Everted sacs of rat ileum[3] tied with thread at both ends and containing 1 ml saline medium show the same effects as the FISHER AND PARSONS preparation (Table II).

The aerobic production of lactic acid by isolated intestinal wall[3,4] is not likely to be an artefact due to inadequate oxygenation, or tissue damage, as it occurs regularly under a variety of different conditions and in isolated intestinal mucosa which is very thin and must be effectively oxygenated[2]. The preferential discharge of the lactic acid in one direction suggests that the formation of lactic acid may play a role in the absorption of glucose. HESTRIN-LERNER AND SHAPIRO[5] have recently reported experiments which suggest that glucose is transported through the intestinal wall in the form of an unidentified derivative which is reconverted into glucose in some other organ. The experiments reported here suggest that the derivative is lactic acid.

Three mechanisms are available for the absorption of glucose *in vivo*: free diffusion of glucose with a concentration gradient, active transport of glucose, as such, against a concentration gradient (perhaps via phosphorylation and dephosphorylation) and conversion in the mucosal cells of glucose to lactate which passes preferentially into the blood to be reconverted into glucose in some other organ in the body. The relative quantitative importance of these three mechanisms varies with conditions *in vitro* and *in vivo* and probably also with different species of animals. The high rate of aerobic glycolysis in the renal medula[6] may be possibly also related to glucose absorption.

The author wishes to thank Dr. R. B. FISHER and Dr. D. S. PARSONS for their generous assistance during the perfusion experiments. Thanks are also extended to Prof. H. A. KREBS and Dr. R. E. DAVIES for advice and criticism.

REFERENCES

1 R. B. FISHER AND D. S. PARSONS, *J. Physiol.*, 110 (1949) 36.
2 F. DICKENS AND H. WEIL-MALHERBE, *Biochem. J.*, 35 (1941) 7.
3 T. H. WILSON AND G. WISEMAN, unpublished.
4 E. LUNDSGAARD, *Fiziol. Zhur.*, 29 (1940) 311.
5 S. HESTRIN-LERNER AND B. SHAPIRO, *Nature*, 171 (1953) 745.
6 F. DICKENS AND H. WEIL-MALHERBE, *Biochem. J.*, 30 (1936) 659.
7 S. B. BARKER AND W. H. SUMMERSON, *J. Biol. Chem.*, 138 (1941) 535.

Received May 13th, 1953

THE AMINO-ACID SEQUENCE IN OXYTOCIN*

by

H. TUPPY

Zweites Chemisches Laboratorium, University of Vienna (Austria)

Degradative studies involving partial hydrolysis of oxidized oxytocin with mineral acid and with enzymes suggest the presence in ocytocin of the following amino-acid sequence:

CyS–Tyr–Ileu–Glu(NH_2)–Asp(NH_2)–CyS–Pro–Leu–Gly(NH_2)

The oxytocin preparation used was obtained from a commercial concentrate ("Pituisan", kindly supplied by SANABO, Vienna) by a process based largely on the 2-butanol extraction method of LIVERMORE AND DU VIGNEAUD[1]. It was shown to be virtually free from other peptide or protein material by paper electrophoresis[2], using the high voltage method of MICHL[3] and the bromophenol blue stain of KUNKEL, TAYLOR, AND DU VIGNEAUD[2]. On hydrolysis, only the eight amino acids known to be present in oxytocin[4] could be detected: leucine (Leu), *iso*leucine (Ileu), tyrosine (Tyr), proline (Pro), glutamic acid (Glu), aspartic acid (Asp), glycine (Gly), cystine ($(CyS)_2$). The preparation did, however, still contain some non-peptide material.

* This work was aided by a grant from the Rockefeller Foundation to Prof. F. WESSELY.

The oxytocin preparation was oxidized with performic acid[5] and hydrolyzed with 5.7 *N* HCl for 8 days at 37° C. The resulting amino-acid and peptide mixture was investigated by two-dimensional paper chromatography[6] using essentially the same methods as those reported by SANGER AND TUPPY[7]. The following peptides could be identified: $CySO_3H$–Tyr, Ileu–Glu, Asp–$CySO_3H$, $CySO_3H$–[Pro, Leu], and Leu–Gly.

Further information was obtained by subjecting oxidized oxytocin to the action of the proteolytic enzyme from Bacillus subtilis, which LINDERSTRÖM-LANG AND OTTESEN[8] had found to transform ovalbumin to plak-albumin. This proteinase was recently crystallized by GÜNTELBERG AND OTTESEN[9] and a sample was generously presented to us. The enzymic digest of oxidized oxytocin was fractionated in a four-compartment ionophoresis cell[7]. The acidic fraction was found to contain two main peptides, each yielding, on hydrolysis, four amino acids, [$CySO_3H$, Tyr, Ileu, Glu] and [Asp, $CySO_3H$, Pro, Leu], the N-terminal residues being $CySO_3H$ and Asp, respectively. From the basic fraction a product was obtained which developed a yellow colour with ninhydrin; it revealed, after hydrolysis with HCl, glycine as the sole constituent amino acid and was chromatographically indistinguishable from glycine amide.

From these findings the structure of oxytocin can be deduced, taking into account DU VIGNEAUD's earlier results showing that the oxytocin molecule is composed of eight amino acids in peptide linkage, each occurring once, and of three residues of ammonia in amide linkage.

The peptides identified in the partial acid hydrolysate of oxydized oxytocin establish the presence of the amino-acid sequences $CySO_3H$–Tyr–Ileu–Glu and Asp–$CySO_3H$–Pro–Leu, respectively, in the two acidic tetrapeptides of the enzymic digest. In these tetrapeptide sequences, glutamic acid and aspartic acid are likely to be present in their amide forms, as glutamine and asparagine residues, thus accounting for 2 of the 3 ammonia residues in oxytocin. The third ammonia residue is accounted for by the basic split product glycine amide which must be assigned the C-terminal position in the oxytocin molecule. As indicated by the dipeptide Leu–Gly, glycine amide is linked to leucine so that Asp(NH_2)–$CySO_3H$–Pro–Leu must be derived from the middle part of the peptide chain. $CySO_3H$–Tyr–Ileu–Glu(NH_2) is then left to represent the N-terminal sequence. This is in perfect agreement with the fact established earlier that cystine occupies a N-terminal position in oxytocin[10].

In the peptide chain of oxidized oxytocin $CySO_3H$–Tyr–Ileu–Glu(NH_2)–Asp(NH_2)–$CySO_3H$–Pro–Leu–Gly(NH_2) the two cysteic acid residues arise from the two halves of one single cystine residue in oxytocin, through oxidative breakage. We must therefore assume that oxytocin is represented by a single peptide chain bound together into a loop through the sulfur atoms of the two half-cystine (CyS) residues.

Six of the eight amino acids found in hydrolysates of oxytocin have also been reported to be present in hydrolysates of purified vasopressin, only leucine and *iso*leucine being replaced by phenylalanine and arginine[11]. Nevertheless a comparison of the amino-acid sequence in oxytocin as formulated above with the order of some amino-acid residues in vasopressin as worked out by ACHER, CHAUVET AND FROMAGEOT[12] shows that a very close structural relationship between these two posterior pituitary hormones does not exist.

A detailed report of this work will be published in *Monatshefte für Chemie*.

REFERENCES

1 A. H. LIVERMORE AND V. DU VIGNEAUD, *J. Biol. Chem.*, 180 (1949) 365.
2 H. G. KUNKEL, S. P. TAYLOR, AND V. DU VIGNEAUD, *J. Biol. Chem.*, 200 (1953) 559.
3 H. MICHL, *Monatsh. Chem.*, 82 (1951) 489.
4 J. G. PIERCE AND V. DU VIGNEAUD, *J. Biol. Chem.*, 182 (1950) 359.
5 J. M. MUELLER, J. G. PIERCE, H. DAVOLL, AND V. DU VIGNEAUD, *J. Biol. Chem.*, 191 (1951) 309.
6 R. CONSDEN, A. H. GORDON AND A. J. P. MARTIN, *Biochem. J.*, 41 (1947) 590.
7 F. SANGER AND H. TUPPY, *Biochem. J.*, 49 (1951) 463.
8 K. LINDERSTRÖM-LANG AND M. OTTESEN, *Comptes rend. trav. lab. Carlsberg, Sér. chim.*, 26 (1949) 403.
9 A. V. GÜNTELBERG AND M. OTTESEN, *Nature*, 170 (1952) 802.
10 H. DAVOLL, R. A. TURNER, J. G. PIERCE AND V. DU VIGNEAUD, *J. Biol. Chem.*, 193 (1951) 363.
11 R. A. TURNER, J. G. PIERCE AND V. DU VIGNEAUD, *J. Biol. Chem.*, 191 (1951) 21.
12 R. ACHER, J. CHAUVET, AND P. FROMAGEOT, *Biochim. Biophys. Acta*, 9 (1952) 471.

Received June 4th, 1953