of polypeptide hydrazides offers the advantage that a potential hydrazide group is introduced into the peptide moiety at the mono-amino acid stage, thus avoiding the exposure of sensitive complex peptides to the action of hydrazine. The systematic application of the amino acid carbobenzoxyhydrazides could be expected to facilitate the synthesis of complex polypeptides which may be difficult to prepare by presently available procedures. These possibilities are now under investigation in this Laboratory.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF PITTSBURGH
PITTSBURGH, PENNSYLVANIA
KLAUS HOFMANN
MARGARET Z. MAGEE
ADOLF LINDENMANN

RECEIVED MAY 15, 1950

PREPARATION OF PEPSIN DIGESTS OF FOLLICLE-STIMULATING HORMONE (FSH) POSSESSING FOLLICLE-STIMULATING ACTIVITY

Sir:

The fact that pituitary adrenocorticotropic hormone (ACTH) can be degraded to peptide fragments which possess hormonal activity,[1,2] led to investigations of the hydrolysates of other protein hormones. This communication concerns the hydrolysates of the follicle-stimulating hormone (FSH) obtained by the enzymic digestion with pepsin.

The follicle-stimulating hormone was prepared from sheep pituitary glands by the method previously described.[3] The preparation was shown to be a homogeneous protein by ultracentrifuge, electrophoresis and diffusion studies. It has a molecular weight of 69,000 and an isoelectric point at *p*H 4.5.

In a typical experiment, 50 mg. of FSH was dissolved in 10 cc. of *p*H 4.0 0.03 *M* acetate buffer containing 2 mg. of crystalline pepsin. After the solution was kept at 30° for 300 minutes, it was found that the hormone had hydrolyzed to the extent of about 65% as estimated by trichloroacetic acid precipitation. The free amino nitrogen content increased from 1.4 to 4.1% as determined by the Van Slyke nitrous acid method. When the hydrolysate was assayed in hypophysectomized female rats for hormonal activity,[4] a total dose of 0.10 mg. administered during three days gave a minimal stimulation of follicular development. This is the same minimal effect dose as that for the pure protein hormone.

In order to ascertain that the hormonal activity resides in the hydrolyzed fragments (peptide residues), the hydrolysates were dialyzed in cellophane bags against distilled water. It was found that the dialysates had the same potency as the original FSH protein, and that no activity was demonstrable in the non-dialyzable material. It is, therefore, clear that the non-protein fraction of the hydrolysates retains the follicle-stimulating activity. It is hoped that these observations may lead to a possible synthesis of biologically active peptide(s).

(1) Li, *Trans. Macy Conf. on Metabolic Aspects of Convalescence*, **17**, 114 (1948).
(2) Li and Pedersen, *Arkiv Kemi*, **1**, 533 (1950).
(3) Li, *Vitamins and Hormones*, **7**, 223 (1949).
(4) I am indebted to Dr. Miriam E. Simpson for biological assays.

DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA
CHOH HAO LI

RECEIVED APRIL 14, 1950

ACTIVATION OF ADRENOCORTICOTROPIC HORMONE (ACTH) WITH ACID-HEAT TREATMENT

Sir:

It is an established fact that adrenocorticotropic hormone (ACTH) possesses certain remarkable properties.[1] For instance, the non-protein fraction of the hormone, after pepsin or acid digest, contains adrenal-stimulating activity.[2,3] In this communication, we wish to report that the activity of both ACTH protein and peptides can be enhanced in dilute acid solution by heat.

The ACTH peptide mixture was prepared from pepsin digest of the hormone by the method[2] previously described. It has an average molecular weight of 1200 and contains an average of 8 amino acid residues.[4] Five mg. of the ACTH peptides was dissolved in 1 cc. of 0.025 *m* HCl, and the solution was put into a boiling water-bath for thirty minutes. After cooling, the solution was diluted with *p*H 7.0 phosphate buffer and assayed[5] with hypophysectomized rats. The procedure of Sayers, *et al.*,[6] was employed for the estimation of adrenocorticotropic activity. It may be seen in Table I that the ACTH potency increases 2 times, as compared with the unheated controls.

TABLE I

ACTIVATION OF ACTH WITH ACID-HEAT TREATMENT

ACTH	Prepn.	Expt.	Rats	Average ascorbic-acid depletion per 100 g. adrenal,[a] mg.	ACTH equivalent, microgram	Ratio
Protein	L2010A	Control	9	102.0 ± 7.6[b]	2.9	4.4
	L2010A	Treated	5	146.4 ± 13.5	12.7	
	L1607M	Control	13	121.4 ± 8.1	5.0	1.6
	L1607M	Treated	8	131.8 ± 11.2	8.0	
Peptides	L2019S	Control	6	111.0 ± 5.5	3.9	2.2
	L2019S	Treated	3	133.7 ± 15.4	8.5	
	L2026MS	Control	10	102.1 ± 9.7	3.0	1.9
	L2026MS	Treated	8	122.6 ± 9.3	5.8	

[a] Assay at 5 microgram dose per 100 g. body weight of hypophysectomized male rats (operated at 40 days of age, 1 day postoperative). [b] Mean ± standard error.

Similar experiments using the whole ACTH protein gave the same results. In one case (L2010A) four-fold activation was observed. The ACTH protein (in 0.025 *m* HCl) became somewhat more

(1) Li, *Ann. Rev. Biochem.*, **16**, 291 (1947).
(2) Li, *Trans. Macy Conf. on Metabolic Aspects of Convalescence*, **17**, 114 (1948).
(3) Brink, Meisinger and Folkers, THIS JOURNAL, **72**, 1040 (1950).
(4) Li and Pedersen, *Arkiv Kemi*, **1**, 533 (1950).
(5) The bioassays were carried out by I. I. Geschwind and B. Williams.
(6) Sayers, Sayers and Woodbury, *Endocrinology*, **42**, 379 (1948).