Lack of Correlation of Eosinopenic and Adrenal Ascorbic Acid-Depleting Activities of Various Adrenocorticotropic Preparations.* (19864)

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Evidence has been presented(1-3) suggesting that eosinopenic activity (EA) is not directly correlated with the adrenal ascorbic acid-depleting activity (AAA) of various ACTH preparations. The following experiments present additional evidence that EA and AAA are not directly correlated, and further, that marked reduction of the AAA of ACTH preparations as produced by alkali-heat treatment does not proportionately inactivate the EA.

Methods. Normal and hypophysectomized male rats of the Long-Evans strain, 60-80 days of age, were employed. The hypophysectomized animals were used for eosinophil counts during the first 7 postoperative days, the animals being used repeatedly at daily intervals. Normal animals were reused at weekly intervals. Direct eosinophil counts were taken from tail vein blood using the diluent described by Speirs and Meyer(4). The blood was diluted 1:20, and eosinophil counts were made on two chambers of a Fuchs-Rosenthal hemocytometer (0.2 mm deep). An initial count was taken, followed immediately by intraperitoneal injection of the solution to be tested and a second count was taken four hours later. All animals with initial eosinophil counts lower than 100/mm^3 were discarded. Care was taken to control environmental and temporal variables in order that data from all experimental groups could be directly compared. Adrenal ascorbic acid-depletion activity in hypophysectomized rats was determined for each ACTH preparation by the method of Sayers *et al*(5) and all activities are expressed in terms of the U.S.P. standard. The ACTH preparations tested were prepared by methods previously described, a reference number for each preparation being given in Tables I and II. In addition, two ACTH preparations, L 2317 M and L 2337 M, were each treated with either HCl or NaOH as follows: a) ACTH was dissolved in 0.1 N NaOH, heated immediately on the boiling water bath for 10 minutes, immediately brought to pH 5 with HCl, and diluted to volume with 0.9%, *i.e.*, NaCl, and b) ACTH was dissolved in 0.2 M HCl, refluxed for 3 hours on the boiling water bath, brought to pH 5 with NaOH, and diluted to volume with 0.9% NaCl.

Results. The data presented in Table I for the normal rat show that ACTH preparations of widely different AAA may exhibit EA of such varying degrees as to suggest the existence of an inverse relationship between AAA and EA. On the other hand, the results for the same preparations assayed for EA in the hypophysectomized rats show (Table I) even greater discrepancies between EA and AAA suggesting that non-specific responses in normal rats may completely vitiate conclusions drawn from eosinophil assay of ACTH in normal rats.

Table II summarizes data which demonstrate that the treatment of ACTH preparations with alkali-heat under the prescribed conditions markedly reduces the AAA of the ACTH preparations, but does not alter the EA in hypophysectomized rats at the dose level tested. In order to demonstrate that the 2.0 mg dose level of NaOH treated-ACTH was not so excessive as to mask a reduction of EA of this preparation, the original material (L 2337 M) was tested in a separate group at the 0.1 mg level which was calculated to approximate the AAA unitage of the NaOH-heat treated ACTH preparation. It was found that

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TABLE I. Comparison of Eosinopenic and Adrenal Ascorbic Acid-Depleting Activity (AAA) of Various ACTH Preparations in Normal (N) or Hypophysectomized (H) Rats.

Preparation tested*	Reference No.	No. of rats	Type of rat	Mean† eosinophil change, % ± S.E.	Estimated AAA depleting activity, IU/mg
Control groups					
Saline		62	N	—17 ± 5	
		46	H		
Exp. ACTH groups					
L 2262 A	8	13	N	—66 ± 7	.2
		22	H	—31 ± 9	
2010 M	7	13	N	—53 ± 9	1
2262 B	8	9	N	—49 ± 9	30
2269 B	8	4	N	—39 ±14	120
		5	H	+79 ±36	
2170 C	9	5	H	—13 ± 7	7

* All preparations tested at 1 mg dose level after single intrap. inj. except L 2170 C which was tested at the 5 mg level.

† The mean initial eosinophil count of 50 normal rats chosen at random from the groups above was 270 ± 28 cells/mm³, of 26 hypophysectomized rats on post-operative day 1: 518 ± 56, day 2; 475 ± 45, day 3; 557 ± 61, day 6; 545 ± 57.

TABLE II. Comparison of Eosinopenic and Adrenal Ascorbic Acid-Depleting Activity (AAA) of Alkali- and Acid-Treated ACTH Preparations in Hypophysectomized Rats.

Preparations tested*	Reference No.	No. of rats	Mean eosinophil change, % ± S.E.	Estimated AAA depleting activity, IU/mg
Control groups				
Saline		46	— 5 ± 4	
Growth hormone		5	— 5 ±14	
Exp. ACTH groups				
L 2317 M	8			
Untreated		9	—66 ± 4	60
NaOH treated		13	—54 ± 6	2
HCl "		8	—56 ± 4	60
L 2337 M	8			
Untreated		9	—76 ± 8	40
" †		7	—25 ± 4	
NaOH treated		9	—63 ± 9	2
HCl "		4	—77 ± 4	40

* All preparations tested at 2 mg dose level after single intrap. inj. except (†) which was tested at the .1 mg level.

at this equivalent unitage level, a distinctly lower EA was found in the untreated material, suggesting that a masking of the EA of the NaOH-heat treated material had not occurred at the 2.0 mg dose level. It is of interest that the HCl-heat treatment of the ACTH as described did not significantly alter the EA of the preparations employed.

Discussion. Evidence has been presented to indicate that there is a lack of correlation between EA and AAA of ACTH preparations assayed in normal rats. An even greater lack of correlation is noted when the same preparations are tested in both normal and hypophysectomized rats. Further, it is shown that alkali-heat treatment of ACTH preparations, under the conditions employed here, reduced the AAA(6), but not the EA of such preparations. It is of interest to note, in this connection, that it has been reported that ACTH is almost completely inactivated, as judged by the adrenal repair test in hypophysectomized rats, after treatment for 30 minutes in 0.1 N NaOH at 100°C(7). On the other

hand, it has been shown(6) that treatment of ACTH in 0.1 N NaOH for 10 minutes in the boiling water bath causes approximately 90-95% inactivation of AAA, suggesting that alkali-heat inactivation of AAA proceeds in a manner independent of EA inactivation during this time period.

It must be emphasized that preparation L 2262 A is an unadsorbed fraction obtained from oxycellulose treatment(8). As shown in Table I, this fraction shows minimal AAA, yet retains some EA, as tested in both normal and hypophysectomized rats. This finding adds increased weight to the possibility that AAA and EA may not be identical.

It has previously been emphasized(2) that, since ACTH fractions lacking in AAA are usually discarded, it is not surprising that we have not found EA in the complete absence of AAA in any of the preparations tested. On the other hand, ACTH preparations highly active in AAA (L 2269 B) may exhibit no EA in hypophysectomized rats.

It is worthy of note that certain ACTH preparations are eosinophilic in action in normal mice and in hypophysectomized rats. An instance of this is recorded in Table I. It is also of note that the administration of 1 to 5 mg doses of ACTH to hypophysectomized rats has not duplicated the maximal depletion of eosinophils noted in hypophysectomized animals with hydrocortisone (~95% on the average for dose levels of 1.0 mg or more)(11). Such a discrepancy between an ACTH induced eosinophilia, and the failure of large doses of ACTH to duplicate in degree the effects of hydrocortisone carries the implication that an eosinophilic component may be present in the ACTH preparations, thus blocking or masking EA. It remains to be shown, however, that this eosinophilia in the hypophysectomized animal is not a non-specific protein effect. Although growth hormone was used as a control material, without effect on the eosinophil level in the hypophysectomized rat, it is felt that still other inert proteins must be tested for possible eosinophilic activity at comparable dose levels. A further possibility exists that certain ACTH preparations may cause the secretion of such small amounts of eosinopenic steroids as to cause an eosinophilia. It has been reported, for example, that in adrenalectomized mice, doses of cortisone acetate in amounts smaller than 1 μg may cause an eosinophilia(10), and in this laboratory, cortisone acetate at a dose level of 250 μg has been found to cause an eosinophilia in hypophysectomized rats(11).

Summary. Equivalent dose levels by weight of various ACTH preparations have been compared on the basis of their eosinopenic activity (EA) in normal and hypophysectomized rats with the adrenal ascorbic acid-depleting activity (AAA) in hypophysectomized rats. The data presented have been interpreted as indicating that: 1. No correlation exists between the EA and AAA of various ACTH fractions as tested either in normal or hypophysectomized rats. 2. EA in normal rats is not necessarily correlated with EA of the same preparations as tested in hypophysectomized rats. 3. Reduction of AAA by treatment with NaOH-heat under the conditions employed does not inactivate EA, suggesting that EA may be a separate and more stable component of the ACTH preparations. Neither AAA nor EA were influenced by HCl-heat treatment in these experiments. 4. No ACTH compound has been tested which is completely free of AAA and yet exhibits EA, although the converse has been observed. 5. Some evidence suggests the presence of an eosinophilic component in certain ACTH preparations.

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Normal Iodine Uptake and Anoxia Resistance Accompanying Apparent Hypometabolism in Hereditary Obese-Hyperglycemic Syndrome.*† (19865)

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The hereditary obese-hyperglycemic syndrome of mice is a recessive Mendelian entity characterized by an adult weight up to 3 to 4 times the normal, sterility(1), high blood sugar levels and glucosuria associated with insulin resistance(2), atrophy and ulcers of the skin, enlarged islets of Langerhans(3), increased food intake and modified food choices (4), decreased spontaneous activity(5), relatively low oxygen consumption(6), and a number of atypical reactions to hormonal and dietary treatments(7). The obese animals also show decreased oxidation of administered acetate and an increased conversion of acetate to fatty acids(8). The possible relation of hyperglycemia to a primary biochemical block has been discussed(6). Its etiological significance as regards hyperphagia has also been examined(9,10). Of special relevance to the study reported here are the following observations: the total basal oxygen consumption of obese mice is not higher than that of the non-obese animals, in spite of the fact that they weigh twice or 3 times the non-obese average(6). On a body surface basis, the basal metabolic rate of the obese animals can be calculated as 40 to 50% below normal. The low oxygen consumption of the obese animals is accompanied by normal fasting respiratory quotients, somewhat elevated non-fasting respiratory quotients(7). Obese animals are unusually sensitive to thyroxine from the standpoints of increased oxygen consumption, weight loss and toxicity(7). Serum and tissue cholesterol levels are high(6,7). As noted above, there is decreased spontaneous activity(5). These findings could be construed as suggesting hypothyroidism. On the other hand, the histological picture of the thyroid of obese animals is normal(3). Administration of thiouracil to non-obese animals, although it decreases their oxygen consumption, fails to reproduce any part of the obese-hyperglycemic syndrome(7). Finally, neither hyperphagia nor insulin resistance are generally associated with hypothyroidism.

Because of the conflicting lines of interpretation with respect to thyroid function in these obese mice, it appeared of importance to directly assess the physiological status of the thyroid by determination of radioactive iodine uptake. It also seemed of interest to compare metabolic rates of obese and non-obese animals on a basis more independent of body size. The propriety of comparing metabolic rates on a body surface basis when dealing with animals of widely different fat content has been questioned in a previous study(6). The anoxia method(11-14) which compares the relative degree of dependence of animals on oxygen tension has been shown to be de-

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