### References and Notes

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## Action of 1,1,Dichloro-2-p-chlorophenyl-2-o-chlorophenylethane on Dog Adrenal Cortex

*Abstract.* A single intravenous injection of *op'*DDD (1,1,dichloro-2-*p*-chlorophenyl-2-*o*-chlorophenylethane) has an acute effect on the adrenal cortex of the dog. Within 2 hours after intravenous injection of the drug, there is a decrease in the in vitro response of the adrenal cortex to stimulation by adrenocorticotrophic hormone and an inhibition of glucose-6-phosphate dehydrogenase activity. The inhibition of glucose-6-phosphate dehydrogenase activity might explain the effect of *op'*DDD on corticosteroid production.

Since Nelson and Woodward reported that DDD [1,1,dichloro-2,2-bis(chlorophenyl) ethane] causes atrophy of the adrenal cortex (*1*), there have been numerous reports dealing with the therapeutic possibilities of this adrenocorticolytic drug. However, the mechanism of action, as far as we know, has not yet been described. We have found that a single injection of *op'*DDD (1,1,dichloro-2-*p*-chlorophenyl-2-*o*-chlorophenylethane) causes a reduction in the response to in vitro stimulation with ACTH (adrenocorticotrophic hormone) and a partial inhibition of adrenal glucose-6-phosphate dehydrogenase. The inhibition of this enzyme suggests one possible mechanism of action for the drug.

Table 1. Effect of *op'*DDD on in vitro response of adrenal gland to ACTH.

| Porter and Silber chromogens (μg/100 mg wet weight) | | *t* | *p* |
|---|---|---|---|
| Control dogs | Treated dogs | | |
| | *No additions* | | |
| 3.0 | 1.3 | | |
| | 5.4 | | |
| 7.9 | 3.2 | | |
| 7.5 | 1.8 | | |
| 7.9 | 1.1 | | |
| 14.2 | 5.4 | | |
| 4.2 | 1.4 | | |
| Mean ± S.E.: | | | |
| 7.6 ± 1.69 | 2.8 ± 0.72 | 2.62 | .022 |
| | *ACTH (0.2 unit per flask)* | | |
| 21.1 | 1.9 | | |
| 21.1 | 7.7 | | |
| 15.1 | 4.4 | | |
| 12.3 | 1.4 | | |
| 21.4 | 7.0 | | |
| 28.9 | 8.9 | | |
| 13.5 | 1.4 | | |
| Mean ± S.E.: | | | |
| 19.0 ± 2.20 | 4.7 ± 1.21 | 5.7 | < .001 |

Eighteen mongrel dogs weighing 12 to 15 kg were used; nine were injected with *op'*DDD (*2*) (60 mg/kg body weight), and the other nine received only solvent [6 ml of ethanol and propylene glycol (1:1)]. After 2 hours, the adrenals were removed under pentobarbital anesthesia and cleaned of adherent fat; one adrenal of each dog was sliced, placed in Warburg flasks (40 to 60 mg of tissue per flask), and incubated for 1 hour in 3 ml of Krebs-Ringer solution with bicarbonate. Then the slices were incubated for another hour in a medium of Krebs-Ringer solution, glucose, and bicarbonate with nothing added or with ACTH added (0.2 unit per flask). After this final incubation, the medium was removed and Porter and Silber chromogens were determined (*3*). The activity of glucose-6-phosphate dehydrogenase was determined (*4*) in cell-free extracts prepared from the adrenals that had not been incubated.

The results (Table 1) show that a single intravenous injection of *op'*DDD decreases the in vitro corticosteroid response of the adrenals to ACTH. We feel that this response is a further confirmation of Nichols' work (*5*) and that it is evidence for a specific site of action of *op'*DDD. Table 2 shows that the activity of glucose-6-phosphate dehydrogenase is partially inhibited in dogs injected with *op'*DDD. The activity of 6-phosphogluconic dehydrogenase and the formation of lactic acid were not influenced by *op'*DDD (*6*).

Dogs treated with DDD for 5 days, besides showing the well-established diminution of Porter and Silber chromogens, show a decrease in the urinary excretion of 17-keto-steroids (*7*). A possible interpretation of this decrease is that the biosynthetic pathways of steroids were blocked at an early stage. The inhibition of glucose-6-phosphate dehydrogenase would be a confirmation of this hypothesis, since the inhibition would result in decreased production of reduced triphosphopyridine nucleotide, which is necessary for the breakdown of the cholesterol side chain (*8*). Moreover, glucose-6-phosphate dehydrogenase, which is very active in the adrenal cortex (*9*), is preferentially located in the inner zones (*10*); and it is in these zones that *op'*DDD has most of its effect (*11*).

Table 2. Effect of *op'*DDD on glucose-6-phosphate dehydrogenase activity of adrenal gland. The unit of activity is change in optical density of 0.001 per milligram of nitrogen per minute, at 340 mμ.

| Activity | | *t* | *p* |
|---|---|---|---|
| Control | *op'*DDD | | |
| 844 | 515 | | |
| 700 | 590 | | |
| 886 | 400 | | |
| 565 | 214 | | |
| 792 | 410 | | |
| 813 | 473 | | |
| 913 | 507 | | |
| 1275 | 340 | | |
| 1287 | 498 | | |
| Mean ± S.E.: | | | |
| 897 ± 80.4 | 438 ± 37.3 | 5.18 | < .001 |

When *op'*DDD was added in vitro to the Warburg flasks, instead of being injected in vivo, it had no effect (*6*). This lack of response in vitro suggests that the *op'*DDD did not reach the intracellular space or that *op'*DDD must be converted into another active product or that the dosage was insufficient (0.8 μmole per flask), because of poor solubility of the drug in the medium. Experiments to elucidate these possibilities are being carried out (*12*).

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