cells and paramecia grown in media including sodium acetate containing tritium. Measured radioautographic images of endoplasmic concentrations of radioactivity in paramecium and of individual yeast cells of 3 μ have been delineated. Since the average beta particle from tritium is absorbed by a micron or less of nuclear track emulsion, from the standpoint of its range, this isotope approaches the ideal for radioautographic localization.

Yeast grown in tritium-containing medium as described and exposed for a shorter time have shown differential localization of tritium in polar and peripheral extranuclear areas. Some of these foci showed only one or two grains of reduced silver (each about 0.3 μ to 0.4 μ) in the emulsion. This probably represents the limit of resolution of radioautography with the present emulsions available for biological experiments.

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Apparent Discrepancies in Evaluation of Adrenocorticotrophic Hormone (ACTH) Activity by Two Assay Methods. (18732)

W. O. REINHARDT AND CHOH HAO LI.*†

From the Division of Anatomy and Department of Biochemistry, University of California, Berkeley.

The availability of a rapid and sensitive method for the assay of ACTH preparations by the adrenal ascorbic acid depletion test in the hypophysectomized rat(1) has made it possible to carry out comparative assays to an extent not allowed by the more time consuming procedure of assay by the adrenal maintenance test in the hypophysectomized rat(2). The lack of internationally accepted test procedures and preparations has, however, prevented the direct comparison of the level of activity of different samples of ACTH. Further than this, no detailed study has been made to date of: (a) the relationship between the potency of preparations in terms of ability to deplete ascorbic acid in the adrenal of the hypophysectomized rat, as compared with various morphological assay methods (adrenal repair and maintenance in the hypophysectomized rat), and (b) the relationship between the ascorbic acid depleting potency and the actual secretory activity of the adrenal gland in terms of the many and known experimental and clinical effects.

It is also known that the criteria (electrophoresis, solubility curves) for establishing the chemical homogeneity of hormone preparations are not strict enough to rule out trace contaminants (either anterior or posterior lobe) which may be pharmacologically active. Under such circumstances, it is obligatory that comparisons be made between various methods of assay, in order that the potency of various preparations can be evaluated in terms of types of responses, which may not necessarily be directly related to each other quantitatively. The present communication describes three series of experiments in which various types of ACTH preparations assayed by the ascorbic acid depletion test did not manifest a proportionally equivalent response in the adrenal maintenance test, or in secondarily manifested effects on the thymus and lymph nodes.

Methods and materials. Five preparations of ACTH were employed: A. ACTH protein prepared according to the method described

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1. Sayers, M., Sayers, G., and Woodbury, L. A., *Endocrinology,* 1948, v42, 379.

2. Simpson, M. E., Evans, H. M., and Li, C. H., *Endocrinology,* 1943, v33, 261.

in 1943(3), B. ACTH protein prepared according to a method to be described(4), C. ACTH peptide digestion mixture prepared from ACTH protein preparation (A), by a method described in 1948(5), (D) ACTH acid peptide in which the peptide is freed from the unhydrolyzed protein(6), and E. ACTH protein (B) treated with 0.2 N-HCl for 30 minutes in the boiling water bath.

Assay of these preparations by the measurement of depletion of adrenal ascorbic acid was carried out on Sprague Dawley or Long-Evans rats,‡ hypophysectomized at 40 days of age, after 1 week in a constant temperature room, according to the technique of Sayers, *et al.*(1), and in similar animals injected for a period of 10 to 15 days beginning immediately after the day of operation, for the maintenance test(2). The latter groups of animals were maintained in a warm room under constant conditions and were fed this laboratory's wet Diet I, *ad lib.*, for the duration of the experiment. At autopsy, one adrenal gland, thyroid, thymus, testes and cervical lymph nodes were weighed on a torsion balance to the nearest milligram. Groups 1 and 2 were given identical ACTH preparations, the groups differing only in the strain of rats employed. Group 3 compares the maintenance responses of the standard (1943) protein at the 0.5 mg/day dose level with a protein preparation (1951) administered at a dose of 1 mg/day and having a higher ascorbic acid potency than the standard (1943) protein preparation. All preparations were tested for antidiuretic and pressor activity. The acid peptide D was substantially free of both activities whereas antidiuretic and pressor amounts in excess of 10 m.u./100 μg were present in the other preparations(7). None of the

TABLE I. Assay of Preparations of ACTH by Adrenal Maintenance and Ascorbic Acid Depletion Tests.

Treatment	Ascorbic acid depletion assay: Test dose, μg	Depletion of ascorbic acid mg/100 g ± SE	Stand. equivalent, μg	Adrenal maintenance test in hypophysectomized rats: Duration of exp., days	Total dose, ACTH, mg	No. of rats	Body wt: Initial, g	Body wt: Gain or (loss), g	Organ wt (and ranges): Adrenal(1) (right), mg	Organ wt (and ranges): Thymus, mg	Organ wt (and ranges): Cervical lymph nodes, mg
Group 1*—Sprague-Dawley rats											
Control (saline)	—	———	—	10	—	3	104	9.3	9.3(7-10)§	268(187-328)	91 (61-113)
ACTH protein (B)	5	118.5 ± 5.1(14)†	5	10	5.25	5	110	10	16.2(14-19)	125 (95-234)	69 (51-103)
" Pepsin peptide (C)	2	112 ± 5.8(22)	3.9	10	5.25	5	102	(1.2)	13.2(11-15)	62 (32-142)	48 (44-61)
" acid peptide (D)	2	148 ± 11.1 (9)	13	10	5.25	4	107	9.5	11.2(11-13)	207(138-267)	122(101-149)
Group 2*—Long-Evans rats											
Control (saline)	—	———	—	11	—	7	132	(16)	4.4 (3.7)	210(171-243)	150(104-249)
ACTH protein (B)	5	118.5 ± 5.1(14)	5	11	5.5	6	133	(19)	14.8(12-17)	81 (27-139)	100 (80-130)
" Pepsin peptide (C)	2	112 ± 5.8(22)	3.9	11	5.5	6	131	(20)	13.6(12-16)	69 (30-91)	108 (81-156)
" acid peptide (D)	2	148 ± 11.1 (9)	13	11	5.5	6	135	(14)	10.8(10-12)	144 (82-189)	126 (76-170)
Group 3*—Long-Evans rats											
Control (uninj.)	—	———	—	15	—	6	117	(20)	3.7 (3.4)	143 (34-229)	119 (82-152)
ACTH protein (A)	5	119 ± 5.3(11)	5	15	6.5	6	122	(11.5)	12.2(11-14)	96 (58-112)	91 (65-132)
" (E)	5	141 ± 12 (7)	10.5	15	13.0	5	120	(15)	12.2 (9-15)	105 (41-225)	78 (68-87)

* All rats hypophysectomized at 40 days of age; intraperitoneal injections of 0.25 mg of each preparation were given twice daily (Groups 1 and 2) or once daily (0.5 or 1.0 mg daily) for 13 days (Group 3). Controls injected with buffered saline in equivalent amounts. Animals were examined under binocular microscope at time of autopsy for completeness of hypophysectomy.

† Parenthetical figures = number of individual determinations in hypophysectomized rats. § Parenthetical values = ranges for the group.

3. Li, C. H., Evans, H. M., and Simpson, M. E., *J. Biol. Chem.*, 1943, v149, 413.

4. Li, C. H. and Reinhardt, W. O., In preparation, 1951.

5. Li, C. H., *Trans. Macy Conf. on Metabolic Aspects of Convalescence* 1948, v17, 114.

6. Li, C. H., *J. Amer. Chem. Soc.*, In press, 1951.

‡ No difference in the response of these strains to this test has been noted in this laboratory.

7. Reinhardt, W. O. and Li, C. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 836.

preparations exhibited thyrotropic or gonadotropic effects. The preparations were not tested specifically for growth, lactogenic or oxytocic activity.

Results. Table I presents the data for assay of ACTH preparations A-E by the ascorbic acid depletion and the adrenal maintenance tests in hypophysectomized rats. Test doses of ACTH preparations (B-E) are evaluated in terms of equivalent ascorbic acid depleting activity in micrograms of the standard protein A. The average weight of the right adrenal is presented in the table for each group of rats. These values can be compared by extrapolation with the standard curve employed in this laboratory(2) for dose-adrenal weight response and will be seen in every instance to show definite maintenance activity.

Examination of Table I will elicit the information that in groups 1 and 2, the ACTH protein (B) preparations were most active in the maintenance of adrenal weights, the acid hydrolysate (D) least active (although significantly greater than the controls), this in spite of a significantly greater ascorbic acid activity in the latter preparation. The most active preparation in terms of thymolytic and lympholytic activity, but with intermediate adrenal weight effect was the ACTH pepsin digest mixture (C) in groups 1 and 2. The ascorbic acid potency of this preparation is about twice that of the standard (1943) protein, although significantly less active ($p < .01$) than the acid peptide preparation.

In Group 3, 2 preparations are compared: the standard protein (A) at a dose level of 0.5 mg/day, as compared with a different protein preparation (E) of higher ascorbic acid activity and at a dose of 1.0 mg/day. The 2 treated groups show approximately the same effects on adrenal weight, thymus and lymph nodes, in spite of the difference in dose level and ascorbic acid depletion potency.

It may be noted that the preparation (D) exhibiting the highest ascorbic acid depleting potency is least active in causing reduction in weights of thymus and cervical lymph nodes. The suggestion must be made that high ascorbic acid depleting potency is not necessarily directly related to the secretion by the adrenal of substances causing reduction in thymus and lymph node weight.

The standard ACTH preparation described in 1943 was purified according to its assay either in the repair or maintenance of the hypophysectomized rat adrenal whereas recent ACTH preparations have been prepared using the ascorbic acid depletion test as a measure of purification.

The present data are interpreted to indicate the lack of a direct correlation between these assay methods.

The evidence suggests the necessity for parallel assays of ACTH preparations (proteins or peptides) by at least 2 methods until such a time as a correlation can be more clearly defined between (a) clinical and experimental observations and (b) activity as determined by various biochemical and morphological assay methods. The possibility must be noted that isolation by various chemical procedures of components of ACTH protein may result in the purification of a peptide exhibiting activity only in one or the other of the various test methods. Questions must also be raised as to (a) possible presence of two or more active factors, (b) differences in rate of absorption, utilization, and excretion, and, (c) role of trace contaminants.

Summary. Evidence is presented to indicate that the assessment of the potency of various types of ACTH preparations may vary according to the assay method employed. The suggestion is advanced that ACTH preparations be routinely assayed by more than one method (ascorbic acid depletion, adrenal repair and maintenance, effect on thymus, lymph nodes, etc.) in order to avoid misinterpretation of the nature of the activity and biological responses to a particular preparation.

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