Comparison of Biologic Effects of ACTH Protein and ACTH Peptide Given by Continuous Injection.* (19295)

DWIGHT J. INGLE AND CHOH HAO LI.

From the Research Laboratories, Upjohn Co., Kalamazoo, Mich., and the Department of Biochemistry, University of California, Berkeley.

Reinhardt and Li(1) have reported on discrepancies in the bioassay of ACTH protein and ACTH peptide by the ascorbic acid depletion test(2) and the adrenal weight maintenance test(3). ACTH protein was more potent than ACTH peptide per unit weight in the adrenal weight maintenance test whereas ACTH peptide was more potent than ACTH protein in the ascorbic acid depletion test. These same preparations of ACTH were used in the present studies.

As was shown by Ingle(4), the biologic response to ACTH protein is much greater when it is administered by continuous rather than intermittent injection. It is possible that the apparent differences in the biologic activities of ACTH protein and ACTH peptide are based upon physical differences and hence upon differences in efficiency of utilization under different test procedures. In the present study we have compared some of the metabolic and morphologic effects of ACTH protein and ACTH peptide when given to normal rats by continuous subcutaneous injection. Under these conditions ACTH protein was more effective than ACTH peptide in causing signs of hypercorticalism.

Methods. Male rats of the Sprague-Dawley strain, having an initial weight of approximately 300 g, were used in these experiments. A medium carbohydrate diet (Table I) was fed by stomach tube each morning (8:30 to 9:15 A.M.) and afternoon (4:15 to 5:00 P.M.). The technics and diets were modifications of those described by Reinecke, Ball and Samuels(5). The rat was placed in a metabolism cage which restricted activity so that the animal was unable to reverse its position. A 21-gauge hypodermic needle, having a small barb attached to its shank to prevent withdrawal, was placed subcutaneously. Sterile needles were used for replacement every 48 hours. ACTH protein and ACTH peptide were made up in sterile solutions of 5% degraded gelatin (Plasmoid, Upjohn) at pH of 2.5 and were administered simultaneously in volumes of 2 cc per rat per day by a 6-place continuous injection machine. The gelatin was found to protect ACTH against precipitation and partial inactivation which sometimes occurs when solutions containing it are passed through a polyethylene tubing as was done in these experiments. In separate (unpublished) studies under similar conditions, it was shown that gelatin alone in the amounts used in these experiments is without any significant biologic effect upon the experimental animals. The experiments were carried out in an air-conditioned room at a temperature of 74° to 78°F. Twenty-four-hour samples of urine were collected at the same hour (8:00 to 8:30 A.M.) each day and were preserved with toluene and citric acid (1 g per sample) to insure the acidity of the urine for nitrogen analyses. Urine glucose was determined by the ferricyanide electrode method of Shaffer and Williams(6) and non-protein nitrogen (NPN) was determined by the micro-Kjeldahl pro-

TABLE I. Composition of Fluid Diet.

	g
Cellu flour (Chicago Dietetic Supply House)	60
Osborne-Mendel salt mixture	40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Mazola oil + 100 mg vit. K	10
Mazola oil	190
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Water to total of	2000

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TABLE II. Gross Changes in Rats Given ACTH Protein and ACTH Peptide for 10 Days. 6 rats in each experiment.

Preparation ACTH	Dose, mg/day	No. showing change			Wt 1 adrenal, mg (avg, range)	Wt thymus (avg, range)
		Died	Gross lesions: Kidneys	Gross lesions: Stomach ulcers		
Protein	.5	0	0	1	39 (31-48)	69 (34-135)
Peptide	.5	0	0	1	35 (32-40)	140 (96-225)
Protein	1	0	5	5	60 (45-74)	36 (24-61)
Peptide	1	0	1	2	38 (34-46)	89 (28-194)
Protein	2	1	4	5	101 (72-124)	28 (22-37)
Peptide	2	0	2	3	70 (50-80)	30 (14-42)

cedure as follows. Proteins were precipitated as the salts of tungstic acid by the Folin-Wu procedure; the organic matter was oxidized by sulfuric acid and hydrogen peroxide; the ammonia was distilled off into a standard acid solution and the latter titrated with standard base. ACTH protein was prepared according to the procedure of Li, Evans and Simpson (7) and ACTH peptide was prepared according to the procedure of Li(8). Following an adaptation period of 2 weeks, the rats were given continuous subcutaneous injections of the hormones for 10 days. At the end of this time the animals were anesthetized with cyclopal, exsanguinated and necropsy performed.

Experiments and results. In Exp. 1, 6 rats were given 0.5 mg daily of ACTH protein and six rats were given 0.5 mg daily of ACTH peptide for 10 days. Experiments 2 and 3 were identical except that the daily doses were 1 mg and 2 mg respectively. In each experiment the biologic responses to ACTH protein were greater than were obtained with the peptide.

Survival (Table II). All of the rats survived the experiments except one animal given 2 mg of ACTH protein daily which was found to be dying on the 10th day of injection.

Gross renal damage (Table II). Some of the rats showed gross pathologic changes in the kidneys which were manifest as a mottled appearance with occasional gray patches and tiny nodules on the surface of the kidney. The nodules represent hypertrophy of the renal tubules. The mottled appearance of the kidneys is believed to represent infection but routine microscopic examination of the tissues was not done in this study. Such changes occurred more frequently among the ACTH protein series.

Stomach ulcers (Table II). Ulcers occurred more frequently in the rats given ACTH protein than in the rats given peptide. The lesions varied from multiple tiny ulcers which covered the pyloric mucosa to a few deep ulcers which caused hemorrhage into the gut.

Urinary NPN and body-weight (Fig. 1, 2 and 3). All of the rats remained in positive nitrogen balance and gained some weight during the control periods. All of the rats lost weight and developed a negative nitrogen balance during the administration of ACTH protein and ACTH peptide, but the peak of nitrogen loss was not sustained. The extent of average change in weight and urinary NPN was proportional to the dose, and the average change elicited by ACTH protein was greater than that caused by ACTH peptide at each of the 3 dose levels.

Glycosuria (Fig. 1, 2 and 3). None of the rats excreted measurable amounts of reducing substances during the control periods. At a dose of 0.5 mg daily, 4 of 6 rats given ACTH protein and 2 of 6 rats given ACTH peptide excreted glucose on one or more days of the injection period. At a dose of 1.0 mg daily, each of the 6 rats given ACTH protein and 3 of 6 rats given ACTH peptide excreted glucose on one or more days. At a dose of 2.0 mg daily all of the rats on each preparation of ACTH excreted glucose on one or more days. The average level of glycosuria was proportional to the dose and the response to ACTH protein was greater than to ACTH peptide.

Although some correlation between each of the signs of hypercorticalism was evident, there was no perfect correlation between any

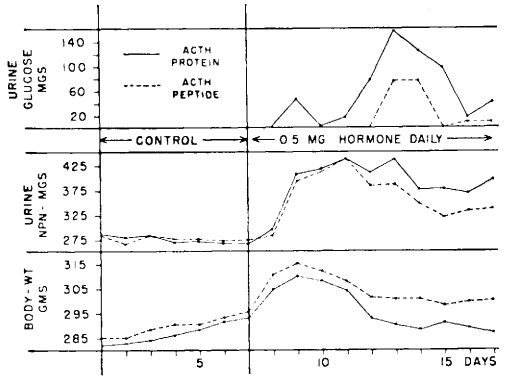

FIG. 1. Comparison of effects of ACTH protein and ACTH peptide given by continuous subcutaneous injection for 10 days. .5 mg/rat/day. Avg 6 rats per group.

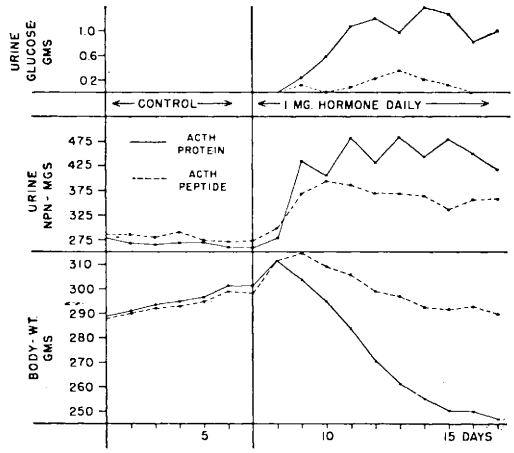

FIG. 2. Comparison of effects of ACTH protein and ACTH peptide given by continuous subcutaneous injection for 10 days. 1 mg/rat/day. Avg 6 rats per group.

two of them, *i.e.*, signs of gross pathology were not limited to the rats with largest adrenals or to animals with the most severe glycosuria, etc.

Discussion. At the time ACTH protein was first isolated(7,9), studies by means of sedimentation, electrophoresis and solubility procedures led investigators to consider it homogeneous. Despite this apparent homogeneity, there is now considerable evidence to indicate that the adrenocorticotropic activity as measured by depletion of adrenal ascorbic acid does not involve the whole protein molecule. It has not yet been possible to characterize fully the active principle or principles in the hydrolysates of the ACTH protein, hence it is not possible to state with certainty that the active component of ACTH protein differs from the active component of ACTH peptide.

The apparent quantitative dissociation in the activities of ACTH protein and ACTH peptide can be interpreted as support for the hypothesis that ACTH represents more than one biologic principle. In the present experiments ACTH protein was found to have about twice the activity of ACTH peptide in causing signs of hypercorticalism. In respect to the adrenal ascorbic acid depletion test(1) the relative potency of these 2 preparations is reversed. At the present time other interpretations of the data cannot be excluded. Differences in the physical properties of ACTH protein and ACTH peptide may cause differences in the efficiency of utilization by the organism and hence in biologic response. The ascorbic acid depletion test is acute whereas the hormone was administered for several days in the present studies. Although the ascorbic acid depletion test has proven useful as a sign of adrenal cortical response to ACTH, its biochemical relationship to the secretory activity of the adrenal cortices is unknown.

In these studies we have controlled one of

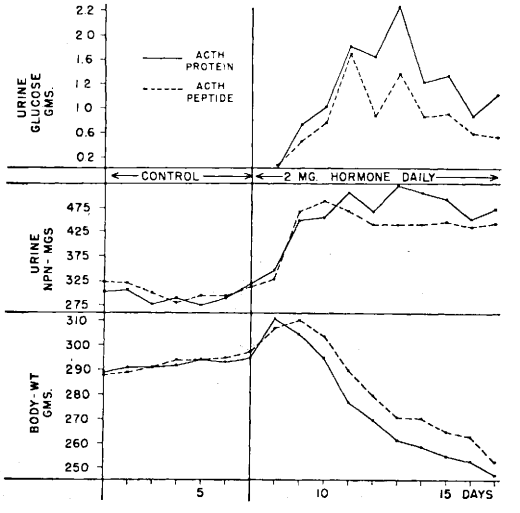

FIG. 3. Comparison of effects of ACTH protein and ACTH peptide given by continuous subcutaneous injection for 10 days. 2 mg/rat/day. Avg 6 rats per group.

the usual variables in bioassays by administering each form of the hormone by continuous injection. Nothing is known of the relative rates of absorption of the two forms of ACTH from the subcutaneous tissues into the blood and of their removal from the blood into the cells of the target organ nor is anything known about the relative proportions of the two forms of ACTH which are inactivated or otherwise wasted before they reach their target organ.

The results of these experiments support the conclusion of Reinhardt and Li(1) that the bioassay of ACTH by the ascorbic acid depletion test does not always correlate with other signs of adrenal cortex response.

Summary. A comparison was made of some of the metabolic and morphologic effects of ACTH protein and ACTH peptide given by continuous subcutaneous injection to normal (300 g) male rats for a period of 10 days. The signs of hypercorticalism were adrenal hyperplasia, thymus atrophy, loss in body-weight, rise in urinary NPN, glycosuria and pathologic changes in the stomach and kidneys. ACTH protein had about twice the biologic activity of ACTH peptide as measured by the above criteria, but was significantly less active than the peptide in causing depletion of adrenal ascorbic acid.

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Is Hypoprothrombinemia Caused by Deficiency of Vitamin K Different from that of Dicumarol?* (19296)

ARMAND J. QUICK, CLARA V. HUSSEY, AND GEORGE E. COLLENTINE.
From the Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wisc.

It is generally accepted that vit. K deficiency causes a true hypoprothrombinemia, and until recently it was believed that Dicumarol likewise reduced only the prothrombin level of blood. One of us(1) obtained findings which were not in accord with this and postulated that the concentration of a factor other than prothrombin might be affected by Dicumarol. This was promptly questioned by Dam(2). Recently, however, he and his associates(3) have postulated that while a decrease of prothrombin occurs in both vit. K deficiency and after Dicumarol, an additional factor is diminished in the first condition, and a second hypothetical agent is decreased after giving Dicumarol. Other workers, likewise, have reached somewhat similar conclusions. MacMillan(4) postulated a decrease of an accelerating factor, while Lein and Lein(5) concluded that Dicumarol causes the formation of an altered prothrombin. Mann and his coworkers(6) recently reported findings which they interpret to mean that Dicumarol decreases a factor, designated "cothromboplastin," much more than prothrombin, while the reverse occurs in vit. K deficiency.

Obviously, there is a need to reinvestigate the problem of whether the hypoprothrombinemia of Dicumarol is essentially different from that of avitaminosis K. Dogs are especially well suited for this study since we(7)

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