J. Physiol. (1974), **241**, *pp.* 271–286
With 2 text-figures
Printed in Great Britain

THE ABSORPTION OF A MIXTURE OF AMINO ACIDS BY RAT SMALL INTESTINE

BY J. R. BRONK AND H. J. LEESE
From the Department of Biology, University of York, York Y01 5DD

(*Received 25 February 1974*)

SUMMARY

1. The absorption of a mixture of amino acids by the small intestine has been studied *in vitro* with mucosal slices from rat jejunum. The mixture contained eighteen amino acids and was used at a concentration of 9·5 mM in the incubation medium. The uptake of fifteen of the amino acids was followed in each sample with the aid of an amino acid analyser.

2. The endogenous amino acid content of the slices decreased during preparation and further substantial losses occurred when the slices were preincubated for 30 sec before the addition of the amino acid mixture to the incubation medium.

3. When the slices were incubated with the amino acid mixture for 4·5 min all of the amino acid studied were accumulated to give concentration ratios of approximately 2·0.

4. The amino acids were taken up rapidly by the tissue so that on average 82% of the amino acids absorbed in 4·5 min had already accumulated after one min of incubation.

5. The data for individual amino acids revealed no obvious competition between the various amino acids, and the extent to which the various amino acids were accumulated by the tissue was proportional to their concentrations in the mixture.

6. When the Na^+ in the incubation medium was replaced by Li^+, the slices accumulated all of the amino acids studied to concentrations significantly higher than those in the incubation medium. However, the concentration ratios obtained with the Li^+ medium were all lower than those obtained with the Na^+ medium and averaged slightly more than 1·5.

7. When the Na^+ in the incubation medium was replaced by K^+ there was little amino acid accumulation, although concentration ratios significantly greater than one were achieved for aspartic acid, glycine, histidine and methionine.

INTRODUCTION

The absorption of amino acids by the mammalian intestine has been studied extensively, but most investigators have been concerned with the uptake of single amino acids, or with the interactions between pairs of amino acids (Wiseman, 1968). Furthermore, the amino acid concentrations used in most experiments have been considerably in excess of those normally found in the lumen of the small intestine *in vivo* (Nixon & Mawer, 1970). In this paper we report the results of an *in vitro* study of the uptake of a mixture of amino acids by rat jejunal mucosa. The amino acid mixture was used at a total concentration of 9·5 mM and with the aid of the amino acid analyser we were able to make simultaneous measurements of the accumulation of fifteen of the eighteen amino acids in each sample. By employing chemical methods to measure the amino acid concentrations we have been able to minimize the effects of exchange diffusion and metabolism which often complicate the interpretation of accumulation measurements made with labelled amino acids. Our work was carried out with slices of rat small intestinal mucosa so that we could study amino acid uptake into the mucosal layer in the absence of the underlying muscle. Artifacts arising from deterioration of the preparation have been minimized by limiting the incubation time to a maximum of five minutes. A preliminary account of some of this work has been given elsewhere (Bronk & Leese, 1974).

METHODS

Measurement of amino acid accumulation

Male Wistar rats of 200–250 g body weight, that had been allowed free access to food, were used in all experiments. Mucosal slices were prepared by the method of Bronk & Parsons (1965) from the jejunum. Experiments were always performed 4–6 hr after the end of the animals' normal feeding time. For each incubation approximately 5 mg dry wt. of slices were placed in 3 ml. of a modified Krebs-bicarbonate Ringer, previously equilibrated with 95 % air, 5 % CO_2 (v/v) at 37° C. The composition of the Ringer was as follows: NaCl 118 mM, $NaHCO_3$ 25 mM, KCl 4·74 mM, $MgSO_4$ 1·19 mM, KH_2PO_4 1·17 mM, $CaCl_2$ 1·70 mM.

In order to study the Na^+ ion requirement for amino acid uptake incubations were also carried out with incubation media in which the NaCl and $NaHCO_3$ were replaced with equivalent amounts of either LiCl and $KHCO_3$, or KCl and $KHCO_3$. In some experiments the mixture of eighteen amino acids was present from the start of incubation, while in others it was added after a 30 sec pre-incubation. The final concentrations of amino acids in the incubation medium are given in Table 1.

Two methods were used for separating the tissue from the incubation medium at the end of each experiment. In the first, the tissue plus medium was transferred to a weighed, round-bottomed 10 ml. centrifuge tube and cooled in ice for 30 sec. The tube was then centrifuged at 4° C for 5 min at 350 *g* in a bench centrifuge and the supernatant poured off. In order to remove any medium adhering to the top

surface of the pellet or to the walls of the tube, 5 ml. ice-cold 0·9 % NaCl were carefully added to the tube, and immediately poured off without disturbing the pellet. The pellet was then extracted with 2 ml. ice-cold perchloric acid (6 %, w/v). In the second method, which has been outlined previously (Leese & Bronk, 1972), the tissue and medium were separated in less than 15 sec by the use of Millipore filters. In both methods the perchloric acid extraction was assisted by agitating the tube containing the mucosal slices for about 10 sec with a loose-fitting Teflon homogenizer driven at 3600 rev/min, before centrifuging at 4° C for 5 min at 350 *g*. The supernatant from this extraction was transferred to a conical, graduated centrifuge tube, and the tube containing the deproteinized tissue pellet was inverted over a filter paper. The small amount of fluid adhering to the inner walls of the tube was removed with a

TABLE 1. Composition of the amino acid mixture

Amino acid	Final concentration in incubation medium (mM)	Molar proportion (glycine = 1)
A Acidic amino acids (isoelectric pH < 3·5)		
Asp	0·60	0·47
Glu	0·94	0·73
B Basic amino acids (isoelectric pH > 7·5)		
Arg	0·27	0·21
His	0·145	0·11
Lys	0·71	0·55
C Other amino acids (isoelectric pH, 5·7–6·5)		
Ala	0·80	0·62
Asn	0·25	0·19
Cys	0·23	0·18
Glu	0·31	0·24
Gly	1·29	1·00
Leu	0·50	0·39
Met	0·39	0·30
Phe	0·35	0·27
Pro	1·07	0·83
Thr	0·55	0·43
Trp	0·20	0·16
Tyr	0·41	0·32
Val	0·47	0·36
Total $A+B+C$	9·485	

rolled filter paper inserted into the tube to within about 2 mm of the pellet. The tube plus pellet was then weighed to obtain the wet weight of the perchloric acid extracted pellet. Excess perchloric acid in the tissue extract was neutralized by the dropwise addition of 30 % KOH, and the precipitated $KClO_4$ spun down at 4° C. 1 ml. of supernatant was removed for amino acid analysis, and re-acidified by the addition of one drop of 6N-HCl. Before being analysed, 0·1 μmol L-norleucine was added to each sample as an internal standard, and the final results were adjusted

with respect to the reading obtained for this standard. The amino acid composition of the extract was determined on a Technicon Autoanalyser, using a continuous elution gradient of sodium citrate buffers, in the range pH 2·88–5·10.

Expression of results

The results for amino acid accumulation are expressed (*a*) as total amount (i.e. including endogenous amino acids) per g initial dry weight of tissue and (*b*) as Tissue/Medium (T/M) values. The latter are obtained by dividing the final concentration of amino acid per ml. tissue water by the initial medium concentration. Calculation of T/M values required estimates of the water content of the tissue and these data were obtained as indicated below. All results are pressed as means ± S.E. of the mean with number of determinations in brackets.

Mucosal slices were weighed on a torsion balance to give the initial wet weight, transferred to a weighed tube, dried to constant weight at 105° C, and re-weighed to give the initial dry weight. This procedure gave ratio (1).

$$\frac{\text{Initial dry wt.}}{\text{Initial wet wt.}} = 0{\cdot}160 \pm 0{\cdot}0031\ (24). \qquad (1)$$

A second series of mucosal slices were weighed on a torsion balance, incubated for 30 sec and extracted with perchloric acid. The dry weight of the pellet from this extraction was then obtained as described for ratio (1) above. These results gave ratio (2) which indicated that the wet weight of the tissue was not significantly altered by incubation and perchloric acid extraction.

$$\frac{\text{Initial wet wt.}}{\text{Wt. after perchloric acid extraction}} = 1{\cdot}04 \pm 0{\cdot}036\ (38). \qquad (2)$$

Another set of slices was used to obtain the ratio of the initial wet wt. as measured by torsion balance, to the wet wt. of the tissue after incubation but before the addition of perchloric acid. The latter value was the weight of the pellet after the initial centrifugation and the saline rinse to remove contaminating incubation medium. These measurements gave ratio (3).

$$\frac{\text{Initial wet wt.}}{\text{Wet wt. from which extract was made}} = 0{\cdot}68 \pm 0{\cdot}016\ (20). \qquad (3)$$

The fact that ratio (3) was less than ratio (2) indicated that extra water became associated with the tissue during the final manipulations, but was subsequently lost during the precipitation procedure. Ratio (1) shows that the true wet wt per g dry wt is 6·25 ml. so that the ratio of tissue water to dry wt. is 5·25. The apparent tissue water in the pellet obtained when the tissue was separated from the medium by centrifugation can be calculated from ratios (3) and (1). This gives:

Wet wt. per g dry wt before perchloric acid addition $= 9{\cdot}19$ g.

Apparent ratio of tissue water to dry wt. after incubation $= 9{\cdot}19 - 1 = 8{\cdot}19$. (4)

When the centrifugation method was used the wet weight of tissue was measured on each sample after perchloric acid extraction. With this value the tissue dry weight was calculated from ratios (1) and (2) and the tissue water from ratio (4). It should be noted that this calculation may give an underestimate of the true intracellular accumulation whenever the concentration of an amino acid in the true tissue water exceeds that in the medium.

When Millipore filters were used to separate the tissue and medium, the tissue wet weight after incubation was found to be the same as that before incubation.

Consequently the tissue dry weight and the tissue water could both be calculated from the wet weight after perchloric acid extraction by ratios (1) and (2). In this case the ratio of tissue water to dry weight was 5·25.

RESULTS

The amino acid content of slices of jejunal mucosa

It was important to know the endogenous levels of amino acids in unincubated mucosal tissue at the start of each incubation since this represented the starting point for the accumulation when there was no preincubation. These values are given in the second column of Table 2. In order to determine the concentration gradients existing immediately after the addition of amino acids to the tissue, the initial intracellular amino acid levels have been divided by the medium levels and the resulting T/M values are included in Table 2. It should be noted that for an amino acid mixture added after 30 sec pre-incubation, the concentrations of amino acids in the tissue were all below those in the incubation medium, while in the case of a mixture added at zero time, there were a variety of inward and outward concentration gradients. Comparison of the first two columns of Table 2 shows that the slices lost some of their endogenous amino acid even during the brief period of manipulation required to prepare the slices for incubation.

The accumulation of amino acids by mucosal slices

The first two columns of Table 3 give the results of experiments in which mucosal slices were incubated with the amino acid mixture without pre-incubation. After 5 min incubation all 15 amino acids had been accumulated by the tissue, and for most of the amino acids the concentration in the tissue water was approximately twice that in the medium.

Comparison of Table 3 with Table 2 shows that the tissue to medium concentration ratio of 2 was reached from quite different starting points in the case of certain of the amino acids in the mixture. For example, the same concentration ratio was observed for both methionine and alanine after the 5 min incubation, although their initial concentration ratios were 0·31 and 3·42, respectively.

Table 3 also shows the results of a second series of experiments in which the tissue was preincubated for 30 sec in substrate-free medium followed by 4·5 min incubation in the presence of the amino acid mixture. Although pre-incubation reduced all the initial concentration gradients to less than 1 (see Table 2), the effect of the pretreatment was to stimulate somewhat some of the subsequent amino acid uptakes. The concentration ratios attained after a 30 sec pre-incubation and a 4·5 min incubation

TABLE 2. The amino acid content of mucosal slices of rat jejunum

	Amino acid	Fresh mucosal slice (μmol/g dry wt.)	Mucosal slice before incubation (μmol/g dry wt.)	*T/M*	Mucosal slice after 30 sec pre-incubation (μmol/g dry wt.)	*T/M*
	No. of observations:	4	6	6	4	4
A	Acidic amino acids					
	Asp	6·9 ±0·61	6·0 ±0·87	1·91±0·28	2·2 ±0·23	0·70±0·073
	Glu	20·7 ±1·4	15·6 ±2·0	3·16±0·41	4·7 ±0·82	0·95±0·17
B	Basic amino acids					
	Arg	2·8 ±0·20	1·6 ±0·13	1·12±0·091	1·11±0·030	0·81±0·22
	His	1·3 ±0·10	1·0 ±0·13	1·31±0·17	0·55±0·41	0·72±0·053
	Lys	2·1 ±0·18	1·8 ±0·12	0·48±0·032	0·92±0·19	0·23±0·048
C	Other amino acids					
	Ala	20·0 ±0·97	14·4 ±1·2	3·42±0·29	3·9 ±0·88	0·94±0·22
	Cys	—	—	—	—	—
	Gly	18·5 ±1·5	11·8 ±1·4	1·74±0·21	3·6 ±0·60	0·53±0·089
	Leu	3·3 ±0·15	2·3 ±0·17	0·88±0·065	1·06±0·23	0·41±0·089
	Met	0·86±0·053	0·63±0·059	0·31±0·029	0·35±0·095	0·17±0·045
	Phe	1·6 ±0·09	1·2 ±0·13	0·66±0·071	0·66±0·17	0·36±0·092
	Pro	4·6 (1)	2·9 ±0·63	0·51±0·11	2·0 (1)	0·34 (1)
	Thr	4·1 ±0·22	3·4 ±0·29	1·18±0·10	1·15±0·25	0·39±0·084
	Tyr	2·1 ±0·09	1·3 ±0·16	0·61±0·075	0·71±0·14	0·32±0·064
	Val	2·9 ±0·18	1·9 ±0·15	0·76±0·06	0·92±0·21	0·36±0·081

were sometimes higher than those found after incubation for 5 min in the presence of amino acids with no pre-incubation of the tissue.

The time course of amino acid accumulation

In order to examine the time course of amino acid uptake, experiments were carried out using the Millipore method to separate the slices from the medium, since this method enabled the incubation time to be defined more precisely. Mucosal slices were preincubated for 30 sec and then

TABLE 3. The accumulation of amino acids by mucosal slices of rat jejunum

Pre-incubation time:	nil		0·5 min	
Incubation time:	5 min		4·5 min	
Amino acid	Amount accumulated (μmol/g dry wt.)	T/M	Amount accumulated (μmol/g dry wt.)	T/M
No. of observations:	12	12	6	6
A Acidic amino acids				
Asp	10·5 ± 0·6	2·14 ± 0·13	12·1 ± 0·87	2·46 ± 0·18
Glu	15·2 ± 0·9	1·98 ± 0·13	17·1 ± 1·1	2·23 ± 0·15
B Basic amino acids				
Arg	4·2 ± 0·05	1·91 ± 0·21	5·0 ± 0·51	2·27 ± 0·23
His	2·3 ± 0·1	1·93 ± 0·12	2·9 ± 0·28	2·44 ± 0·23
Lys	10·5 ± 0·5	1·80 ± 0·09	10·6 ± 0·49	1·82 ± 0·08
C Other amino acids				
Ala	13·8 ± 0·6	2·10 ± 0·08	16·6 ± 1·0	2·53 ± 0·16
Cys	3·0 ± 0·2	1·57 ± 0·12	5·2 ± 0·52	2·76 ± 0·27
Gly	20·4 ± 0·7	1·93 ± 0·07	21·3 ± 0·9	2·02 ± 0·08
Leu	7·6 ± 0·3	1·86 ± 0·07	8·4 ± 0·61	2·05 ± 0·15
Met	6·4 ± 0·4	2·02 ± 0·10	7·4 ± 0·32	2·33 ± 0·10
Phe	5·0 ± 0·3	1·75 ± 0·10	5·1 ± 0·55	1·79 ± 0·19
Pro	13·9 ± 0·8	1·59 ± 0·09	18·6 ± 1·3	2·12 ± 0·15
Thr	8·4 ± 0·5	1·84 ± 0·11	—	—
Tyr	6·2 ± 0·3	1·82 ± 0·10	6·8 ± 0·45	2·02 ± 0·13
Val	7·3 ± 0·4	1·87 ± 0·09	9·8 ± 0·52	2·53 ± 0·13

Tissue and medium separated by centrifugation.

incubated with the amino acid mixture for either 10 sec, 1 min or 4·5 min. The amino acid accumulation data and the corresponding T/M values are given in Table 4. Fig. 1 shows the time course of the accumulation of the amino acids. With the exception of the three basic amino acids which are shown separately the time courses of the uptake of the individual amino acids were very similar and they have been grouped together in the Figure. Table 4 and Fig. 1 show that the amino acids are taken up

TABLE 4. Time course of the accumulation of amino acids by mucosal slices of rat jejunum

Pre-incubation time:	0·5 min		0·5 min		0·5 min	
Incubation time:	10 sec		1 min		4·5 min	
Amino acid	Amount accumulated (μmol/g dry wt.)	T/M	Amount accumulated (μmol/g dry wt.)	T/M	Amount accumulated (μmol/g dry wt.)	T/M
No. of observations:	5	5	6	6	6	6
A Acidic amino acids						
Asp	4·6 ± 0·72	1·48 ± 0·23	6·8 ± 0·70	2·17 ± 0·22	7·6 ± 0·94	2·44 ± 0·30
Glu	7·0 ± 0·36	1·42 ± 0·07	9·7 ± 0·68	1·97 ± 0·14	10·4 ± 0·89	2·12 ± 0·18
B Basic amino acids						
Arg	3·2 ± 0·16	2·24 ± 0·11	3·6 ± 0·14	2·51 ± 0·10	4·2 ± 0·17	2·94 ± 0·12
His	1·1 ± 0·12	1·40 ± 0·16	1·2 ± 0·13	1·61 ± 0·18	2·7 ± 0·31	3·55 ± 0·40
Lys	5·5 ± 0·22	1·48 ± 0·06	6·9 ± 0·33	1·84 ± 0·09	9·9 ± 0·82	2·65 ± 0·22
C Other amino acids						
Ala	7·5 ± 0·40	1·77 ± 0·10	10·6 ± 0·94	2·50 ± 0·22	9·8 ± 0·63	2·32 ± 0·15
Cys	—	—	—	—	2·5 ± 0·02	2·09 ± 0·19
Gly	9·3 ± 0·64	1·37 ± 0·09	14·3 ± 0·70	2·11 ± 0·10	14·6 ± 1·1	2·16 ± 0·16
Leu	—	—	—	—	6·1 ± 0·61	2·31 ± 0·23
Met	2·6 ± 0·25	1·29 ± 0·12	3·3 ± 0·26	1·64 ± 0·13	4·4 ± 0·31	2·16 ± 0·15
Phe	2·3 ± 0·21	1·28 ± 0·11	3·5 ± 0·31	1·89 ± 0·17	4·2 ± 0·35	2·32 ± 0·19
Pro	6·0 ± 0·77	1·06 ± 0·14	11·0 ± 0·65	1·95 ± 0·30	13·2 ± 1·9	2·34 ± 0·33
Thr	3·4 ± 0·52	1·17 ± 0·17	5·3 ± 0·49	1·82 ± 0·17	7·0 ± 0·70	2·41 ± 0·24
Tyr	2·8 ± 0·23	1·30 ± 0·11	3·8 ± 0·34	1·76 ± 0·16	5·0 ± 0·43	2·31 ± 0·20
Val	3·0 ± 0·15	1·20 ± 0·01	4·8 ± 0·32	1·94 ± 0·13	5·5 ± 0·52	2·21 ± 0·21

Tissue and medium separated by Millipore.

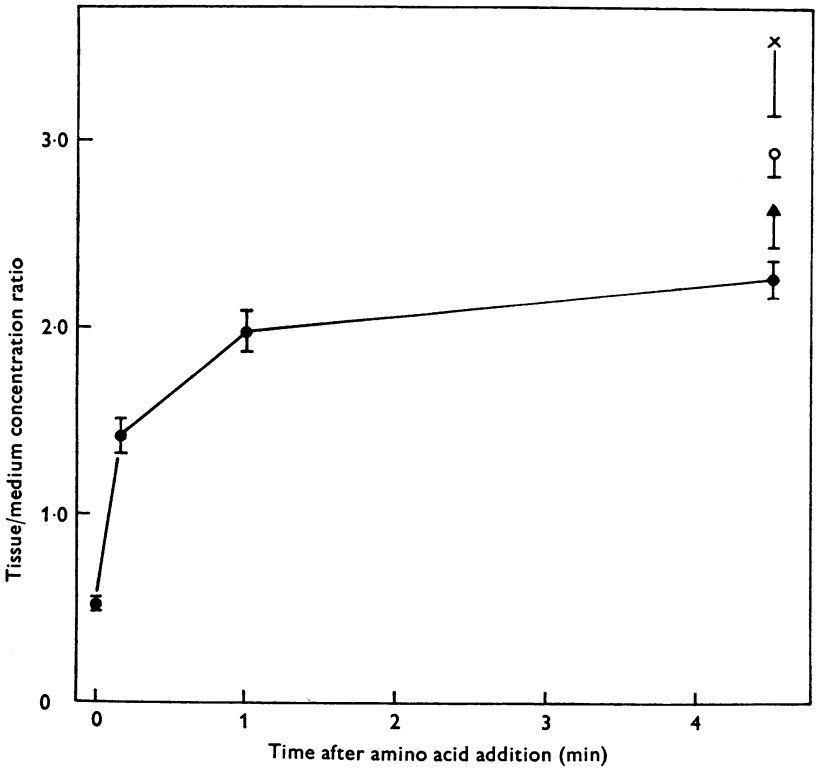

Fig. 1. The time course of the uptake of a mixture of amino acids by mucosal slices of rat jejunum. The points show mean values for the tissue to medium concentration ratios at various times after the addition of the amino acid mixture. The vertical bars show the S.E. of the mean in each case. The mucosal slices were preincubated for 30 sec in the incubation medium before the addition of the amino acids and therefore the value given for the mean concentration ratio at 0 is the average of the fifteen concentration ratios for individual amino acids given in column 5 of Table 2 (these ratios were obtained by dividing the intracellular amino acid concentrations at the end of the 30 sec preincubation period by the initial medium concentrations immediately after amino acid addition). The other values shown in this figure were obtained from the data in Table 4, as follows: the mean concentration ratios after 10 sec and 1 min incubations are the average concentration ratios for all the amino acids reported in columns 2 and 4, respectively, of Table 4. In plotting the results for the 4·5 min incubations the concentration ratios of the three basic amino acids are shown separately from the average of the concentration ratios of the remaining twelve amino acids; the data were obtained from column 6 of Table 4 and are identified as follows: histidine (×), arginine (○), lysine (▲), average of the values for the remaining twelve amino acids (●). In all cases the tissue was separated from the incubation medium by the Millipore filtration method.

very rapidly, since the average value for accumulation at one minute is 82% of that at 4·5 min. The concentration ratios reported in the last columns of Tables 3 and 4 apply to slices incubated with amino acids for 4·5 min after a 0·5 min preincubation, and the two series of experiments differ only in the method used to separate the tissue and medium. The centrifugation and Millipore method gave roughly comparable results although the concentration ratios tended to be higher in the latter case (Table 4). This was particularly true for the basic amino acids. Lower concentration ratios would be expected with the centrifugation method since more extracellular fluid is associated with the slices.

Comparison of the uptake of individual amino acids as a function of their concentrations in the mixture

The similarities between the T/M values obtained for the various amino acids suggested that the extent to which each amino acid was accumulated was a function of its concentration in the mixture. Support for this view is provided by Fig. 2 in which the values for the accumulation of each amino acid (in μmol/g dry wt.) are plotted against the concentration of the amino acid in the incubation medium. The accumulation data for Fig. 2 were obtained from column 5 of Table 4. Each point represents a different amino acid and shows the amount accumulated in a 4·5 min incubation. The line shown in Fig. 2 is the calculated regression of amino acid accumulation on medium concentration, and shows a highly significant linear relationship between the two parameters ($F = 160$, $P < 0{\cdot}001$). The y-intercept of 0·61 μmol/g dry wt. is a measure of the concentrations of each amino acid that might be expected after a 4·5 min incubation in the absence of added amino acid, and it is interesting that this is well below the average amino acid concentration (1·7 μmol/g dry wt.) obtained after a 30 sec pre-incubation (Table 2). Since the ratio of tissue water to dry weight is 5·25 under these conditions the slope of the regression line (11·23 μmol/g dry wt. for each 1 mM increment in medium concentration) suggests that over the range studied the amino acid concentration in the tissue water increases 2·13 mM for each 1 mM increase in medium concentration.

Heat inactivation of amino acid uptake

The data on the time course of amino acid uptake in Table 4 and Fig. 1 show that the accumulation of amino acids occurred progressively during the 4·5 min incubation period. A further indication that the increase in amino acid content represented a true accumulation was provided by a series of experiments in which slices were heated to 100° C for 5 min before incubation. Such treatment prevented any accumulation of amino

acids and after a 5 min incubation all T/M concentration ratios were still well below 1.

The influence of the ionic composition of the medium on amino acid uptake

Since sodium ions have been implicated in the mechanism of amino acid transfer (Wiseman, 1968), it was of interest to determine the effect of

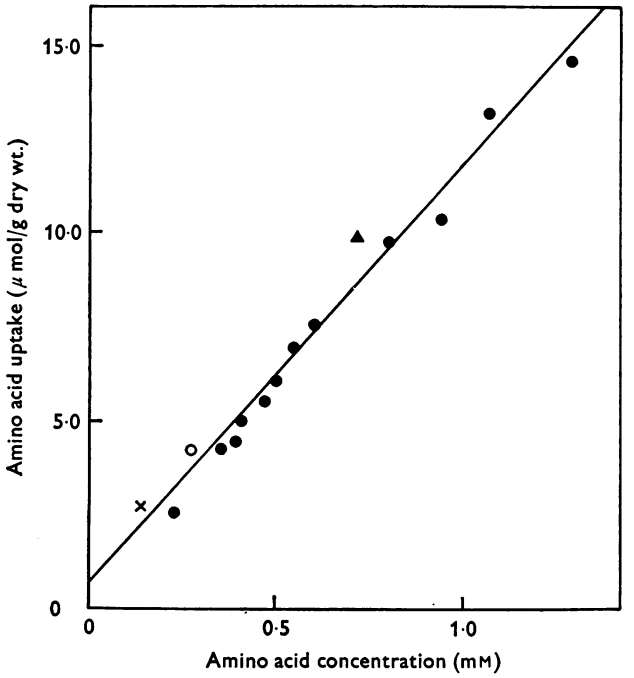

Fig. 2. The uptake of individual amino acids by mucosal slices plotted as a function of the concentrations of the amino acids in the mixture. Each point represents the mean uptake for a particular amino acid during a 4·5 min incubation plotted against the concentrations of that amino acid in the mixture. The uptake values are those given in column 5 of Table 4 and the amino acid concentrations are those shown in Table 1. The basic amino acids are identified as follows: histidine (×), arginine (○), lysine (▲), and the symbol (●) is used for each of the other amino acids. The amino acids were separated from the incubation medium by the Millipore filtration method. The line shown on the graph is the regression of amino acid uptake on amino acid concentration. It has the equation

$y = 11{\cdot}23x + 0{\cdot}61$ and is highly significant ($F = 160$, $P < 0{\cdot}001$).

replacing the Na^+ in the preincubation and incubation media with Li^+ or K^+. For these experiments, the segment of intestine was rinsed with either isotonic LiCl or isotonic KCl rather than isotonic saline. The

results obtained when the tissue was preincubated for 30 sec and then incubated for 4·5 min with the amino acid mixture under these conditions are given in Table 5.

TABLE 5. The accumulation of amino acids by mucosal slices of rat jejunum incubated in media containing Li^+ or K^+ as the predominant cation

Predominant cation	Li^+		K^+	
Amino acid	Amount accumulated (μmol/g dry wt.)	T/M	Amount accumulated (μmol/g dry wt.)	T/M
No. of observations:	12	12	8	8
A Acidic amino acids				
Asp	9·7 ± 0·55	1·99 ± 0·01	7·1 ± 0·53	1·45 ± 0·11
Glu	13·4 ± 0·35	1·75 ± 0·04	9·2 ± 0·52	1·20 ± 0·07
B Basic amino acids				
Arg	3·9 ± 0·42	1·77 ± 0·19	2·4 ± 0·13	1·09 ± 0·06
His	2·4 ± 0·11	2·02 ± 0·10	1·8 ± 0·08	1·51 ± 0·07
Lys	9·7 ± 0·25	1·66 ± 0·04	7·5 ± 0·64	1·30 ± 0·11
C Other amino acids				
Ala	12·3 ± 0·36	1·87 ± 0·06	9·3 ± 0·85	1·41 ± 0·13
Cys	2·4 ± 0·16	1·27 ± 0·09	2·0 ± 0·22	1·06 ± 0·12
Gly	19·2 ± 0·61	1·82 ± 0·06	13·6 ± 0·77	1·35 ± 0·07
Leu	6·9 ± 0·29	1·68 ± 0·07	4·9 ± 0·31	1·22 ± 0·08
Met	5·3 ± 0·16	1·67 ± 0·05	4·1 ± 0·18	1·29 ± 0·06
Phe	4·8 ± 0·15	1·68 ± 0·05	3·5 ± 0·29	1·27 ± 0·10
Pro	13·1 ± 0·65	1·50 ± 0·07	8·8 ± 0·62	1·00 ± 0·07
Thr	8·1 ± 0·22	1·79 ± 0·01	6·1 ± 0·53	1·35 ± 0·12
Tyr	5·9 ± 0·17	1·75 ± 0·05	4·3 ± 0·36	1·31 ± 0·11
Val	6·4 ± 0·20	1·65 ± 0·05	5·0 ± 0·53	1·29 ± 0·13

Tissue pre-incubated for 30 sec; incubated for 4·5 min, separated from the medium by the centrifugation method.

With lithium as the predominant cation, the results of the 4·5 min incubation suggested that the presence of Na^+ in the incubation medium was not obligatory for the uptake process, since, with the exception of cysteine, all of the amino acids were concentrated to a level at least 1·5 times that of the medium. In all cases the T/M concentration ratios were significantly greater than 1.

When the NaCl and the $NaHCO_3$ of the incubation medium were replaced with equimolar amounts of KCl and $KHCO_3$ the normal outward gradient of K^+ ions across the mucosal cell membrane was abolished. The results of 4·5 min incubations under these conditions are also shown in Table 5. In most cases little accumulation took place, and concentration ratios

significantly greater than 1 were only achieved for aspartic acid, glycine, histidine and methionine.

The influence of glucose on amino acid uptake

Actively transported sugars inhibit the simultaneous transport of amino acids but, as far as we are aware, this phenomenon has only been described for single amino acids, and therefore it was of interest to determine whether the same effect could be obtained with a mixture of amino acids. When mucosal slices were incubated with the amino acid mixture plus 11·1 mM glucose after a 30 sec pre-incubation period the results were very similar to those obtained for the amino acid mixture in the absence of glucose (Table 4) and there were no significant differences ($P > 0{\cdot}05$) between the individual amino acid values in these two sets of data.

DISCUSSION

We have shown that isolated mucosal slices of rat jejunum can accumulate the individual amino acids from a physiological mixture to over twice their concentration in the incubation medium. For a given set of experimental conditions the final Tissue/Medium concentration ratios for all the amino acids are remarkably similar, and indicate the absence of important competition effects at these amino acid concentrations. This point is also illustrated by Fig. 2 which shows that the uptake of each individual amino acid was proportional to its concentration in the mixture. Similar findings were reported by Adibi & Gray (1967) for the absorption of an equimolar mixture of eight essential amino acids by human small intestine. They found that the rates of absorption of the amino acids were very similar when the amino acids were presented to the gut at individual concentrations of 1·2 mM. This gave a total amino acid concentration of 9·6 mM in comparison with the total of 9·5 mM in the present work. Significant differences in the uptake of individual amino acids were only apparent at individual amino acid concentrations of 3 mM or more which gave a total amino acid concentration of at least 24 mM. When Adibi, Gray & Menden (1967) studied the uptake of mixtures of eighteen amino acids each used at a concentration of 8 mM the rates of absorption of the individual amino acids differed by more than 100%.

The data in Table 2 indicated that mucosal slices were partially depleted of their endogenous amino acids by manipulation of the tissue prior to incubation, and that substantial losses occurred by subsequent incubation of the tissue for 30 sec in the absence of substrate. No experiments were carried out to determine whether the loss of amino acids on incubation was due to intracellular utilization or leakage into the medium, but the phenomenon provided two different experimental situations from which to

assess the dependence of amino acid uptake on the pre-existing gradients of amino acids across the epithelial cells. The results in Table 3 indicate that higher T/M ratios were obtained after 30 sec pre-incubation, when intracellular amino acid concentrations were all below those of the medium, rather than in the absence of a pre-incubation when the tissue amino acids had not been depleted.

The results in Table 4 show the time course of amino acid entry into mucosal slices followed with the aid of the Millipore separation technique. This method is to be preferred to the centrifugation method for short incubations since the latter method required a longer time to separate the tissue from the medium. The high T/M values obtained for the three basic amino acids, lysine, histidine and arginine, using the Millipore technique contrasted with the results obtained by the centrifugation method, in which the amino acid concentration ratios for each amino acid deviated very little from each other. However, Fig. 2 shows that in relation to the medium concentration the uptake of the basic amino acids did not differ substantially from the general pattern.

The final T/M values for the amino acids leucine and lysine in this study (Table 4) were 2·31 and 2·65 respectively, and they agree fairly well with the values obtained by Bronk & Parsons (1966) for [^{14}C]leucine and [^{14}C]lysine accumulation by rings of rat jejunum incubated in the presence of an amino acid mixture (2·13 and 2·20, respectively). It is interesting to compare these values with those obtained by the same authors for leucine and lysine accumulation in the absence of an amino acid mixture. When added as a single amino acid, leucine (0·12 mM) was accumulated to give a T/M of 14·7, while for lysine (0·06 mM) it was 7·9. Similar differences between the absorption of amino acid in the presence and absence of a mixture of other amino acids have been described by other workers (e.g. Adibi & Gray (1967), Steiner & Gray (1969)). These comparisons suggest that the amino acids in the mixture may compete for the energy required for active transport.

The finding that active transport of the amino acids was not completely abolished by replacing the Na^+ in the incubating medium with Li^+ is contrary to the generally accepted view that Na^+ ions are essential for the transport of amino acids against a concentration gradient (Schultz & Curran, 1970). Whereas the rate of accumulation of the amino acids in the mixture was slower with Li^+ as the predominant extracellular cation, the T/M ratios obtained after 5 min incubation were, on average, only 27 % below those obtained with Na^+. However, when K^+ ions were substituted for the Na^+ ions in the incubation medium (thus providing a Na^+-free medium and eliminating the normal, outward, gradient of K^+ ions across the epithelial cells) active accumulation of the amino acids was virtually

abolished so that the average T/M was only 1·29. These results are in agreement with those obtained by Bronk & Parsons (1966) who studied amino acid accumulation by rings of rat jejunum with Li^+ or K^+ as the predominant cation in the incubation medium. Both the serosal and mucosal poles of the epithelial cells are exposed in the mucosal slices, but only the former is exposed in rings. Therefore, the present results cannot be explained by supposing that the amino acid uptake process loses its Na^+ dependence when the normal asymmetry of the epithelial cells is destroyed by exposing their serosal as well as mucosal faces to the incubating medium. While precise interpretation of our results must await the determination of the sodium and potassium concentrations in the immediate environment of the amino acid transport systems, it seems likely that sodium ions are not obligatory for active accumulation of amino acids by mucosal slices of rat jejunum, provided that an electrochemical gradient of some sort is maintained across the membrane.

We feel our results are of particular significance in the light of recent work by Nixon & Mawer (1970), who found that the free amino acid concentrations in the contents of the small intestine of young adults after a variety of test meals were all below 1 mM. While the situation is complicated by the need to postulate the absorption of peptides, we feel that in studies on the uptake of amino acids *in vitro* it is more meaningful to use low concentrations of amino acids, such as those used in the present work, rather than the unphysiologically high concentrations adopted by most other workers. The present work has also underlined the fact that the uptake of single amino acids by the small intestine is atypical, in the sense that the observed T/M ratios are considerably in excess of those obtained with the mixture of amino acids. The use of single amino acids may mask the co-operative effects whereby the flux of one amino acid stimulates the flux of another in the same direction so that all the amino acids are finally accumulated to a similar extent (Parsons, 1972). Much work has been carried out to classify the amino acids into groups that share the same transport system, and this has required the use of high amino acid concentrations to demonstrate inhibitory effects between the members of a group (for review, see Wiseman (1968)). However, Holdsworth (1972) has expressed the view that competition effects between amino acids (with the possible exceptions of arginine and lysine) are unlikely ever to be rate-limiting during protein absorption under physiological conditions.

We are grateful to the Medical Research Council for the grant which made this work possible.

REFERENCES

ADIBI, S. A. & GRAY, S. J. (1967). Intestinal absorption of essential amino acids in man. *Gastroenterology* **52**, 837–845.

ADIBI, S. A., GRAY, S. J. & MENDEN, E. (1967). The kinetics of amino acid absorption and alteration of plasma composition of free amino acids after intestinal perfusion of amino acid mixtures. *Am. J. clin. Nutr.* **20**, 24–33.

BRONK, J. R. & LEESE, H. J. (1974). Accumulation of amino acids and glucose by the mammalian small intestine. In *Transport at the Cellular Level*, 28th Symp. Soc. Expl Biol., ed. SLEIGH, M. & JENNINGS, D. H. Cambridge: Cambridge University Press (in the Press).

BRONK, J. R. & PARSONS, D. S. (1965). The polarographic determination of the respiration of the small intestine of the rat. *Biochim. biophys. Acta* **107**, 397–404.

BRONK, J. R. & PARSONS, D. S. (1966). Amino acid accumulation and incorporation in rat intestine *in vitro*. *J. Physiol.* **184**, 950–963.

HOLDSWORTH, C. D. (1972). Absorption of protein, amino acids and peptides – a review. In *Transport across the Intestine*, ed. BURLAND, W. L. & SAMUEL, P. D., pp. 136–152. Edinburgh and London: Churchill Livingstone.

LEESE, H. J. & BRONK, J. R. (1972). Glucose accumulation by a rat small-intestinal mucosa after depletion of intracellular adenosine triphosphate. *Biochem. J.* **128**, 455–457.

NIXON, S. E. & MAWER, G. E. (1970). The digestion and absorption of protein in man. 2. The form in which digested protein is absorbed. *Br. J. Nutr.* **24**, 241–258.

PARSONS, D. S. (1972). Summary. In *Transport across the Intestine*, ed. BURLAND, W. L. & SAMUEL, P. D., pp. 253–278. Edinburgh and London: Churchill Livingstone.

SCHULTZ, S. G. & CURRAN, P. F. (1970). Coupled transport of sodium and organic solutes. *Physiol. Rev.* **50**, 637–718.

STEINER, M. & GRAY, S. J. (1969). Effect of starvation on intestinal amino acid absorption. *Am. J. Physiol.* **217**, 747–752.

WISEMAN, G. (1968). Absorption of amino acids. In *Handbook of Physiology*, section 6, Intestinal Absorption, vol. III, ed. CODE, C. F., pp. 1277–1307. Washington, D.C.: American Physiological Society.