phenyl isothiocyanate in peroxide-free dioxane and placed moist into an atmosphere saturated with pyridine, dioxane and water for 2–3 hours at 40°. They are then exhaustively washed with several changes of benzene and a mixture of equal parts of absolute alcohol and peroxide-free ether (until the supernatant after 2–3 hours of gentle shaking shows an o.d. of less than 0.025 at 270 mμ, usually after about 24 hr.). The strips are then dried, and placed in the bottom of a desiccator containing beakers with glacial acetic and 5.7 *N* hydrochloric acid. The desiccator is evacuated to about 100 mm. After 4–16 hours, depending on the protein, the papers are aerated to dissipate the acids and again extracted with the alcohol ether mixture. The PTH's in this solution show the characteristic maximum near 270 mμ, from which their amount and purity can be estimated.[3,5] They are identified either directly by chromatography[6] or after hydrolysis to the amino acids.[2,5] The paper strips are ready for the next cycle of addition of the reagent and release of the PTH.

This technique has been carried through as many as 14 steps with insulin. The yields of PTH are quite similar (about 1.7 equiv. per 6000 g.) through the first five steps and then decrease, sometimes gradually and sometimes more irregularly. Also with other proteins a characteristic amount of PTH is formed at each of several steps. This suggests a clean sequence of reactions. However, chromatographic analysis of the PTH's does not always bear out this conclusion. With insulin, oxytocin, and, according to Harris and Li, α-corticotropin[7] the N-terminal and adjacent peptide bonds are split through the first 5–7 steps, with little evidence for non-specific splitting. Then the chromatographic picture gets rather suddenly much more complex and no further identifications have as yet been possible.

The results (Table I) obtained have confirmed the known amino acid sequences of insulin[8] and oxytocin.[9] One of the remaining questions in the structure of insulin, the location of amide groups, has been answered for the four dicarboxylic residues near the N terminal end of the molecule. Tentative conclusions, in part confirmatory, have been reached for some other proteins. The sequence of eight amino acids of α-corticotropin, as determined by this method, is described by Harris and Li in THIS JOURNAL.[7]

TABLE I

AMINO ACIDS OBTAINED IN STEPWISE DEGRADATION OF PROTEINS[a]

Step no.	I	II	III	IV	V	VI
Insulin	gly, phe	ileu, val	val, asp NH_2	glu NH_2	glu NH_2, his[a]	
Insulin, A chain	gly	ileu	val	glu NH_2	glu[a] NH_2	glu[a]
Insulin, B chain	phe	val[a]	asp NH_2	glu NH_2	his	his[a]
Oxytocin	none[b]	tyr	ileu	glu NH_2	asp NH_2	none
Lysozyme	lys	val[a]	?	gly[a]	many	
Myoglobin	gly	leu[a]	ser[a]	glu[a]		
β-Lactoglobulin	leu	leu[a]	val[a]			
Protamine	pro	arg	arg[a]			

[a] Only the predominating amino acids are listed; cases in which others seemed in amount to approach the main product are indicated by *a*. Identification is then not unequivocal. [b] Cystine PTH is expected to be formed and to remain attached to the peptide.

(5) H. Fraenkel-Conrat and B. Singer, THIS JOURNAL, **76**, 180 (1954); H. Fraenkel-Conrat, in the Chemical Structure of Proteins, p. 102, J. and A. Churchill, Ltd., London, 1953.

(6) J. Sjöquist, *Acta Chem. Scand.*, **7**, 447 (1953).

(7) J. I. Harris and C. H. Li, THIS JOURNAL, **76**, 3607 (1954).

(8) F. Sanger and H. Tuppy, *Biochem. J.*, **49**, 463, 481 (1951); F. Sanger and E. O. P. Thompson, *ibid.*, **53**, 535, 666 (1953).

(9) This sequence, established by du Vigneaud, *et al.* (V. du Vigneaud, C. Roessler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953)), was actually confirmed by these authors by a modification of the Edman method.

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N- AND C-TERMINAL AMINO ACID SEQUENCES OF α-CORTICOTROPIN (ACTH)[1]

Sir:

The isolation from *sheep* pituitary glands of a polypeptide (α-corticotropin) possessing adrenal stimulating activity was recently reported from this laboratory.[2] The hormone was shown to contain 39 amino acid residues with a minimum molecular weight of 4,500. We wish to report the N- and C-terminal sequences obtained when stepwise degradation procedures were applied to α-corticotropin.

The phenyl*iso*thiocyanate (PTC) procedure of Edman[3] as modified by Fraenkel-Conrat[4] was employed for the investigation of the N-terminal sequence. In a typical experiment, 2.8 mg. of α-corticotropin trichloracetate (0.5 μM.) was dissolved in 50 μl. of 2% aqueous ammonia and the solution was applied to a strip (2 × 8 cm.) of Whatman No. 1 filter paper. After it was dried, the strip was treated with 50 μl. of 20% PTC in dioxane, and incubated in an atmosphere of aqueous pyridine for three hours at 40°. The strip was then thoroughly washed successively with benzene and 1:1 absolute alcohol/ether (peroxide-free). Cleavage of the phenylthiohydantoin (PTH) of the N-terminal amino acid was accomplished by placing the washed strip in an evacuated desiccator containing glacial acetic acid and 6 *N* hydrochloric acid for eight hours at room temperature, and the liberated PTH was extracted from the paper strip by shaking for one hour with 10 ml. of ethanol:ether (1:1). In this manner the PTH derivative of the N-terminal residue in α-corticotropin was obtained in a yield of 85%, and was identified as serine PTH by direct chromatography on paper in both the heptane/pyridine and the heptane/*n*-butanol/formic acid systems proposed by Sjöquist.[5]

A second application of the above procedure led to the isolation in 80% yield of a second phenyl-

(1) This investigation was supported in part by research grants from the National Institutes of Health, U.S. Public Health Service (G 20907), and Eli Lilly and Company.

(2) C. H. Li, I. I. Geschwind, A. L. Levy, J. I. Harris, J. S. Dixon, N. G. Pon and J. O. Porath, *Nature*, **173**, 251 (1954).

(3) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).

(4) H. Fraenkel-Conrat, THIS JOURNAL, **76**, 3606 (1954); and in D. Glick, "Methods of Biochemical Analysis," Vol. II, Interscience Publishers, New York, in press. We are indebted to Dr. Fraenkel-Conrat for communicating to us the details of the paper strip procedure prior to its publication.

(5) J. Sjöquist, *Acta Chem. Scand.*, **7**, 447 (1953).

thiohydantoin which was identified chromatographically as tyrosine PTH. In all, the material on the paper strip was submitted to eight successive applications of the PTC procedure; the results obtained are summarized in Table I. The phenylthiohydantoins were subjected to paper chromatography, each in the two-solvent systems described above, and identified by comparison of their R_f's with those of control phenylthiohydantoins which were run concurrently. Furthermore, the PTH derivatives of tyrosine, methionine, glutamic acid and phenylalanine were chromatographically identified as the corresponding amino acids, after hydrolysis in sealed evacuated tubes with 6 N HCl at 150° for 16 hours. From these results it is concluded that α-corticotropin is composed of a single polypeptide chain, having the N-terminal[6] heptapeptide sequence ser.tyr.ser.met.glu.his.phe....

TABLE I

STEPWISE DEGRADATION OF α-CORTICOTROPIN

Step no.	Phenylthiohydantoin Yield,[a] M/4500 g.	Residue[b]
1	0.85	Serine
2	.83	Tyrosine
3	.80	Serine
4	.85	Methionine
5	.81	Glutamic acid
6	.58	Histidine
7	.62	Phenylalanine
8	< .05	Unidentified

[a] Estimated from optical density readings at 270 mμ, assuming ϵ = 16,000. [b] Identified by paper chromatography according to Sjöquist.[5]

The amino acid sequence from the carboxyl end of the peptide was investigated with the aid of carboxypeptidase by the procedure previously described.[7] α-Corticotropin trichloracetate (5.0 mg.) was incubated with diisopropylfluorophosphate-treated carboxypeptidase (0.25 mg.) in a 5.0 ml. solution at pH 8.5 and a temperature of 40°; aliquots of the digest (1.0 ml.) were removed at suitable time intervals and allowed to react with dinitrofluorobenzene at pH 9.0 and 40° for the separation and quantitative estimation[8] of the free amino acids released during the enzymatic reaction. The results indicated that phenylalanine, glutamic acid, and leucine are released successively by the stepwise degradation of the C-terminal sequence[6] . . . .leu.glu.phe in the α-corticotropin molecule. Furthermore, if it is assumed that one mole of C-terminal amino acid is released per mole of α-corticotropin, a value of 4,500 (±200) may be calculated for its equivalent weight which is in excellent agreement with the minimum molecular weight based on amino acid analysis.[2]

The fact that no further significant digestion took place after equivalent stoichiometric amounts of phenylalanine, glutamic acid and leucine had been released in the course of the carboxypeptidase reaction suggested that the fourth amino acid along the chain is one which, failing to conform to the specificity requirements of the enzyme, resists digestion; for example, proline. This prediction was confirmed when the tetrapeptide pro.leu.glu.phe,[6] and the tripeptide pro.leu.glu (R_f's 0.83 and 0.75, respectively, on Whatman No. 1 paper using butanol/acetic acid/H_2O, 4:1:5 as the solvent system) were isolated and characterized from a pepsin digest (enzyme/substrate mole ratio 1:250, pH 2.0, 40°, 8 hours) of α-corticotropin. Since only one residue of leucine is present in the molecule, the tetrapeptide pro.leu.glu.phe is shown to be the C-terminal sequence in α-corticotropin.

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(6) Ser.tyr has been identified as the N-terminal dipeptide (W. A. Landmann, *et al.*, THIS JOURNAL, **75**, 4370 (1953); S. W. Fox, *et al.*, *ibid.*, **76**, 1154 (1954)), and pro.leu.glu.phe as the C-terminal tetrapeptide (W. F. White, *ibid.*, **75**, 4877 (1953)) sequences in Corticotropin A isolated from *hog* pituitary glands.

(7) J. I. Harris in D. Glick, "Methods of Biochemical Analysis," Vol. II, Interscience Publishers, New York, in press; J. I. Harris, C. H. Li, P. Condliffe and N. G. Pon, *J. Biol. Chem.*, **209**, 133 (1954).

(8) A. L. Levy, *Nature*, in press; A. L. Levy and D. Chung, to be published.

ENZYMATIC SYNTHESES OF PYRIMIDINE AND PURINE NUCLEOTIDES.[1] III. FORMATION OF NUCLEOSIDE DIPHOSPHATES AND TRIPHOSPHATES

Sir:

Extracts of yeast and liver which convert orotic acid to uridine-5′-phosphate (U5P) also bring about the synthesis of uridine diphosphate (UDP) and uridine triphosphate (UTP).[2] While several pathways are known for the formation of UTP,[3] no mechanism has been elucidated for its synthesis from U5P. We have now obtained evidence with the use of a partially purified enzyme from yeast for the conversion of U5P to UTP by myokinase-like reactions (equation (1), and illustrated by equations (2, 3)). Unlike muscle myokinase,[4] which acts on adenosine nucleotides only, this yeast enzyme effects a transphosphorylation between uridine and adenosine nucleotides.

(1) Nucleoside-P + *nucleoside*-P-P-P ⇌ nucleoside-P-P + *nucleoside*-P-P

(2) U5P + adenosine triphosphate (ATP) ⇌ UDP + adenosine diphosphate (ADP)

(3) U5P + UTP ⇌ 2 UDP

Like U5P, adenosine-5′-phosphate (A5P) is phosphorylated by the same enzyme preparation by analogous reactions (equations (4, 5)).

(4) A5P + UTP ⇌ ADP + UDP

(5) A5P + ATP ⇌ 2ADP

Preliminary spectrophotometric evidence for the formation of nucleoside diphosphates (equations (2–5)) was obtained by the use of the phosphopy-

(1) This investigation was supported by research grants from the National Institutes of Health, Public Health Service.

(2) A. Kornberg, I. Lieberman and E. S. Simms, THIS JOURNAL, **76**, 2027 (1954); I. Lieberman, A. Kornberg and E. S. Simms, *ibid.*, **76**, 2844 (1954).

(3) A. Kornberg, "Phosphorus Metabolism," Vol. I, edited by W. D. McElroy and B. Glass, The Johns Hopkins Press, Baltimore, Md., 1951; A. Munch-Petersen, H. M. Kalckar, E. Cutolo and E. E. B. Smith, *Nature*, **172**, 1036 (1953); P. Berg and W. K. Joklik, *ibid.*, **172**, 1008 (1953).

(4) S. P. Colowick and H. M. Kalckar, *J. Biol. Chem.*, **148**, 117 (1943); H. M. Kalckar, *ibid.*, **148**, 127 (1943).