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.

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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.

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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).

soluble in 5% trichloroacetic acid solution after being kept in boiling water for thirty minutes: the solubility increased from 5 to 10%, indicating that some degradation occurred during the acid-heat treatment. The mechanism of the enhancement of activity is being studied. We are also investigating the chemical changes in the ACTH peptides after activation with acid-heat treatment.

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PREPARATION AND PROPERTIES OF AN ACID GLYCOPROTEIN PREPARED FROM HUMAN PLASMA

Sir:

An acid glycoprotein has been isolated and crystallized from Fraction VI[1] of pooled normal human plasma. Its preparation and some properties are described herewith.

The solution (Fractions VI + VII) from which the major human plasma proteins[1] have been precipitated from an ethanol–water mixture of mole fraction 0.066, at −5°, at *p*H 5.8 was adjusted to *p*H 7.5 using an ammonium hydroxide–ammonium chloride buffer of *p*H 10. The proteins in solution were adsorbed and carried down by the zinc hydroxide formed. After centrifuging, the precipitate was resuspended in an equal volume of 0.066 mole fraction ethanol at −5° and the *p*H readjusted to 5.8. Insoluble material was removed and barium acetate added to the solution to a concentration of 0.02 *M*, and the *p*H brought to 6.1. The precipitate which formed was separated. An α_1-globulin constituted 93% of the protein remaining in the solution. It was precipitated by increasing the ethanol to mole fraction 0.136 and decreasing the temperature to −18°.

This protein was further purified by precipitation of other proteins concentrated by the above procedure in 0.066 mole fraction ethanol at *p*H 5.8, 0.02 *M* zinc and 0.02 *M* barium at −5°. The protein remaining in the concentrated solution was homogeneous by electrophoresis between *p*H 2.3 and 8.6, and in the ultracentrifuge at *p*H 2.3 and 6.1. Certain properties of this protein are given in Table I.

Hexuronic acid, fatty acid, cholesterol, phospholipid, free SH-groups and esters of sulfuric acid were not found. The acid glycoprotein could be precipitated from aqueous solution by saturation with ammonium sulfate or monosodium phosphate or by addition of 5% phosphotungstic acid in 2 *N* hydrochloric acid. It was not precipitated by addition of 1.8 *M* perchloric acid, 0.06 *M* sulfosalicylic acid, 20% trichloroacetic acid, or by boiling.

TABLE I
CONSTANTS FOR THE ACID GLYCOPROTEIN

Nitrogen, %	10.7
Hexose, %	17
Hexosamine, %	12
Phosphoric acid, %	1.2
$E^{1\%}_{1\ cm.}$ at 278 mμ	8.93
Isoelectric point, *p*H	2.9–3.0
Electrophoretic mobility, sq. cm./volt sec. × 10^{-5}	
*p*H 8.6, Γ/2 0.1, barbiturate	−5.2
*p*H 4.0, Γ/2 0.1, acetate	−2.0
Sedimentation constant, $S_{20,w}$, at infinite dilution	3.5

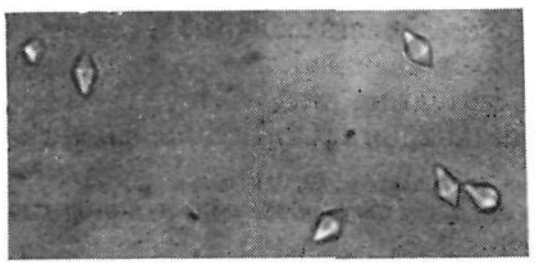

Fig. 1.—Crystals of acid glycoprotein (magnification × 660).

Crystals of this acid glycoprotein (Fig. 1) were obtained under the following conditions: protein 6%, 0.0072 *M* lead acetate, *p*H 5.4, Γ/2 0.02, methanol 10% and acetone 10% at 0°.

Further details of these investigations will be reported subsequently.

I am indebted to Professor E. J. Cohn for his generous advice throughout these studies.

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CONDENSATION OF METHYLGLYOXAL WITH β-OXO ACIDS

Sir:

The paper of M. S. Schechter, N. Green and F. B. LaForge on "Constituents of Pyrethrum Flowers. XXIII. Cinerolone and the Synthesis of Related Cyclopentenolones,"[1] describes condensations of methylglyoxal with β-oxo acids in aqueous solution at *p*H 4.9–8.0 and room temperature, *i.e.*, under so-called "physiological conditions," whereby 2-hydroxy-1,4-diketones are formed under decarboxylation. The authors say: "We have found that the decarboxylation proceeds spontaneously under the conditions of the reaction, the final product being the hydroxydiketone,..."

The fact that spontaneous decarboxylation occurs when condensing aldehydes with β-oxo acids within *p*H range 5–11 was reported first by us in 1932 when we condensed *o*-aminobenzaldehyde with acetoacetic acid, β-oxo-caprylic acid and benzoylacetic acid.[2] We also showed at that time that in a more alkaline solution at

(1) E. J. Cohn, *et al.*, THIS JOURNAL, **72**, 465 (1950).

(1) Schechter, Green and LaForge, THIS JOURNAL, **71**, 3165 (1949).

(2) Schöpf and Lehmann, *Ann.*, **497**, 11 (1932).