ous procedures is increased because of greater sensitivity, better accuracy in lower ranges, stability of test organism and micromethod applicability. 2) With this improved assay, the overlap of serum folic acid activity levels among normal subjects and folic acid deficient patients is so great that this determination has no value as an index of folic acid deficiency.

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Effects of DDD on Steroid Response and Blood Flow Through the Adrenal After ACTH.* (25901)

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The effects of ACTH in increasing corticosteroid secretion of the adrenal cortex and blood flow through the gland have been reported. That these 2 responses are not directly related does not seem to be commonly known. This paper records the dissociation of these phenomena and sets forth some quantitative measurements on vascular reaction. Administration of DDD (2,2-bis(parachlorophenyl)-1,1-dichloroethane) causes a decreased secretion of 17-hydroxycorticosteroids and the adrenal becomes refractory to stimulus from ACTH. However, in doses sufficient to prevent steroid formation, DDD does not affect vascular responses of the gland. These findings are considered important because some isomers of DDD are being used currently in human therapy(1).

Method. Data were derived from 185 mongrel dogs of both sexes. The preparation of DDD used was purchased commercially, and reportedly contains approximately 90% of the p,p'-isomer and 5-7% of the o,p'-isomer(2).

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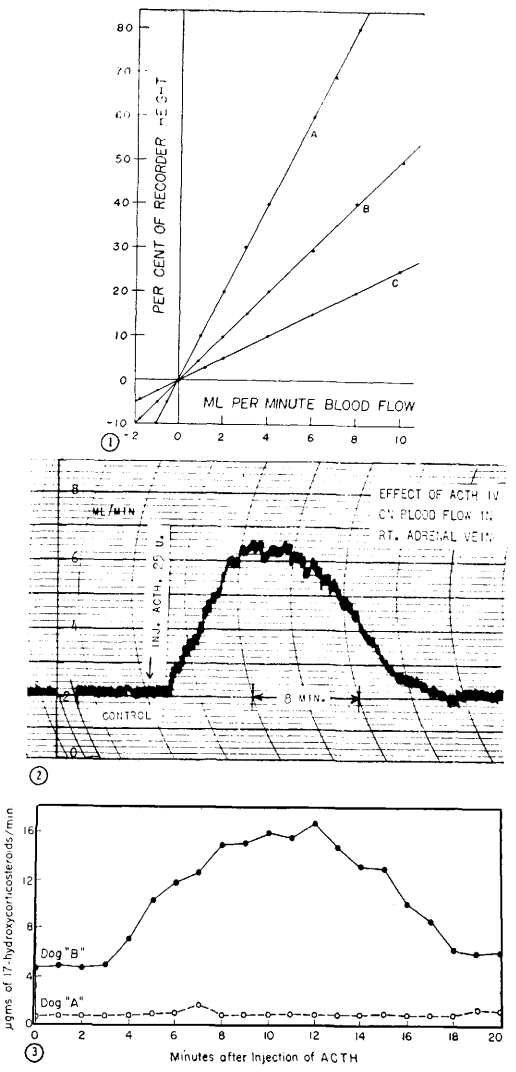

FIG. 1. Calibrations of electromagnetic blood-flow meter, using 3 gain settings shown as plots A, B, and C. Gain setting for these studies approximated plot A.

FIG. 2. A typical adrenal blood flow response to administration of ACTH (25 units) after administration of sufficient DDD (condition A) to prohibit release of 17-hydroxycorticosteroids. This flow response to ACTH was not significantly different from that of dogs untreated by DDD, or from that of dogs treated by DDD in condition B as described.

FIG. 3. Graphic representation of 17-hydroxycorticosteroid response calculated as μg secreted per min. by adrenal of dogs after inj. of 25 units of Armour ACTH lyophilized powder. Dog "A" has been pretreated with 200 mg/kg of DDD once daily for 3 days. Note that low resting rate does not change after ACTH though there occurs a marked increase in blood flow (Fig. 1) and the gland is histologically normal. Dog "B" represents a similar response of a dog pretreated 3 days with 200 mg/kg of DDD and allowed 18 days to recover. This is a normal steroid response to ACTH despite the fact that adrenal shows definite histological changes characteristic of DDD.

In our experience the more potent o,p'- and m,p'-isomers do not have great advantage over commercial preparations in the dog(3). Eight male dogs weighing 10-15 kg were fed 200 mg/kg of DDD powder in gelatine capsules once daily for 3 days. They were then anesthetized with Surital† and the adrenal vein cannulated by the method of Hume and Nelson(4). On the day following operation the dogs were awake and in apparent good condition. They were again lightly anesthetized, the indwelling cannula flushed with heparin-saline solution, and the choker on the caval side of adrenal closed. All blood emerging from the cannula in the lumbo-adrenal vein was effluvent having passed through the adrenal. The dog was then heparinized and adrenal effluvent blood directed through an electromagnetic flow meter(5). To achieve the high flow sensitivity required in these studies, the flowmeter output was taken through a Brush DC amplifier into an Esterline-Angus 1500 ohm, 1 milliampere, recorder by using an impedance matching network. As a result of the modification, for 30 minutes on some occasions, the zero base line was known to shift from 0.1 to 3% of full scale allowing an error of less than 0.3 ml/min in actual measurement. For a flow of 6 ml/minute this entailed a possible error of 5% or less. Since all errors of zero shift were apparent by a zero check, this did not influence our measurements, but it was an inherent factor in the observations. All blood flow measurements reported here were calibrated and rechecked by an actual venous outflow technic through the cannula pickup, both for zero setting and flow responses, rather than chance employment of a synthetic flow system, alone. Representative samples of flowmeter calibrations are shown as 3 plots in Fig. 1. Experimental data were obtained in both DDD-fed and normal dogs by collection of one-minute flow samples of blood into centrifuge tubes which subsequently were analyzed for 17-hydroxycorti-

† Surital kindly provided by Parke Davis and Co.

costeroids by a modification of the Nelson and Samuels(6) method. After about 5 minutes of control flow 25 units of Armour lyophylized ACTH powder (U.S.P.) was dissolved in saline and immediately injected into a peripheral vein. Collection of minute samples of blood was continued for the next 20 minutes as the graphic pattern of blood flow was recorded.

Results. Blood flow results in all 8 dogs were remarkably similar, *i.e.,* within 2-5 minutes blood flow markedly increased to attain a maximum within about 8-15 minutes to 260-400% of control blood flow, whereupon a gradual decline was observed requiring 5-10 minutes to attain pre-ACTH values, a total reaction time of 15 to 30 minutes. A representative tracing of this phenomenon (Dog-A) is shown in Fig. 2. Similar data were observed in control dogs. In contrast, 17-hydroxycorticosteroid secretory rate (0.6 μg/minute) continued unchanged at a basal rate throughout entire period of vascular response (Fig. 3). Thirty minutes after injection of ACTH the dog was killed, adrenals fixed in Zenker's solution, sectioned, stained and examined microscopically.

Five male dogs weighing 10-15 kg were fed 200 mg/kg of dry technical DDD powder in gelatine capsules once daily for 3 days only and 16-18 days later were treated as the dogs in the previous group. When 25 units of ACTH were injected a marked vascular response was obtained in each dog which simulated that of untreated dogs. Blood 17-hydroxycorticosteroids showed the usual normal resting secretory rate of 4.5 μg/minute which increased to 17 μg/minute at the end of 12 minutes. Return to essentially normal secretory rates of 7.1 μg/minute was effected in 20 minutes. This is a normal response as to time and magnitude when compared with 25 normal untreated dogs. Microscopic examination of adrenals from this group of dogs showed a uniform mild-early-atrophic change, consisting of a slight lymphocytic infiltration of the outer layers of the zona fasciculata together with an accumulation of lipids in large vacuoles of the parenchymal cells which pushed the nuclei of many cells to the periphery. Nuclei were small, somewhat pyknotic, and stained deeply basophilic. These are the very definite changes of early response of adrenal cortex to this compound.

Discussion. The foregoing shows that 3 days of administration of DDD to dogs resulted in the adrenals secreting 17-hydroxycorticosteroids at a very low rate, which did not increase with administration of ACTH, while the gland responded to ACTH with the usual increase of blood flow. These glands were histologically "normal" hemodynamically normal, but dysfunctional biochemically. Administration of 3 similar doses of DDD to dogs resulted in adrenals showing early histological change after 18 days on normal diet, yet these glands were secreting 17-hydroxycorticosteroids at a normal rate and responded in a normal fashion to ACTH. The anatomical change in these latter glands did not prevent the usual vascular response, suggesting that site of vascular response to ACTH may be in the vessels of the medulla or in the extracapsular vessels. It also would suggest that ACTH may have an extra "vasodilating component" separate from the "steroid releasing and/or steroid forming component." Such conclusions must be guarded, however, because the ACTH used was the relatively impure clinical preparation and doses were grossly pharmacologic. However, in separate experiments(unpublished), occasional increases up to 150% of control blood flow have been obtained with dog ACTH in doses of 0.005 unit. This would tend to minimize the dose and species problem. The vasodilator effect must be relatively specific for adrenal vasculature because a separate set of studies has been carried out by injecting standardized units of ACTH in the femoral artery of dogs and comparing response with the action of mecholyl. In the periphery, ACTH acts as a mild vasodilator, but its response is of low magnitude and low duration. Femoral flow increased less than 15% (115% of control) and lasted less than 20 seconds with the standardized dosage. Any detectable response in the dog limb is completed in less than 30 seconds. Intravenous injection has less effect on peripheral blood flow response

than intra-arterial injection, as would be anticipated. On the other hand, standardized intravenous injection of epinephrine (1-20 μg) and mecholyl (.1-1.0 μg) produced little or no detectable flow response through the adrenal gland (mecholyl demonstrated a barely discernible flow increase at higher dosages of 10 μg). This readily apparent difference in vascular response from ACTH, epinephrine, and mecholyl in the adrenal vasculature as compared to the periphery deserves a more thorough study.

The histological finding that a "normal" gland, as revealed by microscopic examination of a hematoxylin and eosin preparation, does not secrete 17-hydroxycorticosteroids and is refractory to ACTH raises doubts as to the validity of many conclusions based on only microscopic appearances. It indicates that action of DDD has a latent period and emphasizes the uncertainty of judging the secretory status of the adrenal from microscopic examination. The finding that after 18 days a normal secretory pattern and response to ACTH is present in dog adrenals with cytological change is particularly interesting since Vilar and Tullner(7) have shown that after 4 days treatment with the o,p'-isomer histological changes are apparent, and secretion of corticosteroids is depressed, indicating that secretory capacity recovers while mild histological alteration continues. This delayed histological change from 3 doses of technical DDD has not heretofore been reported.

Conclusions. Technical DDD fed to dogs in 200 mg/kg doses once daily for 3 days resulted in histologically normal adrenal glands which responded to ACTH with the expected increase in blood flow, but demonstrated a low resting secretion rate of 17-hydroxycorticosteroids, which did not increase after administration of ACTH. Dogs fed a similar 3 doses and allowed to continue normal diet for 18 days showed adrenals with definite changes in the cortex which did respond to ACTH with the expected increase of steroid secretion but showed usual increase in blood flow. These data are interpreted to mean that the vasodilating factor of commercial ACTH is separate from steroid formation and/or releasing factor(s) and possibly the vasodilating factor may act on medullary or extracapsular vasculature. Capacity of the cortical parenchymal cells to form and/or release 17-hydroxycorticosteroids, as influenced by DDD, does not parallel histological changes observed in standard hematoxylin and eosin preparations.

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Interaction of Chlorpromazine with Strandin.* (25902)

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LeBlanc(1,2) has shown that chlorpromazine can destroy the mast cell, and has suggested that some of the effects of this drug may be due to release of histamine and serotonin from that cell. The mast cell is also considered to be the site of heparin production(3). During recent study concerned with metachromasy of brain ganglioside, strandin

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