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DIFFERENT MECHANISMS FOR NEUTRAL AMINO ACID UPTAKE BY NEW-BORN PIG COLON

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SUMMARY

1. Mucosal amino acid uptake by pig proximal colon, measured independently for fourteen different amino acids each used at a concentration of 1 mM, ranged from 0·6 to 8·6 n-mole.cm^{-2}.min^{-1} in the new-born to 0 to 0·3 n-mole.cm^{-2}.min^{-1} in the 2-day-old animal. Long chain amino acids entered the mucosa of new-born pig proximal colon much more readily than did short chain amino acids.

2. Glycine was used extensively to inhibit the uptake of other neutral amino acids. The degree of maximal inhibition produced depended on the amino acid used. The relative inability of glycine to inhibit the uptake of long chain amino acids suggested that these compounds could cross the brush border on a carrier inaccessible to glycine. The glycine-sensitive uptake remained more or less constant for all amino acids tested (1–2 n-mole.cm^{-2}.min^{-1}); the glycine-insensitive uptake varied from 0 to 7 n-mole.cm^{-2}.min^{-1} (glycine and methionine respectively).

3. It is suggested that at least two mechanisms exist for the entry of neutral amino acids into pig proximal colon, one showing specificity for hydrophobic amino acids and the other having broad specificity. The mechanism responsible for the uptake of long chain essential amino acids predominates over the less specific mechanism.

4. These results are discussed in relation to previous work carried out on the rabbit ileum where two similar systems for neutral amino acid entry have been shown to be present. Both tissues transport hydrophobic amino acids on their own specific carrier at approximately the same rate; the ability of the pig colon to transport amino acids on the broad specificity carrier is eight times less than in the rabbit ileum. The possibility is raised that this system is subject to regulation.

INTRODUCTION

It has been shown previously that methionine can be both concentrated within and transported across the colonic mucosa of the new-born pig (Smith & James, 1976; James & Smith, 1976). The ability of the proximal colon to transport methionine declines with age (James, Smith & Wooding, 1976), the time course for decay corresponding approximately to the cell renewal time measured during the immediate post-natal period (Jarvis, Morgan, Smith & Wooding, 1977). The absorption of nutrients by the small intestine shows some temporary inhibition shortly after birth due to the endocytotic uptake of large amounts of colostral proteins (Henriques

de Jesus & Smith, 1974). It could be that the proximal colon performs a useful physiological function at this time, absorbing amino acids not taken up by the small intestine. If this were the case one would predict that the colon should be able to transport a wide range of amino acids; this ability was tested in the first part of the present work.

The results obtained were rather surprising, some amino acids entering the colonic mucosa much more readily than others. The second part of the present work analyses amino acid uptake in greater detail using glycine as a specific inhibitor of one type of uptake mechanism. The technique of analysis is similar to that described previously for rabbit ileum (Sepúlveda & Smith, 1978). The possibility that at least two transport systems exist for the uptake of neutral amino acids in the pig colon and that one of these may be capable of regulation is then discussed in relation to previous results obtained using the rabbit ileum.

METHODS

Animals

Pigs used in the present investigation came from a herd of Large Whites bred at Babraham. Parturition was induced routinely by the intramuscular injection of prostaglandin analogues as described originally by Ash & Heap (1973). New-born piglets, removed from the sow prior to suckling, were used for experiment as soon as possible. The longest period of time elapsing between birth and study was 6 hr.

Experimental procedure

Animals were stunned by a blow on the head and killed by decapitation. The proximal colon, dissected free from connective tissue, was prepared for flux measurements as described below. The colon was cut lengthways and the meconium was removed with absorbant paper. A 10 cm length of cleaned tissue was then pinned, mucosal surface uppermost, to a Perspex baseplate which was clamped to a superfusion apparatus similar to that already described for the short-term measurement of isotope uptake by the small intestine of the new-born pig (Henriques de Jesus & Smith, 1974). The present apparatus was equipped with twelve ports, each of area 0·196 cm^2, for the independent measurement of amino acid uptake. The time of contact between radioactively-labelled amino acid and colonic mucosa was limited to 45 sec. Previous unpublished work had shown uptake of different amino acids by new-born pig colon to remain linear with time during incubation for at least 60 sec. Radioactive choline ([methyl-^{14}C]choline, 2·5 $\mu c.ml.^{-1}$, or [methyl-^{3}H]choline, 3·0 $\mu c.ml.^{-1}$) was always present in solutions containing ^{3}H or ^{14}C-labelled amino acids (5 and 0·75 $\mu c.ml.^{-1}$ respectively), to allow correction of total amino acid uptake for tissue extracellular space (Hénin & Smith, 1976). The mean extracellular space for pig proximal colon, measured after a 45 sec contact of the colonic mucosa with ^{14}C and ^{3}H-labelled choline, was 2·78 ± 0·55 and 2·84 ± 0·5 $\mu l.\ cm^{-2}$ respectively. ^{3}H-labelled choline, used in the presence of 142 mM non-radioactive choline, gave the same space as that found in the presence of 143 mM-Na and tracer amounts of choline ($t = 0{\cdot}05$, $P > 0{\cdot}9$, 32 paired comparisons). It was concluded from these experiments that both isotopes were equally effective markers of extracellular space in this tissue.

The experiments to be reported below involved brief superfusion of pig colonic mucosa with bicarbonate saline (Krebs & Henseleit, 1932) gassed with 95% O_2 + 5% CO_2 at a temperature of 37 °C, followed by a 45 sec incubation in the same medium containing radioactive amino acid and choline together with concentrations of glycine varying from 5 to 50 mM. Mannitol was added to these solutions to maintain constant tonicity. Initial experiments were carried out to test whether mannitol could, by itself, affect the uptake of labelled amino acid. Alanine and methionine were chosen to represent hydrophilic and hydrophobic classes of neutral amino acid respectively. Uptake of alanine, measured at mannitol concentrations of 0, 24 and 48 mM was 3·44 ± 0·42, 3·44 ± 0·43 and 3·31 ± 0·62 n-mole.cm^{-2}.min^{-1} respectively, means ± S.E. of four comparisons

using alanine at a concentration of 0·5 mM. Uptake of methionine at mannitol concentrations of 0, 24 and 48 mM was $5{\cdot}21 \pm 0{\cdot}22$, $5{\cdot}23 \pm 0{\cdot}25$ and $5{\cdot}44 \pm 0{\cdot}22$ n-mole.cm^{-2}.min^{-1} respectively, means ± S.E. of four comparisons at a methionine concentration of 1 mM. Mannitol does not affect the rate of uptake of alanine or methionine. The above experiments also show the colon to be relatively insensitive to changes in tonicity up to 48 mM hypertonic to the bicarbonate saline of Krebs & Henseleit.

Materials

The following radioactive isotopes were purchased from the Radiochemical Centre, Amersham, Bucks.: [1-^{14}C]glycine; L-[3,4-^{3}H]proline; L-[U-^{14}C]serine; L-[U-^{14}C]threonine; L-[U-^{14}C]alanine; L-[3,4(n)-^{3}H]valine; L-[methyl-^{14}C]methionine; L-[U-^{14}C]isoleucine; L-[1-^{14}C]leucine; L-[U-^{14}C]tyrosine; L-[U-^{14}C]histidine; L-[U-^{14}C]phenylalanine; L-[U-^{14}C]lysine monohydrochloride; L-[5-^{3}H]arginine monohydrochloride; [methyl-^{14}C]choline chloride and [methyl-^{3}H]choline chloride.

Non-radioactive amino acids came from the following sources: L-proline, L-serine, L-threonine, L-alanine, L-valine, L-isoleucine, L-tyrosine, L-phenylalanine and L-arginine HCl (British Drug Houses Ltd, Poole, Dorset); L-methionine and L-histidine (Hopkin & Williams Ltd, Chadwell Heath, Essex); L-lysine HCl (Cambrian Chemicals Ltd, Croydon, Surrey); glycine (Fisons Scientific App. Ltd, Loughborough, Leics.) and L-leucine (Koch-Light Labs, Colnbrook, Bucks.). All other reagents were of analytical grade.

RESULTS

Amino acid uptake by new-born pig colon

It has been reported previously that the trans-tissue flux of methionine is high in the pig colon at birth and that the capacity to transport methionine declines with age (Smith & James, 1976). An initial series of experiments was carried out to test whether these characteristics of changing transport would also apply to initial fluxes measured across the brush border membrane of the pig colon. A large number of neutral and basic amino acids was used for these experiments. The concentration of each amino acid was maintained constant at 1 mM and uptake measured in either high or low Na-containing medium (145 and 1 mM-Na respectively). Choline was used as a replacement for Na and the Cl-concentration was maintained constant throughout.

There was over a tenfold variation in the rates at which different amino acids entered the colonic mucosa of new-born pig (J_{mc} 0·6–8·6 n-mole.cm^{-2}.min^{-1} measured using 145 mM-Na, Fig. 1*A*). Over-all uptake, but not the range of specificity, was reduced in the virtual absence of Na (0·05–3·2 n-mole.cm^{-2}.min^{-1} measured using 1 mM-Na). Amino acid uptake measured using 145 mM-Na in contact with a 2-day-old pig colon, was extremely low and there was then no obvious preference for any particular amino acid (Fig. 1*B*). There appeared to be a definite pattern in the way in which different neutral amino acids entered the new-born pig colon. Hydrophobic neutral amino acids entered the mucosa much more readily than either the more hydrophilic neutral (glycine, proline, serine, threonine, alanine) or basic (histidine, lysine, arginine) amino acids. The possible significance of these differences will be discussed later.

Inhibition of amino acid uptake into pig proximal colon

The technique developed by Christensen (1969) to detect the presence of different amino acid transport systems, by measuring inhibition characteristics of different

chosen amino acids, was used in the present work to test for the presence of more than one uptake mechanism. The method relies on the generally accepted hypothesis that the effectiveness of a large number of non-radioactive amino acids as inhibitors of the uptake of any two (or more) radioactive analogues will be correlated provided that all the amino acids share the same entry system. Lack of correlation denotes the presence of more than one mechanism of entry.

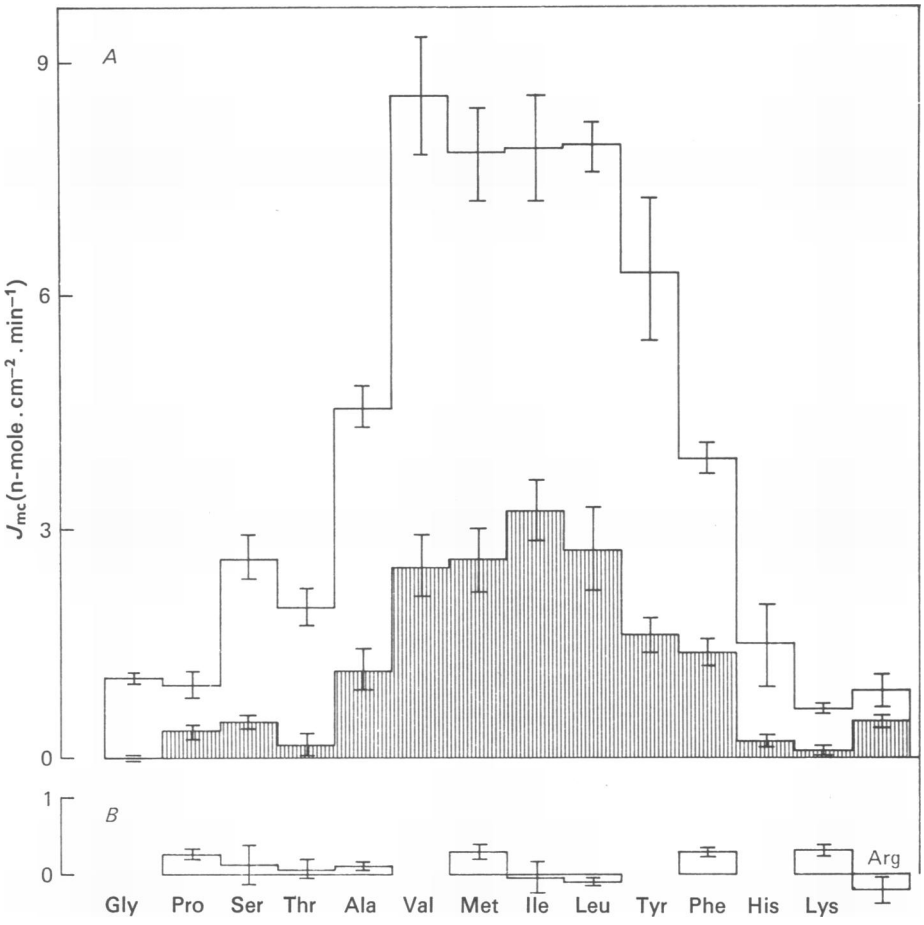

Fig. 1. Amino acid uptake by pig proximal colon. Uptake of each amino acid was measured separately using methods described in the text. Open histograms give uptakes measured in the presence of 145 mM-Na; shaded histograms, uptakes in the presence of 1 mM-Na. Each amino acid was present at a concentration of 1 mM. Values are mean uptakes ± S.E. of from eight to fifty-eight determinations on new-born (Fig. 1*A*) and 2-day-old (Fig. 1*B*) pig proximal colons.

Four radioactively labelled amino acids (leucine, phenylalanine, alanine and glycine) were chosen for study. Uptake of each was measured independently (substrate concentration 1 mM) in the presence and absence of a number of different non-radioactive amino acids used as inhibitors. The results obtained are shown in Table 1. Non-radioactive methionine, leucine, isoleucine, valine and phenylalanine, used at a concentration of 5 mM, were approximately equi-active as inhibitors of radioactively

labelled leucine, phenylalanine, alanine and glycine uptake. Non-radioactive amino acids, alanine, threonine, serine and glycine were very poor inhibitors of leucine uptake, though they continued to be effective inhibitors of glycine uptake. Non-radioactive glycine, used at a concentration of 20 mM, was pre-eminent in this respect, being over ten times more effective at inhibiting its own uptake relative to that of radioactively labelled leucine. This divergent pattern of inhibition suggests that neutral amino acids enter the colonic mucosa of the new-born pig by more than one mechanism. Further experiments were designed to investigate this possibility in greater detail.

TABLE 1. Inhibition of amino acid uptake into the colonic mucosa of new-born pigs. Uptake of leucine, phenylalanine, alanine and glycine was measured into new-born pig proximal colon in the presence and absence of one of a number of inhibiting amino acids, concentration of inhibitors given in mM in parentheses. Each test amino acid was used at a concentration of 1 mM. Numbers give the mean percentage inhibition of uptake ± S.E., each value being derived from at least four comparisons

Inhibiting amino acid (mM)	Percentage inhibition of uptake of: L-Leucine	L-Phenylalanine	L-Alanine	Glycine
L-Methionine (5)	79·5 ± 1·3	96·7 ± 1·7	92·6 ± 2·2	93·7 ± 2·2
L-Leucine (5)	73·9 ± 2·3	94·1 ± 3·6	84·7 ± 2·6	86·7 ± 13·4
L-Isoleucine (5)	68·3 ± 3·0	80·8 ± 2·8	87·6 ± 1·4	98·5 ± 1·5
L-Valine (5)	52·9 ± 1·6	74·2 ± 2·7	70·6 ± 7·4	79·9 ± 9·5
L-Phenylalanine (5)	41·0 ± 1·1	49·8 ± 4·2	44·4 ± 11·0	87·0 ± 5·8
L-Alanine (5)	17·5 ± 7·1	32·5 ± 7·9	36·7 ± 9·7	45·8 ± 4·2
L-Threonine (5)	2·9 ± 3·1	9·6 ± 3·4	15·4 ± 3·3	40·4 ± 4·6
L-Serine (5)	12·5 ± 1·7	13·4 ± 6·9	36·1 ± 3·4	66·7 ± 8·1
Glycine (20)	8·1 ± 3·1	16·1 ± 6·2	31·9 ± 3·7	95·0 ± 7·9

Glycine inhibition of amino acid uptake by pig proximal colon

It is shown in Table 1 that even a relatively high concentration of glycine has only a minimal effect on the uptake of leucine (20 mM-glycine produces an 8% inhibition of leucine uptake). From this it seemed possible that the bulk of leucine transport and perhaps the transport of other hydrophobic side-chain amino acids, might take place through a mechanism inaccessible to glycine. This was tested for by measuring the uptake of isoleucine, another hydrophobic amino acid, in the absence and presence of increasing concentrations of glycine. Comparisons are made with glycine used as an inhibitor of its own uptake (radioactively labelled glycine being assumed always to represent a concentration of 1 mM). The results obtained are shown in Fig. 2.

Glycine was an effective inhibitor of its own uptake, there being very little uptake of isotopically labelled glycine for concentrations of non-radioactive glycine in excess of 30 mM. Some estimates of glycine uptake appeared to be negative when measured in the presence of high concentrations of non-radioactive glycine. This is not caused by using hypertonic medium or by using mannitol to maintain tonicity constant (see Methods). It probably arises from choline giving a very slight over-estimate of the extracellular space. Glycine used at a concentration of 50 mM produced only a 15% inhibition in the uptake of isoleucine measured at a concentration of 1 mM. It is not possible to determine the kinetic characteristics of such a small inhibitory effect. It can be concluded, however, that the vast proportion of isoleucine

uptake by pig proximal colon takes place through a mechanism having negligible affinity for glycine.

The inhibition of uptake of radioactively labelled glycine by increasing concentrations of non-radioactive glycine could be fitted by a hyperbolic curve (Fig. 2*A*). This is given in Fig. 2*B* as a Hanes plot in the form described previously by Muflih & Widdas (1976). The kinetics are consistent with simple competitive inhibition taking place between uptake of labelled and non-labelled glycine. The intercept on

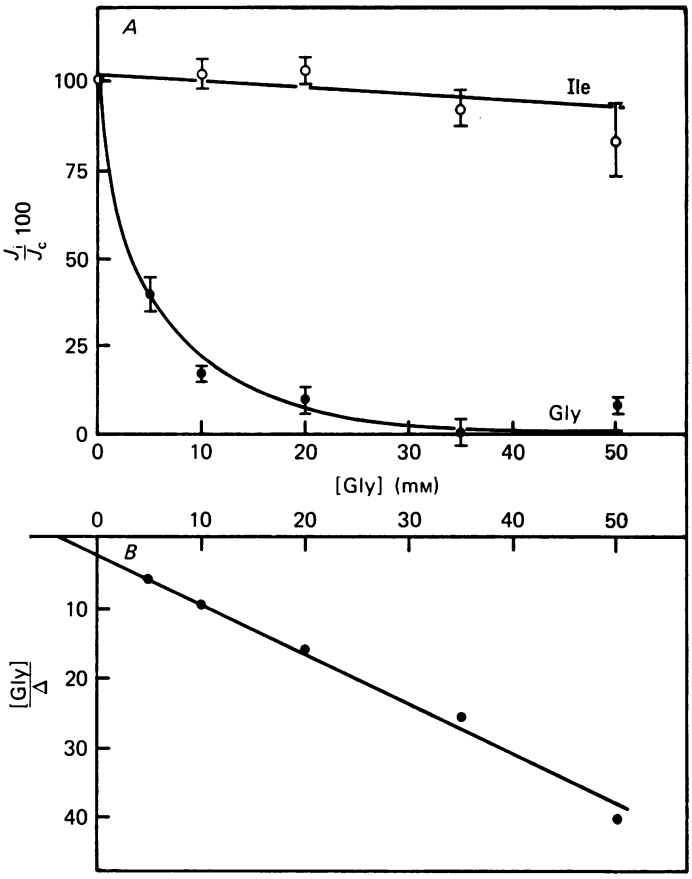

Fig. 2. Glycine inhibition of amino acid entry into new-born pig colonic mucosa. *A*, the ability of glycine to inhibit its own uptake (—●—), and the uptake of isoleucine (—○—), is plotted as a function of glycine concentration. Values are means ± S.E. of four and eight determinations of glycine and isoleucine uptakes respectively. *B*, Hanes plot of glycine inhibition of glycine uptake. The affinity of glycine for its carrier is calculated assuming $K_i = K_m$. The equation showing the relation between K_m and K_i is given in the text. Δ is the inhibited portion of glycine uptake measured for each concentration of glycine used. Each value is the mean of four determinations.

the abscissa in Fig. 2*B* is equal to $-K_i(1+S/K_m)$, where K_i is the inhibitor constant, K_m is the affinity of substrate for its carrier and S is the substrate concentration. For the special case of glycine acting as its own inhibitor K_i can be taken to equal K_m (Christensen, 1975). The K_m of glycine for its uptake mechanism, calculated by this

method, is 2·3 mM, a value in close agreement with that of 1·7 mM for the system one uptake of glycine by rabbit ileum (Sepúlveda & Smith, 1978).

Further experiments were then carried out using glycine as inhibitor to test its effectiveness in blocking the uptake of other neutral amino acids. In this case paired comparisons have been made between the uptake of different neutral amino acids, each being measured separately at a concentration of 1 mM, in the presence and absence of 50 mM non-radioactive glycine. A further analysis has been carried out,

TABLE 2. Glycine inhibition of amino acid uptake. Uptakes of different amino acids were measured at a constant concentration of 1 mM in the absence (J_c) and presence (J_i) of 50 mM-glycine. Numbers give mean uptakes ± S.E., the number of paired comparisons being given in parentheses. Fractional inhibition of uptake ($1-J_i/J_c$), calculated for the actual concentration of glycine used (50), is compared with the maximal possible inhibition (∞), obtained by extrapolation using the iterative technique of Bliss & James (1966). *, **, ***; inhibition significant at the 5, 1 and 0·1 % probability levels assessed by paired *t* test

	Uptake (n-mole.cm^{-2}.min^{-1})		$1-J_i/J_c$	
Amino acid	J_c	J_i	50	∞
Glycine (7)	1·31 ± 0·14	0·21 ± 0·05	0·84***	1·0
L-Serine (7)	2·47 ± 0·16	1·37 ± 0·15	0·45***	0·57
L-Threonine (8)	1·83 ± 0·28	0·97 ± 0·14	0·47**	0·55
L-Alanine (9)	4·73 ± 0·49	3·35 ± 0·27	0·29**	0·34
L-Phenylalanine (10)	4·85 ± 0·43	3·33 ± 0·31	0·31*	—
L-Tyrosine (8)	4·93 ± 0·69	3·64 ± 0·36	0·26*	—
L-Valine (8)	8·25 ± 0·84	6·43 ± 0·80	0·22***	—
L-Isoleucine (7)	6·02 ± 1·21	5·21 ± 1·03	0·13	—
L-Leucine (8)	7·96 ± 0·78	7·03 ± 0·58	0·12	—
L-Methionine (10)	7·65 ± 0·61	6·98 ± 0·40	0·09	—

using the range of glycine concentrations shown in Fig. 2, in cases where glycine inhibition of amino acid uptake is sufficiently great to allow a hyperbolic function to be fitted to the inhibition curve. This is possible when measuring the inhibition of uptake of glycine, serine, threonine and alanine. The summarized results are given in Table 2. The last column shows the fractional inhibition of amino acid uptake produced by glycine. This represents maximal inhibition when calculated from the asymptote of a rectangular hyperbola fitted to the uptake values measured at increasing glycine concentrations. These extrapolated values are compared with the inhibition caused by 50 mM-glycine. The difference between the two sets of figures is about 15 % for glycine, serine, threonine and alanine. The fractional inhibition of amino acid uptake by glycine was reasonably high for the small-chain hydrophilic amino acids. Glycine inhibition of phenylalanine, tyrosine and valine uptake, though small, was still statistically significant. Glycine inhibition of isoleucine, leucine and methionine uptake was too small to be measured accurately.

Each fractional inhibition of amino acid uptake can be converted to an actual amount of amino acid inhibited from entering the mucosa via a glycine-sensitive pathway, by reference to total uptakes plotted in Fig. 1. Both glycine-sensitive and glycine-resistant portions of different amino acid uptakes are plotted separately in Fig. 3. Maximal uptakes by the glycine-sensitive mechanism, measured at substrate

concentrations of 1 mM, are shown for glycine, serine, threonine and alanine (Fig. 3*B*). The glycine-sensitive uptakes for the remaining amino acids, measured using 50 mM-glycine as inhibitor, underestimate transfer rates through the glycine sensitive system.

The K_m for glycine can be used to estimate the degree of inhibition caused by 50 mM-glycine. With the range of K_m values found previously in the rabbit ileum (Sepúlveda & Smith, 1978) it can be calculated that 50 mM-glycine will cause between 83 and 90 % of its maximal inhibition.

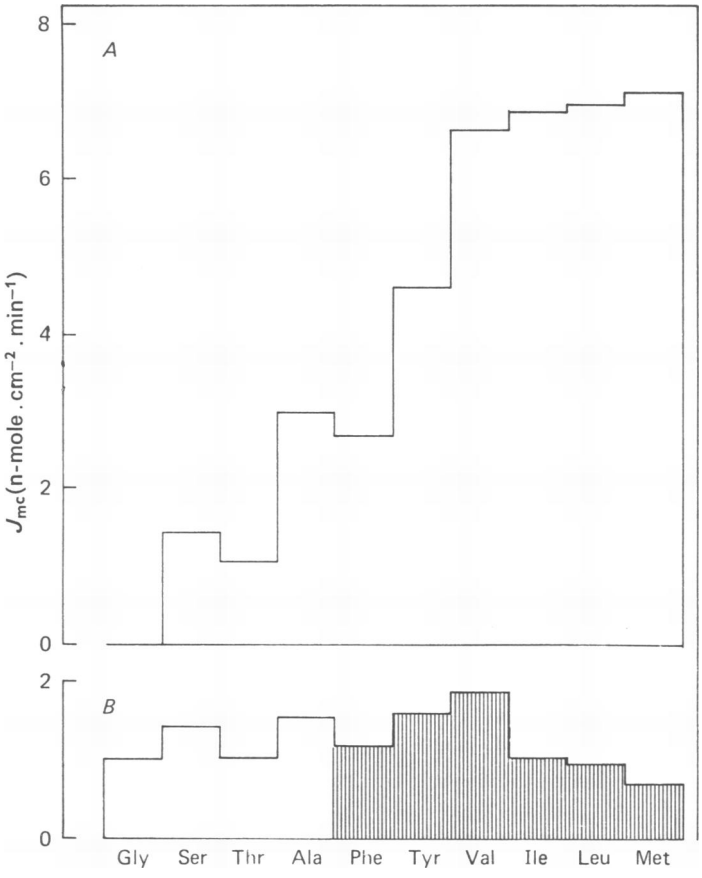

Fig. 3. Two-system uptake of neutral amino acids by pig proximal colon. Total uptake of an individual amino acid is divided into that portion which is resistant or sensitive to inhibition by glycine (Fig. 3*A* and *B* respectively). These values have been obtained, as indicated in the text, by reference to the fractional inhibitions shown in Table 2 and the total uptakes shown in Fig. 1. Open histograms in Fig. 3*B* give uptakes derived from analysis of glycine inhibition curves; shaded histograms give estimates based on inhibition of uptake produced by the presence of 50 mM-glycine in the incubation medium.

Uptake of amino acids by the glycine-sensitive system is generally low and there is no particular specificity of this system for individual amino acids. It should be remembered, however, that the glycine-sensitive uptake of isoleucine, leucine and methionine was not statistically significant.

The amounts of amino acids entering the mucosa through the glycine-resistant mechanism were high for long chain (valine, isoleucine, leucine, methionine) and low for short chain (serine, threonine, alanine) amino acids. None of the glycine uptake took place through this mechanism. The uptakes of phenylalanine and tyrosine by this system were intermediate between those of small and big side-chain amino acids. The ability of an amino acid to enter the pig colonic mucosa on a glycine-resistant system seems to be largely determined by the relative hydrophobicity of the amino acid in question. This statement may, however, need further qualification in cases where the hydrophobicity of the amino acid arises from the presence of an aromatic side-chain (e.g. tyrosine and phenylalanine).

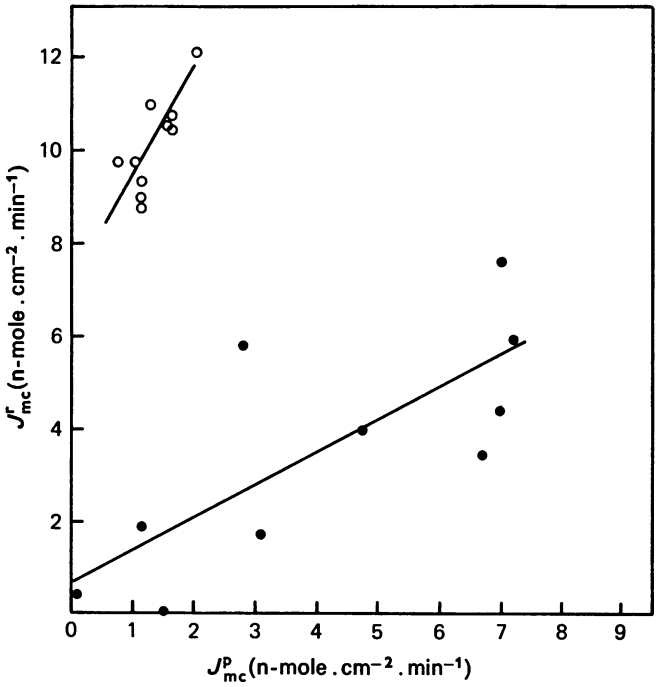

Fig. 4. Intestinal uptake of different neutral amino acids, estimated according to carrier specificity, for rabbit ileum (J^r_{mc}) and new-born pig colon (J^p_{mc}). System 1 (○) refers to uptake inhibitable by serine in the rabbit and glycine in the pig. System 2 (●) refers to uptake resistant to inhibition by serine in the rabbit and glycine in the pig. Results for rabbit ileum have been extracted from the paper by Sepúlveda & Smith (1978).

DISCUSSION

The present work set out to test the prediction that the new-born pig colon would be capable of transporting a whole range of amino acids. This prediction was only partly verified. All the amino acids tested were taken up by the proximal colon, but the tissue showed a marked preference for the long-chain hydrophobic amino acids. These amino acids have been shown previously to enter rabbit ileal mucosa via a system not shared by glycine or serine (Sepúlveda & Smith, 1978). The interesting possibility then arose that the neonatal pig colon might possess only one of the two neutral amino acid transporting systems identified previously in the rabbit.

The precise interrelationship between tissues and species can best be seen by plotting the glycine-dependent and glycine-independent fluxes of neutral amino acids for pig proximal colon against the respective serine-dependent and serine-independent fluxes determined in the rabbit small intestine. The results obtained are shown in Fig. 4. There appears to be a good correlation between the serine- and glycine-dependent fluxes on the one hand ($r = 0{\cdot}78$, $P < 0{\cdot}01$) and the serine- and glycine-independent fluxes on the other ($r = 0{\cdot}75$, $P < 0{\cdot}01$). The actual amounts of neutral amino acids entering the intestinal mucosa of rabbits and pigs via the serine/glycine-resistant pathway were equal (mean ratio of uptake equal to one). What distinguishes the pig colon from the rabbit ileum at this stage in development is its relative inability to transport amino acids by a glycine/serine-sensitive mechanism (mean ratio of influx rabbit to pig equal to $8{\cdot}58 \pm 0{\cdot}75$).

The high degree of correlation between systems distinguished only by their sensitivity or insensitivity to inhibition by glycine and serine is interesting. It suggests, at first sight, that both amino acids share the property of being unable to interact with a carrier system specific for the long-chain hydrophobic amino acids. This is almost certainly true for the rabbit (Sepúlveda & Smith, 1978), but it is not entirely true for the pig. Additional work suggests that serine has a small, but nevertheless significant, affinity for the system handling long-chain amino acids. This makes glycine the better probe for identification of different amino acid transport systems in the pig. In summary therefore one can conclude that there is a great deal of similarity between systems transporting amino acids in rabbit ileum and pig colon, but that the system responsible for uptake of short-chain hydrophilic amino acids has only a small activity in the pig colon. Finally, though these systems show many cross-species similarities, their *precise* selectivity for different neutral amino acids is likely to vary slightly from tissue to tissue.

The suggestion that neutral amino acids might cross cell membranes using two systems, one preferring short-chain and the other long-chain amino acids is not, of course, a new one. Such systems have already been identified and characterized in many different types of cell. One characteristic of the so-called A and L systems in cells such as the Ehrlich ascites tumour cell is a two-way overlap of specificities (Oxender & Christensen, 1963). The advantage of the pig colon and rabbit ileal preparations lies in the fact that glycine and/or serine appear to be completely excluded from participation in L-system type transport. This further example of microheterogeneity in carrier selectivity for different amino acids is particularly useful, since it allows one to analyse the glycine/serine inhibitable system in greater detail. This has already been carried out for the rabbit ileum, but the relative deficiency of the system in the pig colon limits detailed analysis. Why this system should be virtually absent is itself an interesting question which is currently being investigated. Up till now the only other tissue showing absence of transport for small neutral and basic amino acids is the capillary system of the mammalian brain (Yudilevich & Sepúlveda, 1976). The possibility that it is subject to induction and repression, as has been reported for other cell types, cannot be excluded.

The mechanism for transporting long-chain hydrophobic amino acids into newborn pig colonic mucosa has many of the characteristics of the L-system described originally for the Ehrlich cell (Oxender & Christensen, 1963). It has been suggested

that amino acids bind to this system through a three-point attachment, the α-carboxylic group, the α-amino group and the side chain (Oxender, 1965). The specificity of attachment of the side chain is said to be critical by Tager & Christensen (1971), but this view has been challenged recently by Matthews & Zand (1977), who suggest that an apolar bond formed between side chain and carrier has only loosely defined steric requirements. Transport of amino acids by pig colon through the glycine-resistant system (analogous to the L-system) increases with increasing chain length but the fluxes of tyrosine and phenylalanine are less than would be predicted were polarity the only characteristic which determined the rate of entry. Both these amino acids contain benzene rings and it may be that these are too bulky or too restricted in motion relative to the rest of the molecule for optimal bonding to occur with the putative carrier.

The presence of only one major transport system in the pig colon at birth leaves the question of its physiological significance unanswered. The system that is present is capable of transporting all the essential neutral amino acids and it may be of greater potential importance than the system capable of transporting glycine, serine, threonine and alanine (as well as the other neutral amino acids). On the other hand the uptake of basic amino acids is also relatively inefficient in the pig colon and these are also essential amino acids. The fact that young pigs are always at risk from lysine deficiency (see for instance McDonald, Edwards & Greenhalgh, 1973) might reflect additional inadequacies in an ability to absorb lysine, particularly if the pattern of transport seen in the colon were also to apply to the small intestine.

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