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Comparison of Eosinophil and Circulating 17-Hydroxycorticosteroid Responses to Epinephrine and ACTH.* (19960)

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Since relatively pure adrenocorticotropin (ACTH) has become available, the eosinophil response test has emerged as a commonly used and simple method of assessing adrenocortical function. The usual response to the injection of ACTH, Compound E, Compound F. epinephrine or insulin, and to non-specific stress stimuli is characterized by a reduction in the number of circulating eosinophils(1). This eosinopenic response to the administration of ACTH has been used as a major criterion indicating an intact adrenal cortex, with the absence of eosinopenia interpreted as indicating adrenal cortical insufficiency. It has been assumed that in order to elicit an eosinopenic response to epinephrine or non-specific stress stimuli an intact anterior pituitary and adrenal cortex must be present. The postulation has been advanced that these stimuli act through the hypothalamus upon the anterior pituitary causing release of ACTH, which in turn stimulates the adrenal cortex to secrete its hormones(2). The hormones secreted by the adrenal cortex which are generally believed to effect the eosinopenia are the 17-hydroxycorticosteroids(3-7). However, because epinephrine eosinopenia occasionally has been induced in states of impaired function or absence of the adrenal cortex(1,8) the validity of this assumption has been questioned.

Recent development of methods allowing direct measurement of circulating adrenal hormones provides a technic permitting a more definitive evaluation of the relationship between eosinophil and adrenal steroid responses to a stimulating agent. In the present study the circulating plasma concentration of 17-hydroxycorticosteroids has been measured and the effects of ACTH or epinephrine injection on the levels of these steroids and of eosinophils have been compared in children. It should be emphasized that the influence of specific disorders on the type of adrenal response is of secondary interest only, and that the point of primary concern is the comparison of the responses of circulating eosinophils and 17-hydroxycorticosteroids.

Methods. Healthy children and children with various disorders have been used in this study. Responses of eosinophils and 17-hydroxycorticosteroids to the injection of .01 mg/K of epinephrine hydrochloride or 25 I.U. ACTH were studied. Eosinophils were determined by the method of Randolph(9) and circulating plasma 17-hydroxycorticosteroids by Nelson and Samuels' modification(10) of their method for whole blood(11). Total eosinophil counts were done at 0, 1, 2 and 4 hours. The minimum value obtained, expressed as per cent decrease from the control value, is used in the data of the tables. The circulating plasma 17-hydroxycorticosteroid values were obtained at 0 and 2 hours, because

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TABLE I. Mean Response of 17-Hydroxycorticosteroids and Eosinophils to Epinephrine.

Group	Eosinophils: No. pts.	Eosinophils: No. response tests	Eosinophils: % change, mean ± SE_M	17-hydroxycorticosteroids (μg %): No. pts.	17-hydroxycorticosteroids (μg %): No. response tests	17-hydroxycorticosteroids (μg %): Mean 0 hr value	17-hydroxycorticosteroids (μg %): Mean 2 hr value	17-hydroxycorticosteroids (μg %): Change, mean ± SE_M
Congenital adrenal hyperplasia	5	17	−59 ± 3.5	5	8*	6.6	7.8	1.2 ± 1.56
Rheumatic diseases	13	19	−21 ± 9.9	13	18	15.5	15.4	−.1 ± 2.73
All cases except congenital adrenal hyperplasia	41	48	−52 ± 4.7	30	35	11.7	12.6	.9 ± 1.37

* Some of these patients were receiving maintenance cortisone therapy at the time of these measurements. Since the technic employed does not differentiate between endogenous and exogenous 17-hydroxycorticosteroids the values, which are higher than expected, probably reflect measurements of the exogenous hormone(13).

TABLE II. Mean Response of 17-Hydroxycorticosteroids and Eosinophils to ACTH.

Group	Eosinophils: No. pts.	Eosinophils: No. response tests	Eosinophils: % change, mean ± SE_M	17-hydroxycorticosteroids (μg %): No. pts.	17-hydroxycorticosteroids (μg %): No. response tests	17-hydroxycorticosteroids (μg %): 0 hr	17-hydroxycorticosteroids (μg %): 2 hr	17-hydroxycorticosteroids (μg %): Mean change, μg %
Congenital adrenal hyperplasia	5	35	−26 ± 4.9	5	12*	6.3	6.3	0 ± 1.23
Rheumatic disease	11	18	−36 ± 8.4	13	19	15.8	41.8	26 ± 4.71
All cases except congenital adrenal hyperplasia	58	72	−49 ± 3.7	19	30	13	36.5	23.5 ± 3.64

* These levels also include those obtained during periods of cortisone administration and thus reflect the influence of the exogenous hormone. (See footnote, Table I.)

concentrations of these steroids reach peak values within 2 hours after injection of ACTH(12).

Results. Table I shows the responses of eosinophils and 17-hydroxycorticosteroids to epinephrine in different groups of children. In none of the groups shown is there any significant mean elevation in the concentration of the 17-hydroxycorticosteroids in response to epinephrine. In contrast to this there is a satisfactory eosinophil response in all groups except that group with rheumatic disease. In this latter group the mean reduction of eosinophils is only 21 ± 9.9%.

The group of patients with congenital adrenal hyperplasia is separated from the other groups in this table because these patients apparently are unable to produce elevations of their 17-hydroxycorticosteroid concentrations even in response to ACTH injection (13). However, even in the group excluding these patients with congenital adrenal hyperplasia there is no response of 17-hydroxycorticosteroids to epinephrine.

The general responses of eosinophils and of 17-hydroxycorticosteroids elicited by ACTH in different groups of patients are illustrated and compared in Table II. In patients with congenital adrenal hyperplasia there is a mean 26% reduction of eosinophils but no elevation of 17-hydroxycorticosteroids. In the group of patients with rheumatic disease there is a most satisfactory elevation of 17-hydroxycorticosteroids in response to ACTH but an average eosinophil reduction of only 36%. The average of all ACTH response tests done, with the exception of the adrenal hyperplasia group, shows an eosinophil reduction of 49% and an adequate elevation of the 17-hydroxycorticosteroid level.

In Table III selected cases are presented to show that an eosinopenic response to ACTH may occur in the absence of 17-hydroxycorticosteroid elevations. In all of these children there is an adequate eosinopenic response without a corresponding elevation of 17-hydroxycorticosteroid concentrations.

Table IV shows data of 4 children whose

TABLE III. Response to ACTH. Illustrative cases (8 patients) showing adequate eosinopenia but poor 17-hydroxycorticosteroid response.

Eosinophils (% change)	17-hydroxycorticosteroids (μg %) 0 hr	2 hr
−64	11	5.4
−58	7.7	6.5
−45	13.9	11.1
−42	11.9	7.8
−69	2.5	3.1
−66	17.4	13.3
−66	13.6	14.4
−60	7.6	10
Mean −59%	10.7	9

TABLE IV. Response to ACTH. Illustrative cases (4 patients) showing poor eosinophil response with adequate 17-hydroxycorticosteroid elevation.

Eosinophils (% change)	17-hydroxycorticosteroids (μg %) 0 hr	2 hr
+33	12.6	55
+37	10.7	29.9
−12	5.2	63.5
−14	13.5	50.5
Mean +11%	10.5	49.7

responses to ACTH are opposite to those shown in Table III. Here are seen, on simultaneous determinations, poor eosinophil responses but adequate 17-hydroxycorticosteroid responses to ACTH. Thus, even following the administration of ACTH, under certain circumstances an eosinopenia need not be accompanied by a rise in 17-hydroxycorticosteroids; conversely, 17-hydroxycorticosteroid elevations need not be accompanied by an eosinopenia.

Discussion. The following evidence has been used to support the general belief that ACTH and epinephrine induced eosinopenia is mediated by the secretion of 17-hydroxycorticosteroids by the adrenal cortex: 1) Administration of ACTH, 17-hydroxycorticosterone or cortisone to the intact animal or man causes an eosinopenic response, whereas administration of desoxycorticosterone acetate does not(4), 2) *in vitro* incubation of blood with cortisone results in an eosinopenia(5), and 3) in man the adrenal cortical hormone secreted consists primarily of 17-hydroxycorticosterone(6,7). This evidence is highly suggestive, but confirmation by the use of direct technics is lacking.

In general, following the injection of ACTH in human subjects there is an adequate response of both eosinophils and circulating 17-hydroxycorticosteroid plasma concentrations, a reduction of the former and an elevation of the latter. However, certain exceptions to this generalization have been observed; some patients have exhibited a satisfactory eosinopenia without a concomitant increase in circulating 17-hydroxycorticosteroid concentrations while others have shown no eosinopenic response despite adequate elevations of 17-hydroxycorticosteroids. On the basis of these exceptions, the question is raised whether the eosinopenic response to ACTH *need* be mediated by an increased secretion of 17-hydroxycorticosteroids by the adrenal cortex.

In contrast to the generally adequate response of 17-hydroxycorticosteroids to ACTH, usually no elevation of these steroids occurs in response to epinephrine, despite an adequate eosinopenia. On the basis of these data there is a strong suggestion that the eosinopenia which occurs in response to epinephrine *is not mediated* by an increased circulating concentration of 17-hydroxycorticosteroids.

Summary. 1. A comparison of the responses of eosinophils and of circulating 17-hydroxycorticosteroid plasma concentrations is presented. 2. In general, responses of eosinophils and 17-hydroxycorticosteroids to the injection of ACTH are adequate, with a reduction of the former and an elevation of the latter. 3. Certain exceptions to this generalization are noted. On the basis of these exceptions the question is raised whether the eosinopenic response to ACTH need be mediated by the mechanism previously accepted. 4. There is a strong suggestion that the eosinopenia in response to epinephrine is not mediated by an increased circulating concentration of 17-hydroxycorticosteroids secreted by the adrenal cortex.

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Effect of a Series of Ethylenimine Derivatives against Metastasizing Mammary Adenocarcinoma of the Rat. (19961)

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Two N-ethylene substituted phosphoramides were reported by Buckley *et al.*(1), Burchenal *et al.*(2) and Lewis *et al.*(3) to be as effective as Triethylene melamine in retarding the growth of a number of experimental tumors. The inhibitory effect of a large series of ethylenimine derivatives against a wide tumor spectrum has been reported independently by this laboratory(4,5).

The purpose of the studies reported here was to select ethylenimine derivatives* which markedly inhibit the metastasizing tendency of the mammary adenocarcinoma of the Hooded Brown Rat.

Materials and methods. Approximately 2 x 2 mm fragments of mammary adenocarcinoma, R2426, were implanted aseptically by trocar into the subcutaneous tissue of the right side of inbred Hooded rats weighing 100-120 g. The rats were weighed, housed individually and arranged 7 females and 8 males per group. Treatments were started 48 hours after implantation. Included in each experiment were one saline-treated group that served as a negative control, and one Triethylene-melamine-treated group that served as a positive control. A synthetic diet(5) and water were given *ad libitum*. The compounds were prepared in buffered saline and the concentrations used as indicated in Table I. Injections were administered intraperitoneally daily or every other day in a volume of 0.5-1.0 ml. The period of treatment was approximately 42 days. At the end of this time all animals were sacrificed and the lungs excised. Observations were made on the gross metastatic lesions of the lungs and a section† was fixed for histological study. The inhibitory effects on the primary tumor have been reported previously(5).

Results. The pooled results of several experiments in Table I indicate that the 5 compounds at the levels tested markedly reduce the incidence of metastases to the lungs. Fig. 1 illustrates the effect of these compounds on the gross metastatic tumors of the lung. The control lungs on the left show the typical metastatic growths. On the right is shown typical treated lungs with no visible metastatic lesions. Histologically the control lungs show tumor masses so extensive that the normal architecture of the lungs has been nearly destroyed. The lungs of the treated animals

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