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Comparative Assays on Adrenocorticotrophic Hormone Preparations. (19253)

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It has been established that substances isolated from the pituitary gland, when injected into hypophysectomized rats, will induce an increase in adrenal weight(1) and a depletion of adrenal ascorbic acid(2). These findings led to the development of the adrenal maintenance(3), adrenal repair or weight increase (AWI)(3) and ascorbic acid depletion (AAD)(2) tests for the assay of adrenocorticotrophic hormone (ACTH). It has been reported that ACTH preparations subjected to peptic digestion or acid hydrolysis do not give proportionally equivalent responses in assays by the adrenal maintenance and the ascorbic acid depletion tests(4), and it has been suggested that there are two separate adrenocorticotrophic factors, one which induces an increase in adrenal weight and another which depletes ascorbic acid(5). The present report is concerned with the comparative activity of unhydrolyzed and partially hydrolyzed preparations of ACTH as measured by both the adrenal weight increase and the adrenal ascorbic acid depletion technics.

Methods. ACTH was prepared from swine pituitaries by either acetone-HCL extraction

TABLE I. Comparison of Two Methods of Assay.

Preparation	% hydrolysis	% increase adrenal wt by 5 mg total dose*	Ascorbic acid depletion, mg/100 g adrenal wt	
			2 μg	1 μg
A. ACTH extracted with acid acetone		89.9		
B. Peptic digest of A, #1	43	28.9		
#2	48	17.5		
C. ACTH extracted with glacial acetic acid		92		
D. Peptic digest of C, #1	29	36.7		
#2	46	34.7		
#3	52	6.1		
E. ACTH extr. with glacial acetic acid (Batch 2)		70.6	127	77
F. Same as E, except admin. intrav.		61.4		
G. pH 4.7 precipitate from E after acid hydrolysis		10.5	133	
H. Supernatant from G		11.4	124	
I. Peptic digest of E, #1	46	22.4	200	
#2	51	26.5	135	
#3	70	32.3	154	
#4	75	16.8	142	79
J. Same as I-4, except admin. intrav.	75	20.3		

* Administered intraperitoneally, except F and J administered intravenously.

(6) or glacial acetic acid extraction (7a and b). These preparations were partially hydrolyzed by peptic digestion or by refluxing for several hours in 0.001 N hydrochloric acid. The amount of nitrogen soluble in 5% trichloroacetic acid was employed as a measure of the degree of hydrolysis. The preparations were assayed by the AAD technic of Sayers *et al.*(2), and by the AWI method, which was modified after the description of Sayers *et al.* (8). In the latter method, rats weighing 100 to 120 g each were injected intraperitoneally (except for a few groups which were injected intravenously) three times a day on the 10th, 11th, and 12th days after hypophysectomy. The animals were sacrificed on the 13th day and the adrenals were cleaned and weighed to the nearest 0.1 mg. The AAD technic was modified in that in some cases all of the left adrenals of each group were made into one pool and all of the right adrenals were made into another pool before ascorbic acid determination. All assays were done on groups of 4 to 6 rats.

Results. An acid-acetone extract, designated A in Table I, showed a high degree of activity in the unhydrolyzed state when assayed by the AWI method but two samples of this extract, both hydrolyzed less than 50% by peptic digestion (see B in table), suffered a marked loss of activity when assayed by the same method. Similarly, a glacial acetic acid extract, labelled C in the table, sustained a great loss in activity upon peptic digestion over the range of 29 to 52% hydrolysis as indicated by the AWI method of assay (see D in table). In contrast, it will be seen that when both the AWI and AAD methods are used to assay the same preparation, full activity can still be demonstrated after either acid or peptic digestion when assay is performed by the AAD method, but potency is markedly impaired when the AWI method is employed (see E through I in table). The data in the table (F compared with E, and J compared with I No. 4) show also that similar results are obtained by intravenous and intraperitoneal injections when the AWI method is used.

Discussion. A number of plausible explanations of the results may be presented. There may be two separate and distinct substances possessing adrenocorticotrophic activity(5): one which induces adrenal weight increase and another which depletes adrenal ascorbic acid. If this is correct, then it follows that the adrenal weight factor is significantly less resistant to hydrolysis than the adrenal ascorbic acid depletion factor.

Apparently, difference in absorption rate is not a factor, since similar results with the AWI method were obtained by intravenous and intraperitoneal injection. It is possible that a difference in the inactivation rate of the

preparations in the animal body may be a factor in the results. It is also possible that results may be affected by contamination of the preparations with hormones other than ACTH. Pinto(9) reported that estrogen alone does not increase the weight of the adrenals of the hypophysectomized rat, but when administered along with ACTH induces an increase in adrenal weight greater than that produced by ACTH alone.

Brink *et al.*(10) have reported that ACTH peptides prepared by peptic digestion and tested both clinically in man and by the AAD assay in rats showed activity fully as great as unhydrolyzed ACTH concentrates. Our results, coupled with their observations, suggest that values obtained by the AAD assay will show closer correlation to clinical response induced than will values obtained by the AWI assay.

Summary. In the experiments presented, hydrolysis by peptic digestion or acid-heat treatment induced a marked loss of activity of ACTH as measured by adrenal weight increase, but these treatments had little or no influence upon the activity of the hormone as measured by adrenal ascorbic acid depletion.

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Effect of Cell Concentration on Oxygen Consumption of Leukocytes under Varying Conditions.* (19254)

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Conflicting reports exist in the literature concerning the effect of cell concentration on the metabolism of leukocytes. Barron and Harrop(1) studied the metabolism of human leukocytes in autogenous plasma and found that neither glycolysis nor oxygen consumption increases in proportion to an increase in cell concentration. Soffer and Wintrobe(2) also found that oxygen consumption and leukocyte concentration do not increase proportionally. Ponder and MacLeod(3), however, reported that the oxygen consumption of rabbit polymorphonuclear leukocytes, suspended in buffered saline, is very nearly proportional to the number of cells.

The following work is offered as an attempt to reconcile these two seemingly opposing results and to clarify the extent of the disproportion existing between the cell concentration and the metabolism of leukocytes.

Materials and methods. Exudate leukocytes were collected from normal guinea pigs. Each guinea pig was injected intraperitoneally with 60 ml of a 1% sterile sodium chloride

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