B)). Digestion with prostatic phosphomonoesterase[9] gave thymidine-5′ thymidine-3′ phosphate, identical on paper chromatograms (Solvent A) with a sample of this substance synthesized earlier.[1] The dithymidine phosphate produced above was degraded by the snake venom diesterase[8] fraction to give approximately equivalent amounts of thymidine and thymidine 5′-phosphate (Solvent A).

By the above synthetic procedure but using so far only *p*-toluenesulfonyl chloride as the reagent, we have prepared 3′-(2′-deoxyadenylyl)-thymidine 5′-phosphate (I, R = thymine; R′ = adenine).

Some progress has been made in the identification of the products which result when thymidine 5′-phosphate alone is treated with *p*-toluenesulfonyl chloride or DCC in anhydrous pyridine.[1] One product, present in the dialysable fraction,[10] travels faster than thymidine 5′-phosphate in Solvent A but slower in Solvent B. The substance which is more stable than thymidine dinucleotide, (I, R = R′ = thymine) to hydrochloric acid, gives thymidine 3′,5′-diphosphate on hydrolysis. Incubation with crude snake venom gives thymidine and inorganic phosphate whereas the diesterase[8] fraction slowly yields thymidine 5′-phosphate (Solvent B). An electrometric titration shows the absence of any groups titratable in the *p*H range 5–8. The total evidence indicates that this substance has the cyclic structure II (R = thymine). This finding demonstrates that in the thymidine 5′-phosphate "polymerization reaction" *inter*molecular and *intra*molecular phosphorylations are competing reactions.

It is clear that the above approach offers promise for the synthesis of the higher oligonucleotides.

Acknowledgments.—This work has been supported by grants from the National Research Council and National Cancer Institute of Canada.

(9) G. Schmidt, "Methods in Enzymology," Vol. II, Academic Press, Inc., New York, N. Y., 1955, p. 523-530.

(10) Dialysis against distilled water; a large proportion of the nucleotidic material is non-dialysable under these conditions.

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THE ISOLATION AND STRUCTURE OF A MELANOCYTE-STIMULATING HORMONE FROM BOVINE PITUITARY GLANDS

Sir:

The purification, isolation and structure of a porcine melanocyte-stimulating hormone (β-MSH) have recently been reported by various investigators.[1,2,3,4] We now wish to describe the isolation and structure of a melanocyte-stimulating peptide from the posterior-intermediate lobes of bovine pituitaries. In its countercurrent distribution behavior and amino acid composition, this peptide differs considerably from the bovine preparation.[2]

The isolation procedure was the same as that previously described for β-MSH,[4,5] with commercially available bovine posterior pituitary powder used as the starting material. Zone electrophoresis on starch[6] was carried out with pyridine–acetic acid buffers at *p*H 4.9 and at *p*H 6.4; under these conditions, it was observed that the MSH activity[7] possessed a cathodic mobility greater than that of β-MSH. When the active fraction obtained by zone electrophoresis was further purified by countercurrent distribution at 20° in the system 0.5% trichloroacetic acid *vs. sec*-BuOH (266 transfers), a main peak with a partition coefficient of 0.52 was obtained (Fig. 1); the MSH activity

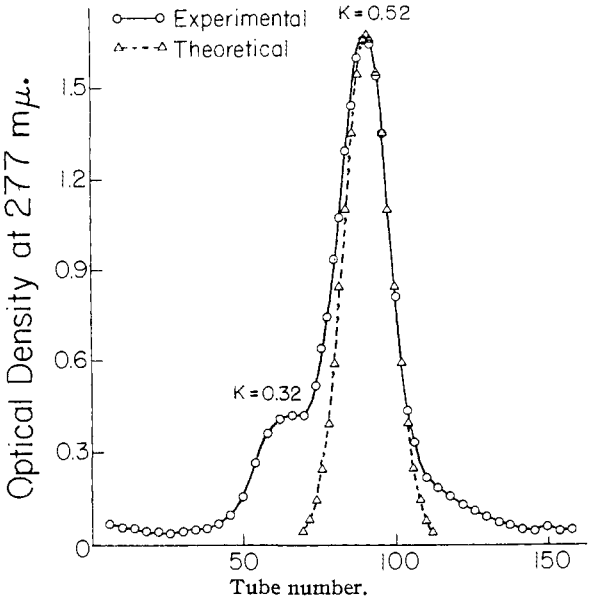


Fig. 1.

was concentrated in this peak. The material falling within the theoretical distribution curve for a partition coefficient of 0.52 (tubes 80–104) was recovered and submitted to zone electrophoresis on starch (*vide infra*) at *p*H's 4.9, 5.7, 6.4, 6.9, 7.3 and 8.4; at each *p*H the material migrated as a single zone which contained all the MSH activity. Moreover, after reaction of the material with carboxypeptidase and fluorodinitrobenzene, the sole amino acid found at either terminus was aspartic acid. Finally, quantitative amino acid analysis of the 24-hour acid hydrolysates by the paper-fluorodinitrobenzene method[8,9] gave the following molar ratio for the constituent amino acids

$Asp_{2.0}$, $Glu_{1.0}$, $Ser_{1.7}$, $Gly_{2.0}$, $Pro_{3.0}$, $Met_{1.1}$, $Phe_{1.0}$, $Tyr_{1.0}$, $Lys_{1.8}$, $His_{1.0}$, $Arg_{0.9}$, Try_1

(1) J. Porath, P. Roos, F. W. Landgrebe and G. M. Mitchell, *Biochim. et Biophys. Acta*, **17**, 598 (1955).

(2) B. G. Benfey and J. L. Purvis, *Biochem. J.*, **62**, 588 (1956).

(3) J. I. Harris and P. Roos, *Nature*, **178**, 90 (1956). We wish to apologize for the omission in our earlier communication[4] of mention of the independent work of Harris and Roos, giving the complete structure of β-MSH (porcine). A discussion of the lack of purity of the β-MSH preparations of other workers[1,2] also was not included because of space limitations. Reference to all of the above work has been made in two detailed papers on this subject.[5,15]

(4) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **78**, 4494 (1956).

(5) I. I. Geschwind and C. H. Li, *ibid.*, **79**, 615 (1957).

(6) H. G. Kunkel and R. J. Slater, *Proc. Soc. Exper. Biol. Med.*, **80**, 42 (1952).

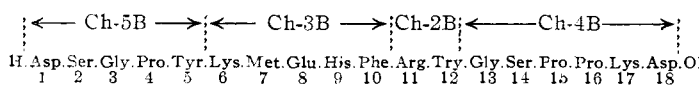
(7) The melanocyte-stimulating potency of each fraction was estimated by the method described by K. Shizume, A. B. Lerner and T. B. Fitzpatrick (*Endocrinology*, **54**, 553 (1954)).

(8) A. L. Levy, *Nature*, **174**, 126 (1954).

(9) Tryptophan was determined by the method of T. W. Goodwin and R. A. Morton (*Biochem. J.*, **40**, 628 (1946)).

No other amino acids were detected even as trace contaminants. No amide-NH_3 was detected in the peptide, and the isoelectric point was found to be 7.0 as determined by zone electrophoresis on starch.[10] It may be noted that there is one additional residue of serine and one less residue of glutamic acid in the bovine MSH than in β-MSH.[3,4,11] Hence, as would be expected, the isoelectric point[4,5] (pH 5.8) of β-MSH is lower than that of the bovine hormone.

By the paper-strip modification[12] of the phenyl isothiocyanate method,[13] the amino acid sequence at the N-terminus was established as Asp.Ser.Gly.-Pro.Tyr.Lys.Met. A portion of this N-terminal sequence was confirmed by means of kinetic studies with leucine aminopeptidase.[14] Aspartic acid was identified as the C-terminal amino acid by reaction with carboxypeptidase. Digestion of the peptide hormone with chymotrypsin gave rise to four peptides in accordance with the specificity of the enzyme:



The separation and analysis of these peptide fragments in the chymotryptic hydrolysate were carried out as previously described.[4,15] The amino acid sequence in peptide Ch-3B was also elucidated by the action of leucine aminopeptidase. These data permit the formulation of the structure shown above for the bovine MSH.

It will be seen that this structure differs from that proposed for the porcine hormone[3,4] only at position 2, where a seryl residue replaces a glutamyl. As far as we are aware, the replacement of an uncharged residue (serine) by a charged one (glutamic acid) has not previously been encountered in connection with species variations among biologically active peptides.

(10) I. D. Raacke and C. H. Li, *J. Biol. Chem.*, **215**, 277 (1955).
(11) P. Roos, *Acta Chem. Scand.*, **10**, 1061 (1956).
(12) H. Fraenkel-Conrat, THIS JOURNAL, **76**, 3606 (1954).
(13) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).
(14) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955). We are indebted to Dr. L. K. Ramachandran for a gift of this enzyme.
(15) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **79**, 620 (1957).
(16) This work was supported in part by grants from the U. S. Public Health Service (G-2907) and the Albert and Mary Lasker Foundation.

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RECEIVED DECEMBER 26, 1956

FLUOROTELLURATES

Sir:

We have studied the behavior of tellurium hexafluoride and of selenium hexafluoride toward various inorganic fluorides. Neither of these hexafluorides showed evidence of strong interaction or of fluorine exchange with covalent fluorides; however, the tellurium compound did react with certain ionic fluorides to form the new fluorotellurate salts. Selenium did not form fluoroselenates; apparently, the requisite orbitals in selenium lie at too high an energy level.

The fluorotellurates, which have the limiting composition $2MF \cdot TeF_6$, were prepared by heating a metal fluoride with an excess (100%) of tellurium hexafluoride in a pressure vessel to 250° and cooling this reaction mixture over a period of 24 hours to room temperature. After the excess gas was released, the solid was recovered. In the case of cesium the solid product analyzed closely for $2CsF \cdot TeF_6$. The X-ray pattern of the cesium compound consisted of many weak lines which were not indexed; no crystalline cesium fluoride was present. Rubidium and potassium fluorides gave products that had the empirical compositions $2RbF \cdot 0.52TeF_6$ and $2KF \cdot 0.32TeF_6$. X-Ray diffraction patterns of these solids showed the presence of the alkali metal fluoride and a second crystalline phase. The fluorides of sodium, lithium, and barium were inactive under the experimental conditions. Under comparable conditions, selenium hexafluoride gave no sign of reaction with cesium and potassium fluorides. The F^{19} magnetic resonance spectra of binary systems of liquid tellurium (and selenium) hexafluoride with arsenic trifluoride and with boron trifluoride showed no evidence of compound formation or of a measurable fluorine exchange rate up to 60°.

The fluorotellurates dissociated at elevated temperatures to yield the starting materials. Crude measurements of the dissociation pressure of cesium fluorotellurate yielded an average value for the heat of dissociation of 0.4 kcal. over the temperature range 200–250°. Addition of the fluorotellurates to water resulted in evolution of tellurium hexafluoride and dissolution of the alkali metal fluoride. The fluorotellurates displayed no stability outside the solid state, and the stability of the lattice appeared to be inversely related to the polarizing power of the cation. The lattice could either be composed of M^+ and $TeF_8^=$ or of M^+, F^-, and TeF_7^- aggregates. A unique solution of the crystal structure is being sought in analyses of the X-ray diffraction pattern and the F^{19} magnetic resonance spectrum of the cesium compound.

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FORMATION OF 6β-HYDROXY AND 6-KETO DERIVATIVES OF ESTRADIOL-16-C^{14} BY MOUSE LIVER MICROSOMES

Sir:

Further study of the protein-binding of estradiol by fortified liver preparations[1] has revealed that mouse liver microsomes in the presence of reduced triphosphopyridine nucleotide and oxygen convert estradiol-16-C^{14} to at least five new metabolites[2] extractable into an acetone-benzene mixture (3:4). Preliminary chromatographic analysis on

(1) I. L. Riegel and G. C. Mueller, *J. Biol. Chem.*, **210**, 249 (1954).
(2) G. Rumney, *Fed. Proc.*, **15**, 343 (1956).