## Inhibition of Steroid-Induced Adrenal Hypofunction. (21290)

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There is considerable evidence to support the view that the administration of adrenal cortical hormones suppresses adrenal function and induces adrenal atrophy in the rat(1-3). Clinical evidence indicates that patients on cortisone therapy also develop a functional and anatomical depression of the glands(4-8). Since it is generally agreed that the depressant effects of cortisone are the result of decreased secretion of ACTH by the anterior pituitary gland and that the adrenal hypofunction results from the lack of endogenous stimulation of the adrenal cortex, it appeared possible to employ a combination of cortisone and ACTH to eliminate or minimize the adrenal hypofunction induced by cortisone. Furthermore, it was felt that the ACTH would probably have a therapeutic action of its own through induced secretion of cortical steroids. Therefore, a study was undertaken to determine the effects of cortisone acetate and hydrocortisone on the adrenals of the intact dog and to determine whether concurrent ACTH administration would eliminate or minimize any adrenal hypofunction produced by the steroid hormones. The estimation of adrenal potential was made by observing the degree of eosinopenia evoked by test doses of ACTH before and after the course of treatment.

It was demonstrated that ordinary therapeutic doses of cortisone acetate and hydrocortisone in the intact dog cause a functional depression of the adrenal cortex which persists to some degree two weeks after cessation of treatment. Concurrent administration of ACTH lessens the steroid-induced adrenal depression, as evidenced by an earlier eosinopenic response in ACTH-steroid-treated animals than in animals receiving the steroid alone.

*Materials and Methods.* The animals used were laboratory acclimated male dogs 13 to 20 kg body weight at the beginning of the experiment. Eosinophil counts were determined by a modification of the technic of Spiers and Meyer(9). The counts were made on venous blood obtained without stasis and prevented from coagulating by addition of heparin, 50 units per ml of blood. *Adrenal response* to ACTH was determined on each dog in the following manner: (1) a blood sample was taken at 10:00 A. M.; (2) the "test dose" of ACTH, 0.5 USP unit per kg body weight in physiological saline, was injected intramuscularly immediately after the bleeding; (3) a second blood sample was taken four hours after the ACTH injection. The eosinopenic response was expressed by per cent difference between the zero-time count and the 4-hour count. An eosinopenic response of a 50% decrease in number over the pre-injection level was considered significant. The "test dose" response was carried out prior to and at intervals after cessation of treatment. Each dog received a single daily 0.5 ml intramuscular injection of the test preparation for a period of 28 days in the cortisone and cortisone-ACTH-treated animals and 21 days in the hydrocortisone and hydrocortisone-ACTH-treated group. Each ml of the preparations used contained the following:

Exp. #1

a) 10 units ACTH
   25 mg cortisone acetate aqueous susp.
b) 25 mg cortisone acetate aqueous susp.
c) 25 units ACTH
   25 mg cortisone acetate aqueous susp.

Exp. #2

a) 10 units ACTH
   25 mg hydrocortisone aluminum monostearate peanut oil gel
b) 25 mg hydrocortisone aluminum monostearate peanut oil gel

Exp. #3

a) 10 units ACTH
   25 mg hydrocortisone
   16% Knox Gelatin
   0.5% phenol
b) 10 units ACTH-pectate
   25 mg hydrocortisone
   16% Knox Gelatin
   0.5% phenol
c) 25 mg hydrocortisone
   16% Knox Gelatin
   0.5% phenol

*Results.* Exp. #1. It will be noted (Table I) that 3 of the 4 animals treated with the mix-

TABLE I. Eosinophil Response to ACTH of Dogs before and after Treatment with Adrenal Steroids Alone or in Combination with ACTH.

| Exp. No. | Preparation and daily dose | Dog No. | % change of eosinophils in response to "test dose" of 0.5 u/kg ACTH: Pre-treatment | Days post-treatment: 2 | 3 | 4 | 5 | 6 | 7 | 8 | 10 | 11 | 12 | 13 | 14 | 15 | 17 | 19 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1a | Cortisone acetate, 12.5 mg; | 1 | 77 | 15 | | | | | 33 | | | | | | 48 | | | |
| | aqueous suspension; | 2 | 45 | 3 | | | | | 11 | | | | | | 41 | | | |
| | once daily/28 days | 3 | 73 | 7 | | | | | 61 | | | | | | 51 | | | |
| | | 4 | 64 | 6 | | | | | 74 | | | | | | 74 | | | |
| 1b | Cortisone acetate, 12.5 mg; | 5 | 61 | 45 | | | | | 30 | | | 49 | | | | 57 | | |
| | ACTH, 12.5 units; aqueous | 6 | 85 | 56 | | | | | 61 | | | 81 | | | | 54 | | |
| | suspension; once daily/28 | 7 | 37 | 6 | | | | | 15 | | | 23 | | | | 44 | | |
| | days | 8 | 78 | 58 | | | | | 53 | | | 80 | | | | 54 | | |
| 1c | Cortisone acetate, 12.5 mg; | 9 | 90 | 60 | | | | | 65 | | | | | | 82 | | | |
| | ACTH, 5 units; aqueous | 10 | 70 | 24 | | | | | 41 | | | | | | 67 | | | |
| | suspension; once daily/ | 11 | 70 | 5 | | | | | 55 | | | | | | 65 | | | |
| | 28 days | 12 | 39 | 5 | | | | | | | | | | | 38 | | | |
| 2a | Hydrocortisone, 12.5 mg; | 13 | 68 | | 30 | | 4 | | | 1 | 3 | | 44 | | | | | 45 |
| | aluminum monostearate | 14 | 59 | | 2 | | 32 | | | +70 | 10 | | 11 | | | | | 24 |
| | peanut oil gel; once | 15 | 60 | | 16 | | 52 | | | 17 | 52 | | | | | | | 61 |
| | daily/21 days | 16 | 88 | | 31 | | 37 | | | 51 | 38 | | | | | | | 40 |
| | | 17 | 58 | | 3 | | 57 | | | 28 | 26 | | | | | | | 48 |
| 2b | Hydrocortisone, 12.5 mg; | 18 | 52 | | 56 | | 63 | | | | | | | | | | | |
| | ACTH, 5 units; aluminum | 19 | 88 | | 89 | | 85 | | | | | | | | | | | |
| | monostearate peanut oil | 20 | 85 | | 84 | | 64 | | | | | | | | | | | |
| | gel; once daily/21 days | 21 | 64 | | 33 | | 53 | | | 17 | 43 | | | | | | | |
| 3a | Hydrocortisone, 12.5 mg; | 22 | 65 | | | 82 | | 15 | | 29 | | 20 | | 53 | | | | |
| | 16% gelatin; once daily | 23 | 63 | | | 50 | | 32 | | 41 | | 73 | | | | | | |
| | /21 days | 24 | 63 | | | 8 | | 34 | | 35 | | 42 | | 42 | | 51 | | |
| | | 25 | 57 | | | 44 | | 41 | | 16 | | 36 | | 42 | | 57 | | |
| | | 26 | 38 | | | 12 | | 12 | | 25 | | 23 | | 30 | | 35 | 48 | |
| 3b | Hydrocortisone, 12.5 mg; | 27 | 72 | | | 58 | | 21 | | 69 | | 42 | | 52 | | | | |
| | ACTH, 5 units; 16% gel- | 28 | 72 | | | 46 | | 51 | | 36 | | 42 | | 43 | | | | |
| | atin; once daily/21 days | 29 | 60 | | | +5 | | 25 | | 26 | | 25 | | 26 | | 31 | 33 | 35 |
| | | 30 | 72 | | | 22 | | 44 | | 64 | | 72 | | 64 | | | | |
| | | 31 | 20 | | | 50 | | 50 | | 50 | | 50 | | | | | | |
| 3c | Hydrocortisone, 12.5 mg; | 32 | 61 | | | 43 | | 19 | | 40 | | 42 | | 41 | | | | |
| | ACTH-pectate, 5 units; | 33 | 50 | | | 55 | | 53 | | 56 | | | | | | | | |
| | 16% gelatin; once daily | 34 | 55 | | | 57 | | +8 | | 43 | | 39 | | 63 | | | | |
| | /21 days | 35 | 51 | | | 43 | | 37 | | 70 | | 52 | | 70 | | | | |
| | | 36 | 37 | | | 51 | | 52 | | 57 | | 43 | | 57 | | | | |

ture had a normal response to the "test dose" of ACTH by the second day post-therapy. When the dose of ACTH was decreased to 5 units per day, the post-injection sensitivity of the ACTH-steroid-treated animal was inferior to that of the animal receiving the greater dose and was not different from the animal that received cortisone alone.

*Exp. #2.* Three of the 4 ACTH-hydrocortisone-treated animals showed a normal eosinopenic response 3 days post-injection, while none of the hydrocortisone-treated animals showed such a response. The adrenal depression of the hydrocortisone-treated animal persisted from 10 to 20 days after hormonal treatment.

*Exp. #3.* Animals were injected with hydrocortisone alone or in combination with ACTH, or ACTH-pectate, in 16% gelatin. Of the 5 animals that received hydrocortisone alone, 2 were not affected by the 21-day therapy, the other 3 showed depressed adrenal function. Although one of the 3 showed an adequate response to the "test dose" on the fourth day post-injection, such a response did not reoccur until the thirteenth day post-therapy. Complete adrenal recovery in these animals occurred by the fifteenth-seventeenth day post-steroid treatment.

Of the animals that received ACTH, or ACTH-pectate, with the hydrocortisone, 3 animals of each group showed an eosinopenia by the fourth day post-therapy, and complete recovery of all but one animal, #29, was obtained by the eighth day. Animal #29 never responded to the ACTH "test dose" which may have resulted from refractoriness to exogenous ACTH.

*Discussion.* It is apparent that the adrenals of the dog are rendered hypofunctional by exogenous adrenal steroid. This functional depression is not only reversible but appears to be prevented by the simultaneous injection of ACTH.

The adrenal response to ACTH after cortisone or hydrocortisone therapy is not unlike that described by Engleman *et al.*(10), who demonstrated in the human a delay in adrenal responsiveness to corticotropin during treatment with cortisone.

Although response of the dog adrenal gland to exogenous ACTH after simultaneous corticotropin-cortisone or hydrocortisone therapy supports the belief that the adrenal gland has a greater potential than the adrenal gland of the steroid-treated animal, definite evidence for the potential of the adrenal-pituitary axis is not now available. Experiments now in progress are designed to demonstrate whether the steroid-induced adrenal-pituitary axis lethargy can be prevented by simultaneous injection of ACTH with the steroid by subjecting the animals to a stress procedure after hormone treatment.

*Summary.* (1) Adrenocortical function of the dog is suppressed by administration of therapeutic doses of cortisone or hydrocortisone. (2) The suppression is prevented by simultaneous administration of ACTH.

1. Ingle, D. J., Higgins, G. M., and Kendall, E. C., *Anat. Rec.,* 1938, v71, 363.

2. Winter, C. A., Silber, R. H., and Stoerk, H. C., *Endocrinology,* 1950, v47, 60.

3. Stebbens, R. B., *Fed. Proc.,* 1949, v9, 345.

4. Sokoloff, L., Sharp, J. T., and Kaufman, F. H., *Arch. Int. Med.,* 1951, v88, 627.

5. Sprague, R. G., *Am. J. Med.,* 1951, v10, 567.

6. McIntosh, H. W., and Holmes, C. B., *Lancet,* 1951, v261, 1061.

7. Salassa, R. M., Bennett, W. A., Keating, F. R., and Sprague, R. H., *J. Am. Med. Assn.,* 1953, v152, 1509.

8. Fraser, C. G., Preuss, F. J., and Bigford, W. D., *ibid.,* 1952, v149, 1542.

9. Speirs, R. S., and Meyers, R. K., *Endocrinology,* 1949, v45, 403.

10. Engleman, E. P., Krupp, M. A., Johnson, H. P., Welch, J. S., Wrenn, H. T., and King, W. R., *Arch. Int. Med.,* 1953, v91, 1.

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