

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.

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ISOLATION AND STRUCTURE OF HUMAN CORTICOTROPIN (ACTH)¹

Sir:

Preliminary structural work on a corticotropin isolated from acetone dehydrated human pitui-

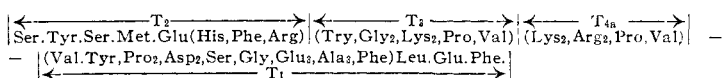


Fig. 1.—Constituent amino acids and possible arrangement of fragments from tryptic digestion of human ACTH. Three additional peptides (T_{4b} , T_5 , T_6) were obtained. Their composition indicated that they were derived from T_{4a} . The relative positions of T_3 and T_{4a} have not been ascertained.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

taries indicated that it is similar to corticotropins from other species.^{2,3,4}

An oxycellulose purified concentrate of MSH and ACTH, from human glands,^{5,6,7} was adsorbed on a diethylaminoethyl cellulose⁸ column at 5°. Gradient elution was established to 0.2 M, pH 5.5, through a mixing flask of 300 ml. of 0.005 M, pH 7.0 ammonium acetate buffer. Since ACTH possesses intrinsic MSH activity,⁹ the *in vitro* frog skin bioassay¹⁰ was used to locate active MSH and ACTH fractions. The major fraction was purified further on a carboxymethyl cellulose⁸ column; stepwise elution was used with 0.05 M, pH 5.9, and 0.25 M, pH 6.9, ammonium acetate. Two active fractions were resolved. Fraction A possessed MSH but no ACTH activity. Fraction B possessed 26 USP units of ACTH¹¹ and 4×10^4 units of intrinsic MSH per mg. and was judged

(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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(11) This preparation was found to be unstable by Dr. J. D. Fisher.

homogeneous when an acid hydrolysate gave nearly integral molar ratios upon amino acid analysis¹²: Ala₃, Arg₃, Asp₂, Glu₅, Gly₃, His, Leu, Lys₄, Met, Phe₃, Pro₄, Ser₃, Tyr₂, Val₃. 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 sequence¹⁴ 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 pH 6.5 and purified further by paper chromatography.¹⁵ The relative positions of each peptide and their constituent amino acids¹⁶ are shown in Fig. 1. Peptides with composition similar to those isolated from human material have been reported for porcine,⁴ ovine³ and bovine³ ACTH.

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(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 chromatography¹² or paper chromatography in the butanol-acetic acid-water (4:1:5) system.

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STRUCTURE OF MELATONIN¹

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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