LACTATE AND HYDROGEN ION GRADIENTS DEVELOPED ACROSS THE RAT INTESTINE *IN VITRO*

by

T. H. WILSON*

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield (England) and Department of Biochemistry, Oxford University, Oxford (England)

When the upper small intestine of the rat is perfused *in vitro* according to the method of FISHER AND PARSONS, fluid is transferred from the mucosal to the serosal side[1]. Within one hour the glucose concentration rises on the serosal side and drops considerably on the mucosal side (see FISHER AND PARSONS[1]). Some glucose disappears from the system and about half of this can be accounted for by lactic acid of which more than 80% appears on the serosal side in the form of lactate ion. Considering that the lactic acid is produced by the mucosa[2], its appearance on the serosal side is unexpected. Another surprising finding is the constancy of the concentration of bicarbonate on the serosal side and the fall on the mucosal side. The result of one such experiment is shown in Table I. The inference is that glucose is converted into lactic acid in the intestinal wall, and whilst the lactate ions appear on the serosal side the H ions are ejected at the mucosal side.

TABLE I

GLUCOSE, LACTATE AND H^+ GRADIENTS IN PERFUSED RAT INTESTINE

62 cm of upper small intestine of rat perfused according to the method of FISHER AND PARSONS[1] with oxygenated (5% CO_2 + 95% O_2) bicarbonated-Ringer containing 0.5% glucose at 38° for 1 h. Lactate determined colorimetrically[7] and bicarbonate manometrically. pH calculated assuming $pK = 6.1$ and $CO_2 = 1.238$ mM.

	Volume	*Glucose*		*Lactate*		*Bicarbonate*		*pH*
	ml	*Conc. mM*	*Total amount µMol*	*Conc. mM*	*Total amount µMol*	*Conc. mM*	*Total amount µMol*	
Initial Mucosal	53.6	26.8	1440	—	—	22.5	1210	7.36
Final Mucosal	38.2	16.3	622	1.07	40.8	13.2	505	7.13
Initial Serosal	40.3	28.2	1140	—	—	24.3	980	7.39
Final Serosal	53.4	31.6	1690	6.33	338	25.0	1340	7.40
Change on Mucosal Side			—820		+ 41		—700	
Change on Serosal Side			+550		+340		+360	
Net Change			—270		+380		—340	

TABLE II

GLUCOSE, LACTATE AND H^+ GRADIENTS IN A SAC OF EVERTED RAT INTESTINE

Everted sac of rat intestine (142 mg dry wt.) shaken for 90 min in bicarbonate-Ringer at 37° C and gassed continuously with 5% CO_2 + 95% O_2. pH calculated as in Table I.

	Volume	*Glucose*		*Lactate*		*Bicarbonate*		*pH*
	ml	*Conc. mM*	*Total amount µMol*	*Conc. mM*	*Total amount µMol*	*Conc. mM*	*Total amount µMol*	
Initial Mucosal	20.0	8.60	172	—	—	20.8	416	7.33
Final Mucosal	18.3	2.32	42.5	7.1	131	8.6	158	6.94
Initial Serosal	1.0	8.60	8.6	—	—	20.8	20.8	7.33
Final Serosal	2.68	11.8	31.6	45	121	28.0	75.0	7.45
Change on Mucosal Side			—130		+130		—260	
Change on Serosal Side			+ 20		+120		+ 50	
Net Change			—110		+250		—210	

* Exchange fellow of the American Cancer Society.

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.