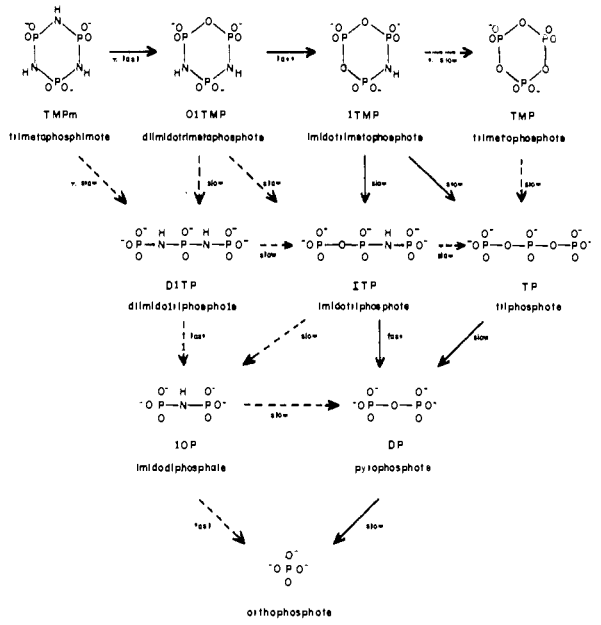
this change was reported recently.[3] Hydrolysis of DITP (*p*H 3, 60°) results in the initial formation of P–NH_2 and P–OH fragments which then condense to an extent of up to 30% of the total phosphorus present. TMPm is converted almost quantitatively into DITMP (*p*H 3).



The ring imidophosphates have been isolated from TMPm hydrolysates in good yields by ion exchange techniques. Although the chain imidophosphates occur in the acid hydrolysates of TMPm to only a small extent, DITP, ITP, and IDP have been prepared in high yields by selectively cleaving the corresponding ring imidophosphates at the oxygen bridges in 30% NaOH (75° or higher). At high NaOH concentrations P–N–P bridges appear to be inert, whereas they are cleaved more readily than P–O–P bridges in the *p*H range 2–11. Thus, P–N–P linkages differ markedly from P–O–P in dependence of hydrolysis rate on *p*H; more quantitative data will be reported soon.

The various compounds involved in this study were characterized by elemental analyses, acid–base titrations, paper chromatographic and ion-exchange separations, and X-ray diffraction (powder patterns). Products obtained by the methods of Stokes and de Ficquelmont had the elemental analyses of the monohydrate of DITMP (some ITMP·H_2O), an acid–base titration curve of a ring polyphosphate (all H's strong), an X-ray pattern nearly identical to that of TMPm·H_2O, and yielded the corresponding DITP·$6H_2O$, ITP·$6H_2O$ mixture in 30% NaOH (75°). The monohydrates of all three ring imidophosphates are isomorphous and form a continuous series of solid solutions. The hexahydrates of DITP, ITP, and TP are similarly isomorphous. A typical analysis of DITP·$6H_2O$ gave 24.3% Na, 19.8% P, 5.92% N (theoretical, 24.26% Na, 19.6% P, 5.91% N), a titration curve with pronounced endpoints at *p*H 5 and 8.6 (spread one H per mole of DITP·$6H_2O$) and a vague endpoint at *p*H 10.8.

A detailed paper on this work is in preparation.

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ISOLATION AND STRUCTURE OF MELANOCYTE-STIMULATING HORMONE FROM PORCINE PITUITARY GLANDS[1]

Sir:

We wish to report herein the isolation in pure form of the melanocyte-stimulating hormone (MSH, intermedin) from the posterior lobes of porcine pituitary glands. The structure of this hormone, a peptide consisting of 18 amino acids, will also be presented in this communication.

A crude fraction of MSH was prepared from porcine posterior pituitary powder by glacial acetic acid extraction and fractional precipitation with acetone and ether, followed by adsorption on oxycellulose (15% of the weight of the ether precipitate).[2] Following elution with 0.1 *N* HCl, a highly potent[3] concentrate was obtained. The eluate was de-acidified with methyldioctylamine[4] and brought to *p*H 6.5–7.0 with dilute ammonia. The precipitate that formed was discarded, and the supernatant fraction, after lyophilization, was submitted to zone electrophoresis on starch[5] with a pyridine-acetic acid buffer of *p*H 4.9. A peak containing the bulk of the MSH activity could be eluted from a very narrow region; this peak was then submitted to countercurrent distribution for 1100 transfers at 20°, in the system 0.5% trichloroacetic acid *vs. sec*-BuOH. One main skewed peak was observed. The peak was divided in half and each half was recovered separately. When both halves were re-run in the same system for 248 transfers, the distribution curve of each was virtually identical to the theoretical, and both possessed *K* values of 0.60. They were also shown to possess practically identical biological activities.

Quantitative amino acid analysis of the 24- and 48-hour acid hydrolysates by the paper-fluorodinitrobenzene method[6] gave the following composition, based on molar ratios of the constituent amino acids:

$Asp_{2.0}$, $Glu_{2.0}$, $Ser_{1.0}$, $Gly_{1.9}$, $Pro_{3.1}$, $Met_{0.7}$, $Phe_{1.1}$, $Tyr_{1.0}$, $Lys_{1.8}$, $His_{1.0}$, $Arg_{1.0}$, $Try_{1.0}$.

Tyrosine and tryptophan were determined by a

(1) This work is supported in part by the U. S. Public Health Service (G-2907) and the Albert and Mary Lasker Foundation. Original manuscript received July 2, 1956.

(2) (a) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950); (b) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951); (c) M. S. Raben, I. N. Rosenberg and E. B. Astwood, *Federation Proc.*, **11**, 126 (1952).

(3) Potency of preparations has been determined by the method described by K. Shizume, A. B. Lerner and T. B. Fitzpatrick (*Endocrinology*, **54**, 553 (1954)). Preparations purified by zone electrophoresis possess activities of 1×10^{10} MSH u./g. The unit is that described by Shizume, *et al.* Though a chemical purification occurs during the counter-current distribution procedure, some inactivation also takes place.

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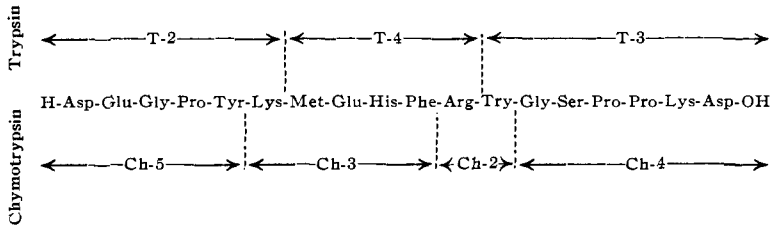

spectrophotometric method.[7] The amide content as determined by two different procedures[8,9] was 0.0. Thus, the minimum molecular weight calculated from these data is 2,177. Moreover, the isoionic point is computed[8,10] to be at *p*H 5.9. This value is in good agreement with the experimentally determined isoelectric point, which was found by zone electrophoresis on starch to be at *p*H 5.8, after correction for electroösmosis.[11]

For structural determinations, 20-mg. aliquots were digested with either crystalline trypsin (Armour) or crystalline chymotrypsin (Armour), and in each case the entire hydrolysate was submitted to electrophoresis on paper in a collidine-acetic acid buffer.[12] All peptides were located by means of guide strips sprayed with ninhydrin, and were subsequently eluted from the paper with dilute ammonia. The homogeneity of each peptide area was demonstrated by paper chromatography of aliquots in two different solvent systems. All peptides were analyzed for the N-terminal amino acid[13] and for quantitative amino acid composition.[6] Aliquots of the peptides arising from chymotryptic digestion were also submitted to digestion with carboxypeptidase to determine the C-terminal sequence. Carboxypeptidase was also employed for C-terminal analysis[14] of the MSH molecule itself, as was stepwise degradation from the N-terminus by the paper-strip modification[15] of the phenyl isothiocyanate[16] reaction. The latter procedure was successfully employed for eight successive steps. From these data, a structure for the MSH molecule is proposed (see above).

The most striking aspect of this formulation is that the sequence . . . Met . Glu . His . Phe . Arg . Try . Gly . . . also occurs, identically, in all the corticotropins[17,18,19] that have been studied hitherto. This, in all probability, explains the intrinsic melanocyte-stimulating activity of pure corticotropin preparations. In the corticotropin molecule, this central sequence apparently acquires, by virtue of a different specific order of amino acids on each side of it, an adrenal-stimulating activity as well. The significance of these results will be the subject of subsequent communications.

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TEMPERATURE EFFECTS ON NUCLEAR MAGNETIC RESONANCE ABSORPTION OF HYDROGENS ATTACHED TO NITROGEN

Sir:

The nuclear magnetic resonance absorption of hydrogens attached to nitrogen (^{14}N) may show sharp, broad or triplet lines. Single sharp lines are obtained with amines and ammonia where exchange is rapid.[1] Broad lines (10–75 c.p.s. at 40 mc.) are observed for many amides and pyrrole,[2] while sharp triplet absorption has been observed for gaseous and completely anhydrous ammonia[2] and ammonium ions in acid solution,[3] where N–H exchanges are slow.[4] Triplet absorption is expected from spin–spin interactions between the protons and ^{14}N ($I = 1$).

The broad lines associated with N–H absorption of amides and pyrrole could be due to intermediate rates of exchange[4] or relaxation of the ^{14}N nuclei by interaction between their quadrupoles and surrounding asymmetrical electrical fields.[5] Indeed, significant sharpening of N–H lines by exchange was achieved through adding small amounts of sodium to pyrrole and concd. ammonia to formamide. However, the broad lines in the pure liquids are *not* the result of intermediate exchange rates because the line widths *decrease* with increasing temperature and, with several substances, triplet N–H absorption has been observed at higher temperatures. This behavior is opposite to that expected for any exchange process[3] having a positive temperature coefficient. That exchange was not involved was demonstrated by the spectrum of an acidified pyrrolidine hydrochloride solution at 0°. This had a broad singlet N–H absorption and showed sharp spin–spin splitting (~7 c.p.s.) of the α-hydrogens by the N–H hydrogens. At 50°, the N–H absorption was a broadened triplet without change in the splitting of the α-hydrogens.

In Table I, approximate temperatures where the N–H absorption was intermediate between singlet and triplet as well as line appearances at 30° are listed for several substances. With several amides,

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(3) R. A. Ogg, *Faraday Society Discussion*, **17**, 215 (1954).
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(5) For refs. see J. E. Wertz, *Chem. Revs.*, **55**, 829 (1955).