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EFFECTS OF AMINO ACIDS, DIPEPTIDES AND DISACCHARIDES ON THE ELECTRIC POTENTIAL ACROSS RAT SMALL INTESTINE

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SUMMARY

1. The effects of a number of amino acids, peptides and disaccharides on the potential difference across the wall of the rat small intestine have been studied.

2. All the L-amino acids tested, except lysine and arginine, and the three D-amino acids tested stimulate the potential when present in the mucosal fluid.

3. The concentration dependence and time course of the potential differs between different amino acids.

4. The results suggest a close correlation between the active transport of an amino acid and the potential change which it evokes.

5. Glycyl-glycine, glycyl-L-alanine and tri-glycine stimulate the potential and this appears to be due to the amino acids liberated by hydrolysis.

6. Maltose and sucrose, but not lactose, stimulate the potential, and the effect appears to depend on the extent of hydrolysis of the disaccharide.

7. The magnitude of the potential varies in different parts of the intestine. For the amino acids tested the maximum potential occurred in the distal ileum, while in the case of hexoses the maximum potential was found in the mid-intestine.

8. The results are discussed in relation to the mechanisms of transfer of hexoses and amino acids.

INTRODUCTION

It is now well established that actively transported hexoses and amino acids in the lumen of the gut, or on the mucosal side of an everted sac

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preparation of small intestine, are able to alter the transmural potential difference (p.d.). The studies on amino acids by Schachter & Britten (1961), Baillien & Schoffeniels (1962) and others has been limited to a few amino acids, and the present study was undertaken to investigate the effects of a wide range of amino acids on the electrical potential difference across the rat small intestine. Since dipeptides can be hydrolysed intracellularly (Newey & Smyth, 1962), it was of interest to examine the effects of glycyl-glycine, glycyl-alanine, and the tripeptide glycyl-glycyl-glycine on the potential. The effects of the commonly utilized disaccharides maltose, sucrose and lactose were also studied. A preliminary account of this work has been given by Kohn, Smyth & Wright (1966).

METHODS

White female rats (220–250 g) of the Sheffield strain were used and before experiment were maintained on an unrestricted diet of rat cubes (Diet 86: Oxoid, London). The techniques used followed closely those of Barry, Dikstein, Matthews, Smyth & Wright (1964). The rats were anaesthetized with pentobarbitone sodium and the combined jejunum and ileum was removed, everted and divided into five equal segments. In most experiments the middle fifth (sac III numbering from the proximal end) was used since it had been shown by Barry *et al.* (1964) to support the largest hexose potential. In some experiments other segments of intestine have been used, and these are designated sacs I, II, IV and V as in previous studies from this department. From the segment selected, two 6 cm sacs were prepared: where it is necessary to distinguish these, *a* and *b* denote the proximal and distal portions of the segment respectively. These 6 cm sacs were used to determine transmural p.d. as described by Barry *et al.* (1964). All experiments were performed in bicarbonate saline (Krebs & Henseleit, 1932).

The effects of various amino acids, peptides and disaccharides were studied by immersing the sac in bicarbonate saline (mucosal fluid), recording the potential and then replacing the mucosal fluid by bicarbonate saline containing the substance to be tested. All sacs were filled with 1 ml. bicarbonate saline (serosal fluid). Preliminary experiments (described in the Results section) showed that this procedure introduced a complication in that addition of the test substance caused an osmotic effect similar to that discussed by Smyth & Wright (1964, 1966), in addition to the specific effect for the particular substance. In most of our experiments this complication has been minimized by preceding the test solution with an equimolar solution of mannitol in bicarbonate saline.

In vivo experiments involved a similar procedure to that described by Barry *et al.* (1964). This utilizes a segment of intestine, with intact blood supply, through which test solutions can be circulated and across which p.d. can be determined.

The p.d. was measured using calomel half-cells connected to the mucosal and serosal solutions by Polythene tubing filled with M-KCl and 2 % agar. The half-cells were connected to a Vibron electrometer (Electronic Instruments Ltd., Richmond, Surrey).

Treatment of results. Following the procedure of Lyon & Crane (1966) the relationship between electric potential and concentration of amino acids has been studied by the application of methods formally similar to those of enzyme kinetics. In these the initial concentration (C) of amino acids in the mucosal fluid has been used, and the steady-state potential generated (P) is substituted for the rate of enzyme reaction. During the time taken to achieve a steady-state potential, a finite amount of substrate transport will occur. From data similar to that quoted in Fig. 10 the resulting change in mucosal substrate con-

centration can be estimated. In our experiments this appears usually to be less than 1% and has been ignored in subsequent calculations. The reciprocals of these quantities have been plotted according to the procedure of Lineweaver & Burk (1934), with $1/C$ as the abscissa and $1/P$ as the ordinate. In enzyme kinetics the intercept of the regression line on the abscissa is the negative reciprocal of the Michaelis constant and the intercept on the ordinate the reciprocal of the maximum velocity of reaction. The corresponding quantities obtained here are affinity constant (K) and the maximum potential (P_{max}). In describing the results the procedure is referred to as the Lineweaver–Burk plot and K and P_{max} are used with the above meanings. The selection of the straight line for the best fit of the experimental points offers some problems. Since the variance of $1/P$ is not independent of $1/C$ and since the values of $1/P$ are concentrated at low values of $1/C$, the calculation of a non-weighted regression line by the method of least squares would not be a valid procedure. In the circumstances a choice of the best fit by eye seems to be as satisfactory as any more complicated statistical device and accordingly has been used.

RESULTS

Preliminary experiments

In these experiments, the preparation was set up with bicarbonate saline as the mucosal fluid and the p.d. continuously monitored. When a steady value was achieved (usually after about 10 min) the mucosal fluid was drawn off and replaced by bicarbonate saline at 37° C to which the amino acid had been added. Figure 1 shows a typical experimental result, using glycine as the test amino acid. After a small discontinuity in the record due to the operation of changing the solutions, a small fall in p.d. was observed, a minimum being reached in about half a minute. This was followed by a fairly rapid rise to a new steady level. Experiments with a number of amino acids showed a similar initial fall in p.d.

Two amino acids, however, gave only the initial fall with no appreciable subsequent rise. These were lysine and arginine, both of which were used as the monohydrochloride. Figure 2 shows the effect of 15 mM lysine. Since lysine and arginine are known to be poorly transported by the intestine, these preliminary experiments suggested that the fall in potential caused by lysine or arginine had a similar origin to that caused by mannitol (Smyth & Wright, 1966) which is probably an osmotic effect. The results with arginine and lysine suggested that the initial fall in potential observed for the other amino acids might have a similar osmotic origin, and thus the observed potential is the sum of two components, an osmotic depression of p.d. and a p.d. rise associated with amino acid transfer.

In order to separate these components, all further investigations were carried out by the technique described in the Methods section in which the test substance replaces mannitol in the mucosal solution. In this way, no change in mucosal osmotic pressure is produced when the test substance is added. (Osmolarities have not been measured directly, and equimolar solutions have been assumed to be iso-osmotic.)

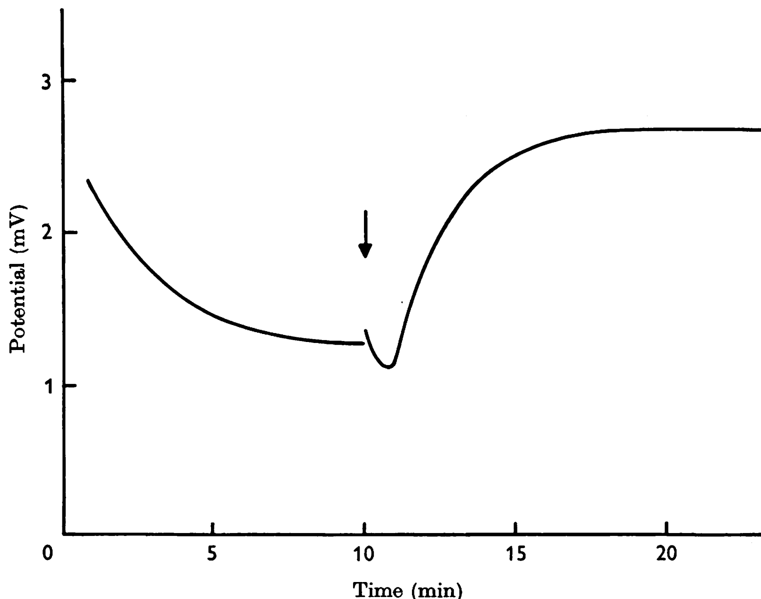

Fig. 1. Time course of the change in potential difference (p.d.) across the wall of an everted 6 cm sac of rat mid-intestine when 15 mM glycine is added to the mucosal solution. Time is given in minutes from setting up the preparation, and p.d. in millivolts. In all graphs positive values for the p.d. indicate that the serosal solution is positive with respect to the mucosal.

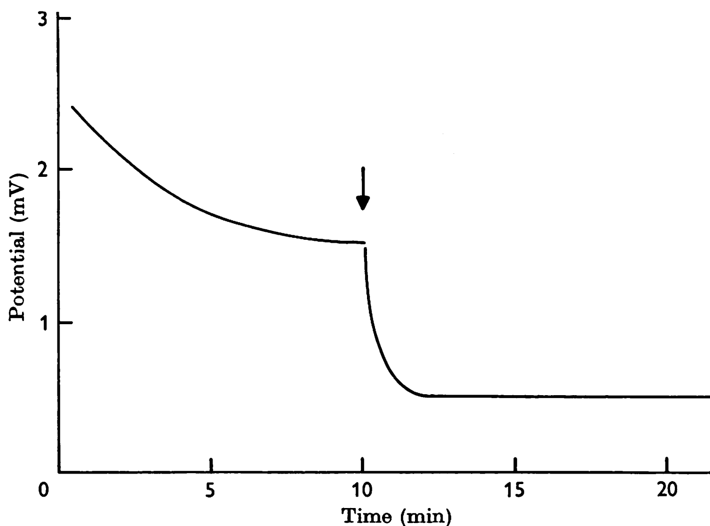

Fig. 2. Time course of the change in p.d. when 15 mM L-lysine monohydrochloride is added to the mucosal solution in an *in vitro* preparation of rat mid-intestine. Time in minutes, p.d. in millivolts.

Potential changes associated with amino acid transfer

By minimizing the osmotic effects, that part of the p.d. change characteristic of the particular test substance can be examined. The result with glycine is shown in Fig. 3 and comparison with Fig. 1 shows that the fall in potential during the first half minute has disappeared and a steady increase is obtained from the time of immersion of the sac in mucosal fluid containing glycine. It is therefore concluded that the initial fall in potential seen in Fig. 1 is of osmotic origin.

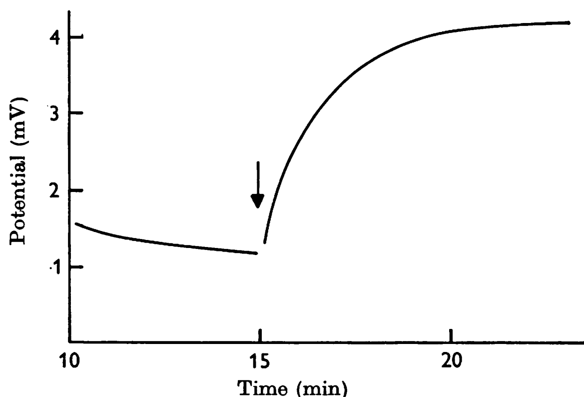

Fig. 3. Time course of the change in p.d. across the wall of the everted rat mid-intestine when 15 mM mannitol in Krebs bicarbonate saline is replaced by 15 mM solution of glycine. Time is given in minutes and p.d. in millivolts.

A study of a number of amino acids showed that there was considerable variation in both the magnitude of the potential and in the rate and pattern of development of the potential. It is not easy to separate these into different groups, but at the risk of over-simplification the following description attempts to make a broad classification. There is, first, a group of amino acids in which the potential rises asymptotically to a maximum value which is maintained for at least 15 min or longer. Within this group there is great variation in the rate of development. Some amino acids, e.g. glycine, alanine and valine, give responses similar to that shown in Fig. 3 in which the potential change approximates to an exponential with a half time of the order of 1–3 min. In other cases the rise is much more rapid and it is difficult to determine whether this is exponential or not. An example of this is leucine, which is illustrated in Fig. 4. A second group can be distinguished in which there are two more or less distinct phases in the rise in potential, the record showing a definite inflexion. A typical example is shown in Fig. 5 which was obtained with glutamine. This complex curve might well be due to the super-position of two separate

components, a rapid one and a slower one, and the amino acids might vary chiefly in the relative size of these. In the amino acids in group 1 it may be that two components are also present, but one component is much larger than the other thus giving the appearance of a smooth curve. A third group can be fairly clearly defined as amino acids which do not show

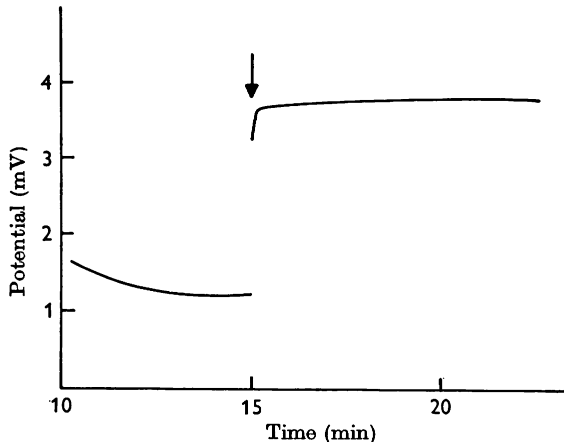

Fig. 4. Time course of the change in p.d. across the wall of the everted rat mid-intestine when 15 mM mannitol in Krebs bicarbonate saline is replaced by 15 mM solution of leucine. Time is given in minutes and p.d. in millivolts.

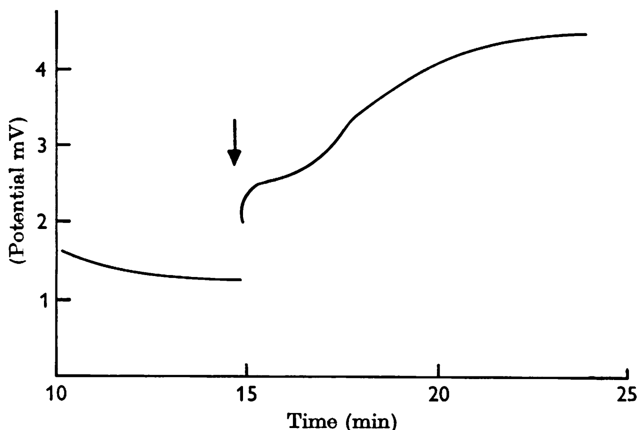

Fig. 5. Time course of the change in p.d. across the wall of the everted rat mid-intestine when 15 mM mannitol in Krebs bicarbonate saline is replaced by 15 mM solution of glutamine. Time is given in minutes and p.d. in millivolts.

any potential and these include arginine and lysine. A fourth group of amino acids shows a potential which is not maintained and rapidly falls off. Figure 6 (phenylalanine) and Fig. 7 (tryptophan) show good examples of this, and cysteine also falls into this group. This fall in p.d. suggests

a failure of the cell to maintain the potential generating mechanism. If the p.d. rise is associated with transfer or uptake of the amino acid, then a subsequent fall might indicate the achievement of equilibrium. Alternatively, it could represent the occurrence of some irreversible change in the cell such as depletion of energy sources or tissue damage. It was found that after exposure to tryptophan or phenylalanine, the ability of the preparation to respond to glycine or glucose is markedly impaired. It seems probable that irreversible changes are produced quite rapidly in the

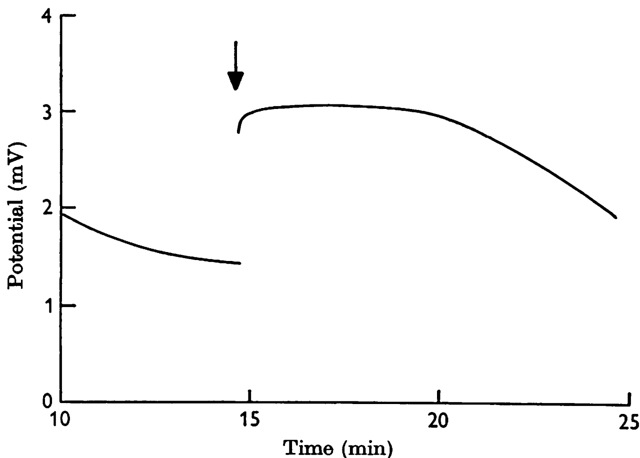

Fig. 6. Time course of the change in p.d. across the wall of the everted rat mid-intestine when 15 mM mannitol in Krebs bicarbonate saline is replaced by 15 mM solution of phenylalanine. Time is given in minutes and p.d. in millivolts.

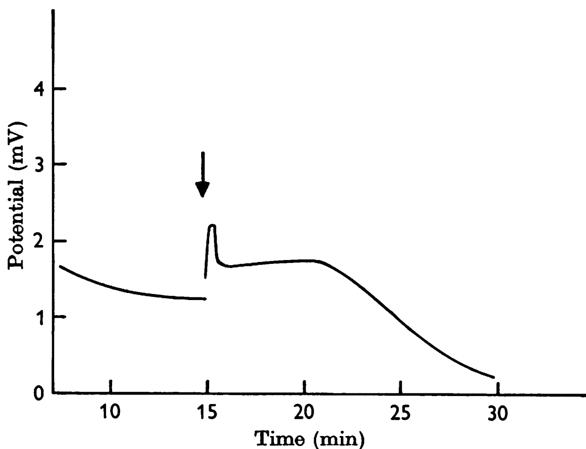

Fig. 7. Time course of the change in p.d. across the wall of the everted rat mid-intestine when 15 mM mannitol in Krebs bicarbonate saline is replaced by 15 mM solution of tryptophan. Time is given in minutes and p.d. in millivolts.

epithelial cells by these two amino acids. An attempt to summarize the different responses for the various amino acids is given in Table 1.

D-*amino acids*. Three amino acids (D-alanine, D-serine and D-leucine) have been examined briefly. In each case a significant rise in p.d. was observed, and for D-leucine this amounted to two thirds of the p.d. change

TABLE 1. Effects of 15 mM concentrations of amino acids on the p.d. across the wall of an everted preparation of rat mid-intestine. The values for p.d. represent means (±S.E.) for at least six sacs of the p.d. change when a 15 mM solution of mannitol in Krebs Ringer bathing the mucosal face of the sac is replaced by an equimolar solution of the amino acid. An indication of the time course for the p.d. change is also given

Amino acid	Potential difference mV	Time course of p.d. rise
Glycine	1·50 ± 0·20 (6)	Fairly close to exponential
L-Alanine	4·35 ± 0·25 (14)	Sometimes initial jump; subsequently exponential
L-Valine	2·75 ± 0·20 (9)	Simple exponential
L-Leucine	2·75 ± 0·20 (6)	Most of the p.d. change immediate; slight further rise
L-Isoleucine	1·90 ± 0·20 (6)	Most of the p.d. change immediate; slight further rise
L-Serine	2·25 ± 0·20 (6)	Marked inflexion; initial rise, not jump
L-Threonine	3·15 ± 0·45 (7)	Approximately exponential with small inflexion
L-β-Phenylalanine	1·70 ± 0·20 (7)	See Fig. 6
L-Tyrosine	2·35 ± 0·25 (6)	Similar to phenylalanine but better maintained
L-Tryptophan	0·85 ± 0·20 (6)	See Fig. 7
L-Cysteine	3·50 ± 0·30 (8)	Very rapid rise. Maximum poorly maintained
L-Methionine	1·75 ± 0·10 (6)	Initial rapid rise; second small exponential step
L-Proline	3·05 ± 0·15 (8)	Fairly rapid approximately exponential
L-Hydroxyproline	1·55 ± 0·20 (6)	Exponential with inflexion (slight)
L-Aspartic acid	1·45 ± 0·15 (6) }	Initial rapid rise followed by smaller second
L-Glutamic acid	1·86 ± 0·20 (6) }	rise (sometimes absent)
L-Glutamine	3·70 ± 0·40 (8)	Two distinct stages—similar to asparagine
L-Asparagine	2·20 ± 0·20 (6)	Two distinct stages, usually separated by minimum
L-Histidine	2·30 ± 0·20 (7)	Initial rapid rise; second smaller exponential step
L-Arginine	0·10 ± 0·10 (6)	No appreciable effect
L-Lysine	0·10 ± 0·15 (6)	No appreciable effect
D-Leucine	2·15 ± 0·16 (6)	Similar to L-leucine
D-Serine	0·42 ± 0·05 (6)	Most of the rise is a rapid exponential
D-Alanine	1·30 ± 0·25 (10)	Similar to L-alanine

for the same concentration (15 mM) of L-leucine. Although slight contamination by L-isomers cannot be ruled out, the result for leucine suggests strongly that D-amino acids may also stimulate transmural p.d. in the intestine.

Magnitude of the p.d. changes with different amino acids. Most of the soluble, naturally occurring amino acids have been tested at a concentration of 15 mM. Exceptions are tyrosine which was used in a saturated solution (about 5 mM) and cystine which was not examined since its solubility is known to be very low. The results, which are also included in

Table 1, show a considerable variation in the magnitude of the response to various amino acids at this concentration, although in every case the effect was considerably smaller than that for glucose or galactose.

Relationship between amino acid concentration and evoked p.d. changes. It seemed possible that the variation in the p.d. response observed might be partly due to a difference in the degree of saturation of the potential generating mechanism. To test this the concentration dependence has been studied for five of the amino acids, viz. glycine, L-alanine, L-methionine, L-histidine and L-asparagine, which represent examples from all the differing types of p.d. response observed. For each amino acid, the general time course of the p.d. change was independent of the concentration, but the maximum p.d. showed a marked dependence.

The results are shown in Figs. 8*a* and 9*a*, each point being the mean of six estimates of p.d. using sacs from three different animals. The standard errors, which in every case were less than ($\pm$ 0·45 mV) and usually less than ($\pm$ 0·25 mV), have been omitted to simplify the figure. For each of the amino acids the potential generating mechanism is saturable but the concentration dependence is very variable. For example, methionine evokes, at low concentrations, a greater p.d. than the other amino acids, but at high concentrations, a smaller p.d. than the others. The Lineweaver–Burk plots for these amino acids are shown in Figs. 8*b* and 9*b*. Glycine, alanine and histidine all have intercepts with the ordinate not very different from each other, indicating similar values for P_{max} while those for asparagine and methionine are very different. All five amino acids have very different intercepts on the abscissa, indicating different K values. The curves for methionine and glycine resemble the relation between transfer and concentration so closely that it is worth while plotting these together. The values for transfer are taken from Dawson (1965) and are plotted with the p.d. values in Fig. 10.

These two sets of data, i.e. relation of transfer and of potential to concentration, clearly show a very close resemblance, suggesting a causal connexion between potential and transfer. Lineweaver–Burk plots were also made of transfer and potential in relation to concentration and the regression lines had very similar intercepts on the abscissa confirming a similar concentration dependence for the two processes.

Effects of glycyl-glycine, triglycine and glycyl-alanine

The effects of the two dipeptides (glycyl-glycine and glycyl-alanine) and the one tripeptide (triglycine) were initially examined using 15 mM concentrations. All three substances had an effect on p.d. very similar to that for alanine or glycine, i.e. rising asymptotically to a well-maintained maximum, although sometimes an inflexion was seen. Typical results are shown in

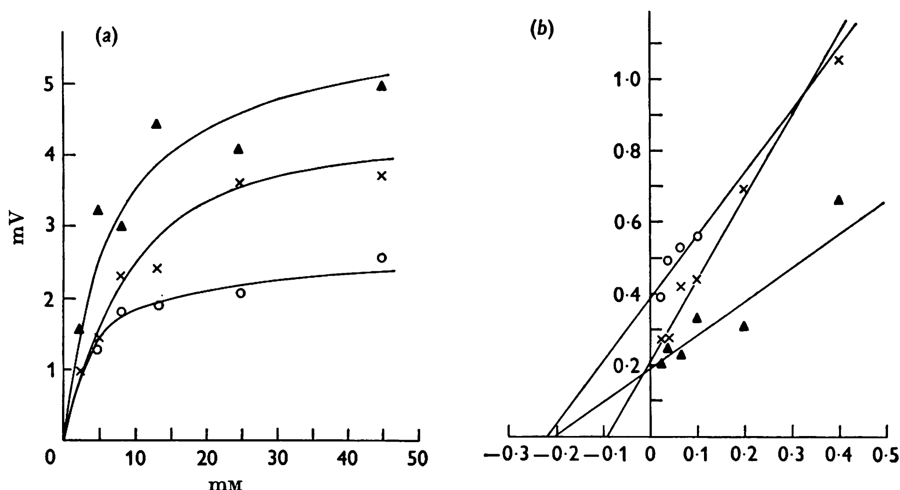

Fig. 8. (*a*) Concentration dependence of the p.d.s generated by alanine (▲), histidine (×) and asparagine (○) in the everted mid-intestine of the rat. The p.d. given is the change produced by replacing a given concentration of mannitol in the serosal solution by the same concentration of amino acid. Amino acid concentration is given as millimolar, p.d. in millivolts.

(*b*) Lineweaver–Burk plot of the same data. The reciprocal of the steady-state p.d. change is plotted on the ordinate against the reciprocal of the amino acid concentration.

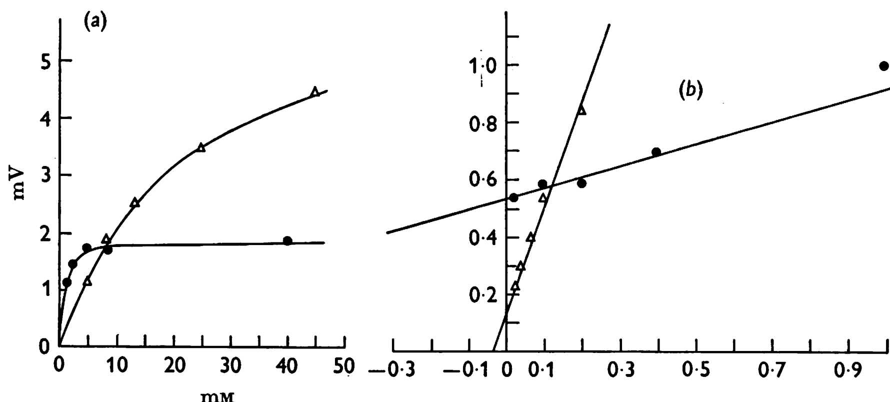

Fig. 9. (*a*) Concentration dependence of the p.d.s generated by glycine (△) and methionine (●) in the everted mid-intestine of the rat. The p.d. given is the change produced by replacing a given concentration of mannitol in the serosal solution by the same concentration of amino acid. Amino acid concentration is given as millimolar, p.d. in millivolts.

(*b*) Lineweaver–Burk plot of the same data. The reciprocal of the steady-state p.d. change is plotted on the ordinate against the reciprocal of the amino acid concentration.

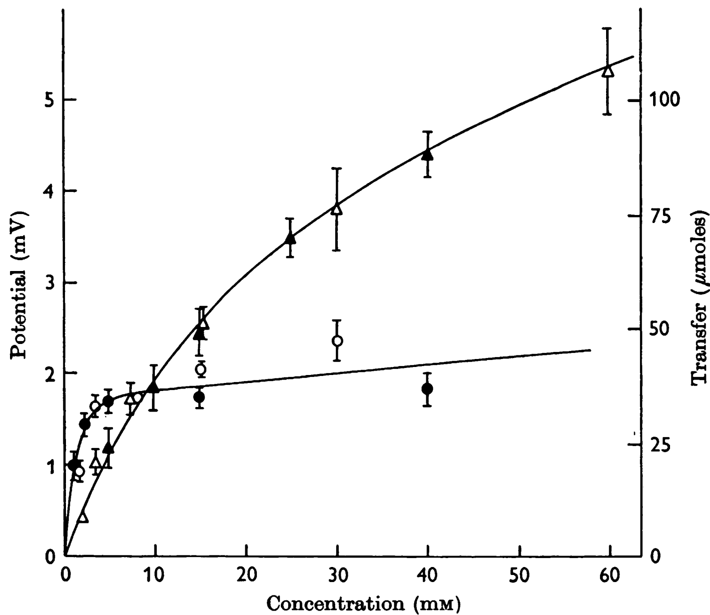

Fig. 10. Comparison between the concentration dependence of transfer and change in transmural p.d. for methionine and for glycine in everted sacs of rat mid-intestine. The p.d. changes for glycine (▲) and methionine (●) are the same as in Fig. 8. The data for transfer from Dawson (1965) for glycine (△) and methionine (○) are given as μmoles amino acid recoverable from gut wall and serosal fluid after 30 min. P.d. is in millivolts and concentration as millimolar.

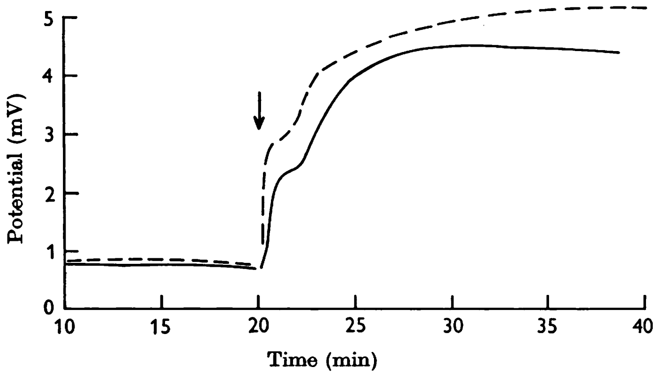

Fig. 11. Time course of the change in transmural p.d. when 15 mM mannitol in the mucosal solution is replaced by 15 mM glycyl-glycine (continuous line) or 15 mM triglycine (dashed line). The test substance added at the point indicated by the arrow. Time is in minutes and p.d. in millivolts.

Fig. 11. The maximum p.d. changes given by the 15 mM concentrations are included in Table 2.

The effects of concentrations have also been studied for these peptides and the results obtained are shown in Table 2. The data for potential and concentration has also been expressed in a Lineweaver–Burk plot in Fig. 12.

TABLE 2. Potential difference changes produced by varying concentrations of peptides when replacing equimolar mannitol solutions in the mucosal fluid. Results as mean ± S.E.

Concentration (mM)	Glycyl-glycine (mV)	Triglycine (mV)	Glycyl-alanine (mV)
0·5	0·25 ± 0·15 (6)	—	—
1	0·60 ± 0·15 (6)	0·80 ± 0·15 (7)	0·90 ± 0·15 (8)
2·5	1·65 ± 0·15 (9)	1·50 ± 0·15 (6)	2·45 ± 0·25 (6)
5	2·45 ± 0·20 (10)	2·30 ± 0·20 (6)	3·40 ± 0·30 (6)
7·5	—	—	3·95 ± 0·20 (6)
10	3·55 ± 0·20 (8)	3·10 ± 0·30 (6)	—
15	3·85 ± 0·20 (6)	5·30 ± 0·30 (6)	5·40 ± 0·25 (6)
20	4·50 ± 0·15 (6)	—	—
25	—	5·00 ± 0·35 (6)	—
40	5·45 ± 0·20 (6)	—	—

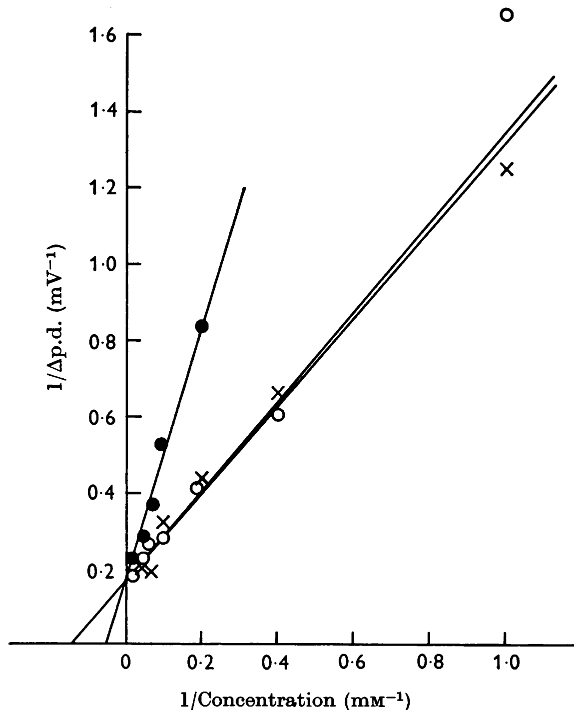

Fig. 12. Lineweaver–Burk plot to compare the effects of glycine (●), glycyl-glycine (○) and triglycine (×) on the transmural p.d. across the everted rat intestine. Ordinate is the reciprocal of change in p.d. $(mV)^{-1}$ and abscissa is the reciprocal of concentration $(mM)^{-1}$.

There is no significant difference between the *K* values for glycyl-glycine and triglycine, but both of these differ from glycine. The three regression lines intersect the ordinate at approximately the same point.

Variation in potential along the gut

Barry *et al.* (1964) studied the variation in hexose stimulated p.d. in the small intestine and showed that the maximum effect for glucose occurs in the mid-intestine. In the present experiments, a study was made of the potential changes caused by glycine, glucose and mannitol in ten different

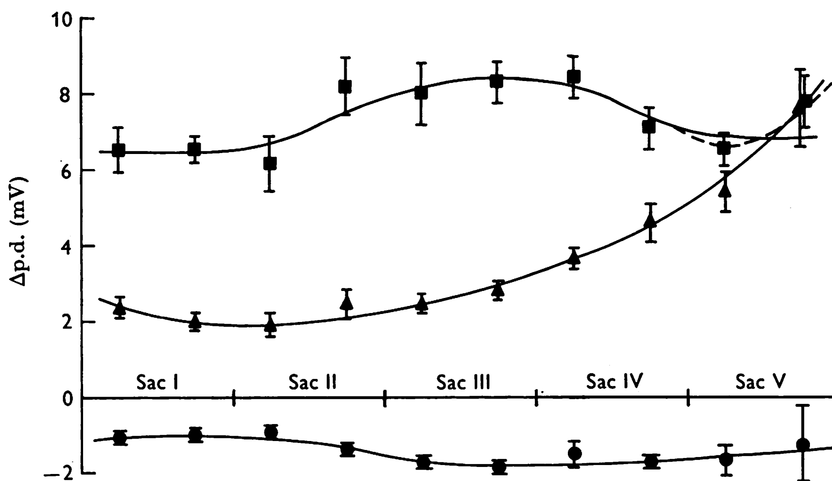

Fig. 13. P.d. changes caused by 25 mM mucosal solutions of glucose (■), glycine (▲), or mannitol (●). The value for mannitol represents the p.d. change when mannitol is added to the mucosal fluid. The other figures represent the p.d. changes when the mannitol solution is replaced by an equimolar glucose or glycine solution. All values are plotted as (±1 S.E.). P.d. changes are in millivolts.

regions of the intestine, i.e. two sacs from each of segments I to V. In all cases, 25 mM concentrations were used and the results are shown graphically in Fig. 13. As the object is to compare the changes along the intestine of the potential generated by the amino acid and hexose transfer mechanism, and also the osmotic potential elicited by mannitol, the three curves have not been obtained in quite the same way. The values for hexose and amino acids show the difference in each case between the value in the presence of hexose and amino acid and the value in the presence of mannitol, whereas the osmotic potential shows the value in the presence of mannitol only.

There is an obvious difference between the effects of glycine and glucose. In confirmation of findings of Barry *et al.* (1964), the maximum p.d. change for glucose occurs in the mid-intestine whereas glycine exerts its maximum effect in the lower ileum. All three potentials were much more variable and

less stable in sac V than in other parts of the intestine and this is emphasized by the larger standard errors, again confirming the findings of Barry *et al.* (1964).

In vivo studies

It might appear that the everted sac is a highly unphysiological preparation. Smyth (1963, 1964) has discussed reasons for thinking this is not the case, and in the work of Barry *et al.* (1964) the p.d. changes associated with hexose transfer were similar in an *in vivo* preparation of rat small intestine to those in the everted sac.

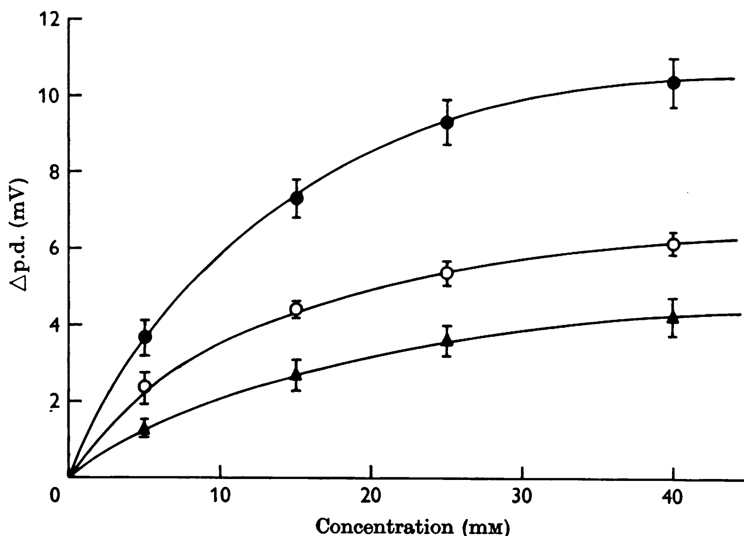

Fig. 14. Effect of various concentrations of L-alanine on the transmural p.d. in rat small intestine *in vivo*. The regions correspond to sac III (▲), sac IV (○) and sac V (●) of the *in vitro* preparation. Each point represents the mean (± S.E.) of data from four animals.

By use of similar *in vivo* preparations a number of amino acids have been tested and good qualitative agreement has been obtained with the results from the everted sac. In addition, a more detailed study of the effects of L-alanine *in vivo* has been made for segments of the intestine corresponding to sacs III–V. The results obtained have been plotted in Fig. 14. Each point represents the mean of four different segments from four animals.

These results confirm the *in vitro* findings (*a*) that the potential increases with increasing concentrations of amino acid up to a maximum value, i.e. it shows saturation kinetics, and (*b*) that the distal end of the small intestine shows a higher potential than the middle at all concentrations of alanine tested. The *in vivo* results have also been treated in a similar

way to the *in vitro* ones, Lineweaver–Burk plots having been made. The results are shown in Fig. 15. It will be seen that the three regression lines (for sacs III, IV and V) have the same intercept on the abscissa, indicating a value for K of about 14 mM. This intercept is, however, significantly different from that in the *in vitro* plot for sac III, which gives a K value of about 7 mM.

Effect of disaccharides on the transmural p.d. The effects of maltose, sucrose and lactose have been examined using the same approach as that adopted for the amino acids and dipeptides. Table 3 shows the effects of

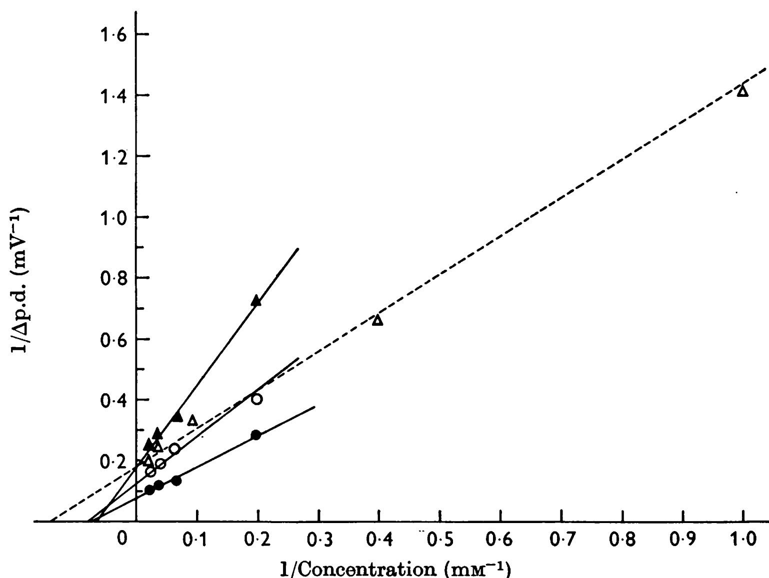

Fig. 15. Lineweaver–Burk plot of the data in Fig. 14, together with the data for L-alanine from Fig. 9. The effects of L-alanine *in vivo* in segments corresponding to sac III (▲), sac IV (○) and sac V (●) are compared with data for sac III (△) from the *in vitro* preparation.

each of these disaccharides on the transmural p.d. together with results for glucose and Fig. 16 shows the time course of the development of the potential. It is evident that there is a great difference between the effects of the three disaccharides. The effect of maltose is very similar to that of glucose, both as regards concentration dependence and time course. The p.d. change for sucrose at low concentrations is much smaller than that for isomolar glucose, but the difference decreases as concentrations are increased. The rate of p.d. rise is also much slower with sucrose than with maltose. With lactose no potential is observed. These results are all explicable in the view that the potential is due to glucose liberated from the disaccharide, and the difference between the three is due to different

rates of hydrolysis. Even allowing for the fact that each molecule of sucrose liberates only half the amount of glucose compared with a molecule of maltose, the hydrolysis of sucrose must proceed at a much slower rate. The hydrolysis of lactose is negligible at least as indicated by the lack of an evoked potential except, possibly, at the highest concentrations tested.

Additive effects of amino acids and hexoses. The different distribution of potential generating capacity for glucose and glycine along the intestine

TABLE 3. The effects of disaccharides on the transmural p.d. in everted rat mid-intestine when replacing equimolar mannitol in the mucosal solution. Figures for glucose are given for comparison. Results as mean ± S.E.

	Potential differences			
Concn. (mM)	Maltose (mV)	Sucrose (mV)	Lactose (mV)	Glucose (mV)
2·5	5·40 ± 0·50 (8)	0·75 ± 0·10 (6)	−0·05 ± 0·10 (6)	4·45 ± 0·40 (12)
5	7·00 ± 0·30 (7)	1·65 ± 0·20 (6)	−0·05 ± 0·15 (6)	6·15 ± 0·75 (6)
10	7·20 ± 0·45 (6)	3·20 ± 0·25 (6)	−0·05 ± 0·20 (6)	7·55 ± 0·40 (12)
15	8·40 ± 0·45 (7)	4·60 ± 0·30 (7)	0·00 ± 0·20 (6)	8·30 ± 0·20 (6)
25	8·15 ± 0·45 (7)	6·70 ± 0·20 (6)	0·25 ± 0·15 (6)	8·60 ± 0·35 (9)
50	9·00 ± 0·35 (6)	8·10 ± 0·20 (6)	0·85 ± 0·25 (7)	9·65 ± 0·35 (8)

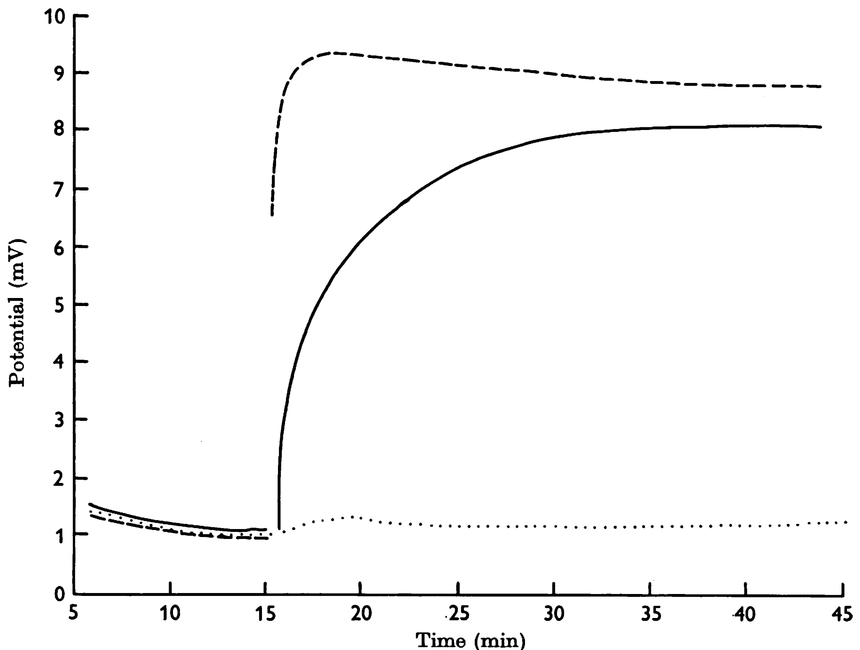

Fig. 16. Time course of the p.d. changes when 25 mM mannitol is replaced by 25 mM maltose (dashed line), sucrose (continuous line) or lactose (dotted line) in an *in vitro* preparation of everted rat mid-intestine. The results are taken from three separate experiments using different animals but under otherwise similar conditions. P.d. is in millivolts and time in minutes.

suggests that they do not share the same mechanism. This point was further examined by testing the additive effects of glucose and glycine in sac III by the following procedure. The sac of intestine was immersed in a mucosal solution of 40 mM mannitol and the potential measured (*a* mV); this was replaced successively by the following solutions and the potential recorded for each: 25 mM mannitol + 15 mM glucose (*b* mV); 25 mM glycine + 15 mM glucose (*c* mV); and 15 mM mannitol + 25 mM glycine (*d* mV). The potential due to 15 mM glucose is *b–a*; the potential due to

TABLE 4. Additive effects of 25 mM solutions of test substances added to 15 mM glucose (by replacement of 25 mM mannitol). Results are given as mean ± S.E. Except where indicated, observations on 6 sacs

Test substance	Potential difference change on addition to 15 mM glucose (mV)	Potential difference change with test substance only (mV)
Glucose	1·00 ± 0·20	8·90 ± 0·75
Galactose	1·20 ± 0·25	7·65 ± 0·40
Glycine	1·85 ± 0·20	2·50 ± 0·15 (7)
Glycyl glycine	3·60 ± 0·15	5·30 ± 0·35

25 mM glycine is *d–a*; the additive effect of 25 mM glycine over and above the potential due to 15 mM glucose is *c–(b–a)*. The procedure was repeated substituting galactose and glycyl-glycine for glycine, and finally for a control sac in which glucose was substituted for glycine. These results give the capacity of 25 mM of the test solution in the presence of 15 mM glucose to increase the potential above that due to 15 mM glucose alone. The results are shown in Table 4, in which 15 mM glucose gives nearly the maximum potential, this being increased by 1·0 mV when an additional 25 mM glucose was added. A very similar effect was produced by adding 25 mM galactose suggesting that glucose and galactose share a common potential generating mechanism. In contrast, glycine, which in 25 mM solution only causes a 2·5 mV change in contrast to the 8·9 mV caused by glucose, when added to 15 mM glucose causes a significantly larger increase than does glucose. This is even more evident with glycyl-glycine. These results suggest that the potential generating mechanism for glycine is different from that for glucose and galactose.

DISCUSSION

The results confirm the work of Baillien & Schoffeniels (1962) and of Zalusky & Schultz (1963) in showing that amino acids as well as hexoses can increase the potential across the wall of the small intestine. They extend the findings to another species, the rat, and also to many more amino acids as well as certain peptides. One very obvious difference

between the potentials evoked by amino acids and those by hexoses in the rat does not appear to have been noted in the tortoise or rabbit, i.e. the much slower rate of onset for many of the amino acids. In general, the hexose potential has acquired at least two thirds of its maximum value within 1 sec whereas the amino acid potential frequently continues to rise for several minutes. The slower development of the amino acid potential reveals that the potential obtained consists of two components: (1) an osmotic component (Smyth & Wright (1964) have shown that increase in mucosal osmotic pressure decreases the transmural potential), and (2) a component which is presumed to be associated with the transfer of the amino acid. Lyon & Crane (1966) have also suggested that there are two similar components in the hexose potential. In general, however, the osmotic component has not been taken into consideration in previous studies, and this may be of considerable importance for substances producing comparatively small changes in potential. The technique described reduces the osmotic component very considerably, but to eliminate it completely, determination of two additional parameters would be required, namely, the osmotic coefficient of the test substance and the reflexion coefficient of the gut wall to that substance.

It is clear from Table 1 that there is a considerable variation of results for the different amino acids. Some amino acids resemble the hexoses in giving the major part of their effect within a few seconds, whilst for others the potential continues to rise over several minutes. The reproducibility of the response for a given amino acid makes it improbable that the differences represent artifacts resulting from the technique. We have made an attempt to divide the amino acids into groups but would regard this as very tentative. Perhaps the most useful hypothesis is that the electrical response of the intestine to amino acids consists of two components (quite distinct from the osmotic one) a rapid and a slow one, and the shape of the curve in each case will depend on the relevant contributions of these two components. This could explain why some curves are inflected and others are not.

In the case of hexoses it is well established that the potential is given only by hexoses which can be moved against a concentration gradient. Does this apply to the amino acids? Of the amino acids found here to produce a potential, the following were found by Wiseman (1951, 1953, 1956) to show movement against a concentration gradient, L-alanine, L-valine, L-leucine, L-isoleucine, L-serine, L-threonine, L-β-phenylalanine, glycine, L-methionine, L-proline, L-hydroxyproline and L-histidine. Tyrosine has been shown to move against a gradient by Lin & Wilson (1960) and tryptophan by Spencer & Samiy (1960). Amino acids found to cause a potential which have not been shown to be moved against a gradient

include aspartic and glutamic acids and the D-amino acids, D-serine and D-leucine. However, it was shown by Matthews & Wiseman (1953) and Neame & Wiseman (1957) that aspartic and glutamic acids were transaminated with production of alanine and either asparagine or glutamine and these could well be the cause of the potential. As regards the D-amino acids, although leucine and serine have not been shown to move against a concentration gradient it does not follow that they do not use a transfer mechanism. Newey & Smyth (1967) have discussed the simultaneous transfer of solute and solvent and pointed out that simultaneous movement of fluid by an independent mechanism can mask the change in concentration which might be expected from active transfer of solute. Furthermore, there is evidence that at least some D-amino acids are actively moved, e.g. D-methionine (Jervis & Smyth, 1959, 1960), D-alanine (Field, Schultz & Curran, 1967). These results with the dicarboxylic amino acids and D-amino acids would agree with the concept that the electrical potential is related to the active transfer of amino acids.

The amino acids found not to cause a potential were the dibasic acids, L-arginine and L-lysine. These amino acids were found by Wiseman not to be moved against a concentration gradient. A later report by Hagihira, Lin, Samiy & Wilson (1961) showed that from very small concentrations there might be some movement against a gradient. Our results are in keeping with the views of Wiseman that the dibasic amino acids do not use a special transport mechanism. They differ from those of Zalusky & Schultz (1963) who found that lysine caused a potential in rabbit ileum. However, this may be the result of a species or regional difference. In general we feel justified in drawing the conclusion that amino acids which use a special transport mechanism generate an electrical potential.

Our results demonstrate that the magnitude of the potential varies for different amino acids and is concentration dependent. In every case there is evidence that the potential generating process is saturable, and the analogy of enzyme kinetics appears to be a valid approach. In applying enzyme kinetics Lyon & Crane (1966) used the plot of Lineweaver & Burk (1934) in which p.d. was substituted for reaction velocity. If, as seems probable, the p.d. reflects changes in the rate of an ion pump, the Lineweaver–Burk plot expresses the dependence of this pump on extracellular amino acid concentration. However, the arguments used merely imply a potential generating site without specifying the nature of this site, and from the Lineweaver–Burk plot we derive a maximum potential (P_{max}) and a constant K characterizing the affinity of the amino acid for the potential generating mechanism. (K is defined as the concentration of amino acid in the mucosal fluid which will generate half the maximum potential.) The actual generating site lies at an unknown region of the cell

and presumably the magnitude of the potential depends on the concentration of the amino acid at that site. K must therefore be controlled by two factors, (*a*) one which expresses the affinity of the mechanism for the amino acid present at the site, (*b*) one which expresses the accessibility of the site to amino acid initially present in the mucosal fluid. Variations in K obtained experimentally could therefore be due to differences in either affinity or accessibility. P_{max} is the maximum potential obtained and therefore is a measure of the ion pumping capacity activated by the amino acid. Different amino acids using the same transport system could have different affinities but could produce the same P_{max}. If the P_{max} varies it must mean that either the tissue resistance is altered or a different number of sites are being used. (In the present work tissue resistance has not been measured and will not be considered further.) A different number of sites in use could occur in two ways. The same mechanism could exist in different parts of the intestine or in different species, but the number of active sites could vary. If, however, the maximum potential differs in the same part of the intestine in one species for two amino acids it must mean that they use different sites and this is another way of saying that they use another mechanism.

With the above considerations in mind, the following conclusions can be drawn from the various Lineweaver–Burk plots described in the experimental section. Figures 8*a* and 9*a* show plots of five different amino acids and it is seen that L-alanine, glycine and histidine have a similar intercept on the ordinate indicating a similar P_{max}. This suggests that they use the same transfer mechanism. In contrast, methionine and asparagine have a different P_{max} suggesting that a different mechanism is involved. This is in keeping with the suggestion of Newey & Smyth (1964) that glycine uses a mechanism which is not available to methionine.

When the same amino acid (alanine) is studied in different parts of the intestine K remains constant while P_{max} changes. This could be due to different numbers of transporting sites in different parts of the intestine, but all with the same affinity, or to differences in tissue resistance. In comparing the same amino acid in the same part of the intestine in *in vitro* and *in vivo* conditions, P_{max} remains constant but K changes. This suggests that there is a change in the accessibility of the site in these conditions. On eversion of the intestine, the villi tend to stand out and be separated, the mixing is more efficient and it is not unreasonable to think that this increases accessibility and hence the apparent affinity. K also differs in experiments where glycine or its peptides are used. The results suggest that the sites are more accessible to glycine when it enters as a peptide. This is in keeping with the views of Newey & Smyth (1962) who showed that glycine and glycyl-glycine enter the intestine at the same rate

but intracellular hydrolysis of the dipeptide gives a greater concentration of glycine in the intestine.

The fundamental question is the precise cause of the potential and although the experiments do not solve this problem they offer some information relevant to it. The first detailed hypothesis was that of Schultz & Zalusky (1964) and it is useful to see how the present findings fit in with this. Briefly, their scheme requires a sodium pump at the serosal pole of the cell which removes sodium from the cell and hence maintains a lower intracellular sodium concentration. As a result of this sodium enters the cell from the intestinal lumen on a ternary carrier, which can also transport amino acid. Thus, the movement of sodium down its concentration gradient enables amino acids to move against their gradient. Schultz & Zalusky believe in fact that the mechanism is similar to that put forward by Crane (1962) for glucose. They further believe that the sodium pump is the cause of the potential. If this view were correct it would be expected that potential and transfer would be related, and in fact the present results show that this is the case. Figure 10 shows the transfers and potentials of glycine and methionine at different concentrations. These results therefore fit in with the Schultz–Zalusky model.

What is more difficult to reconcile with this scheme is the time relations of the potential which are very different from those with hexoses. The hexose potential reaches a maximum within seconds, while the amino acid potential may require minutes. If we elaborate the Schultz–Zalusky model further to attempt to explain this, we must assume that the electrogenic pump at the serosal border of the cell removing sodium from the cell can only maintain a certain sodium gradient. If sodium is entering the cell slowly the gradient will be reached at a much lower level of activity of the pump. In the presence of glucose or amino acid more sodium can enter the cell on the ternary carrier and hence the pump removes more sodium while maintaining the same gradient, and a higher electrical potential will be caused. The time taken to achieve the maximum potential will be that required to reach a new steady state and in the presence of amino acid this time must be greater than in the presence of glucose. The only possible mechanism would appear to be a slower movement of Na on the ternary carrier with amino acids than with glucose.

Other possibilities should perhaps be considered to explain the different time relations of hexoses and amino acids. Newey & Smyth (1960) believe that the dipeptidases might not occupy the same site as the disaccharidases and that the site of transfer of amino acids might be more remote from the luminal border than that of hexoses. If this mechanism is concerned with generating the potential this might explain the different time relations. It is also difficult on this model to explain the inflexions on the potential

curves obtained here for many amino acids. This difficulty might be overcome by assuming that more than one kind of ternary carrier may operate for some amino acids, with different rates of entry. Newey & Smyth (1964) have shown that some amino acids, e.g. glycine and proline, use two carriers, and it is possible that these carriers move through the membrane at different rates.

Another problem which may throw light on the mechanism is the possibility of additive effects of different substances on the potential. If the potential is related to sodium transfer on a hexose or amino acid carrier, then substances moving on different carriers might be expected to give additive effects, while substances moving on the same carrier would not necessarily do so. The findings here show that glucose and galactose do not give additive effects and are consistent with their using the same carrier. On the other hand, amino acid and hexose potentials are, to a considerable extent, additive and this is consistent with the idea that they use separate carriers to facilitate entry of sodium. Thus, these experiments are consistent with the Schultz–Zalusky model.

It is, nevertheless, possible that the generation of the potential is a more complex process than that represented by this model. The link between sodium entry on the ternary carrier and electrogenic sodium transfer at the serosal border is implicitly intracellular sodium concentration, and this may be affected by other factors. Thus Barry, Eggenton & Smyth (1967) have shown that serosal mannose can stimulate sodium transfer without affecting the p.d. in rat jejunum. Furthermore, Taylor, Wright, Schultz & Curran (1967) have demonstrated that active chloride secretion into the mucosal solution may be important in the potential generated by galactose. The possibility of a more direct metabolic control of ion transport cannot be ruled out and Kohn, Newey & Smyth (1967) have recently shown that ATP in the absence of amino acids or hexoses can stimulate the p.d. Newey, Sanford & Smyth (1968) have suggested that intracellular Na concentration might change the affinity of the carrier for the amino acid, and the movement of amino acid against a gradient could thus occur without entry of Na on the same carrier. All these possibilities must be considered before a final conclusion can be drawn about the nature of the electrogenic process, which still seems to us not to be adequately explained by any model yet put forward.

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