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Relative Biological Activity of Some Adrenal Cortical Steroids as Determined by Eosinopenic Test.* (20522)

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Previous studies have demonstrated that any stress which will stimulate adrenal cortical function will cause a fall in the number of circulating polymorphonuclear eosinophilic leukocytes(1,2). Recently, this response of the circulating eosinophils to adrenal cortical stimulation was developed by Speirs and Meyer into a quantitative and specific method for estimating relatively small amounts of corticoids(3-5). The present paper describes the biological activity of various steroids as determined by the Speirs-Meyer test. Several points of technic are also discussed.

Methods. F_1 hybrids of C_{57} Brown female and C_{57} Black male mice were adrenalectomized and implanted with 11-desoxycorticosterone acetate (D.C.A.). The mice were housed in metal cages, and were used for the first time in the assay after an interval of at least 4 days after the operation. On the morning of the test they were injected subcutaneously with 20 μg of epinephrine hydrochloride. Four hours later, a sample of tail blood was taken, immediately after which the test sample was injected. Three hours later another sample of tail blood was drawn. Eosinophil counts of the blood were made in the Fuchs-Rosenthal or in the Speirs-Levy hemocytometer, and the percent fall in the eosinophil count in second sample relative to the first was calculated. The mean percent fall in a group of about 6 mice was used as a measure of the activity of the substance tested. Other details of the method are supplied in the paper of Speirs and Meyer(3-5). Of the first 98 mice who were adrenalectomized and pre-treated with epinephrine only 36 had counts of more than 30 cells per 2 chambers of the Fuchs-Rosenthal hemocytometer, that is, more than 93 cells per cubic millimeter of blood. Inasmuch as Speirs' suggestion of discarding such "low count" mice was followed(5), this meant that a large proportion of mice could not be used in the test. The solution of the *problem of the "low count"* mice was found to depend in part on how much D.C.A. was implanted subcutaneously at the time of adrenalectomy. At first,

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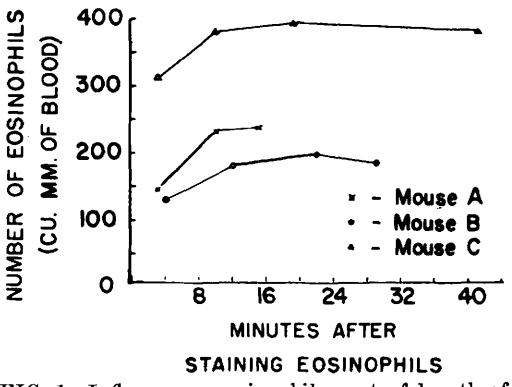
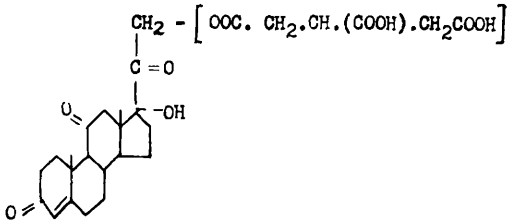


FIG. 1. Influence on eosinophil count of length of time cells were in contact with the diluent.

when a 2½ mg pellet was implanted into each mouse, the rather large proportion of "low count" mice just mentioned was the result. However, when a 5 mg pellet was used 62 out of the next 97 mice utilized had more than 93 eosinophils per cu millimeter. In these experiments, 2-3 month old mice were used. Recently, 5-6 month old mice were used, and the incidence of low count mice was further reduced to about 10% of mice adrenalectomized and implanted with 5 mg D.C.A. The mouse *blood in the Speirs-Myer* test must be in contact with the diluent for at least 10 minutes before stabilized counts can be obtained. Counts taken only 3 to 4 minutes after diluting blood were consistently lower than those counted about 10 minutes later (Fig. 1). The diluent used was that of Speirs and Meyer(3).

Corticosterone (Kendall's Compound B), 11-dehydrocorticosterone acetate (Kendall's Compound A acetate), and 11-desoxy-17-hydroxycorticosterone (Reichstein's Substance S) were tested for activity and compared with that of 11-dehydro-17-hydroxycorticosterone (Kendall's Compound E). All compounds were administered subcutaneously in 0.10-0.25 cc of 10% ethanol solution and tested at several dose levels. Sodium cholate was also tested at 2 dose levels in a few animals. In another experiment, the eosinopenic activity of 11-dehydro-17-hydroxycorticosterone-21-aldehyde (Compound E aldehyde) and of 11-dehydro-17-hydroxycorticosterone-tricarballylate (Compound E tricarballylate) were compared with that of 17-hydroxycorticosterone (Kendall's Compound F). Compound F was administered in solution containing not more than 0.75% methanol. Compound E aldehyde was injected as an aqueous solution containing 15 mg % or less of dextrose, while Compound E tricarballylate was given as an aqueous solution containing not more than 48 mg % mannitol. The structural formula of Compound E tricarballylate is



The 3 compounds were administered via the tail vein at several dosage levels but in the same injection volume—0.20 cc. In this experiment the doses were selected so that the calculations for the factorial analysis according to Bliss and Marks could be carried out (6). The *relative potencies* of Compound E, Compound A acetate, and Compound B, and of Compound F, Compound E aldehyde, and Compound E tricarballylate were estimated by Irwin's method(7). The departure from parallelism of the regression lines of the above steroids was tested by a χ^2 test.

The data of *Compound F, Compound E aldehyde, and Compound E tricarballylate* were also submitted to a factorial analysis, as described by Bliss and Marks(6), to test for the significance of the differences between steroids, for the significance of the slope of the dosage-response curve, for departure from parallelism, for curvature of the combined curve and for opposed curvature of the separate curves. For this test, equal numbers of animals at each dosage of each compound were required. Inasmuch as some groups had more than 6—the lowest number per group—data were removed from those groups with more than this number. However, the values removed were selected so that the mean, standard error and relative potency of the effected data were only very slightly and insignificantly altered by the removal.

Results. Compounds A acetate and B were

TABLE I.
Relative Eosinopenia Activities of Some Adrenal Corticoids, and Precision of the Test.*

Compound	No. of mice	Slope (b)	Index of precision (λ)	Potency ratio	Range of potency (.95%)
Compound E	28	+ 73.67	.256	100	—
" B	61	+ 65.37	.271	11	3 to 14
" A acetate	19	+102.72	.180	12	1 to 22
" F	19	+ 64.33	.402	100	—
" E aldehyde	20	+ 92.32	.337	39	26 to 58
" E tricarballylate	28	+100.37	.337	11	8 to 16

* The potency ratios of Compounds A acetate and B were expressed in terms of Compound E while those of Compounds E aldehyde and E tricarballylate were expressed in terms of Compound F.

TABLE II. Percent Fall in Circulating Eosinophil Cells Produced by Various Steroids in Adrenalectomized Mice. Pre-treated with Epinephrine.

Compound	Amt, μg	No. of mice	% fall in eosinophils, mean ± stand. error
Kendall's Compound E	1.3	5	27 ± 8
	1.6	8	36 ± 10
	2.8	7	51 ± 6
	4.6	8	69 ± 4
Kendall's Compound A acetate	7.5	7	9 ± 3
	10.0	6	26 ± 12
	30.0	6	72 ± 7
Kendall's Compound B	4.3	7	8 ± 5
	4.5	6	1 ± 1
	9.0	7	19 ± 8
	15.0	10	34 ± 7
	22.5	6	45 ± 5
	30	7	48 ± 10
	41.7	12	66 ± 6
	65	6	87 ± 4
Reichstein's Substance S	50	4	4 ± 4
	100	7	2 ± 1
Sodium cholate	2000	2	0 ± 0
	4000	2	0 ± 0
Kendall's Compound F	1.5	6	35 ± 11
	3.0	6	56 ± 8
	6.0	7	74 ± 5
Compound E aldehyde	3.0	7	16 ± 8
	6.0	6	51 ± 11
	12.0	7	72 ± 4
Compound E tricarballylate	12.0	11	22 ± 7
	24.0	9	50 ± 10
	48.0	8	82 ± 3

found to have about 12 and 11%, respectively, the eosinopenic activity of Compound E (Table I). Substance S and sodium cholate were inactive at the dosages tested (Table II). The dosage-response curves of Compounds E, A acetate and B showed a significant positive slope ($P < 0.001$), and no significant departure from parallelism between Compounds E and A acetate, or E and B.

Compound E aldehyde and Compound E tricarballylate had 39% and 11%, respectively, the activity of Compound F (Table I). That Compound E aldehyde was less active than Compound F was apparent from the fact that 3, 6, and 12 μg, respectively, of the former were required to produce an eosinopenia comparable to that obtained with 1.5, 3, and 6 μg, respectively, of the latter, while even more, that is 12, 24, and 48 μg, respectively, of Compound E tricarballylate were necessary to produce the same degree of eosinopenia (Table II). The dose-response curves of these 3 corticoids showed no significant departure from parallelism, indicating that the range of doses was in a useful portion of the curve. In all 3 steroids there was an insignificant amount of curvature and of opposed curvature.†

Discussion. The values obtained for Com-

† The solvents in which the hormones were administered did not appear to be eosinopenically active. Thus, the 10% ethanol solution showed little or no activity when administered with Substance S or with 4.5 μg of Compound B. Similarly, little or no eosinopenia occurred when a 15 mg% dextrose solution containing 1.5 μg of Compound E aldehyde was assayed or a 48 mg% mannitol solution containing 6 μg of Compound E tricarballylate was administered. Only when the same volumes of these solvents containing larger amounts of these corticoids were administered did an eosinopenia occur. Therefore, the solvents themselves were inactive. The eosinopenic inertness of the above solvents agrees with Speirs and Meyer's observation that of a wide variety of substances, including over 60 steroids, only solutions containing certain corticoids with an oxygen at C_{11} and a double bond at C_{4-5} produce an eosinopenia in epinephrine-pretreated adrenalectomized mice.

pound E in the present study were very similar to those reported by Speirs and Meyer(4). Thus, they reported a mean percent fall in eosinophils of 81, 44, and 24, 3 hours after 6, 3, and 1 μg, respectively, of Compound E were administered. Utilizing the data obtained for Compound E in the present study (Table II), the estimated mean percent fall in eosinophils at 3 hours for 6, 3, and 1 μg of Compound E was 77, 55, and 20. Similarly, Speirs and Meyer reported a 79, 60, and 13% decline in circulating eosinophils 3 hours after the administration of 6, 3, and 1 μg, respectively, of Compound F. For the same doses of Compound F, the results in similarly treated mice in our laboratory were 74, 56, and 28%, respectively. Thus, the agreement between these results was satisfactory, especially in view of the fact that each laboratory used a different vehicle for administering these hormones and also, in the case of Compound F, a different route of administration. Thus, Speirs and Meyer injected Compound F subcutaneously, while we administered it intravenously. Therefore, a comparison of the relative eosinopenic activities of the corticoids in the present study would appear to be valid even though some were administered under the skin and others via tail vein.

Of all the compounds so far tested for their eosinopenic effect by Speirs and Meyer and by this laboratory, Compound E, Compound E acetate, and Compound F were the most active. Any alteration in the structure of these steroids has so far resulted only in loss of eosinopenic activity. Thus, saturation of the 3 α, β unsaturated ketone in Compound E to give Kendall's Compound G completely destroyed this activity(4) as did also removal of the C_{11} oxygen from Compound E (see Substance S, Table II). Removal of the 17-hydroxyl from the Compound E acetate or Compound F resulted in a considerable but not total loss of eosinopenic activity (see Compounds A acetate and B, Table II). Oxidation of the C_{21} alcohol to an aldehyde also resulted in a reduction of eosinopenic activity although less so than the other changes mentioned (see Compound E aldehyde, Table II). Finally, although the acetate ester of Compound E was as active as free Compound E(4), Compound E tricarballylate appeared to be less active (Table II) even if allowance was made for the fact that only 2/3 of the molecular weight of Compound E tricarballylate consisted of Compound E.

The order of the potency of the adrenal cortical steroids as measured by the Speirs-Meyer eosinopenic test was very similar to that found by other methods, which measure some aspect of carbohydrate metabolism (11,12) or work performance(13); that is, Compounds E and F were more active than Compounds A, B and Substance S. Furthermore, Compounds A and B were found to have the same order of biological activity, whether investigated by an "eosinopenia" test (Table I), or by a "sugar activity" test (11,12,14,15). Also, Substance S showed little or no biological activity by the Speirs-Meyer test (Table II), by the "sugar activity" or "work performance" assays(16,17). Finally, while in the eosinopenia test, E was as active as F(4,Table II), or even more active(18), F had the greater efficacy in assays which measure sugar activity(11,13) or work performance(19), and in tests which measured a variety of metabolic and morphologic criteria(20).

The indices of precision, λ, were higher for the Speirs-Meyer test than for many other bio-assays of adrenal cortical steroids (Table I) but it had the advantage of being able to measure smaller amounts of the sugar-active corticoids than most other corticoid bio-assays(8). The importance of this fact was observed in a recent study which revealed that rat adrenals produce corticoids *in vitro*, and that corticotropin, added *in vitro*, stimulated this production(9,10). The amount of corticoids produced by the rat adrenals *in vitro* was too low to be detected by most of the other bio-assays but was within the range of the Speirs-Meyer method.

Summary. The eosinopenia test of Speirs and Meyer for determining small amounts of adrenal cortical steroids was found to yield dependable results in our laboratory. A series of corticoids examined for their eosinopenic effect had the following order of activ-

ity: Compound E and equally-active Compound F were most efficacious in reducing the number of circulating eosinophils. Compound E aldehyde was less active, while Kendall's Compound A acetate, Kendall's Compound B and Compound E tricarballylate were still less potent. Reichstein's Substance S had no eosinopenic activity even though it was administered in larger amounts than the other compounds. Except for Substance S, the other corticoids tested showed a significant positive regression of percent fall of circulating eosinophils on the logarithm of the administered dose within the limits of the dosages tested. The slopes of the linear regressions of these corticoids were also more or less similar.

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Immobilization of *Schistosoma mansoni* Miracidia by Immune Serum.* (20523)

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Immobilization of ciliates and free living flagellates by antibodies is well known(1,2). This report concerns the development of an immobilizing factor for the ciliated larval stage (miracidium) of *Schistosoma mansoni* by experimentally infected animals. This factor appears in the serum at about the same time in the course of the infection as do the capacities to form a precipitate around the cercariae (infective larval stage)(3), to agglutinate the cercariae(4), and to form a "hood" around the cercariae(5). It is recognized that these 3 reactions and the one here described may be caused by the same antibody.

Material and methods. The miracidia used in the immobilization tests were obtained from the livers of infected hamsters. A suspension of the infected liver in 0.15 *M* NaCl was prepared by maceration in the Waring Blender for 30-60 seconds; this suspension was allowed to settle in a sedimentation cone for ½ hour. The sediment was suspended

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