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ABSORPTION OF FOLIC ACID FROM THE SMALL INTESTINE OF THE RAT

BY

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The absorption of folic acid from segments of the small intestine of the rat has been measured *in situ* with tritium-labelled folic acid. The fraction absorbed was independent of concentration below 10^{-6} M but was depressed to half at 4×10^{-5} M. Direct measurements of fluxes showed that the mucosal → serosal flux was about 14 times the serosal → mucosal flux, and therefore that uptake of folic acid is an active process. In the ileum but not in the duodeno-jejunum, absorption was depressed by the presence of electrolyte. There was little difference in absorptive capacity between jejunum and ileum nor was there any significant change in animals suffering from a dietary deficiency of folic acid.

Studies in man using both isotopic and microbiological methods for its determination have shown that folic acid given by mouth is absorbed rapidly and nearly completely (Girdwood, 1953; Anderson, Belcher, Chanarin & Mollin, 1960; Kinnear, Johns, Macintosh, Burgen & Cameron, 1962). Over the narrow range of doses studied the fraction absorbed is not dependent on the dose. Although the dietary intake of folic acid is very small (probably no more than 100 μg/day; Toepfer, Zook, Orr & Richardson, 1951) and hence the concentration in the gut lumen is very low, the blood concentration is kept so low that absorption against a concentration gradient is probably never necessary. On the other hand it is legitimate to enquire why a substance so insoluble in lipids should be so readily absorbed. *A priori*, therefore, it seemed likely that a specialized transport system for folic acid existed like those known to transport monosaccharides, amino acids, pyrimidines, and certain electrolytes (Davson, 1959; Schanker, 1961; Schanker & Tocco, 1960; Schanker & Jeffrey, 1961).

In the present study it will be shown that an active transport system for folic acid does indeed exist in the intestine.

METHODS

The perfusion of the gut was carried out by a modification of the procedure described by Curran & Solomon (1957). Male rats of the Wistar strain weighing 250±50 g were fasted overnight and then anaesthetized with intraperitoneal urethane 1 g/kg. A tracheal cannula was inserted and the abdomen opened by a midline incision. A flexible polyethylene tube was then passed down the oesophagus, brought out into the abdominal cavity through a small incision in the stomach, and then inserted into the proximal end of the segment of small

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intestine being studied. A short length of polyethylene tubing was inserted into the distal end of the segment and drained into the collecting vial. The tip of a thermistor probe was placed in the abdomen, and the intestines were covered with a piece of toilet tissue soaked in 0.9% NaCl which in turn was covered by a thin sheet of plastic film (Saran). A 60 W light bulb was fixed about a foot above the rat. The probe was connected to a bridge and relay (Thermistemp Temperature Controller Model 71, Yellow Springs Instrument Co.) which controlled the illumination of the lamp. By this means the abdominal contents of the animal could be maintained at $37.0 \pm 0.2°$ C. Fluid entering the intestine was warmed to body temperature during its passage through the tube traversing the oesophagus.

After cannulation the segment of intestine was gently washed through with normal saline until the washings were clear. It was then flushed with the perfusing solution after which perfusion at a rate of 0.1 ml./min was commenced. After 15 min, collections were made, usually for 1 hr periods for 2 to 4 hr. The rate of flow was selected after preliminary experiments because about half of the standard concentration of folic acid was absorbed at this rate and in this range the accuracy of measurement of folic acid uptake was greatest.

The segments of intestine perfused were (1) from 1 cm distal to the pylorus for a 25 to 30 cm length of duodenum and jejunum, (2) the ileum from 25 to 30 cm proximal to the ileocolic valve to its termination.

Tritiated folic acid was prepared and purified as described by Johns, Sperti & Burgen (1961). The radioactivity of the intestinal perfusion solution and the sample collected was measured by adding 0.1 ml. to 15 ml. of a dioxane-naphthalene scintillator solution (20 g naphthalene, 1 g 2,5 diphenyloxazole, 25 mg 1, 4 bis-2-(5-phenyloxazolyl) benzene (POPOP) in a mixture of 77 ml. dioxane and 23 ml. absolute ethanol). Plasma samples were counted by adding 0.2 ml. Hyamine (Eisenberg, 1958) and gently agitating until solution occurred. 1.5 ml. ethanol was added and then 15 ml. of scintillator solution (0.6 g 2,5 diphenyloxazole and 20 mg 1, 4 bis-2-(5-phenyloxazolyl) benzene (POPOP) in 100 ml. toluene). The samples were counted at $-5°$ C in a Packard Tricarb liquid scintillation spectrometer.

Counting efficiencies were determined by adding tritiated toluene as an internal standard. Perfusion solutions were usually made up by adding folic acid to 0.9% NaCl containing 200 mg/l. of Polyglycol 4000 (Carbide and Carbon Chemicals, Montreal). The latter is known not to be absorbed or broken down in the intestine (Shaffer & Critchfield, 1947; Sperber, Hyden & Ekman, 1953) and served as a volume indicator. Polyglycol 4000 was estimated turbidimetrically as the trichloracetic complex (Hyden, 1955) in a Photovolt fluorometer type 540. The fractional absorption of folic acid was therefore calculated as $1-\frac{F_2 \cdot P_1}{F_1 \cdot P_2}$ where F_1 is the folic acid concentration in the perfusion solution and F_2 the folic acid concentration in the effluent, P_1 the Polyglycol concentration in the perfusion solution and P_2 the Polyglycol concentration in the effluent. In all cases duplicate or triplicate determinations were done.

Deficiency of folic acid was produced by feeding rats weighing 100 g on a diet deficient in folic acid (Nutritional Biochemical Corporation) with the addition of 1.8% succinyl sulphathiazole to suppress intestinal flora capable of synthesizing folic acid (Stokstad, 1954). The animals were maintained on this diet for six weeks at which time mild leucopenia developed; this was taken as functional evidence of folic acid deficiency.

One group of animals was fed on a high gluten diet prepared by providing 25% of the total caloric intake as high gluten flour (MacDowell Brothers, Brockville, Ontario) and the remaining calories from Purina chow.

RESULTS

At a rate of flow of 0.1 ml./min, approximately 40% of the radioactivity was absorbed from the duodeno-jejunal segment when the concentration in the perfusion fluid was between 10^{-8} and 10^{-6} M, but with further increase in the concentration,

TABLE 1

PERCENTAGE OF FOLIC ACID ABSORBED FROM THE PERFUSION SOLUTION

* t=3·60; p=<0·02. † Number of animals

Molar concentration in the lumen	Duodeno-jejunum	Ileum
10^{-8}	45·0±3·68 (5)†	
10^{-7}	38·6*±3·25 (14)	24·4*±2·24 (6)
10^{-6}	40·9±2·90 (5)	
2×10^{-5}	19·6±4·16 (5)	
10^{-4}	14·0±1·78 (4)	
5×10^{-4}	1·7±5·95 (5)	
10^{-3}	9·7±3·82 (8)	

the fractional absorption declined (Table 1). This suggests that a saturable mechanism of absorption is involved. The data fit reasonably well a process following Michaelis-Menten kinetics with a $K_s = 4\times10^{-5}$ M and an absorption maximum of approximately 10^{-7} moles/hr (Fig. 1).

The rate of absorption from the ileum was about 60% of that in the duodeno-jejunum when tested at a concentration of 10^{-7} M. The difference is significant,

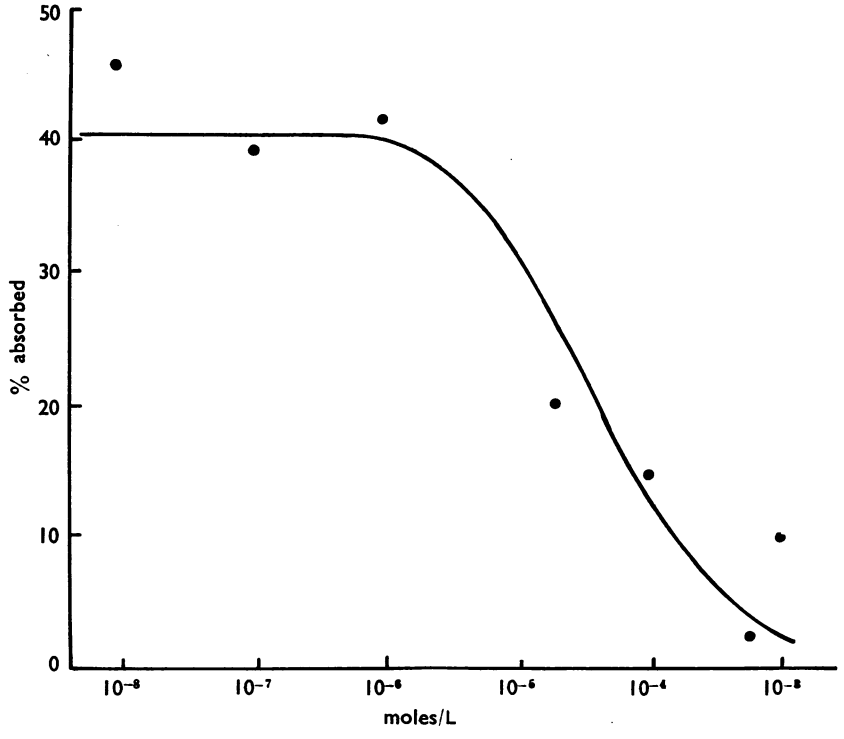

Fig. 1. Percentage of perfused [^{3}H]-folic acid absorbed by the duodeno-jejunal segments at different concentrations. The solid line is drawn for the equation: % Absorbed$=\frac{40}{1+C/Ks}$, where Ks$=4\times10^{-5}$ M. Ordinate: percentage absorbed. Abscissa: concentration in perfusion solution.

but was not striking enough to make worth while a detailed exploration of the response to dose in the ileum.

Absorption against a raised blood folic acid level

In some experiments the ileum was perfused with tritiated folic acid (10^{-7} M) while the blood level of folic acid was increased by infusion of unlabelled folic acid from the endogenous level (<10 μg/l.; 2×10^{-8} M; Toennies, Frank & Gallart, 1956; Cooperman, Luhby & Avery, 1960) to 10–30 mg/l. ($2–7 \times 10^{-5}$ M). The percentage absorption was 26.8 ± 2.24, which was not significantly different from that in the control series. This experiment merely shows that the mucosal → serosal flux of tritiated folic acid is not affected by a several hundred fold adverse mass gradient, a fact of considerable importance in the performance of clinical tests of absorption (Kinnear *et al.*, 1962). It does not indicate that net uptake of folic acid can necessarily proceed against a gradient.

Serosal → mucosal flux of folic acid

In these experiments the ileum was perfused with non-radioactive folic acid at a concentration of 10^{-6} M, while a nearly constant blood level of radioactive folic acid was maintained by infusion. The procedure was to inject folic acid with a specific activity of 3.8 μC/mg in an initial dose of 1.5 mg followed by an infusion of 0.01 mg/min. The blood level was calculated from the serum radioactivity and was steady at a level of $2.9–8.3 \times 10^{-5}$ M (mean 4.5×10^{-5} M). The mean serosal → mucosal flux was 0.37×10^{-8} moles/hr (Table 2).

TABLE 2
FOLIC ACID FLUXES (MOLES/HR) IN THE ILEUM

	Mucosal→Serosal	Serosal→Mucosal
1	$5{\cdot}6 \times 10^{-8}$	$0{\cdot}37 \times 10^{-8}$
2	$5{\cdot}0 \times 10^{-8}$	$0{\cdot}83 \times 10^{-8}$
3	$3{\cdot}5 \times 10^{-8}$	$0{\cdot}18 \times 10^{-8}$
4	$7{\cdot}1 \times 10^{-8}$	$0{\cdot}08 \times 10^{-8}$
	Mean $5{\cdot}3 \times 10^{-8}$	$0{\cdot}37 \times 10^{-8}$

Net influx $4{\cdot}9 \times 10^{-8}$
Influx/efflux = 14·3
Serosal and mucosal concentrations $4{\cdot}5 \times 10^{-5}$ M

In another series of experiments the mucosal → serosal flux was measured. In this case the folic acid concentration in the blood was raised to the same levels as in the previous experiment but with non-radioactive folic acid. The intestine was perfused with radioactive folic acid at a concentration of 4.5×10^{-5} M, i.e. equal to the mean blood concentration in the previous experiment.

The mean mucosal → serosal flux was 5.3×10^{-8} moles/hr which was 14 times the flux in the reverse direction. The net flux in these experiments averaged 4.9×10^{-8} moles/hr.

Similar experiments were not carried out on the duodenum-jejunum because of difficulty in avoiding contamination from radioactivity excreted in the bile and pancreatic juice.

These experiments establish unequivocally that there exists an active absorptive process for folic acid with a large flux ratio and hence capable of sustaining a large concentration ratio.

The effect on absorption of the osmolarity of the gut contents

It is known that the flux of water out of the small intestine is dependent on the osmolarity of the contents of the gut. The flux is higher from water than from isotonic or hypertonic saline, presumably because of the hydrodynamic effect of the net water flux (Lee, Code & Scholer, 1955).

It was of interest to see whether a similar effect existed in the case of absorption of folic acid. In the duodeno-jejunal segment, absorption was unchanged whether the perfusing folic acid was in distilled water, 0.9% NaCl (285 m Osmolar) or 1.2% NaCl (380 m Osmolar). On the other hand absorption from the ileum was increased in water and depressed by increasing the NaCl concentration to 1.2% (Fig. 2).

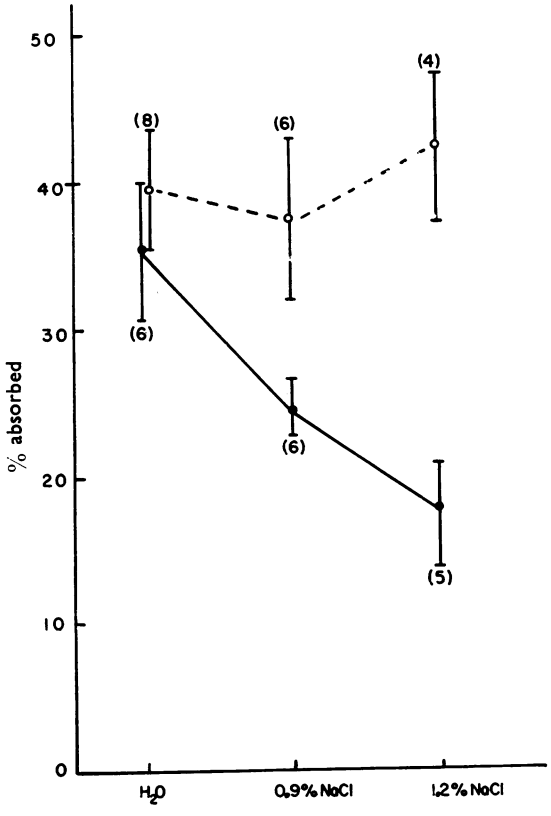

Fig. 2. Effect of the osmolarity of the gut contents in the absorption of folic acid. The folic acid concentration was 10^{-7} M and was made up in water, 0·9% NaCl or 1·2% NaCl. Ordinate: percentage absorbed. Abscissa: solution of folic acid. ●——●: Ileum. ○- - -○: Duodeno-jejunum.

The difference in behaviour of the two segments is all the more surprising since rates of absorption of water were similar in both segments with pure water in the lumen and were similarly depressed by addition of electrolytes.

Absorption in animals deficient in folic acid

Tissue avidity for folic acid given intravenously is increased in both man and rat when there is nutritional deficiency of the vitamin (Chanarin, Mollin & Anderson, 1958).

However, in patients with untreated pernicious anaemia in whom tissue avidity is also increased, intestinal absorption of folic acid is within the normal range (Anderson *et al.*, 1960).

In four deficient animals studied the absorption from a 10^{-7} M solution by the duodeno-jejunal segment was $44.0 \pm 10.0\%$; this was not increased significantly from $38.6 \pm 3.25\%$ in the control preparation. However it must be borne in mind that folic acid deficiency may lead to degenerative changes in the intestinal mucosa and this effect may counter-balance any tendency to an adaptive increase in transport capacity.

Effect of gluten and gliadin

Considerable evidence exists that gluten can cause defects of absorption in the human small intestine in man and it is known that the defective intestinal folic acid absorption in non-tropical sprue can be strikingly reversed by a strict gluten-free diet (Kinnear *et al.*, 1962).

In animals which had been maintained for six weeks on a diet in which flour rich in gluten comprised 25% of the total caloric intake, the absorption from the duodeno-jejunal segment was $27.1 \pm 4.00\%$. This was lower than in the controls, but the difference falls just short of significance ($t=2.24$, $p=0.07$). The result is, however, so suggestive that it would be highly desirable to study this effect further with a higher gluten intake administered for a longer period of time. Saturation of the perfusion solution with wheat gliadin failed to affect absorption in normal animals ($36.7 \pm 5.20\%$).

Effect of methotrexate

Addition of 1.8×10^{-4} M methotrexate to the 10^{-7} M folic acid in the solution perfusing the duodeno-jejunal segment reduced the percentage absorbed to 30.4 ± 2.8, but this is not a significant change ($p=0.2$) and does not establish whether methotrexate combines with the transport system for folic acid. At any rate its affinity for the transport system must be considerably lower than that of folic acid.

DISCUSSION

The experiments reported here show that the rat intestine possesses an active transport system for folic acid of considerable capacity. Taking both upper and lower small intestines into account the maximum absorption per day would be approximately 15 μM/kg (8 mg/kg). This may be compared with the estimated

dietary intake in man of approximately 0.004 μM/kg (1 to 2 μg/kg), or the oral dose used in clinical tests of folic acid absorption, 0.006 to 0.08 μM/kg (3 to 40 μg/kg). It is evident that if the absorptive capacity in man is comparable to that in the rat, neither of these doses comes near to saturating the absorptive capacity. Indeed these doses fall within the range where fractional absorption is independent of dose, which accords with the experimental findings of Anderson *et al.* (1960). These observations emphasize the danger of assuming that an absorptive process is diffusional, merely because a constant fraction is absorbed over a limited range of dose. Depression of uptake is seen only when the highest concentration tested is comparable to the K_s of the transport system. Since the K is frequently in the millimolar range (e.g. uracil; Schanker & Tocco, 1960), the concentration required to demonstrate depression may be high.

While this paper was in preparation, a study by Turner & Hughes (1962) of the transfer of B vitamins in everted sac preparations of the small intestine of the rat has appeared. They were unable to demonstrate any active uptake of folic acid or other B-vitamins. In addition the mucosal → serosal flux found by these authors is much smaller than in our experiments. In their experiments a concentration of 10^{-7} M folic acid was used and a mucosal → serosal flux of 3.6 p.moles/hr found in a sac made from 5 cm of jejunum. To make this comparable with our experiments this would correspond to 22 p.moles/hr for a 30 cm length. On the other hand, in our experiments the mucosal → serosal flux at 10^{-7} M was 232 ± 20 p.moles/hr for the duodenum-jejunum and 146 ± 13 p.moles/hr for the ileum. The lower values in the *in vitro* preparation suggest that the transport process is impaired, which would account for the failure to find the generation of a concentration gradient.

It seems not unlikely that careful study of the absorption of other essential trace nutrients will reveal the existence of specialized transport systems for these substances too. Indeed, while little remains to support the thesis that the intestine selectively excludes undesirable substances, there is increasing evidence of the existence of mechanisms which ensure that desirable dietary constituents will be well absorbed.

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