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INFLUENCE OF VASCULAR FLOW ON AMINO ACID TRANSPORT ACROSS FROG SMALL INTESTINE

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SUMMARY

1. The vascularly perfused intestine of the frogs, *Rana ridibunda* and *R. pipiens*, was used to investigate the transfer of the non-metabolized amino acids α-amino isobutyric acid (AIB) and 1-amino-cyclopentane-1-carboxylic acid (cycloleucine) across the intestinal epithelium from the lumen into the portal vein.

2. The steady-state rate of transfer of cycloleucine was significantly increased with increasing vascular flow rate, both in the presence and absence of Na in the fluid in the intestinal lumen, although at all flow rates the transfer was lower when the Na was replaced by K. The relatively high rate of transfer of cycloleucine seen at high rates of vascular flow when the luminal perfusate was free of Na was almost abolished when leucine was added to the lumen.

3. When the vascular flow is interrupted cycloleucine is taken up from the lumen and accumulates in the tissue. The accumulated amino acid can be measured during the subsequent wash-out when the vascular flow is resumed. The rates of transfer of accumulated cycloleucine as measured during subsequent wash-out were lower than those found at continuous flow; it is suggested that there is a limit to the amount of amino acid that the epithelial tissue can retain when the vascular flow is interrupted. A significant accumulation of cycloleucine occurs when Na is present in the lumen, but the accumulation is negligible when Na is replaced by K.

4. The unloading of the amino acids AIB and cycloleucine from the epithelium into the vasculature was investigated. The two amino acids showed markedly different kinetics of exit, that of AIB being monoexponential while that of cycloleucine was biexponential. Moreover, the rate of exit of cycloleucine was influenced by the presence of Na in the intestinal lumen, and by the rate of vascular perfusion. The apparent diffusion coefficients for the exit were smaller than those expected had unloading taken place by unobstructed free diffusion.

5. It is concluded that specific processes for exit from the epithelium play a significant role in amino acid transfer across the small intestine, and that the exit of AIB is more restricted than that of cycloleucine.

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INTRODUCTION

Whereas experiments using *in vitro* preparations of small intestine or membrane preparations show that concentrative uptake of sugars and amino acids (Riklis & Quastel, 1958; Csáky, 1963; Murer & Hopfer, 1974, 1977) across the brush border is dependent on the presence of Na ions in the mucosal solution, *in vivo* preparations give an equivocal picture. For instance, neither Fleshler & Nelson (1970) using L-alanine, nor Saltzman, Rector & Fordtran (1972) studying glucose, found any marked decrease in uptake on perfusing the lumen with Na-free solution. The most evident difference between the *in vivo* and the *in vitro* methods is the presence of a circulation beneath the contraluminal side of the epithelium. Using a preparation to maintain vascular perfusion with solutions of known composition (Parsons & Prichard, 1968) it has been demonstrated that increasing the rate of vascular flow leads to an increase in the rate of transfer of 3-O-methyl glucose (3MG) in the absence of sodium ions from the luminal perfusate (Boyd, 1976, 1977). Moreover, the rate of transfer of L-leucine is diminished with removal of sodium ions from the lumen (Boyd, Cheeseman & Parsons, 1975). The present study was therefore performed to see whether the diminution of amino acid transfer was dependent on vascular flow rate, and to characterise further the transfer of amino acid out of the lumen into the vasculature.

METHODS

Animals

Rana pipiens and *R. ridibunda* were used; some characteristics of these frogs are given in Table 1. The two species live in different natural habitats and require different tank conditions, for *R. ridibunda* spend most of their time in water, while *R. pipiens* prefer land. The animals were kept in tanks in which the water was constantly exchanged and maintained at 15–18 °C and were fed regularly with maggots or vitaminized crickets (Xenopus Co.). Occasional infection with *Aeromonas hydrophila*, *Staphylococcus epidermis* and *Citrobacter freundii*, all causing a condition generally known as red leg, was treated with antibiotics (5 mg oxytetracycline/30 g body wt. administered by stomach tube twice daily).

Amino acids

α-amino isobutyric (AIB) and 1-amino-cyclopentane-1-carboxylic acid (cycloleucine) were supplied by Sigma Co., and labelled compounds (^{3}H and ^{14}C) by The Radiochemical Centre, Amersham. The rates of transfer across the epithelium were calculated from measurement of radioactivity in the vascular effluent. Radioactivity of [^{3}H]AIB and of [^{14}C]leucine was measured by liquid scintillation spectrophotometry using the scintillation mixture described by Boyd *et al.* (1975). That these amino acids were not significantly metabolized during transfer was confirmed by chromatography (method of Vomhof & Tucker, 1965). Over 95 % of the radioactivity of the venous effluent was found to be in the amino acids.

Preparation of vascularly perfused small intestine

The perfusion system was similar to that described by Boyd *et al.* (1975) but the operative procedure differed in a number of ways.

After removal from the tank, the animal was kept for a time on ice (usually about 30 min, depending on size): it then was weighed alive, and pithed. The ventral abdominal wall was exposed, the anterior abdominal vein ligated at either end and the whole wall removed. The pectoral girdle was cut to expose the heart and the great vessels. All vessels supplying regions of the gastrointestinal tract not to be perfused were then ligated, including the vessels supplying

the spleen. Incisions were made into the stomach and colon, and the lumen of the gastrointestinal tract flushed with about 50 ml Ringer to wash out the small intestine.

The left aorta and the coeliaco-mesenteric artery were completely exposed. Two loose ligatures were tied around the left aorta, one at its most anterior end and the other proximal to the coeliaco-mesenteric artery. A loose ligature was also tied around this artery proximal to the branch supplying the stomach, around which was tied a tight ligature. A loose ligature was placed around the hepatic portal vein.

The anterior thoracic ligature was then tightened, an incision made into the vessel caudal to it, and the arterial cannula with perfusing fluid flowing from it was inserted down the aorta and coeliaco-mesenteric artery to a point below the branch to the stomach. The cranial end of the aorta was then cut to provide an outlet for the fluid entering the heart.

TABLE 1. Characteristics of animals and intestines used for perfusion studies. Note that the whole of the small intestine was employed and the values for intestine are obtained after perfusion

Species (suppliers)	*n*	Weight of frog (g)	Length of intestine (mm)	Dry weight of intestine (mg)	Tissue water (g/kg dry wt.)
R. pipiens (Carolina Biological Supply Co., Burlington, North Carolina, U.S.A.)	11	75·7 ± 5·9	131 ± 4	72 ± 9	4·14 ± 0·20
R. ridibunda (Mavad, Budapest, Hungary)	14	97·2 ± 10·9	240 ± 14	153 ± 19	4·45 ± 0·18

An incision was made for the portal vein at a point between the liver and the first branches of the vein; the venous cannula, selected to be the largest size that would enter the vein, was then inserted and pushed to a point caudal to these branches. The position of the tip of the cannula was then adjusted so that maximal flow was achieved.

The intestinal lumen was then cannulated through the incisions already made, loose ligatures having previously been placed in position. When the lumen of the intestine was completely filled, the portal cannula was finally adjusted to obtain full recovery of the arterial flow. The height of the frog above the lower end of the outflow cannulae was also adjusted if necessary. The venous outflow was regularly at least 95 % of the arterial inflow.

The rate of perfusion through the lumen was chosen to be as near to 15 ml. g^{-1} dry weight intestine min^{-1} as could be predicted from the weight of the frog.

Composition of perfusates

The perfusates were Krebs-Ringer-bicarbonate solution modified for the requirements of frogs (Boyd *et al.* 1975). In 'Na-free' solutions K was substituted for Na. A metabolic substrate (glucose 2 mM) was added to the vascular fluid, together with albumin, necessary for full recovery from the portal vein of the arterial inflow (Parsons & Prichard, 1968). A concentration of albumin of 5 g $l.^{-1}$ was used in *R. ridibunda* (Boyd *et al.* 1975), but it was found that 10 g $l.^{-1}$ was needed in experiments using *R. pipiens* to establish complete portal recovery of the inflow.

Measurement of steady-state rates of transfer at varying rates of flow

The rate of transfer was measured at different rates of vascular perfusion within the physiological range, which taking the data of Weizsäcker (1911) as interpreted by Parsons & Prichard (1968) is 4·6 ml. g^{-1} dry weight intestine min^{-1}. As the rates of transfer were found to vary between different frogs, the results were standardized.

The value of transfer at the average rate of vascular flow for all frogs of one species was deemed to be 100 in each animal. The values of the transfer rates found at other rates of flow were expressed as a percentage of this value, on the assumption (see Results section) that the rate of transfer was linearly related to the rate of vascular flow.

Non steady-state measurements

Measurements of transfer following the abrupt introduction of 'labelled' amino acid into the lumen can be used to calculate the quantity of amino acid required to establish a steady rate of transfer across the tissue into the vascular bed. This quantity we refer to as the 'loading pool'. Similarly, an 'unloading pool' can be calculated from measurements of the transfer after the abrupt removal of the labelled amino acid from the lumen (Boyd & Parsons, 1978). As backflux into the lumen may occur occasionally during unloading from the epithelium, large concentrations (10 mM) of unlabelled amino acid were added to the lumen to replace the labelled amino acid and to inhibit its re-uptake.

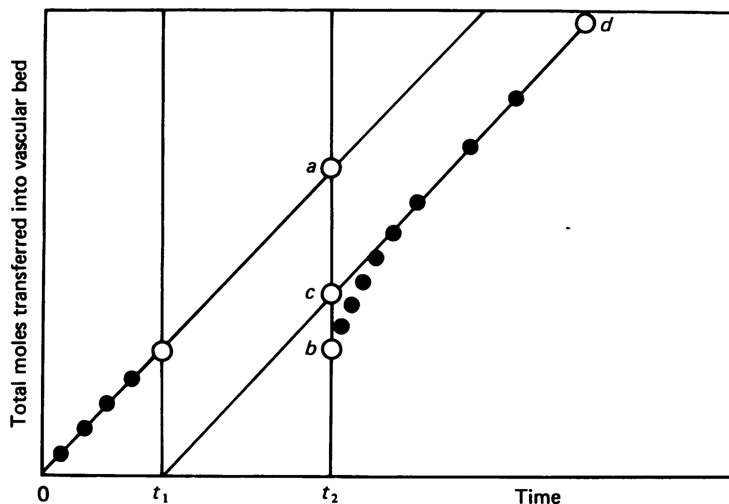

Fig. 1. Diagram to show effects of stopping vascular flow on recovery of absorbed substrate in portal venous effluent. Slopes of lines 0–*a* and t_1–*c*–*d* are measures of rates of absorption in steady state. During interval t_1–t_2 vascular flow stopped. For further details see text.

Vascular stop flow

Influx into the tissue at zero vascular flow rates can be determined using the technique of vascular stop flow, during which the vascular perfusion pump is turned to a minimum setting (Boyd & Parsons, 1978).

The quantity of substrate that is accumulated in the tissue during the period of stop can be measured from the plot of accumulative appearance against time (Fig. 1).

During the period 0–t_1 with vascular flow occurring, transport into the vascular bed occurs at a rate given by the slope of the line 0–*a*. With the resumption of vascular flow after it has been stopped during the period t_1–t_2, transport into the vessels eventually settles down at a constant rate given by the slope of the line *c*–*d*, which in practice is always the same as that before the stop. However, the line *c*–*d* is always displaced below 0–*a* so that the quantity of substrate that can be recovered from the tissue when vascular flow is resumed is given by the difference between the heights of the ordinates *c*–*b*.

This quantity can also be determined from the area beneath a plot of the instantaneous rate of appearance against time after vascular flow is resumed.

A minimum value for the concentration of amino acid in the epithelium accumulated in the tissue during the vascular stop can be estimated if the amount of tissue water is known.

RESULTS

Steady-state experiments

The effects were examined of varying vascular flow on the steady-state rate of transfer of cycloleucine from the lumen (2 mM) into the portal vein in the presence and absence of Na in the lumen.

Both in *R. pipiens* and *R. ridibunda* (Fig. 2 and Table 2) the rate of transfer of cycloleucine is significantly dependent on the vascular flow rate when sodium is present in the lumen. Large rates of amino acid transfer are also seen at minimal rates of vascular perfusion. In experiments without Na in the lumen, the rate of transfer is also significantly dependent on the rate of vascular flow. However, in this case the rates of transfer are lower at all vascular flow rates; indeed, rates of transfer at low rates of flow are minimal. Thus unit increase in flow produces a large fractional change in the transfer rate. Comparing values of transfer in the presence and absence of Na in the lumen, the proportionate change in transfer after adding or removing the sodium will be greater at smaller flow rates than at larger (Fig. 2).

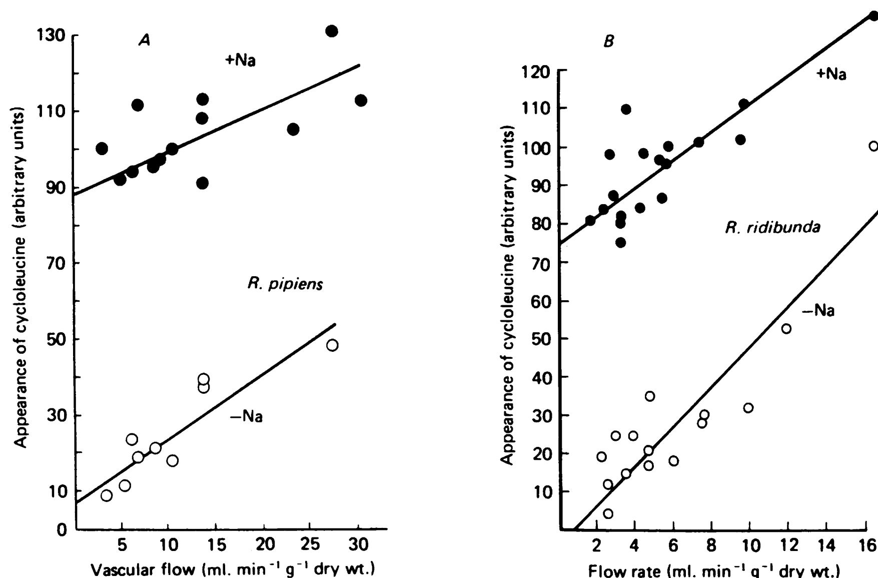

Fig. 2. Influence of flow rate on rate of transfer of cycloleucine from lumen into portal venous effluent. Filled circles, Na present in bulk phase in lumen. Open circles, Na absent from bulk phase in lumen. Rates of transfer standardised as described in the Methods section. *A*, *R. pipiens*. *B*, *R. ridibunda*.

Vascular stop flow experiments

The rates of transfer of cycloleucine predicted from steady-state experiments for zero rates of vascular flow were compared with data derived from vascular flow experiments (Table 3). The rates predicted for zero flow, by measuring the intercept of the regression relating flow and transport on the abscissa and the rates derived

from stop flow experiments, differ according to whether sodium is present or absent in the lumen. With Na present in the lumen less amino acid is recovered after vascular stoppage than is predicted from the steady-state experiments. On the other hand, transfer of amino acid is small when there is no flow through the epithelial vasculature and no Na is present in the lumen.

TABLE 2. Linear regressions relating rate of transfer of cycloleucine into portal venous effluent and rate of vascular flow. Transfer rates are standardised to a value of 100 at vascular flow rates of 7·4 ml. min^{-1} g dry wt.$^{-1}$ (*R. ridibunda*, six animals) and 10·44 ml. min^{-1} g dry wt.$^{-1}$(*R. pipiens*, four animals)

Na in bulk phase in lumen	*n*	Slope ± S.E.	Intercept at zero flow	*P* for significance of slope from zero
R. ridibunda				
Present	19	3·42 ± 0·58	75·8	$P < 0.001$
Absent	16	5·09 ± 0·70	−2·0	$P < 0.001$
R. pipiens				
Present	18	1·08 ± 0·35	88·6	$P < 0.01$
Absent	9	1·73 ± 0·31	7·0	$P < 0.001$

TABLE 3. Effects of Na in bulk phase in lumen on appearance of cycloleucine (2 mM in lumen) in vascular effluent following vascular stop. The extra amino acid expected to appear after the stoppage of flow is taken to be the rate of appearance in steady state of vascular flow × duration of stop. The extra amino acid appearing after vascular flow is resumed is that appearing over and above the steady-state rate of transfer

		Amount of extra amino acid expected to appear after stop (µmole g dry wt.$^{-1}$)		Amount of extra amino acid actually appearing after stop (µmole g dry wt.$^{-1}$)	
Species	(Stop: min)	Na present	Na absent	Na present	Na absent
R. pipiens	(30)	25·6	4·8	12·8	2·4
R. pipiens	(30)	21·0	—	10·5	—
R. ridibunda	(5)	17·7	0·6	1·5	0·1

To investigate the possibility that in the presence of Na in the lumen there is an upper limit to the amount of amino acid that the epithelium can retain, the effects of different lengths of vascular stoppage were investigated in a single animal (Table 4). It is seen that depending on the duration of the interruption of vascular flow different values of the subsequent rate of transfer are found; the longer the stop the lower the subsequent rate of transfer of the substrate accumulated during the stop. For instance, after 5 min stopped flow the subsequent wash-out of the accumulated cycloleucine is as large as that predicted from steady-state experiments. However, after the 10 min stop, less cycloleucine is recovered than would be expected from the steady-state rate and extending the stop to 30 min yields no further increase in the amount of amino acid held in the epithelium.

Evidence that cycloleucine is 'actively' transferred

In addition to the stimulating effects of Na in the lumen on the transfer of cycloleucine, especially at low flow rates as described above, other evidence is as follows.

First, inhibition of transfer in the presence of a competing amino acid was demonstrated. This was done with Na absent from the lumen, the condition in which simple, passive, diffusion would be most likely. L-leucine was added at high concentration (10 mM) to the lumen after the rate of transfer of labelled cycloleucine had been determined with cycloleucine alone. If the effect of flow on the transfer rate could be

TABLE 4. *R. ridibunda*. 2 mM-cycloleucine in lumen. Appearance of cycloleucine in vascular effluent following three different periods of vascular stop flow in a single animal. The extra amino acid expected to appear after the stoppage of flow is taken to be steady state rate of appearance × duration of stop

Length of stop (min)	Amount of amino acid expected to appear after stop (μmole g dry wt.$^{-1}$)	Amount of amino acid actually appearing after stop (μmole g dry wt.$^{-1}$)
5	5·5	5·5
10	12·5	8
30	40	7·5

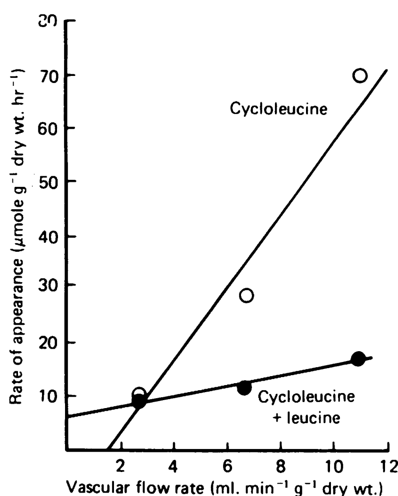

Fig. 3. Influence of L-leucine, 10 mM in lumen and of vascular flow rate on transfer of cycloleucine into portal venous effluent of *R. ridibunda*. Na in bulk phase of lumen fluid replaced by K.

explained by simple diffusion alone, then the addition of leucine would not be expected to have any effect. In fact it was found that at all vascular flow rates investigated (Fig. 3) there is a reduction in transfer of labelled cycloleucine into the portal venous effluent when L-leucine was also present in the lumen. Secondly, an estimation of the minimum concentration of amino acid in the tissue was made from stop flow data from two experiments with *R. pipiens*. This minimum concentration is that found on the supposition that the amino acid was uniformly distributed throughout all the tissue water. The values found, 3·3 and 2·7 mM are both greater than the concentration present in the lumen (2 mM-Na present).

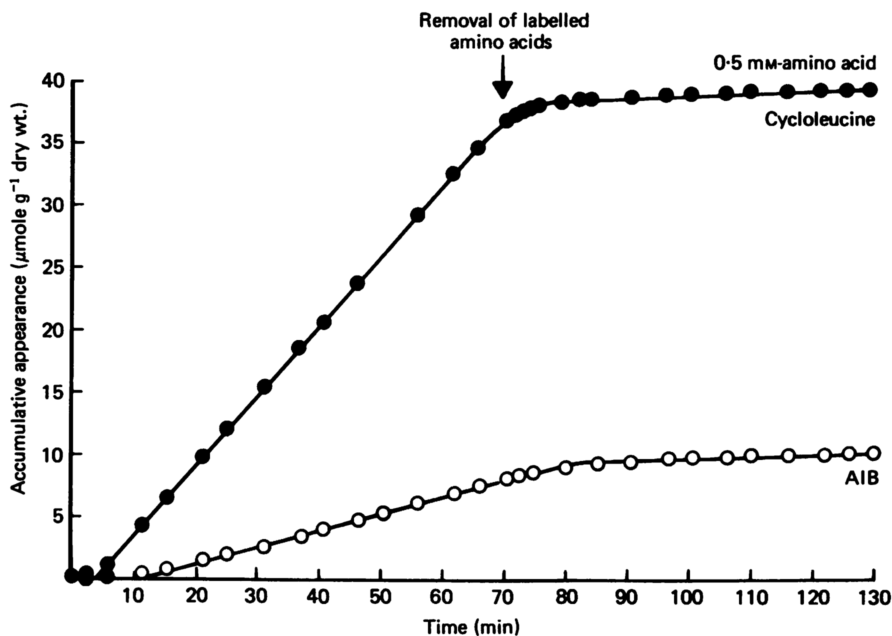

Fig. 4. Comparative rates of transfer of cycloleucine and AIB, each 0·5 mM, from intestinal lumen to vascular bed in *R. ridibunda*. After the steady state of transfer had been established for about 60 min, absorption was stopped by removing the labelled amino acid and at the same time substituting 10 mM unlabelled amino acids.

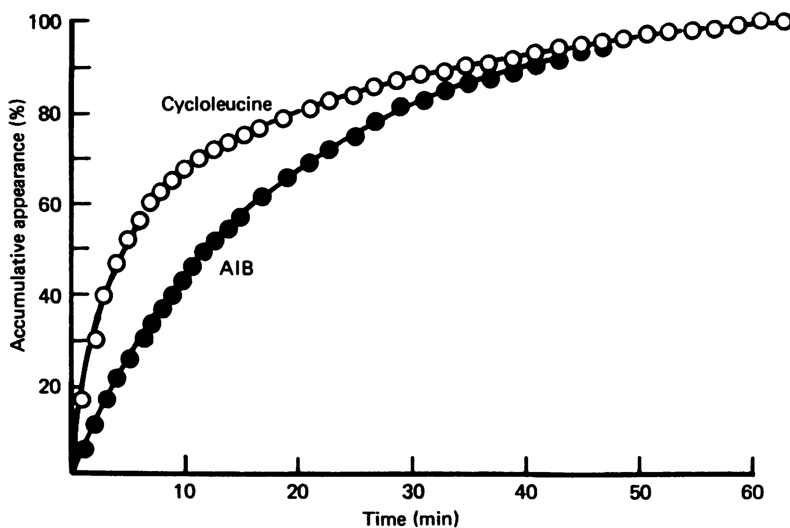

Fig. 5. Comparative unloading into portal venous effluent of cycloleucine and AIB previously absorbed from intestinal lumen. *R. ridibunda*. In each case total amount accumulated deemed to be 100 u.; ordinate, linear scale.

The exit of amino acids from the epithelium into portal venous effluent

(*a*) *Exit of cycloleucine and AIB compared.* The exit of cycloleucine and AIB from the epithelium into the vascular bed were compared in *R. ridibunda*. In order to minimize possible effects of competition, the concentrations used in this study were lower than those used previously. Each amino acid was introduced into the lumen at

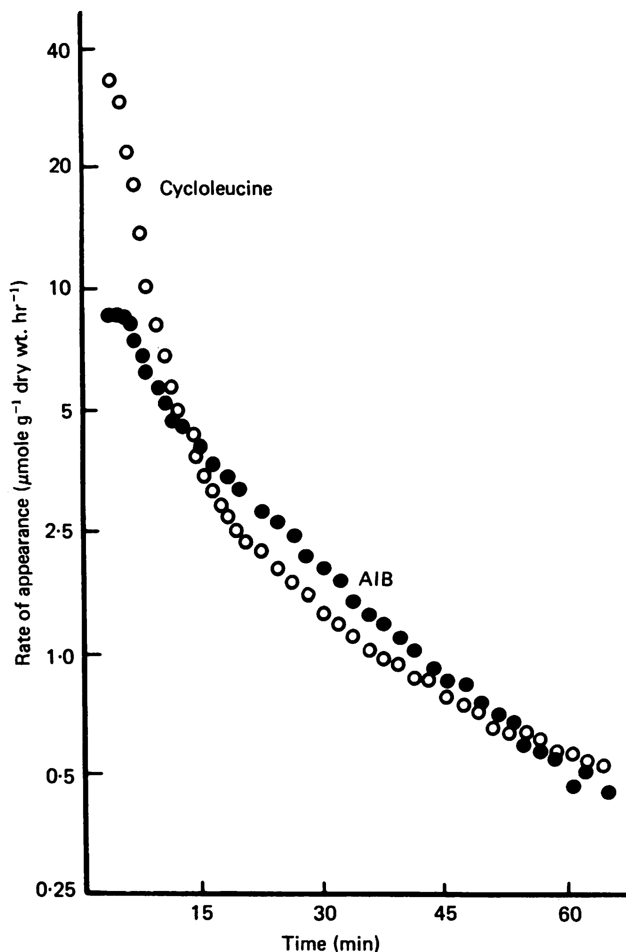

Fig. 6. Wash-out into portal blood of previously absorbed cycloleucine and AIB. *R. ridibunda*. Ordinate on logarithmic scale. Open circles, cycloleucine. Closed circles, AIB. Rate constants for cycloleucine, 0·255 and 0·222 min^{-1}. For AIB, 0·048 min^{-1}.

a concentration of 0·5 mM. After the steady-state rate of transfer had been achieved, the labelled amino acids were rapidly withdrawn and replaced by 10 mM unlabelled amino acid. The results are shown as an accumulative plot in Fig. 4. The time needed to reach the steady-state of transfer for AIB is more than 3 times greater than that for cycloleucine, for which the steady-state rate of transfer is almost twice as high.

The differences between the rates of wash-out of the two amino acids from the

epithelium is demonstrated in Fig. 5, in which the accumulated appearance for each amino acid in the portal venous effluent at the beginning and at the end of the experimental period was deemed to be 0 and 100 respectively for each amino acid. It can be seen that cycloleucine empties from the epithelium at a much faster rate. Indeed after 10 min, when only 45 % of the AIB has left the epithelium, nearly 70 % of the cycloleucine has been unloaded.

Fig. 6 shows that when the instantaneous rates of appearance in the vasculature are plotted on a logarithmic scale, the wash-out of AIB is monoexponential whilst that for cycloleucine is better fitted with two exponential components. Moreover, the

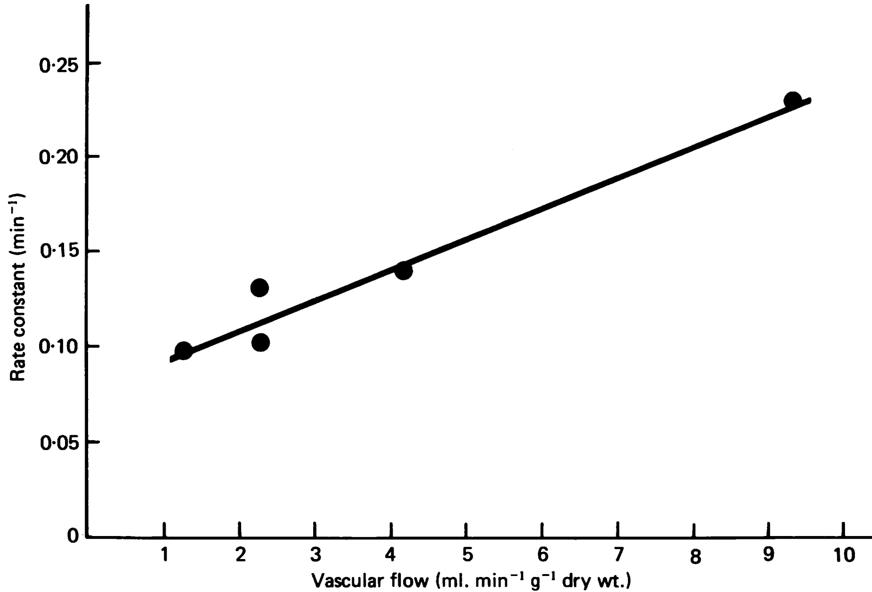

Fig. 7. Influence of rate of vascular perfusion on rate constant of 'fast' component of wash-out of cycloleucine into portal vasculature. Four separate observations on the unloading were made on a single animal. *R. ridibunda*. For the regression line, slope: $1{\cdot}55 \pm 0{\cdot}02 \times 10^{-2}$. For significance of slope, $t = 8{\cdot}46$, $P < 0{\cdot}01$.

rate constant at the earlier part of the washout is greater for cycloleucine. However, the second rate constant of cycloleucine exit is smaller than the mono-exponential rate constant of AIB, although by this time the amino acids are appearing in the portal venous effluent only in trace amounts.

From these studies, estimations of pool sizes can be attempted. The size of the 'loading' pool can be estimated from the plot of total amino acid transported against time. Because the rate of transfer in the steady state rate and time required to reach steady state are counterbalancing factors in the determination of pool size, the pool sizes for loading of AIB and cycloleucine are similar at 1·8 and 2·0 μmol g^{-1} dry weight of intestine respectively.

The unloading pool sizes can be determined from the exponentials of wash-out. This is calculated to be 2·95 μmol g^{-1} dry weight for AIB. The fact that cycloleucine shows a double exponential wash-out means that two pools are unloading; the relative

sizes of these pools depends on the model adopted (Huxley, 1960). If the two pools are assumed to be completely independent, they are found to be 2·5 μmol and 24·9 μmol g^{-1} dry weight. It can be seen that the pool size for the unloading of cycloleucine is larger than that for loading.

(*b*) *Effects of changing the rate of vascular flow on the exit.* The extent to which values of the rate constant for exit depend upon vascular flow was investigated for cycloleucine. It can be seen (Fig. 7) that the rate constant is flow-rate dependent.

DISCUSSION

In experiments on the epithelial transport of the non-metabolised sugar, 3-O-methyl glucose (3MG), Boyd & Parsons (1978) examined the effects of changing the rate of mesenteric blood flow through the absorbing segment. With transport in the steady-state, increasing the rate of blood flow increased the rate of transport, and with Na ions present in the lumen there was a significant rate of monosaccharide transport, even at low rates of vascular perfusion. The relationship between transport from the lumen into the vascular bed and the rate of vascular perfusion was substantially linear, and extrapolation of the regression line back to zero flow yielded a significant transfer. The transport was inhibited by the presence of other sugars or of phlorizin in the intestinal lumen.

The present experiments with cycloleucine show exactly analogous features. Thus with sodium present in the lumen, the transport of cycloleucine is also dependent upon vascular flow; it occurs at high rates even at relatively low rates of vascular perfusion and the transport is inhibited by the presence in the lumen of another amino acid (leucine). With the Na ions in the lumen replaced by K, the present experiments show that at low rates of vascular flow the transport of cycloleucine occurs very slowly but increases substantially as the rate of mesenteric blood flow increases. However, the rates of transport are always less than the values found with sodium present. Similar effects were found for 3MG by Boyd (1977) at low rates of vascular perfusion; the transfer of 3MG from K-Ringer solutions in the lumen increased markedly from very low values as the vascular flow rate was raised.

These observations help to explain the lack of agreement between experiments using brush border membranes or the intestinal mucosal epithelium *in vitro* (e.g. everted sacs) where Na ions in the lumen are certainly required for the uphill transport of monosaccharides and amino acids, and the findings for the intestine *in vivo* where the dependence of amino acid and monosaccharide transport can be less marked (Fordtran, 1975; Förster, 1972). Cheeseman & Parsons (1976) showed that amino acid transport from lumen to blood in the frog was reduced, but not abolished, at low rates of vascular perfusion when Na ions in the lumen fluid were replaced by K.

Although we have no evidence that with vascular perfusion the amino acid is accumulated, e.g. by a Na-dependent process operating across the brush border membrane during the transfer from lumen into the vasculature, such an accumulation may still occur even with Na ions nominally absent from the bulk of the fluid in the lumen. The vascular fluid contains Na ions and one possibility is that with continuing mesenteric perfusion, especially at higher flow rates, Na ions can escape from

the extracellular tissue across the epithelium to enter the fluid in the lumen in the region of the brush border membranes (region of laminar flow: unstirred layer; Nernst diffusion layer; see Winne, 1977) and recirculate. In this case, the bulk phase of fluid in the lumen would be Na-free, but the composition of the fluid in the luminal microenvironment of the brush border membranes could permit the accumulative uptake of amino acids, driven by Na ions, across the brush border. This view would be in accord with the earlier observations of Boyd *et al.* (1975) who found effects of blood Na on epithelial transport.

Another effect of blood flow on epithelial transport of monosaccharides has been described by Boyd & Parsons (1979). Previously absorbed 3MG is washed out from the epithelium into the blood in a biexponential fashion; the rate constant of the 'fast' component of the wash-out for 3MG is related to the blood flow such that the faster the flow the bigger the rate constant. The present experiments show a similar effect for cycloleucine. The wash-out of this amino acid into the vasculature is described by a biexponential function and the 'fast' rate constant, which accounts for about 80 % of the wash-out, is flow dependent.

It appears that the processes underlying the exit of amino acids from the epithelium distinguish between AIB and cycloleucine, the latter being discharged from the epithelium at a higher rate, while AIB is discharged at a lower rate, and in monoexponential fashion. These features for the two amino acids are analogous to the cases of 3MG and α-methylglucoside (αMG) (Boyd & Parsons, 1979). Thus the exit of 3MG exhibits biexponential kinetics, while for αMG the exit is monoexponential in form, but from a very large pool.

The results of the vascular stop flow studies confirm the view discussed earlier that, in the absence of Na ions in the lumen, the transfer of cycloleucine is negligible with vascular flow arrested. However, it seems that the amount of cycloleucine that can be retained in the tissue is limited. Unless the period of vascular stop is of short duration, the amount retained in the tissue is always less than that predicted from the product of the steady state rate of transfer and the duration of the vascular stoppage. A limiting factor to the accumulation in these experiments may be unloading and recycling of cycloleucine between the epithelium and the lumen.

In the experiments reported here for cycloleucine and in those reported earlier for 3MG (Boyd & Parsons, 1978), altering the vascular flow rate produces changes in the rate constant for exit into the vasculature of the bulk of previously absorbed substrate. One way of interpreting this effect is in terms of changes in the diffusive pathway between cells and vasculatures. Thus if D' is the effective diffusion coefficient and the mean path length over which diffusion is occurring is L, then

$$D' = 4kL^2/\pi^2,$$

where k is the rate constant for the final two thirds of the wash-out (Hill, 1928; eqn. (42). See also Keynes, 1954; Caldwell & Keynes, 1969).

Thus with a rate constant for wash-out of AIB of 0·048 min^{-1} and for cycloleucine of 0·255 min^{-1}, the effective diffusion coefficient for a mean path length of 25 μm can be calculated as 2×10^{-9} for AIB (mol wt., 103) and $1 \times 10^{-8} \text{cm}^2 \text{ sec}^{-1}$ for cycloleucine (mol wt., 129). The expected values for the diffusion coefficient would be of the order of 10^{-6} (D for alanine $\times$ mol wt. 89 is 9×10^{-6} and for glucose, mol.

wt. 180 is 6×10^{-6} cm^2 sec^{-1}) so that the apparent diffusion coefficient in the exit pathway appears to be around two orders of magnitude smaller than that for diffusion in free solution. Thus, increasing the rate of vascular perfusion increases the values of the rate constant and hence increases the effective permeability of the exit process, e.g. by making the apparent diffusion coefficient larger, or by reducing the length of the diffusive pathway or by both. Similar conclusions can be drawn for the exit of sugars (see data of Boyd & Parsons, 1979).

We therefore conclude that exit from the epithelium into the blood of the vascularly perfused preparation involves exit from the epithelial cells and diffusive movement along a relatively restricted pathway. At some step, possibly at the cell membrane, exit is highly specific, cycloleucine being much more permeable than AIB; the effects of increasing the mesenteric blood flow include an increase in the apparent permeability of the exit path.

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