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THE UPTAKE OF PTEROYLGLUTAMIC ACID BY THE RAT JEJUNUM

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SUMMARY

1. The uptake of pteroylglutamic acid (folic acid) from the lumen of the rat jejunum was studied using the everted sac technique with [^{3}H]pteroylglutamic acid. Transport into the serosal solution and uptake into the tissues were determined separately.

2. The rate of uptake of pteroylglutamic acid was studied over a concentration range of $1 \cdot 10^{-8}$–$1 \cdot 10^{-7}$ M. Indications were obtained that a rate-limiting process was involved for uptake of this vitamin into the tissues but transport into the serosal solution was shown to be proportional to concentration over this range.

3. The tissues were shown to accumulate pteroylglutamic acid with respect to the incubating solutions at concentrations of $1 \cdot 10^{-7}$ M and below when sacs were incubated for 1 h at 37 °C. This accumulation could be demonstrated when pteroylglutamic acid was present initially on the mucosal side only, and when it was present on both sides of the intestine with no initial concentration gradient between the mucosal and serosal solutions. In the latter case pteroylglutamic acid was removed preferentially from the mucosal solution although some uptake from the serosal solution occurred.

4. The uptake process could be inhibited by 4-amino-10-methylpteroylglutamic acid (methotrexate) and by fluoride when a phosphate-buffered solution (pH 6.2) was the incubating medium but not when Krebs–bicarbonate saline was the incubating medium.

5. It is suggested that the process whereby pteroylglutamic acid is accumulated in the tissues is not apparent at $1 \cdot 10^{-6}$ M and higher concentrations because of the greater contribution by passive diffusion and solvent drag to its total transport. Transport of pteroylglutamic acid into the serosal solution may be largely independent of the special uptake process described.

INTRODUCTION

The absorption of pteroylglutamic acid in the mammalian small intestine has been widely studied but no clear picture of its mode of transport has emerged. Evidence has been presented by several workers to indicate that the transport of this vitamin is mediated by simple passive diffusion[1–4], whilst others have found indications that a more specialised mechanism or an "active" process is involved[5–12].

In a previous report[13] evidence was given to show that at a concentration of $1 \cdot 10^{-6}$ M the transport of pteroylglutamic acid in the small intestine is predominantly due to both passive diffusion and solvent drag with the water flow. In this

report evidence is presented to show that an additional, more specialised mechanism, can be detected at lower pteroylglutamic acid concentrations, for the uptake of this vitamin from the lumen of the jejunum. This finding could explain some of the contradictory reports on the transport of this vitamin which have appeared in the literature.

MATERIALS AND METHODS

Male rats, Wistar strain, weighing approximately 170 g were starved for 24 h. The everted sac technique of Wilson and Wiseman[14] was used for this study. The preparation and incubation of the everted sacs and the estimation of [^{3}H]pteroylglutamic acid in the tissues and in the bathing solution have been described earlier[13]. The jejunal region of the small intestine was used since the proximal part of the small intestine has been shown to transport pteroylglutamic acid more efficiently than the other regions[6,10,11,13]. The incubating solutions were Krebs–bicarbonate saline (pH 6.0) containing 11 mM glucose or a modified phosphate buffer containing 0.11 M sodium phosphate buffer (pH 6.2), 35.7 mM NaCl, 5.5 mM KCl, 1.8 mM $MgSO_4$ and 11 mM glucose. Pteroylglutamic acid transport has previously been shown to be optimum at pH 6.0[13]. When the modified phosphate buffer was used no change in pH occurred during the incubation. However, when Krebs–bicarbonate saline was the incubating solution there was a tendency for the serosal and mucosal solutions to become less acid during the incubation, a phenomenon which has been described by other workers[15,16]. Since the absorption of pteroylglutamic acid in these experiments includes pteroylglutamic acid taken up into the tissues of the sacs as well as that transported to the serosal side, both of these components of the total transport were measured.

RESULTS

Variation with concentration

The uptake of pteroylglutamic acid by the tissues was studied over the concentration range $1 \cdot 10^{-8}$–$1 \cdot 10^{-7}$ M. Transport into the serosal solution was studied in the same experiments. The incubating medium was Krebs–bicarbonate saline, (pH 6.2). Fig. 1a shows the variation of the uptake into the tissues and the transport across the wall of the jejunum with concentration. Transport of pteroylglutamic acid to the serosal solution was proportional to concentration over the range studied, but uptake of pteroylglutamic acid into the tissues appeared to involve a rate-limiting process. The Eadie–Hofstee plots of the values are shown in Fig. 1b.

Concentration of pteroylglutamic acid by the jejunal tissues

The uptake of pteroylglutamic acid was measured over the concentration range $4.5 \cdot 10^{-9}$–$1.6 \cdot 10^{-6}$ M in the mucosal solution only. In these experiments the sacs were incubated for 1 h, and the incubating solution used was the modified phosphate buffer, pH 6.2. Table I shows that at concentrations below $1 \cdot 10^{-6}$ M the tissues concentrate pteroylglutamic acid with respect to the mucosal solution; at $4.5 \cdot 10^{-9}$ M the ratio was 3.5 ± 0.2. At high initial concentrations ($1 \cdot 10^{-6}$M and above) no such concentration occurred and the ratio was approximately 1.0.

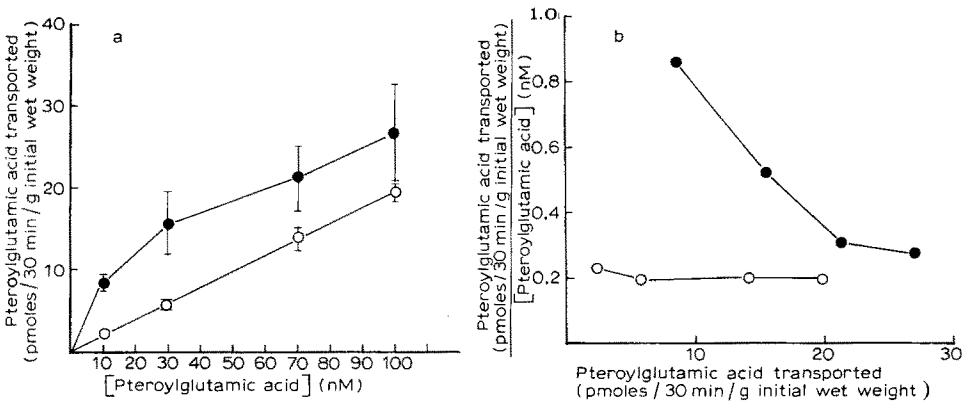

Fig. 1. Variation of rate of pteroylglutamic acid transport with concentration. Jejunal sacs were incubated for 30 min at 37 °C in Krebs–bicarbonate saline containing 11 mM glucose. (a) Variation of rate of pteroylglutamic acid transport with increasing pteroylglutamic acid concentration. Values are given as the means of 4 experiments with the standard error of the mean. ○, pteroylglutamic acid transported to the serosal solution, ●, pteroylglutamic acid taken up into the tissues. (b) Eadie–Hofstee plot for pteroylglutamic acid transport. ○, pteroylglutamic acid transport to the serosal solution, ○, pteroylglutamic acid uptake by the tissues.

TABLE I

VARIATION OF PTEROYLGLUTAMIC ACID ACCUMULATION WITH CONCENTRATION

Sacs were incubated for 1 h at 37 °C in 0.11 M sodium phosphate buffer (pH 6.2) containing 35.7 mM NaCl, 5.5 mM KCl, 1.8 mM $MgSO_4$ and 11 mM glucose. The tissue concentration in nmoles/g final wet weight are compared with the initial mucosal concentration in nmoles/ml. Values are given as the means with the standard error of the mean and the number of experiments in parentheses.

Initial concentration (M)	*Tissue concn/mucosal concn*
$4.5 \cdot 10^{-9}$	3.5 ± 0.2 (4)
1.10^{-8}	3.3 ± 0.2 (6)
$2 \cdot 10^{-8}$	3.0 ± 0.3 (6)
$1 \cdot 10^{-7}$	2.5 ± 0.2 (9)
$6 \cdot 10^{-7}$	1.5 ± 0.2 (3)
$1 \cdot 10^{-6}$	1.0 ± 0.1 (9)
$1.3 \cdot 10^{-6}$	1.1 ± 0.2 (3)
$1.6 \cdot 10^{-6}$	1.1 ± 0.1 (3)

Table II shows the concentrating ability of the tissues when pteroylglutamic acid was initially present on both sides of the sac at the same concentration, over the range $1 \cdot 10^{-8}$–$1 \cdot 10^{-6}$M. In these experiments the incubating solution was Krebs–bicarbonate saline. It can be seen that at $1 \cdot 10^{-7}$ M and below the tissues concentrate pteroylglutamic acid with respect to both the mucosal and serosal solutions. In these experiments, the final serosal solution concentration was higher than the final mucosal solution concentration. However, no net transport to the

TABLE II

PTEROYLGLUTAMIC ACID TRANSPORT, WITH NO INITIAL CONCENTRATION GRADIENT BETWEEN THE MUCOSAL AND SEROSAL SOLUTIONS

Sacs were incubated in Krebs–bicarbonate saline (pH 6.0) containing 11 mM glucose, for 1 h at 37 °C. Serosal and mucosal concentrations in nmoles/ml are compared with tissue concentrations in nmoles/g final wet weight. Values are given as the means with the standard error of the mean with the number of experiments in parentheses.

Initial concn (M)	*Serosal concn/ mucosal concn*	*Tissue concn/ mucosal concn*	*Tissue concn/ serosal concn*
$1 \cdot 10^{-8}$	1.13 ± 0.03 (6)	3.27 ± 0.20 (6)	2.90 ± 0.19 (6)
$2 \cdot 10^{-8}$	1.48 ± 0.10 (6)	2.97 ± 0.29 (6)	2.29 ± 0.17 (6)
$1 \cdot 10^{-7}$	1.35 ± 0.15 (6)	2.43 ± 0.28 (6)	1.81 ± 0.10 (6)
$1 \cdot 10^{-6}$	0.87 ± 0.05 (6)	0.89 ± 0.11 (6)	0.97 ± 0.11 (6)

serosal solution had occurred, but the uptake of pteroylglutamic acid had occurred preferentially from the mucosal solution although some uptake from the serosal solution had also occurred. At the higher pteroylglutamic acid concentration ($1 \cdot 10^{-6}$M) again no tissue concentration occurred and the ratio again remained at approximately 1.0.

Effect of glucose and inhibitors

Omission of glucose from the incubating solutions had no significant effect on the uptake of pteroylglutamic acid at a concentration of $1 \cdot 10^{-7}$ M. Attempts were made to inhibit the uptake of pteroylglutamic acid with 4-amino-10-methylpteroylglutamic acid or with F^-. When Krebs–bicarbonate saline was the incubating solution no inhibition was observed with either of these substances, but when the modified phosphate buffer was the incubating medium inhibition was observed with high concentrations of either substance. When pteroylglutamic acid was present on the mucosal side at $1 \cdot 10^{-8}$ M, the uptake was depressed by 46% by $5 \cdot 10^{-4}$ M 4-amino-10-methylpteroylglutamic acid. Inhibition of uptake at $4.5 \cdot 10^{-9}$ M pteroylglutamic acid by $1 \cdot 10^{-3}$ M NaF was 68%.

DISCUSSION

The results show that at low pteroylglutamic acid concentrations ($1 \cdot 10^{-7}$M and lower) the tissues of the rat jejunum have the ability to concentrate it with respect to the bathing solutions. In a previous report[13], it was shown that at $1 \cdot 10^{-6}$ M, pteroylglutamic acid was transported by passive diffusion and by solvent drag with the water flow. At this concentration, the special uptake process described in this paper was not evident because of the greater transport by the other process. Fig. 1b indicates that the tissue uptake process was not solely passive diffusion; the values for the uptake of pteroylglutamic acid into the tissues may represent the sum of two processes, *i.e.* a specialised saturable process and a passive diffusion of the vitamin into the tissues. The relative contributions of these processes would therefore be different for different concentrations; at the higher concentrations the

contribution of passive diffusion to the total uptake would appear to be greater than at the lower concentrations. It is possible that the concentrating process is an uptake of pteroylglutamic acid onto a protein or proteins within the tissues or onto the cell surfaces. Other workers have shown that folate binds to a specific protein in milk[17,18], and Hoffbrand and Peters[19] found that it was concentrated in the mitochondrial fraction of guinea-pig intestinal mucosa. It is possible that pteroylglutamic acid binds to an enzyme involved in folate metabolism such as dihydrofolic acid reductase which has been demonstrated in rat intestine[20]. This however seems unlikely since 4-amino-10-methylpteroylglutamic acid has been shown to be a very potent inhibitor of this enzyme[20,21], whereas in the present study, this substance inhibited only at high concentrations and only when the modified phosphate buffer was the incubating medium. The reasons for the different effects of the inhibitors in the two buffers is not clear.

Transport of pteroylglutamic acid to the serosal side of the sac was shown to be proportional to its concentration in the mucosal buffer over the range $1 \cdot 10^{-7}$–$1 \cdot 10^{-6}$ M. Thus the pteroylglutamic acid transported to the serosal side in these experiments is probably not derived to any large extent from that taken up into the tissues by the concentrating process. It is possible that pteroylglutamic acid taken up by the rate-limiting process is released and transported across the remaining cell barriers at a rate much slower than that for transport by diffusion. The accumulation of pteroylglutamic acid in the intestinal tissues could represent a mechanism for its efficient uptake when diets low in this vitamin are presented to the animal or for its storage when food is rich in this vitamin.

It is noteworthy that some previous workers who have suggested that pteroylglutamic acid is transported by a rate-limiting process in the gut[9–11] have used techniques necessitating calculation of its transport from measurements of its levels in the mucosal buffer only. These workers therefore may have obtained values for the absorption of pteroylglutamic acid which included pteroylglutamic acid transported into the tissues as well as that transported to the serosal side. Some workers who have advocated passive diffusion as the sole mode of transport measured the transport into the serosal solution[1], which has also been shown to be proportional to concentration in this study.

Thus the major mechanism for removal of pteroylglutamic acid from the mucosal side of the jejunum depends on its concentration in the mucosal solution. At high concentrations ($1 \cdot 10^{-6}$ M and above) pteroylglutamic acid is transported mainly by passive diffusion and solvent drag,[13] but at lower concentrations ($1 \cdot 10^{-7}$ M and below) another mechanism can be detected which is rate-limiting and therefore of quantitatively less importance at $1 \cdot 10^{-6}$ M concentration.

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