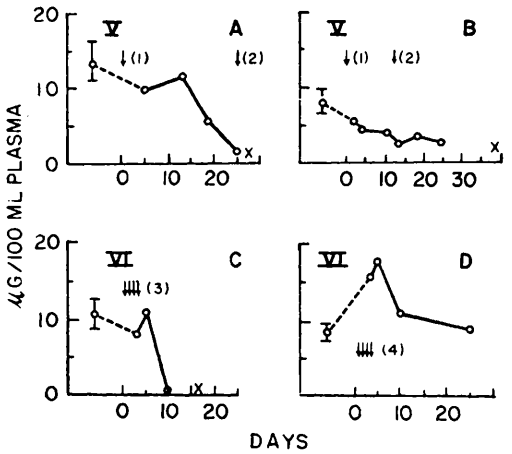



FIG. 3. Effect of Compounds V and VI in dogs on the level of plasma 17-hydroxycorticosteroids 2 hr after inj. of ACTH. ♀ represents control values; ↓ indicates oral administration of compounds in corn oil as follows: (1) 50 mg/kg/day started and stopped at (2); (3) 200 mg/kg/day; (4) 100 mg/kg/day. Animals died or were sacrificed at ×.

A showed moderate atrophy of the zona fasciculata at autopsy and Dog B, marked atrophy of the zona fasciculata.‖

*Compound II,* different from Compound I in that it has no para-phenyl substitution, markedly decreased the ability of the adrenal cortex to secrete corticoids in the 2 dogs studied (Fig. 2, II A, B). The fall of plasma corticoids obtained with this compound is similar to that previously demonstrated for

The authors wish to thank Dr. G. R. Hennigar for making the histopathological interpretations and to gratefully acknowledge the technical assistance of Angela Cahn and Jean Sadler in doing the plasma corticoid determinations.

Compound I and DDD(3), but recovery from its effects was somewhat more rapid than from Compound I. This is in line with the observation of Larson *et al.*(2) that Compound I produces a greater degree of atrophy than Compound II. As shown with *Compound III,* the substitution of bromine for chlorine on the para-phenyl positions of the DDD molecule did not materially alter its effects on the adrenal. This compound caused a decrease in adrenal responsiveness (Fig. 2, III C, D, E) comparable to that observed with Compounds I and II. Dogs D and E were sacrificed for autopsy studies. The adrenal of Dog D showed moderate atrophy of both the zona reticularis and zona fasciculata, while the adrenal of Dog E was reported as showing "atrophy of the fasciculata."

Another bromine derivative, *Compound IV,* in which the aliphatic chlorine of DDD was replaced by bromine, caused a fall in the plasma corticoids from 16 to 0 μg per 100 ml plasma following treatment (Fig. 2, IV F) in the one dog studied. Insufficient material precluded further experiments. When this dog was sacrificed almost 3 months after dosing, no evidence of adrenal damage was found on histopathological examination. In this instance the adrenal cortex had apparently been physiologically blocked as far as elaboration of corticoids was concerned without showing any evidence. of an irreversible character at least, of anatomical alterations.

*Compound V* (FW-152) is DDD hydroxylated on the 2-position of the ethane moiety. It caused a depression of adrenal cortical function (Fig. 3, V A, B), in the 2 dogs studied. This is in confirmation of a preliminary experiment already reported(3). Because of its relative toxicity as compared to other members of the series, Compound V was given at a dosage level of 50 mg/kg body weight. Even at this low dosage the 2 dogs developed elevated plasma bilirubin levels with total values of 2.9 and 1.6 mg per 100 ml after 18 days, and each of the animals lost 3 kg of weight during the experiment. Larson *et al.*(2) had found that this compound produced moderate to marked adrenal cortical hypertrophy. At autopsy the 2 dogs in the present study showed deeply jaundiced tissues. Unfortunately autolytic changes made histopathological examination in one dog impossible, and in the case of the other, the specimen was lost.

When Compound VI, an ethylene analogue of DDD, was administered to one dog at a level of 200 mg/kg body weight for 4 days, the plasma corticoids fell to zero at the tenth day and the animal died on the sixteenth day (Fig. 3, VI C). In the next experiment the dosage was decreased to 100 mg/kg for 4 days; at this dosage the animal became very ill, but recovered. The plasma corticoids of this dog *rose* from 8 to 18 μg per 100 ml plasma on the fifth day and then gradually returned to normal level (Fig. 3, VI D). Due to our inability to obtain more of this compound it was not possible to repeat these experiments. Whether this apparent reversal in corticoid response following the smaller dose of Compound VI was due simply to a natural periodic variation in this dog the adrenals of which were unaffected by this dose of Compound VI, to a synergism between Compound VI at this dose level and ACTH, or to a combination of these and/or other factors, is not known. However, with reference to the possibility that this might be an instance of natural variation, it should be pointed out that both our present control experiment (see below) and past experiences(3) indicate that this is quite unlikely. Larson *et al.*(2) had previously found that this compound produces moderate hypertrophy of the adrenal cortex involving both the zona glomerulosa and zona fasciculata.

*Compounds V and VI* were of special interest because, in the absence of demonstrable pathology in other organs, Larson *et al.*(2) found marked or moderate adrenocortical hypertrophy in 3 dogs which survived for 2 or 3 weeks of daily administration of Compound V, and moderate adrenocortical hypertrophy in 2 dogs which survived 2 weeks of daily administration of Compound VI. Furthermore, in one of the 2 dogs treated with Compound V by Cobey *et al.*(3), plasma 17-hydroxycorticosteroid levels fell from 14 to 2 μg % after treatment. It was therefore considered probable that Compounds V and VI blocked steroidogenesis in, or release of steroids from an

hypertrophied adrenal cortex. The fall in corticoids following ACTH (Fig. 3, A, B, C) is consistent with this thesis. However, development of jaundice in the present studies with Compound V, and the lack of pathological study of the 4 animals receiving Compounds V and VI prevents acceptance of the attractive conclusion that the hypertrophied gland is a blocked gland incapable of responding to ACTH. Although not proven, it can be suggested, nevertheless, that Compounds V and VI do act in this manner, that is, cause hypertrophy of the adrenal cortex while blocking steroidogenesis or release of corticoids. Another compound known to have such properties is amphenone, a substance which produces hypertrophy with diminution of function. In rats amphenone produces marked accumulation of lipoid and hyperplasia of the adrenocortical cells(5). In dogs it causes hypertrophy and lipoid accumulation in the zona fasciculata and zona reticularis which is accompanied by diminution in adrenal venous blood content of 17-hydroxycorticosteroids and failure to respond to ACTH (6). From the revised formula of amphenone (7) it appears that the DDD derivatives and amphenone have structural similarities.

*Compound VII* represents DDD in which the chlorine on the 1-position is replaced with hydrogen. In the one dog studied, its administration, in a dose of 200 mg/kg daily for 4 days, produced no changes in the plasma corticoid level. Lack of material prevented further testing. This compound was shown by Larson *et al.*(2) to cause moderate adrenal cortical hyperplasia in the one dog used.

Compound VIII, benzophenone, was investigated because of the possibility that it might occur in the body as a metabolite of DDD. It produced no significant change in the plasma corticoids in 3 dogs given 200 mg kg per day for 4 days.

One dog was maintained as a control for 23 weeks and determinations of the plasma corticoids were made at intervals (paired with an experimental dog) following the procedure used for the experimental animals. Capsules containing corn oil were given in the same manner (daily, for 4-day periods) in which the DDD derivatives were administered. Sixteen determinations were made and no significant variations were observed in the plasma corticoid levels in this control dog receiving corn oil.

*Summary.* 1) The effect of 8 derivatives of 2,2-bis-(p-chlorophenyl) - 1,1-dichloroethane (DDD, TDE) on responsiveness of the adrenal cortex to ACTH, as determined by changes in plasma 17-hydroxycorticoids, has been studied in dogs. The following derivatives decreased the corticoid response: those with para-phenyl modifications; bromine substitution of the aliphatic chlorines; hydroxylation on the 2-position of the ethane moiety. No change in response was noted with a derivative in which hydrogen replaced chlorine in the 1-position of the ethane moiety, and with benzophenone, a possible DDD metabolite. Somewhat equivocal results were obtained with an ethylene analogue. 2) The relationship between these biochemical findings and the histopathologic effects recorded for this series of compounds is discussed.

1. Nelson, A. A., Woodard, G., *Arch. Path.*, 1949, v48, 387.

2. Larson, P. S., Hennigar, G. R., Finnegan, J. K., Smith, R. B., Jr., Haag, H. B., *J. Pharmacol. and Exp. Ther.*, 1955, v115, 408.

3. Cobey, F. A., Taliaferro, I., Haag, H. B., *Science*, 1956, v123, 140.

4. Nelson, D. H., Samuels, L. T., *J. Clin. Endocrinol. and Metab.*, 1952, v12, 519.

5. Hertz, R., Tullner, W. W., Schricker, J. A., Dhyse, F. G., Hallman, L. F., *Recent Progress in Hormone Research*, 1955, v11, 119, Academic Press, N. Y.

6. Tullner, W. W., Jackson, S. J., *The Endocrine Society, Program of 39th meeting*, 1957, 73.

7. Bencze, W. L., Allen, M. J., *J. Org. Chem.*, 1957, v22, 352.

Received January 2, 1958. P.S.E.B.M., 1958, v97.