Reduction of Antidiuretic and Pressor Activity Associated with Adrenocorticotropic Hormone (ACTH) Preparations. (18648)

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The preparation of purified ACTH from whole pig or sheep pituitary glands, or from dissected beef anterior lobe material, may involve contamination of the final product by substances having antidiuretic and pressor activity similar to that found in posterior lobe extracts. Since clinical and experimental use of ACTH preparations requires relative freedom from posterior lobe contaminants in order to avoid undesirable side effects of these substances, it is thought to be of value to present a method which reduces to markedly low values the antidiuretic and pressor activity found in various preparations of sheep ACTH.

Materials and methods. Four types of ACTH preparations have been tested: (A) ACTH protein prepared according to the method of Li, Evans and Simpson(1); (B) ACTH peptide mixture prepared by pepsin digestion of ACTH protein (A) according to Li(2); (C) ACTH protein prepared according to a method to be reported by Li and Reinhardt(3); and (D) ACTH acid peptides prepared from ACTH protein preparation (C), according to a method described by Li(4), in which the acid hydrolysate is freed from the unhydrolyzed protein and isolated. These various types of preparations were dissolved in, and treated with varying concentrations of hydrochloric acid, and heated for different time intervals in a boiling water bath. No measurements were made of pH values after dissolving the ACTH in the acid solutions. The solutions (10 mg/ml) were then brought to pH 3 by the addition of 1 N sodium hydroxide and diluted 1:100 in isotonic saline for injection purposes. Pitressin (Parke Davis and Co.) was employed as a standard for assay purposes, the commercial preparation being diluted from the original concentration of 20 units/ml to 1, 10, or 100 milliunits/ml in isotonic saline. Tests for antidiuretic activity in these various preparations were made in the manner described by Birnie *et al.*(5), using the hydrated rat. Adult, male, Long-Evans rats, weighing 200-350 g, were fasted, but allowed access to tap water for 18 hours. They were then given 3 ml of water/100 sq. cm surface area for each of 3 successive hours. At the time of the third dose of water, the urine excretion for the previous 2 hours was measured, the test substance was injected intraperitoneally, and urine collections were begun for the 90-minute test period. The urine excreted in this 90-minute period of time was calculated as the percent of the water administered in the hydration period minus the volume excreted up to the time of administration of the test preparation. All solutions were assayed in groups of 3 or 4 animals, individually handled, accompanied by saline and/or pitressin control groups. Pressor tests were made on sodium pentobarbital anaesthetized and heparinized unfasted 400-500 g male rats by direct cannulation of the internal carotid artery using the mercury manometer. These animals exhibited no pressor reaction to the intravenous administration of physiological saline solution and showed minimal (10 mm Hg) pressor responses to 2-4 milliunits of Pitressin. The log dose-response curve was linear in the range 4 to 100 milli-

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TABLE I. Comparison of Water Excretion by the Hydrated Rat Given Various Preparations of ACTH, Pitressin or Saline.

Preparations tested	No. of different samples tested	No. of rats	Treatment: Hydrochloric acid, normality	Treatment: Duration of treatment, min.	Avg % water excreted†
Isotonic saline	7	21	0	0	65 (48-86)
Pitressin					
1 milliunit	3	13	0	0	52 (38-63)
10 "	7	21	0	0	15 (6-29)
100 "	7	19	0	0	19 (2.5-29)
ACTH protein* (A) and (C)					
1	7	31	0	0	16 (9-26)
2	1	2	.1	30	18
3	1	3	.2	30	8
4	1	3	.2	60	67
5	1	3	.2	120	60
6	2	7	.2	180	62
7	1	4	.2	360	63
8	3	15	.5	30	56 (40-76)
9	3	10	1	30	57 (51-60)
ACTH pepsin digestion mixture (B)					
10	2	12	0	0	12 (8-16)
11	1	4	.2	60	54
11	1	4	.2	120	75
11	1	4	.2	180	65
ACTH acid peptide (D)					
12	2	7	.2	60	55 (54-56)

* See text for description of preparations (A)-(D). Test injections were made intraperitoneally in 1 ml of isotonic saline, pitressin in the dose levels indicated, and ACTH preparations in 100 μg per test dose.

† Parenthetical figures indicate range of averages of 3 or 4 animals per group.

units. Test substances were injected intravenously (external jugular vein) in levels of 100 micrograms in 0.1-0.2 ml solution. Pressor responses were graded as positive or negative at the dose level employed.

Results. The results of testing 12 different ACTH preparations on 107 animals in the antidiuretic test are presented in tabular form (Table I) to compare the per cent of water excretion after various ACTH preparations as compared with saline and pitressin controls. The averages and ranges (for each group of 3 or 4 rats) are presented for the various types of preparations employed. It will be noted that the non-acid treated and unheated preparations uniformly contained at least 10 milliunits of activity per 100 micrograms as compared with the pitressin controls. No attempt was made to determine the total contamination by further titration, although it was evident that many of these preparations contained far in excess of this amount of contaminating antidiuretic activity (unreported assays). On the other hand, treatment of the ACTH protein (A) or (C), or ACTH peptides from pepsin digestion with acid and heating reduced the antidiuretic activity by a factor of at least 10. The exact figure cannot be stated because the original materials were not assayed for their total contamination. It will further be noted that pepsin digestion of protein does not reduce antidiuretic activity, whereas added treatment with acid and heat does affect this result. It will be remarked that antidiuretic activity was reduced in preparations heated either in 0.5 N HCl for 30 minutes or for at least one hour in 0.2 N HCl. This latter concentration of acid would appear to be most useful because the solutions can be neutralized to pH 3 without the formation of excess NaCl in the injection mixture.

Pressor tests were conducted on various

types of ACTH preparations. In 6 of the preparations treated with acid and heat as indicated above, pressor effects were absent in the 100 μg dose levels tested, whereas it was uniformly present in the nontreated preparations. In the instance of the pressor tests, no effort was made to assay for total original contamination. It was thought to be of importance that antidiuretic and pressor activities were found to parallel each other, both in qualitative and in quantitative tests insofar as these were conducted.

The assumption is made in these experiments that the anti-diuretic and pressor activities noted in these ACTH preparations is of posterior lobe origin. No note was made for the 90-minute intervals reported, of any distinct difference in the form of the urinary excretion curves obtained after injection of: (1) ACTH test substances; (2) pitressin; or (3) ACTH tested with added pitressin. No tests have been carried out routinely for oxytocic or chloruretic activity.

The acid and heat treatment described does not inactivate the ACTH activity of these preparations as tested by their capacity to produce lowering of the ascorbic acid level of the adrenals of the hypophysectomized rat(6). Under certain conditions, this activity may be actually enhanced by this treatment (7).

Summary. Treatment of sheep ACTH protein or peptide with 0.2 N hydrochloric acid in the boiling water bath for at least one hour, under the conditions described, produces a substantially complete reduction in antidiuretic and pressor activities originally present.

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Azorubin-Binding Capacity of Normal and Pathological Sera.* (18649)

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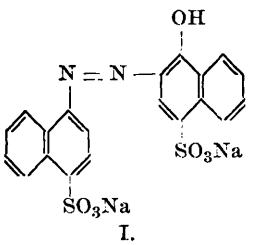
The ability of serum proteins to bind certain organic dyestuffs, has been studied by means of various methods, and certain differences have been found between normal and pathological sera(1-6). A chromatographic method for the quantitative determination of the capacity of serum albumins to bind azorubin, an acidic dye, has been described by Westphal, Gedigk and Meyer(7). In the present study, this method has been used to compare the azorubin-binding capacity (ABC) of normal with that of pathological sera.

Methods. (For details see reference(7)). The azorubin (formula I) preparation used



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