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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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]

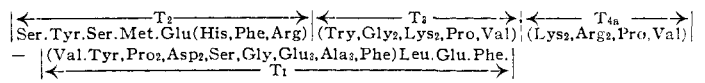

Fig. 1.—Constituent amino acids and possible arrangement of fragments from tryptic digestion of human ACTH. Three additional peptides (T_{4b}, T_5, T_6) were obtained. Their composition indicated that they were derived from T_{4a}. The relative positions of T_3 and T_{4a} have not been ascertained. By 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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(16) Amino acid composition was determined by ion-exchange chromatography[12] or paper chromatography in the butanol–acetic acid–water (4:1:5) system.

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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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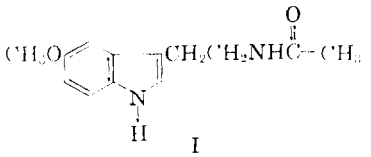

I

I was eluted from a silicic acid column and subjected again to countercurrent and column purification; 40 μg. of I was obtained from 100 g. of lyophilized bovine pineal glands.[6]

I and II possessed similar ultraviolet spectra (I, λ_{max} 2780 Å., shoulders at 2970, 3090 Å.; II, λ_{max} 2760 Å., shoulders at 2960, 3080 Å.); fluorescence spectra (when $\lambda_{excitation}$ = 304 mμ, I λ_{max} = 333 mμ, II λ_{max} = 338 mμ). These data and similar blue color with Ehrlich reagent suggested that I is also a substituted 5-hydroxyindole. O-Methylation at position 5 was suggested by: failure to migrate as a cation in electrophoresis at *p*H 11; lack of acid–base shift of ultraviolet absorption maxima; greatly increased lightening ability of many 5-methoxyindoles over their parent 5-hydroxyindoles.

The presence of an ester or amide in the side chain at position 3 was indicated by: detection of a carbonyl group (by infrared)[7] not conjugated with the indole nucleus (by ultraviolet absorption and fluorescence); neutrality of I and negative color reactions for aldehydes and ketones. An alcohol ester was unlikely because on hydrolysis of I with acid or base II was not detected. From these findings, together with observations that acetylcholine but not choline has lightening ability, we guessed that I might be N-acetyl-5-methoxytryptamine.

Synthetic N-acetyl-5-methoxytryptamine (III, 40 mg.) was prepared by reducing 100 mg. of 5-methoxyindole-3-acetonitrile[7] with 160 mg. of sodium and 2 ml. of ethanol,[8] then acetylating the product with 4 ml. of both glacial acetic acid and acetic anhydride at 100° for 1 minute. Purification was achieved by countercurrent distribution and silicic acid chromatography as for I.

III and I were found identical with respect to: countercurrent distribution (peak tube 12); elution curve from silicic acid column; ultraviolet and fluorescence maxima; biologic activity (minimal lightening of isolated frog skin at 10^{-12} gram/ml.); R_f in descending chromatography systems 2-propanol:concd. NH_3:water 16:1:3 (R_f 0.83); 1-butanol:acetic acid:water 4:1:5 (R_f 0.9); 1-butanol:acetic acid:water:pyridine 15:5:12:10 (R_f 0.9); heptane:pyridine 7:3 (R_f 0.10); heptane:pyridine 6.5:3.5 (R_f 0.80); benzene:ethyl acetate:water 20:1:20 (R_f 0.39).

The increased lightening ability of I over N-acetyl - 5 - hydroxytryptamine[9] suggests that O-methylation of hydroxyindoles forms substances of increased biologic activity, in contrast to O-methylation of catechol amines. The exquisite sensitivity of frog melanocytes to I indicates a neurohormone function, since melanocytes reflect their neural origin by responding to compounds, including acetylcholine and noradrenaline, which stimulate neurons.

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(6) We are grateful to the Armour Laboratories for supplying us with many kilograms of bovine pineal glands.

(7) We wish to thank Drs. A. Krivis and J. Szmuszkovicz and Mr. W. C. Anthony of The Upjohn Company for carrying out microinfrared studies, preparing numerous model compounds, supplying us with 5-methoxyindole 3-acetonitrile and offering many helpful suggestions.

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WATER SUBEXCITATION ELECTRONS IN AQUEOUS FORMIC ACID RADIOLYSIS[1]

Sir:

The sole γ-ray radiolysis and 1860 Å. photolysis products of dilute formic acid are equal amounts of carbon dioxide and hydrogen.[2,3] Carbon monoxide, however, is produced by formic acid excitation when the light-absorbing species is changed from water to formic acid at 1860, 2537 and 2669 Å.[3,4] Although electron capture mechanisms have been suggested[5,6,7] and may account for carbon monoxide, water subexcitation electrons[8,9] having a kinetic energy $\epsilon < E_w$, the lowest excitation potential of water, provide a more likely exciting species for this reaction.

$G(CO)$ for pure 26.6 *M* formic acid irradiated by γ-rays is 1.25 and consequently direct ionization and dissociation also produce carbon monoxide.[4] But direct ionization cannot account for the unexpectedly high $G(CO)$ of 0.25 and 0.50 in 0.1 and 1.0 *M* formic acid respectively. Over this concentration range, $G(CO_2)$ increases and $G(H_2)$ remains unchanged. Direct action $G(CO_2)$ here will be below 0.005 and 0.05. In view of carbon monoxide formation by light, its absence in dilute solution radiolysis and the expected negligible direct ionization action in 0.1 *M* solutions, direct dissociation of excited formic acid by water subexcitation electrons is postulated.

Although the probability of molecular excitation is proportional to the appropriate optical constant only for fast electrons,[10,11] the ratio of probabilities of two different excitations is approximately equal to the ratio of oscillator strengths down to fairly low values of ϵ.[12] Therefore we assume that all electrons including the subionization electrons excite water and formic acid in the ratio of $k_w c_w$ to $k_f c_f$ at any given energy transfer (k_w, k_f; molar

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