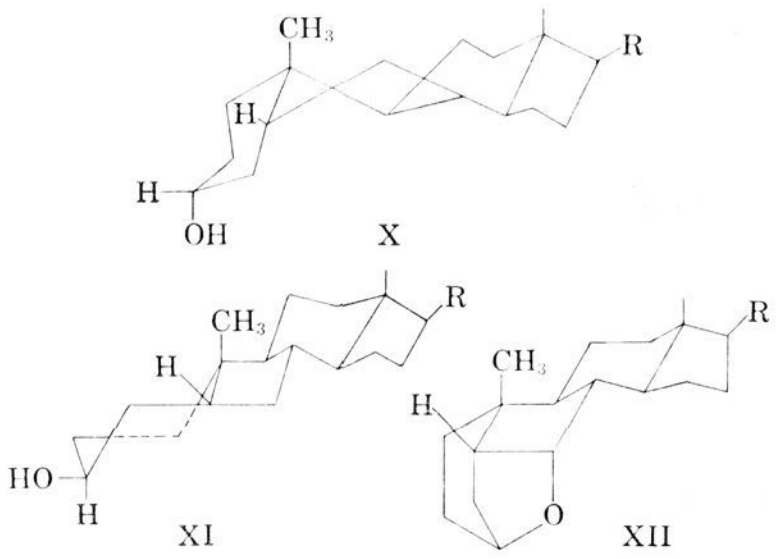

for the reasons given. First, revision of our hydride results clearly indicates that the 3α configuration is equatorial. Second, from a study of models, it is clear that an oxide derived from conformation XI is impossible and that ring A must be in a boat conformation (XII) in the oxide.[6] Thirdly, using the "octant rule,"[7] it is predicted[8] that the rotatory dispersion curve of conformation X should be negative and of conformation XI should be positive; the dispersion curve of B-norcoprostanone is negative.

Acknowledgment.—Investigation supported, in part, by U. S. Public Health Grant CY-4284.

(6) Prof. L. F. Fieser has informed us that he is in agreement with this conclusion as regards the conformation of the oxide.

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(8) R. B. Woodward, private communication.

(9) General Electric Co. Fellow in Chemistry, 1958–1959.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA
BERKELEY 4, CALIFORNIA

WILLIAM G. DAUBEN
GEORGE A. BOSWELL, JR.[9]
GILBERT H. BEREZIN

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ENZYMATIC CONVERSION OF GLUCOSE-6-PHOSPHATE (G6P) TO A NEW HEPTULOSE PHOSPHATE BY RAT LIVER[1]

Sir:

Rat liver preparations and hemolysate have been reported to convert G6P to sedoheptulose-7-phosphate on the basis of color reactions[2] and paper chromatography[3] of the free heptulose. We have now found another product of G6P metabolism, a new heptulose phosphate which can be readily distinguished on paper from sedoheptulose phosphate (Fig. 1). In addition, the migration of the anhydride of the new heptulose was more rapid than sedoheptulosan in acetone, butanol, water (7:2:1). However, the free sugars cannot be separated on paper, which accounts for the delay in observing the new heptulose. Evidence for its structure will be reported in a later communication.

(1) Aided by research grants from the American Cancer Society, Inc., New York (P-106, P-107), and the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, Bethesda, Md. (C-3213).

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(3) A. Bonsignore, S. Pontremoli, G. Fornaini and E. Grazi, *Boll. Soc. ital. biol. sper.*, **33**, 555, 558 (1957).

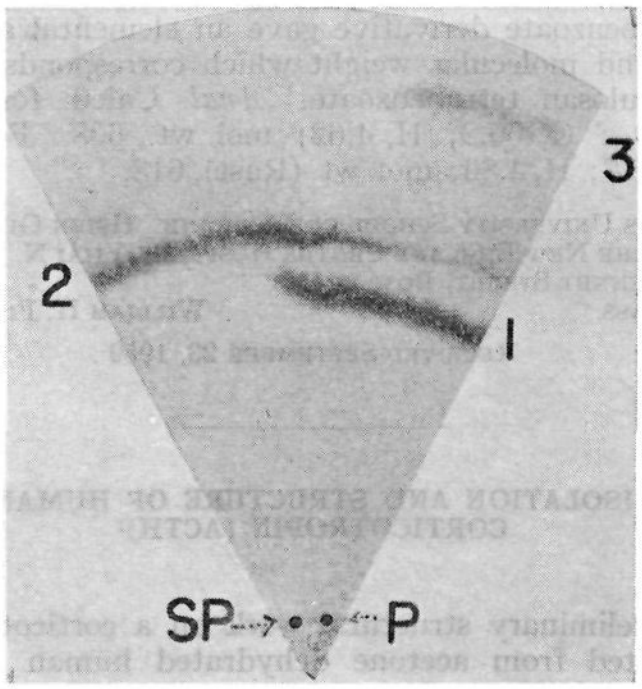

Fig. 1.—A sector of a circular paper (Whatman no. 3 MM) chromatogram (radius 14 cm.) showing the difference in mobility between S7P and the heptulose phosphate from the G6P reaction mixture. Solvent: ethyl acetate, pyridine, acetic acid, water (5:3:1:1) as developing solvent and ethyl acetate, pyridine, water (11:40:6) for saturation of the chromatographic chamber[7]; time, 10 hours. Multiple development was carried out[8]: spraying reagent, 0.5% orcinol in 15% TCA solution in water-satd. butanol, specific for ketoheptoses only.[9] SP = Sedoheptulose-7-phosphate (a gift from Dr. B. L. Horecker) travelled 7.2 cm. to position 2 and the product (P) of the deproteinized and concentrated reaction mixture of G6P, 6.1 cm. to position 1. Position 3 is the location of free heptuloses.

In a typical reaction, 0.2 ml. of 0.1 *M* G6P solution plus 0.5 ml. of Tris buffer, *p*H 7.2, was incubated with 0.3 ml. of a supernatant solution obtained by centrifuging a 10% liver homogenate for 30 minutes at 18,000 × *g*. After 4 hours at 37° in the presence of toluene the mixture was deproteinized either by adding 1 ml. of 95% ethanol or by heating at 100° for 2 minutes and the supernatant was analyzed for heptulose by the Dische orcinol reaction.[4]

Additional evidence for the existence of a new ketoheptose is provided by the isolation from a large scale digest[5] of heptulosan anhydride which failed to crystallize like sedoheptulosan but gave a crystalline tetrabenzoate (20 mg.) (spheroids) whose melting point (75–76°) and optical rotation (no rotation at 2.9% in $CHCl_3$) differed from sedoheptulosan tetrabenzoate[6] (hexagonal plates) melting point (164–165°) and $[\alpha]^{25}D$ −188°. The

(4) Z. Dische, *J. Biol. Chem.*, **204**, 983 (1953).

(5) The protein free filtrate from 250 ml. of digest (substrate; 1 g. G6P) was chromatographed on a cellulose column and developed with methyl Cellosolve, methyl ethyl ketone, ammonia water (7:2:3). Heptose-phosphate rich fractions were pooled and their aqueous solutions hydrolyzed with potato phosphatase. In each case the free heptose was further purified by cellulose column chromatography (acetone, butanol, water, 7:2:1) until a single sugar (paper chromatography) was the product. By treatment with hot mineral acid the heptose was converted to the anhydride.

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polybenzoate derivative gave an elemental analysis and molecular weight which corresponds to a heptulosan tetrabenzoate. *Anal.* Calcd. for $C_{35}H_{28}O_{10}$: C, 69.2; H, 4.62; mol. wt., 608. Found: C, 69.7; H, 4.86; mol. wt. (Rast), 613.

TUFTS UNIVERSITY SCHOOL OF MEDICINE HSIEN-GIEH SIE
AND THE NEW ENGLAND CENTER HOSPITAL VIJAI N. NIGAM
30 BENNET STREET, BOSTON 11,
MASS. WILLIAM H. FISHMAN

RECEIVED SEPTEMBER 23, 1959

ISOLATION AND STRUCTURE OF HUMAN CORTICOTROPIN (ACTH)[1]

Sir:

Preliminary structural work on a corticotropin isolated from acetone dehydrated human pituitaries indicated that it is similar to corticotropins from other species.[2,3,4]

T_3 (Ser.Tyr.Ser.Met.Glu(His,Phe,Arg)) | T_2 (Try,Gly_2,Lys_2,Pro,Val) | T_{4a} (Lys_2,Arg_2,Pro,Val) – T_1 ((Val.Tyr,Pro_2,Asp_2,Ser,Gly,Glu_3,Ala_3,Phe)Leu.Glu.Phe.)

Fig. 1.—Constituent amino acids and possible arrangement of fragments from tryptic digestion of human ACTH. Three additional peptides (T_{4b}, T_5, T_6) were obtained. Their composition indicated that they were derived from T_{4a}. The relative positions of T_3 and T_{4a} have not been ascertained. By analogy with ACTH from other species, the structure shown seems to be correct.

An oxycellulose purified concentrate of MSH and ACTH, from human glands,[5,6,7] was adsorbed on a diethylaminoethyl cellulose[8] column at 5°. Gradient elution was established to 0.2 *M*, *p*H 5.5, through a mixing flask of 300 ml. of 0.005 *M*, *p*H 7.0 ammonium acetate buffer. Since ACTH possesses intrinsic MSH activity,[9] the *in vitro* frog skin bioassay[10] was used to locate active MSH and ACTH fractions. The major fraction was purified further on a carboxymethyl cellulose[8] column; stepwise elution was used with 0.05 *M*, *p*H 5.9, and 0.25 *M*, *p*H 6.9, ammonium acetate. Two active fractions were resolved. Fraction A possessed MSH but no ACTH activity. Fraction B possessed 26 USP units of ACTH[11] and 4×10^4 units of intrinsic MSH per mg. and was judged homogeneous when an acid hydrolysate gave nearly integral molar ratios upon amino acid analysis[12]: Ala_3, Arg_3, Asp_2, Glu_5, Gly_3, His, Leu, Lys_4, Met, Phe_3, Pro_4, Ser_3, Tyr_2, Val_3. One mole of tryptophan was found.[13] Its homogeneity was further confirmed by finding, upon tryptic digestion, the number of peptides containing tryptophan, histidine, methionine, tyrosine and arginine was consistent with the amino acid composition and the specificity of trypsin.

Carboxypeptidase digestion of the hormone and of the C-terminal octadecapeptide isolated from a tryptic digest indicated the C-terminal sequence to be Leu. Glu.Phe. The N-terminal sequence[14] of the hormone was found to be Ser.Tyr.Ser.Met.Glu.

Tryptic digestion split the hormone into four major and three minor fragments. They were separated by ionophoresis in pyridine–acetate buffer at *p*H 6.5 and purified further by paper chromatography.[15] The relative positions of each peptide and their constituent amino acids[16] are shown in Fig. 1. Peptides with composition similar to those isolated from human material have been reported for porcine,[4] ovine[3] and bovine[3] ACTH.

(1) This investigation was supported by grants from the American Cancer Society and the United States Public Health Service.

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(5) We wish to express our gratitude to Dr. M. S. Raben for the generous donation of human ACTH crude concentrate, to Dr. J. D. Fisher for ACTH assays, and to Dr. W. F. White for the generous donation of a highly purified carboxypeptidase preparation.

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(12) S. Moore, D. H. Spackman and W. H. Stein, *Anal. Chem.*, **30**, 1185 (1958).

(13) A. B. Lerner and C. P. Barnum, *Arch. Biochem.*, **10**, 417 (1946).

(14) H. Fraenkel-Conrat, J. I. Harris and A. L. Levy, "Methods of Biochemical Analysis," edited by D. Glick, Interscience Publishers, Inc., New York, N. Y., 1955, pp. 383–397.

(15) The systems used were either 1-butanol–acetic acid–water (4:1:5) or 1-butanol–acetic acid–pyridine–water (30:6:24:20).

(16) Amino acid composition was determined by ion-exchange chromatography[12] or paper chromatography in the butanol–acetic acid–water (4:1:5) system.

SECTION OF DERMATOLOGY TEH H. LEE
DEPARTMENT OF MEDICINE AARON B. LERNER
YALE UNIVERSITY SCHOOL OF MEDICINE
NEW HAVEN 11, CONNECTICUT VINA BUETTNER-JANUSCH

RECEIVED AUGUST 25, 1959

STRUCTURE OF MELATONIN[1]

Sir:

Melatonin, I, found in bovine pineal glands and in smaller amounts in peripheral nerves of man, monkey and cattle, is the most effective known lightening factor of frog (*Rana pipiens*) skin.[2,3,4] Unfortunately, I exists in pineal glands in such minute quantities that conventional approaches to studying its structure were impossible. We wish to report experiments that led to the conclusion that I is N-acetyl-5-methoxytryptamine.

I and 5-methoxyindole-3-acetic acid, II, also present in pineal glands, were isolated by a procedure previously described.[5] With 1.5% methanol

(1) This investigation was supported by grants from the United States Public Health Service.

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