REFERENCES

1 D. B. MELVILLE, *Vitamins and Hormones*, 17 (1959) 155.
2 L. GROSSMAN AND N. O. KAPLAN, *J. Biol. Chem.*, 231 (1958) 727.
3 T. AKAZAWA AND E. E. CONN, *J. Biol. Chem.*, 232 (1958) 403.
4 J. B. MUDD AND R. H. BURRIS, *J. Biol. Chem.*, 234 (1959) 2774.
5 S. J. KLEBANOFF, *J. Biol. Chem.*, 234 (1959) 2480.
6 H. G. WILLIAMS-ASHMAN, M. CASSMAN AND M. KLAVINS, *Nature*, 184 (1959) 427.
7 H. G. WILLIAMS-ASHMAN AND M. KLAVINS JOHNSON, *Enzymologia*, 22 (1960) 18.
8 S. J. KLEBANOFF, *Federation Proc.*, 19 (1960) 31.
9 S. J. KLEBANOFF, *Biochim. Biophys. Acta*, 48 (1961) 93.
10 H. HEATH AND G. TOENNIES, *Biochem. J.*, 68 (1958) 204.
11 S. J. KLEBANOFF, *J. Biol. Chem.*, 234 (1959) 2437.
12 W. H. MCSHAN AND R. K. MEYER, *Arch. Biochem.*, 9 (1946) 165.
13 S. J. KLEBANOFF, *Biochim. Biophys. Acta*, 44 (1960) 501.
14 H. THEORELL AND B. SWEDIN, *Naturwissenschaften*, 27 (1939) 95.
15 G. BARGER AND A. J. EWINS, *J. Chem. Soc.*, (1911) 2336.
16 I. E. BALABAN AND H. KING, *J. Chem. Soc.*, (1927) 1858.
17 I. FRIDOVICH AND P. HANDLER, *Federation Proc.*, 19 (1960) 29.

Biochim. Biophys. Acta, 56 (1962) 460–469

SOME CHARACTERISTICS OF THE PYRIMIDINE TRANSPORT PROCESS OF THE SMALL INTESTINE*

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(Received June 26th, 1961)

SUMMARY

Uracil is actively transported across the wall of the rat, hamster, and frog small intestine *in vitro*. The transport process requires oxygen, is blocked by a number of substances which interfere with cell metabolism, and is inhibited by pyrimidines with chemical structures similar to that of uracil.

INTRODUCTION

An earlier study of pyrimidine absorption in the intact rat revealed that uracil and thymine cross the intestinal epithelium by both active transport and passive diffusion[1]. Active transport is the predominant mode of absorption at low concentrations of the pyrimidines, whereas passive diffusion predominates at high concentrations at which the active transport process is saturated. The transport mechanism appears to be different from those responsible for the active transfer of sugars and amino

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acids since high concentrations of D-glucose and L-histidine do not depress thymine absorption.

The present investigation of pyrimidine transfer across the wall of the small intestine *in vitro* describes some characteristics of the active transport process, and shows that the process exists in the hamster and frog as well as in the rat.

METHODS AND MATERIALS

Experimental procedure

Male Sprague-Dawley rats weighing 125 to 140 g, golden hamsters of either sex weighing about 110 g, and frogs (*Rana catesbiana*) of either sex weighing about 400 g were fasted for 20 h prior to the experiments but were allowed free access to water. The animals were killed by decapitation, and the upper part of the small intestine was removed. Sacs of everted intestine were prepared according to the method of WILSON AND WISEMAN[2] except that the sacs were considerably longer (20 cm) and were filled with a relatively small volume of fluid which did not distend the intestinal wall. Two millilitres of bicarbonate–saline solution[3], containing 1 g of glucose per litre and various concentrations of [2-^{14}C]uracil, was placed in each intestinal sac, and the sac suspended in 15 ml of the same fluid contained in a 50-ml beaker. The beakers were shaken in a Dubnoff metabolic shaker (90 oscillations/min) at 37° in an atmosphere of oxygen–carbon dioxide (95:5). After 1 h, the mucosal and serosal solutions were collected, and the concentration of uracil estimated from the radioactivity of the solutions. Uracil was previously shown to be stable under the conditions of these experiments[1].

Analytical procedures

[2-^{14}C]Uracil and [*carboxy*-^{14}C]inulin (New England Nuclear Corp., Boston, Mass.) were measured by the liquid counting technique of COTLOVE[4]. Aniline was estimated according to the method of BRATTON AND MARSHALL[5], and *m*-nitrophenol by a method described in an earlier publication[6].

RESULTS AND DISCUSSION

Transfer of uracil across the intestinal epithelium of various species

When sacs of everted small intestine of the rat, hamster, and frog were filled with a solution of 0.02 m*M* uracil and incubated in the same medium, the pyrimidine was transported, from the mucosal to the serosal solution, against a concentration gradient (Table I). For example, the rat intestine developed a serosal/mucosal concentration ratio of 3.77, the hamster intestine a ratio of 1.81, and the frog intestine a ratio of 1.28. Ratios decidedly lower than these resulted when the concentration of uracil was increased to 0.05 m*M*; and the ratios declined to values of slightly less than unity when the concentration was raised to 0.5 m*M*.

The decline in the serosal/mucosal ratio with increasing initial concentrations of uracil is evidence of saturation of the active transport mechanism. For example at low concentrations of uracil, the number of molecules actively transported across the gut wall adds considerably to the relatively small number of molecules present in the serosal solution, resulting in the establishment of a marked serosal/mucosal

TABLE I

TRANSFER OF URACIL ACROSS THE INTESTINAL WALL OF VARIOUS SPECIES

Sacs of everted small intestine of the rat, hamster and frog were filled with solutions containing various concentrations of [2-^{14}C]uracil, suspended in the same solutions, and incubated at 37° for 1 h. The final concentrations of the mucosal and serosal solutions are expressed as the mean ± the range of values.

Species	*Number of animals*	*Concentration of uracil (mM)*			*Serosal to mucosal concentration ratio after 1 h*
		Initial, mucosal and serosal solutions	*Final, mucosal solution*	*Final, serosal solution*	
Rat	29	0.020	0.013 ± 0.001	0.049 ± 0.007	3.77
	8	0.050	0.040 ± 0.002	0.077 ± 0.009	1.93
	8	0.500	0.480 ± 0.005	0.442 ± 0.012	0.92
Hamster	6	0.020	0.016 ± 0.001	0.029 ± 0.003	1.81
	4	0.050	0.042 ± 0.002	0.054 ± 0.005	1.29
	4	0.500	0.476 ± 0.007	0.432 ± 0.010	0.91
Frog	5	0.020	0.018 ± 0.001	0.023 ± 0.002	1.28
	4	0.050	0.047 ± 0.001	0.043 ± 0.003	0.92

concentration gradient. In contrast, when the concentration of uracil is far greater than that which saturates the active transport mechanism, the number of molecules transferred into the serosal solution does not appreciably increase its concentration. Accordingly as the initial concentration of pyrimidine is raised, the serosal/mucosal ratios would be expected to decline to values approaching unity.

The failure of WILSON AND WILSON[7] to observe a concentration gradient of uracil across the wall of the everted hamster intestine is thus explained by the fact that these workers studied the pyrimidine at relatively high concentrations[8] at which the transport mechanism is saturated and passive diffusion predominates as the mode of transfer.

The question arose as to why ratios significantly less than unity (0.92) resulted at the higher concentrations of the pyrimidine. A possible explanation is that water,

TABLE II

DISTRIBUTION OF VARIOUS SUBSTANCES ACROSS THE WALL OF THE RAT SMALL INTESTINE

Sacs of everted small intestine were filled with a solution containing either [*carboxy*-^{14}C]inulin (70 μg/ml) alone, or the inulin together with aniline·HCl (75 μg/ml) or *m*-nitrophenol (450 μg/ml). The sacs were suspended in the same solution and incubated at 37° for 1 h. Serosal to mucosal concentration ratios are expressed as the mean ± the range of values.

Number of animals	*Compound*	*Serosal to mucosal concentration ratio of compound after 1 h of incubation*
4	[*carboxy*-^{14}C]Inulin	0.89 ± 0.02
3	[*carboxy*-^{14}C]Inulin	0.89 ± 0.01
	Aniline	0.99 ± 0.01
3	[*carboxy*-^{14}C]Inulin	0.90 ± 0.02
	m-Nitrophenol	1.00 ± 0.01

transported from the mucosal to the serosal solution[9,10], dilutes the latter solution so rapidly that the rate of passive transfer of uracil is insufficient to maintain the initial concentration ratio of unity. Evidence for this view was obtained on comparing the serosal/mucosal ratio of uracil (0.92) with the ratios of substances having either very slow or very rapid rates of passive transfer (Table II). For example inulin, a substance which diffuses across the isolated gut wall very slowly[11], gave a ratio similar to that of uracil, 0.89. In contrast, aniline and *m*-nitrophenol, compounds known to diffuse across the intestinal epithelium at very rapid rates[6,12] gave ratios that were close to unity (0.99–1.00).

Depression of uracil transport by various metabolic inhibitors and by the absence of oxygen

The active transport of uracil across the intestinal wall of the rat was markedly inhibited by several substances which interfere with cell metabolism (Table III). For example, 1 m*M* *p*-chloromercuribenzoic acid, iodoacetic acid, 2,4-dinitrophenol and phlorizin depressed the concentration gradient of uracil from a control value of 3.8 to values ranging from 1 to 1.65. Fluoride ion (1 m*M*) also depressed the transport of uracil, but the degree of inhibition was considerably less than that produced by the other substances.

Uracil transport was also blocked under anaerobic conditions. Thus, substituting nitrogen–CO_2 (95:5) for the usual oxygen–CO_2 atmosphere depressed the serosal/mucosal concentration ratio of the pyrimidine to a value of 1.0.

TABLE III

EFFECT OF METABOLIC INHIBITORS ON THE TRANSPORT OF URACIL ACROSS THE RAT INTESTINAL WALL

Sacs of everted small intestine were filled with a 0.02 m*M* solution of [2-^{14}C]uracil containing 1 mmole/l of a metabolic inhibitor. The sacs were suspended in the same solution and incubated at 37° for 1 h. The final concentrations of uracil in the mucosal and serosal solutions are expressed as the mean ± the range of values in 5 to 7 animals.

Metabolic inhibitor	*Concentration of uracil (mM) after 1 h*		*Serosal to mucosal concentration ratio of uracil after 1 h*
	Mucosal solution	*Serosal solution*	
—	0.013 ± 0.001	0.049 ± 0.007	3.77
p-Chloromercuri-benzoic acid	0.020 ± 0.002	0.020 ± 0.002	1.00
Iodoacetic acid	0.019 ± 0.001	0.023 ± 0.002	1.21
2,4-Dinitrophenol	0.018 ± 0.001	0.025 ± 0.004	1.39
Phlorizin	0.017 ± 0.002	0.028 ± 0.003	1.65
Sodium fluoride	0.014 ± 0.002	0.040 ± 0.005	2.86

Inhibition of uracil transport by other pyrimidines

The active transport of uracil by the rat intestine was completely inhibited in the presence of high concentrations of thymine (5-methyluracil), 5-fluorouracil, or 5-bromouracil, pyrimidines known to be actively transferred across the gut wall[13] (Table IV). In contrast, 6-azauracil and 6-azathymine produced a relatively slight degree of inhibition, and barbital (5,5-diethylbarbituric acid) no significant inhibition of uracil transport.

Thus, although changes in the substituent at the 5-position of the uracil molecule

TABLE IV

EFFECT OF VARIOUS PYRIMIDINES ON THE TRANSPORT OF URACIL ACROSS THE RAT INTESTINAL WALL

Sacs of everted small intestine were filled with a 0.02 m*M* solution of [2-^{14}C]uracil containing 5 mmoles/l of another pyrimidine. The sacs were suspended in the same solution and incubated at 37° for 1 h. The final concentrations of uracil in the mucosal and serosal solutions are expressed as the mean ± the range of values in 5 to 7 animals.

Compound added to the uracil solution	*Concentration of uracil (mM) after 1 h*		*Serosal to mucosal concentration ratio of uracil after 1 h*
	Mucosal solution	*Serosal solution*	
—	0.013 ± 0.001	0.049 ± 0.007	3.77
Thymine	0.020 ± 0.001	0.018 ± 0.002	0.90
5-Fluorouracil	0.020 ± 0.002	0.018 ± 0.001	0.90
5-Bromouracil	0.020 ± 0.001	0.018 ± 0.002	0.90
6-Azauracil	0.014 ± 0.001	0.038 ± 0.004	2.72
6-Azathymine	0.014 ± 0.002	0.041 ± 0.004	2.93
Barbital	0.013 ± 0.001	0.047 ± 0.004	3.62

appear to have little or no effect on the affinity of the compound for the transport process, an alteration of the structure or degree of saturation of the pyrimidine ring results in a marked loss of affinity.

CONCLUSION

The process by which a number of pyrimidines are transported across the intestinal epithelium has the same general characteristics as the processes responsible for the active intestinal transfer of sugars and amino acids. Thus, the substrate is transferred against a concentration gradient; the transport mechanism becomes saturated at high concentrations of substrate; there is competition for transport among compounds of closely related structure; the mechanism shows specificity for a particular molecular structure; and the transport process is inhibited by substances which interfere with cell metabolism.

REFERENCES

1 L. S. SCHANKER AND D. J. TOCCO, *J. Pharmacol. and Exptl. Therap.*, 128 (1960) 115.
2 T. H. WILSON AND G. WISEMAN, *J. Physiol. (London)*, 123 (1954) 116.
3 H. A. KREBS AND K. HENSELEIT, *Z. physiol. Chem., Hoppe-Seyler's*, 210 (1932) 33.
4 E. COTLOVE, *Federation Proc.*, 14 (1955) 32.
5 A. C. BRATTON AND E. K. MARSHALL JR., *J. Biol. Chem.*, 128 (1939) 537.
6 L. S. SCHANKER, *J. Pharmacol. and Exptl. Therap.*, 126 (1959) 283.
7 T. H. WILSON AND D. W. WILSON, *J. Biol. Chem.*, 233 (1958) 1544.
8 T. H. WILSON, personal communication.
9 G. WISEMAN, *J. Physiol. (London)*, 133 (1956) 626.
10 D. H. SMYTH AND C. B. TAYLOR, *J. Physiol. (London)*, 141 (1958) 73.
11 D. CHALFIN, I. L. COOPERSTEIN AND C. A. M. HOGBEN, *Proc. Soc. Exptl. Biol. Med.*, 99 (1958) 746.
12 L. S. SCHANKER, D. J. TOCCO, B. B. BRODIE AND C. A. M. HOGBEN, *J. Pharmacol. and Exptl. Therap.*, 123 (1958) 81.
13 L. S. SCHANKER AND J. J. JEFFREY, *Nature*, 190 (1961) 727.