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AMINO ACID TRANSPORT IN THE GOLDFISH INTESTINE

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SUMMARY

1. The serosal transfer of the following eight amino acids: threonine, alanine, serine, histidine, valine, methionine, phenylalanine and leucine, was measured using everted sacs of anterior intestine taken from goldfish acclimatized to 8° C and incubated at 25° C.

2. All eight amino acids were actively transported and the serosal transfer correlated with the steady potential ($P < 0{\cdot}001$) and with the amino acid-evoked potential ($P < 0{\cdot}05$) measured on the same preparations.

3. The goldfish rectum actively transported alanine and the steady potential was raised when alanine bathed the mucosa of the everted preparation.

4. L-aspartic acid was partly transaminated to alanine by the goldfish anterior intestine; the rectum transaminated alanine to an unidentified amino acid which might have been serine, asparagine or glutamine or some mixture of these three.

5. It is suggested that L-amino acids increase the ease by which sodium enters the mucosal cell but that it is the rate at which this sodium is transported across the basal membrane which determines the net serosal transfer of amino acids.

INTRODUCTION

The pattern of nitrogenous excretion in the goldfish is by now well established (Smith, 1929; Maetz & Romeu, 1964) but there is still a total ignorance of mechanisms which control the absorption of nitrogen-containing substances from the intestine. Very little is in fact known about this aspect of absorption in any species of fish (Brown, 1957) and it is still not certain whether protein is absorbed after complete break-down to amino acids as suggested by Al-Hussaini (1949), or whether the final digestion of dipeptides occurs only within the mucosal cell (Schlottke, 1939). Amino acids can be transported actively across the intestine of the flounder, a marine teleost (Rout, Lin & Huang, 1965); this probably represents a physiological process, but it need not be incompatible with

intracellular proteolysis since, in the rat, both processes can be shown to take place in the same tissue (Newey & Smyth, 1962).

Work reported here was designed mainly to establish whether the goldfish intestine could actively transport amino acids. This was demonstrated, the fish intestine behaving in many ways like a mammalian intestine, but its ability to concentrate certain amino acids was found to be different from that reported previously for mammals. The possible significance of these differences for certain aspects of temperature acclimatization is discussed.

Goldfish intestine has been used previously to demonstrate the active transport of sodium and glucose (Smith, 1964*a*, *b*) and more recently to analyse the control which glucose exerts over the transport of sodium (Smith, 1966*a*, *b*). In the terminal ileum of the rabbit both glucose and amino acids change the net transport of sodium (Schultz & Zalusky, 1964, 1965). A further reason for the present work was therefore to test the prediction that amino acids would alter sodium transport in the goldfish intestine in the same way as glucose.

METHODS

Goldfish, weighing approximately 50 g, were obtained from Pet-Reks (Anglia) Ltd., Melbourn, Cambs. They were stored at room temperature in a large aquarium where the water was constantly aerated. Periodically, fish were transferred from this aquarium to a smaller one where the water was maintained at 8° C. They stayed in the cold water for at least 2 weeks and were then used for experiments as required. Fish were fed once each day up to the time of the experiment.

Fish were decapitated and the folds of the intestine separated in bicarbonate saline (Krebs & Henseleit, 1932) which was gassed at room temperature with 95 % O_2 + 5 % CO_2 and contained glucose (1 mg/ml.). Pieces of anterior intestine or rectum were everted and made into open-ended sacs as described previously (Smith, 1966*a*). The whole operation took less than 10 min. Bicarbonate saline (0·1 ml.) containing the amino acid under test in a concentration of 10 mM was added to the empty sac which was then placed in 15 ml. of fresh bicarbonate saline which also contained the amino acid in the same concentration. The sac was incubated at 25° C for a total of 125 min and 95 % O_2 + 5 % CO_2 bubbled through the medium bathing the mucosa.

During incubation the transmural potential was recorded with a Vibron electrometer through agar bridges containing 0·9 g/100 ml. NaCl. The output from the Vibron electrometer was taken to a 'Xactrol' pen recorder (Ether Ltd., Stevenage) to obtain a permanent record of changes in potential. The transmural potential rose steadily during the first 30 min of incubation and then became stable. This will be called the 'steady potential'. At some time during the remaining 95 min of incubation, the sac was transferred to fresh medium containing no amino acid. Five minutes later the sac was replaced in the original medium. Potentials were recorded throughout these manipulations. The immediate increase in potential which was seen when the sac was replaced in the amino acid-containing medium will be called the 'amino acid-evoked potential'.

At the end of incubation the contents of the sac were removed and the volume estimated by weight. The intestine was also weighed after blotting to remove excess fluid. Samples of sac contents were analysed for ninhydrin-positive material on an automatic amino acid

analyser of the type described by Spackman, Stein & Moore (1958). In some cases, where the samples contained traces of protein, a preliminary deproteinization with picric acid was carried out before analysis (Moore & Stein, 1954). The final results were expressed both as millimolar concentrations of amino acid found in serosal fluid collected at the end of incubation, and as net serosal transfer of amino acids/2 hr incubation/100 mg intestine used.

RESULTS

Control experiments. Before measuring the transport of amino acids across goldfish intestine it was first necessary to estimate the amount of ninhydrin-positive material coming from sacs incubated in bicarbonate saline containing no added amino acids. Everted sacs from goldfish anterior intestine and rectum, each containing 0·1 ml. bicarbonate saline, were incubated and samples taken for amino acid analysis 2 hr later. The amino acids found are shown in Table 1. The actual concentration in each

TABLE 1. Amino acids appearing in fluid bathing the serosa of everted sacs of goldfish anterior intestine and rectum. Sacs were incubated for 2 hr at 25° C in bicarbonate saline containing 1 mg/ml. glucose. Bicarbonate saline (0·1 ml.) was placed in the sac at the start of incubation. Each value is obtained from the pooled samples of six experiments

	Serosal appearance (μ-moles/100 mg tissue/2 hr)	
Amino acid	Anterior intestine	Rectum
Aspartic acid	0·01	Trace
Threonine	0·03	0·03
Serine + amides	0·09	0·12
Glutamate	0·03	0·01
Glycine	0·04	0·02
Alanine	0·12	0·07
Valine	0·06	0·03
Methionine	0·02	Trace
Iso-leucine	0·05	0·04
Leucine	0·11	0·06
Tyrosine	0·05	0·03
Phenylalanine	0·05	0·03
Lysine	0·08	0·03
Histidine	0·02	0·02
Arginine	0·06	0·04
Taurine	0·04	0·03
Ammonia	0·07	0·12

case depended on the individual amino acid and on whether anterior intestine or rectum was the tissue used. The amino acids might have been derived from blood present in the tissues at the start of incubation or some might have been synthesized during incubation.

In experiments where single amino acids were incubated with segments of intestine to determine the serosal transfer, the total transfer was corrected by subtraction of the corresponding mean control value. The method of analysis did not permit separation of serine from glutamine and asparagine, and the control subtracted from the total transfer of serine has

been calculated on the assumption that glutamine, asparagine and serine were present in equal amounts. The control values were in all cases small in comparison with those obtained with added amino acids, the highest (leucine) being about 10% of its own serosal transfer.

In addition to the common amino acids, ammonia and taurine were present in all the samples analysed.

Transport of L-*alanine by anterior intestine and rectum.* Different parts of mammalian intestine show different capacities for transporting L-amino acids (Wiseman, 1964). The transport of L-alanine by goldfish rectum and anterior intestine was studied to see whether this was true also for the goldfish. The transmural potentials, with or without L-alanine present, were also recorded and are shown in Fig. 1. Both the steady and

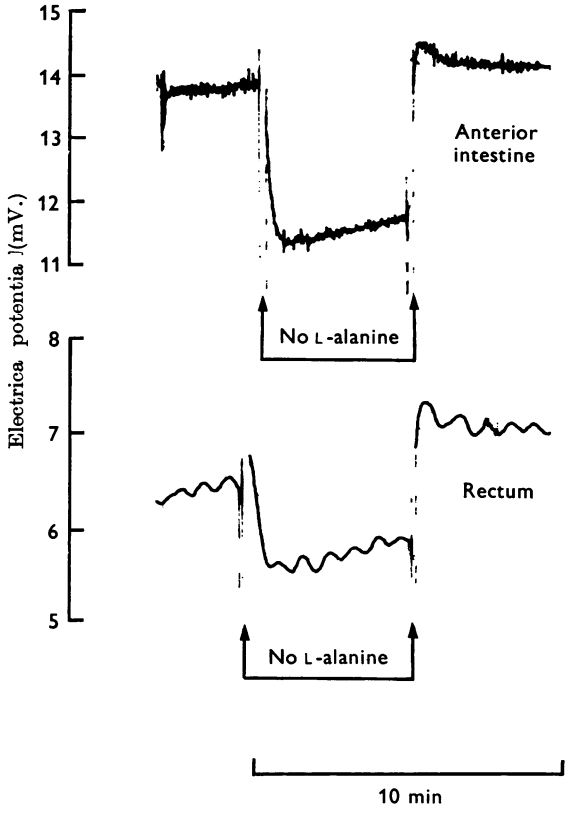

Fig. 1. Transmural potentials of everted goldfish anterior intestine and rectum recorded in the presence and absence of L-alanine. The everted preparations were incubated at 25° C in bicarbonate saline gassed with 95% O_2 + 5% CO_2. L-Alanine (10 mM) was present initially in both mucosal and serosal solutions (15 and 0·1 ml. respectively). During incubation the sac was transferred to medium with no alanine for a period of 5 min.

the L-alanine-evoked potentials were higher for the anterior intestine; so was the serosal transfer of L-alanine (3·32 μ-moles/100 mg/2 hr for the anterior intestine and 1·4 μ-moles/100 mg/2 hr for the rectum). At the end of incubation the serosal fluid collected from sacs of everted rectum showed an increased 'serine + asparagine + glutamine' peak on the chromatogram trace. However, even if this increase were due to transamination reactions involving alanine, the theoretical transfer for alanine would still be considerably less than that for the anterior intestine. All subsequent experiments were done with everted sacs of anterior intestine.

Serosal transfer of L-*amino acids by goldfish anterior intestine.* Eight amino acids were incubated individually with everted sacs of anterior intestine. The starting concentration of amino acid on both sides of the sac was 10 mM and conditions of incubation were as stated above. The corrected transfer values, together with the final concentrations of amino acids found within the sacs, are shown in Table 2. All were transported against their concentration gradients but at widely different rates. The steady potentials in the presence of amino acids, and the amino acid-evoked potentials, were also measured during each experiment and these are plotted against the serosal transfers of individual amino acids in Fig. 2. There was a positive correlation between the serosal transfer of amino acids and both the steady transmural potential ($P < 0{\cdot}001$) and the amino acid-evoked potential ($P < 0{\cdot}05$). The scale of electrical potential in Fig. 2*b* has been expanded for clarity, but the μ-moles of amino acid transferred/mV potential are in fact the same for both Fig. 2*a* and *b* ($t = 2{\cdot}38$, $n = 30$, $P < 0{\cdot}05$).

The mean steady potential at 25° C in the presence of glucose and absence of amino acids is 5 mV (Smith, 1964*a*). Subtraction of this value from the steady-state transmural potentials moves the curve drawn in Fig. 2*a* to the left making it approximately equal to the curve drawn in Fig. 2*b*, over the lower range of serosal transfers. At higher serosal transfers (e.g. alanine shown by an arrow in Fig. 2) there was a very high steady transmural potential compared with the evoked potential. We have at present no explanation for this discrepancy. It seems to indicate that an amino acid can change the steady potential in some way not affected by its removal from the mucosal surface of the intestine.

When L-aspartic acid was incubated under the same conditions the mean steady transmural potential was 4·4 mV and there was no aspartic acid-evoked potential. Aspartic acid was not transported against a concentration gradient.

Wiseman (1956) determined a series of concentration gradients for different amino acids using everted sacs of hamster intestine. Starting with the amino acid in equal concentration on both sides of the sac, the

serosal concentration was determined after incubation for 1 hr. His values are plotted against those for goldfish anterior intestine in Fig. 3. Aspartic acid has been included in the figure to show that it is not transported against a concentration gradient. This is true for both the rat intestine (Wiseman, 1953) and the goldfish intestine (see below). The concentration gradients developed by all eight amino acids which were

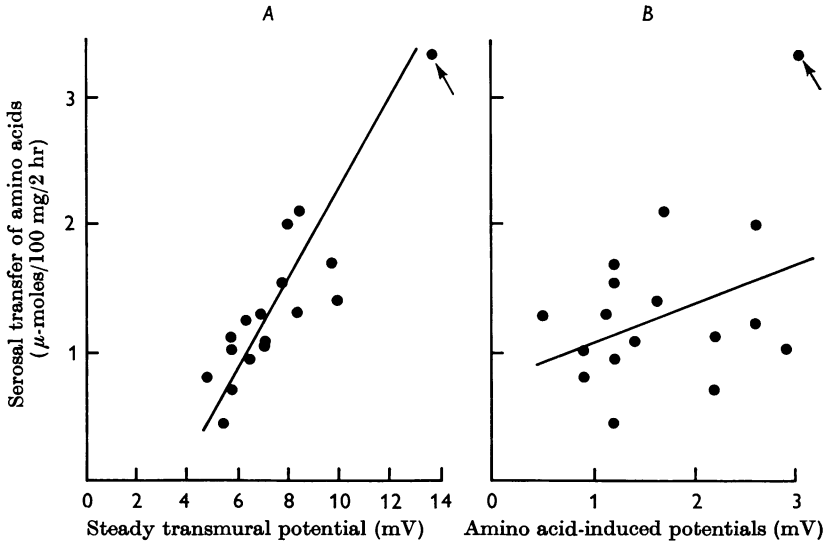

Fig. 2. Serosal transfers of eight different L-amino acids by goldfish anterior intestine plotted against both steady (*A*) and amino acid-evoked potentials (*B*), determined on the same preparations. Conditions of incubation are as stated in Fig. 1. The eight amino acids used were: threonine, alanine, serine, histidine, valine, methionine, phenylalanine and leucine; each amino acid was tested at least twice. Points show single or averaged results depending on the design of particular experiments. The arrows show one experiment with alanine referred to in the text.

TABLE 2. Mean serosal transfers of L-amino acids by goldfish anterior intestine. The initial amino acid concentration in mucosal and serosal fluids was 10 mM. The conditions of incubation were as stated in Table 1. The numbers in brackets show the number of experiments from which mean values were calculated

Amino acid	Serosal transfer (μ-moles/100 mg/ 2 hr)	Final serosal concentration (mM)
Alanine	2·4 (2)	25·8
Histidine	0·7 (3)	15·9
Leucine	1·0 (3)	19·9
Methionine	1·3 (3)	20·1
Phenylalanine	1·2 (2)	20·0
Serine	1·1 (2)	20·5
Threonine	1·8 (2)	26·9
Valine	1·6 (3)	24·9

actively transported were greater for the goldfish than for the hamster intestine and in addition there appeared to be at least two distinct groups of amino acids. Leucine, phenylalanine, methionine and valine were concentrated by goldfish intestine to nearly twice the values found for hamster intestine whereas histidine, serine, alanine and threonine were only concentrated by the goldfish intestine to about 1·2 times the values found for hamster. The time and temperature of incubation and the initial concentrations of amino acid used were different for the two series of

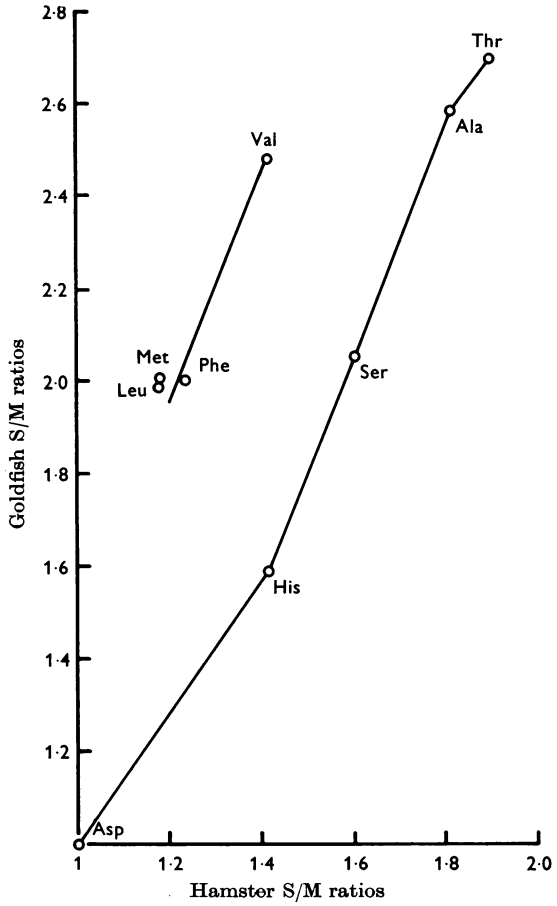

Fig. 3. Concentration gradients produced by goldfish and hamster intestines while absorbing L-amino acids. The S/M (serosal/mucosal) ratios for hamster intestine are taken from Wiseman (1956). The S/M ratio for aspartic acid has been taken from rat intestine (Wiseman, 1953) assuming no transport against a concentration gradient. Each S/M ratio for goldfish intestine is the mean result of two to three determinations. Conditions of incubation for goldfish intestines are as described in Fig. 1.

experiments but these differences were constant throughout. The variation found within the series is probably due to differences between the two species.

Transamination of L-*aspartic acid by goldfish anterior intestine.* Bicarbonate saline containing 10 mM L-aspartic acid was placed within everted sacs of goldfish anterior intestine and the sacs then incubated in the normal way in a large volume of bicarbonate saline also containing 10 mM aspartic acid. After incubation the medium within the sac was found to

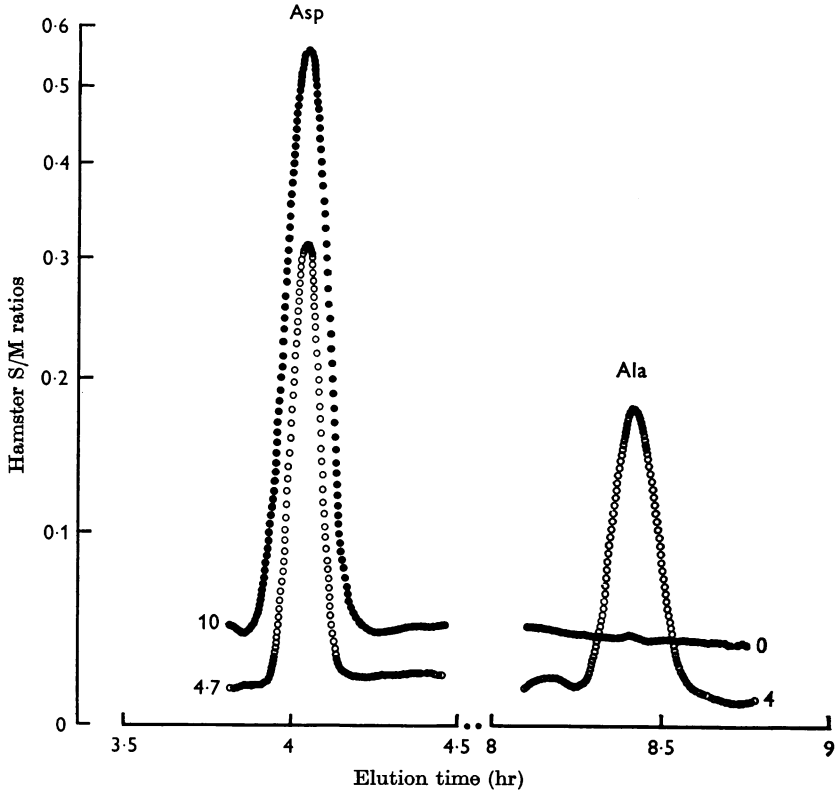

Fig. 4. Transamination of L-aspartic acid by goldfish anterior intestine. An everted sac of goldfish intestine was incubated in the presence of 10 mM L-aspartic acid as described in Fig. 1. The optical density of ninhydrin-positive colour from the automatic amino acid analyser is plotted against the time of elution. Asp: aspartic acid; Ala: alanine. Numbers show μ-molar concentrations of the two amino acids measured at the beginning ● and end ○ of incubation.

contain alanine in addition to a reduced amount of aspartic acid. One experiment is shown in Fig. 4. The concentration of aspartic acid was reduced from 10 to 4·7 mM and alanine appeared in a concentration of 4 mM. The quantities of amino acids present at the end of incubation,

corrected for the amounts of alanine and aspartic acid which come from sacs incubated in the absence of added amino acids, are shown in Table 3. If the amount of alanine which appeared is added to the amount of aspartic acid remaining, the sum becomes very nearly equal to the total aspartic acid present at the start of incubation. It could not be established whether any aspartic acid had itself been transferred across the intestine from mucosa to serosa during the time of incubation.

TABLE 3. Transamination of L-aspartic acid by goldfish anterior intestine. The sac weighed 116 mg and the conditions of incubation were the same as in Table 1

Amino acid	μ-moles added	μ-moles found	μ-moles from control intestine	μ-moles found corrected for control
Aspartic acid	1·0	0·6	0·01	0·59
Alanine	0	0·51	0·14	0·37
Total added	1·0	—	—	—
Total found	—	—	—	0·96

DISCUSSION

The intestines of goldfish and higher vertebrates transport and metabolize amino acids in very similar ways. Of the nine amino acids tested with everted sacs of goldfish intestine, eight were concentrated at the serosal surface and the ninth, aspartic acid, was transaminated to alanine. The same eight amino acids are transported actively by everted sacs of hamster intestine (Wiseman, 1956) and by many other mammalian intestines (see Wiseman, 1964), and the formation of alanine by transamination from aspartic acid has been described for both rat and dog intestine (Matthews & Wiseman, 1953; Neame & Wiseman, 1957). The goldfish rectum also actively transports alanine, but less readily than the anterior intestine, and similar quantitative differences exist along the intestines of higher vertebrates, though in these cases the particular area showing maximal absorption depends on the amino acids used and the species chosen for study (see Wiseman, 1964). Several amino acids increase the transmural potential of rabbit ileum (Schultz & Zalusky, 1965) and the same effect is seen when amino acids are brought into contact with the goldfish mucosa. It is apparent from these comparisons that transport mechanisms of higher vertebrates are fully operative in the fresh-water fish.

When the transport of eight amino acids by goldfish intestine was compared with that found for the hamster, valine, methionine, leucine and phenylalanine appeared to form a separate group, being concentrated at the serosal surface to a much greater extent that would have been predicted from the results of Wiseman (1956). This might be due to differences in the conditions of incubation but more probably it reflects a difference between the two species. It is possible that the differences are associated

with the ability of goldfish to acclimatize to different temperatures, a process which has been shown to be accompanied by changes in the levels of certain enzymes (Kanungo & Prosser, 1959; Hochachka, 1965). Some amino acids control specifically the synthesis of enzymes, for example, in poliovirus (Lwoff, 1965) and in HeLa cell cultures (DeMars, 1958) and valine, methionine, leucine and phenylalanine may perform a similar function in the acclimatizing goldfish. An analogy may exist here with *E. coli* where protein synthesis is thought to be regulated partly through amino acids brought into the bacterium by permeases which are present as normal constituents of the bacterial cell wall. These permeases are stereospecific and at least three are known to occur, one for phenylalanine, one for methionine and one for valine and leucine (Cohen & Monod, 1957). It may be more than a coincidence that these four amino acids are the ones readily transported by the goldfish intestine.

The formation of alanine by transamination from aspartic acid has been described above and it appeared that transamination also occurred in the rectum incubated with alanine where an increased 'serine + asparagine + glutamine' peak was obtained. Enzyme systems have been described which could catalyse the formation from alanine of any one of these amino acids (see Dixon & Webb, 1964).

The relation which the transport of sodium bears to the transport of amino acids and sugars in the intestine is a complicated one, capable of several different interpretations (Csáky, 1964; Schultz & Zalusky, 1964, 1965; Crane, 1964; Esposito, Faelli & Capraro, 1964; Barry, Dikstein, Matthews, Smyth & Wright, 1964). That both amino acids and sugars increase the net transport of sodium has been shown by direct measurements (Schultz & Zalusky, 1964) and by measurements of potential and short-circuit current (Clarkson, Cross & Toole, 1961; Schultz & Zalusky, 1965; Asano, 1964). It is also true for goldfish intestine (Smith, 1966*a* and this paper). The increase in sodium transport is thought by some to be caused by changes within the luminal membrane of the mucosal cell but this need not directly implicate sodium in the active transport of sugars and amino acids (Smith, 1966*a*) as has been suggested by Crane (1964) and by Schultz & Zalusky (1965).

Gilles-Baillien & Schoffeniels (1965) found the increase in steady potential across tortoise intestine, caused by glucose or alanine, to be situated at the basal membrane of the mucosal cell. It may be that the amino acid-evoked potential is initiated at the luminal membrane and subsequently replaced by a basal membrane potential. If this is so the correlation between the transfer of amino acids and both the amino acid-evoked potential and the steady potential is explained since the steady state potential would be largely composed of the transposed amino acid-

evoked potential. The replacement of a transient potential at an outer diffusion barrier by a steady potential across a transporting membrane is also thought to take place under special conditions in the frog skin (Kidder, Cereijido & Curran 1964).

There is reason therefore to think that it is the pumping of sodium across the basal membrane of a mucosal cell which controls the exit and therefore indirectly the entry of amino acids and sugars. The function of amino acids and sugars at the luminal membrane could be merely to increase the rate of entry of sodium, possibly by causing allosteric changes in a postulated carrier molecule (Smith, 1966*b*).

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