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TRANSFER OF PROPIONATE BY RAT SMALL INTESTINE *IN VITRO*

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SUMMARY

1. The transfer of propionate by sacs of rat everted intestine has been investigated in relation to a number of physico-chemical factors which affect movement of weak electrolytes.

2. Neither the observed movement nor the distribution of propionate can be accounted for by the theory of non-ionic diffusion or by modifications of it, such as the microclimate hypothesis or partial permeability to ions.

3. It is not possible to account for the observed propionate movement by the electrical potential across the gut or by solvent drag.

4. The most satisfactory explanation for the observations is a transfer process in the gut for volatile fatty acids, and some features of this are discussed.

INTRODUCTION

Smyth & Taylor (1958) found that the volatile fatty acids were transferred across the small intestine of the rat *in vitro* against a concentration difference, and these results were confirmed by Barry & Smyth (1960). One possible explanation of these findings is a specific transfer mechanism for the volatile fatty acids, but other less specific processes have not been excluded. An alternative explanation is that offered by Hogben, Tocco, Brodie & Schanker (1959), who showed that an unequal distribution of weak electrolytes across the intestine may be due to a difference in pH on the two sides of the membrane, combined with the higher lipid solubility of the non-ionized form relative to the ionized form. The experiments described here were designed to test the effects of various factors, including pH, on the transfer of a volatile fatty acid, propionic acid, across the intestine. A preliminary account of this work has been given by Barry, Jackson & Smyth (1964).

METHODS

The *in vitro* preparation was the sac of rat everted small intestine (Wilson & Wiseman, 1954). White rats of the Sheffield strain, maintained on a diet of Oxoid cubes (diet no. 86) were used, and the animals were anaesthetized with pentobarbitone sodium. Two types of

experiments were done, (*a*) 'transfer' experiments, (*b*) 'metabolism' experiments. The procedure followed closely that described by Parsons, Smyth & Taylor (1958). In the transfer experiments, transfer of propionate was studied in various conditions. In the metabolism experiments, the metabolism of glucose was studied in the same conditions, as this might affect the transfer of propionate. Metabolism of propionate was not studied, as Barry & Smyth (1960) have shown that this is not appreciably metabolized by the rat intestine.

Transfer experiments. Sacs were made from the first 30 cm of the jejunum, except where stated otherwise, and were filled with 2 ml. of saline. They were shaken in flasks containing 30 ml. saline in equilibrium with a gas phase of 5 % CO_2 and 95 % O_2 for 1 hr at 37° C. The saline was bicarbonate saline (Krebs & Henseleit, 1932) modified by altering the concentration of bicarbonate and of chloride. The relation between the pH and the bicarbonate and chloride concentration is shown in Fig. 1; the saline used in particular experiments will

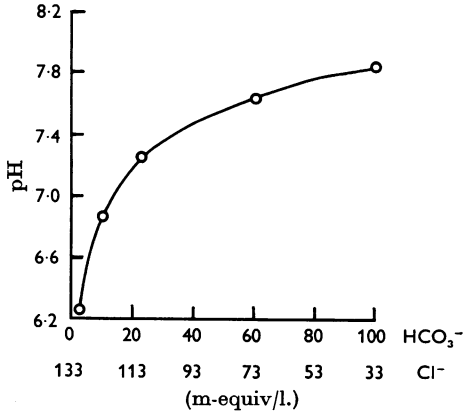

Fig. 1. Relation of pH to composition of saline. This is modified from that described by Krebs & Henseleit (1932) by addition of sodium propionate (20 mM) and adjustment of bicarbonate and chloride concentrations to vary the pH, while maintaining a Na concentration of 145 m-equiv/l.

subsequently be referred to in terms of its pH. Propionate, glucose, fructose and phlorrhizin were initially present in the mucosal and serosal fluids as described subsequently. At the end of the incubation period the mucosal and serosal fluids were collected and samples taken for determination of pH and propionate concentrations.

Metabolism experiments. These were carried out as described for transfer experiments up to the end of the incubation period. The flasks with their contents were then plunged into boiling water for 5 min. The contents were homogenized and samples taken for glucose determination.

Measurement of pH. Samples of mucosal and serosal fluids were taken in a hypodermic glass electrode at the end of the experiment for pH determination on a Vibron Electrometer with pH-measuring unit (Electronic Instruments Ltd.). Although this procedure is open to the objection that the sample is allowed contact with an altered gas phase, it was felt to be preferable to collection under paraffin, which might preferentially remove propionate. Preliminary tests showed that the rate of equilibration between fluid and atmosphere was extremely slow in the conditions used.

Radioactive experiments. In some experiments [^{14}C]-labelled propionate was used to estimate the loss of propionate from the serosal fluid. [^{14}C]Sodium propionate was obtained

from the Radiochemical Centre, Amersham, and was diluted with non-labelled propionate to give a specific activity of 2·5 nc/μmole. This was used in the serosal fluid in a concentration of 20 mM with 20 mM non-labelled propionate in the mucosal fluid. At the end of the incubation period the serosal fluid was collected and deproteinized with 7 % copper sulphate and 10 % sodium tungstate, and a sample of the filtrate counted in a Packard Tricarb scintillation counter.

Chemical. Propionate was determined by steam distillation by a technique essentially similar to that used by Friedemann (1938). Glucose was determined by the method of Nelson (1944) as modified by Somogyi (1945).

Expression of results. The terms used are those defined by Barry, Matthews & Smyth (1961), but for convenience of the reader are repeated here. The *mucosal fluid* is the fluid in which the sacs are suspended, and the *serosal fluid* is the fluid inside the sac. The *mucosal fluid transfer* is the diminution in the volume of fluid on the mucosal side during the course of the experiment; the *serosal fluid transfer* is the increase in volume inside the sac; the *initial mucosal propionate concentration* and *initial serosal propionate concentration* are the concentrations in the mucosal and serosal fluids at the beginning of the experiment. The *final mucosal* and *serosal propionate concentrations* are the corresponding concentrations at the end of the incubation period. The *mucosal propionate transfer* is the amount of propionate which disappears from the mucosal fluid during the experiment; the *serosal propionate transfer* is the increase in propionate in the serosal fluid. The *propionate concentration transferred* is the propionate transfer divided by the fluid transfer. The term 'mucosal propionate concentration transferred' implies that a certain amount of propionate leaves the mucosal side in a certain volume of fluid, and that this relation can be expressed as a concentration. We must, however, consider the possibility that fluid and propionate do not necessarily leave the mucosal fluid by the same route, i.e. fluid may leave by pores, while propionate may be able to penetrate the lipid membrane. This could make the concentration transferred a somewhat abstract concept, but it does not invalidate its usefulness in showing the relation between fluid and propionate movement. Barry & Smyth (1960) have considered this parameter in some detail, and have pointed out that a mucosal concentration transferred greater than the initial mucosal concentration is not necessarily evidence of active transfer of solute. On the other hand much more weight can be attached to a high serosal concentration transferred, in considering active solute transfer.

RESULTS

Changes in pH during incubation

A series of experiments was carried out in which the initial pH of the saline was varied between 6·24 and 7·87. At the end of the incubation the pH of the mucosal and serosal fluids was determined and the results are given in Table 1. During the incubation the pH fell in both mucosal and serosal fluids and the final serosal pH was lower than the final mucosal pH.

In most experiments shown in Table 1 the saline contained glucose, which is known to stimulate the transfer of propionate. One set of experiments was performed in the absence of glucose to test whether this stimulation could be explained by a change in the final pH gradient. The result of these experiments is given at the foot of Table 1. The changes in pH are smaller than those found in the presence of glucose, but again the final serosal pH is lower than that in the mucosal fluid.

Effect of pH on propionate distribution

Table 1 also shows the final concentrations of propionate found in the mucosal and serosal fluids. Apart from the experiment at the highest pH tested (7·87) the final serosal concentration is always higher than the final mucosal concentration, i.e. the higher concentration is found in the fluid of lower pH.

From the propionate concentration and the pH the concentrations of ionized and non-ionized propionic acid can be calculated. These figures are given in Table 2 for the experiments in which glucose was present in the incubation medium. In all cases, except that at pH 7·87, both ionized and non-ionized forms are at a higher concentration in the serosal fluid than in the mucosal fluid.

TABLE 1. Changes in pH and concentration of propionate in mucosal and serosal fluids during incubation. Sacs of rat everted intestine containing 2 ml. saline with 20 mM propionate and 28 mM glucose were incubated for 1 hr at 37° in 30 ml. of the same solution, the initial pH being that shown in the first column. One series of experiments, shown at the foot of the table, was carried out in the absence of glucose. The values given are the means ± S.E. of mean

Initial pH of mucosal and serosal fluid	No. of expts.	Final pH		Final concentration of propionate (mM)	
		Mucosal fluid	Serosal fluid	Mucosal solution	Serosal solution
6·24	5	5·81 ± 0·03	5·63 ± 0·02	18·4 ± 0·2	20·6 ± 0·6
6·67	6	6·16 ± 0·02	5·81 ± 0·01	18·5 ± 0·1	23·3 ± 0·2
6·86	5	6·23 ± 0·01	5·89 ± 0·04	17·9 ± 0·2	27·3 ± 0·1
6·94	5	6·34 ± 0·01	6·06 ± 0·01	17·2 ± 0·1	29·2 ± 0·2
7·25	5	6·67 ± 0·03	6·38 ± 0·01	16·8 ± 0·2	32·9 ± 0·4
7·44	5	7·00 ± 0·03	6·56 ± 0·02	17·7 ± 0·3	26·8 ± 0·5
7·64	5	7·17 ± 0·02	6·65 ± 0·03	19·3 ± 0·2	22·1 ± 0·3
7·87	5	7·49 ± 0·03	6·91 ± 0·03	19·3 ± 0·1	18·2 ± 0·5
		No glucose present			
7·22	6	7·31 ± 0·01	7·20 ± 0·01	19·4 ± 0·1	26·3 ± 0·2

TABLE 2. Final concentrations of ionized and non-ionized fractions of propionic acid in the mucosal and serosal fluids in the experiments shown in Table 1. The values given are calculated from the mean values for concentration and pH in Table 1

Initial pH	Initial concentration (mM) mucosal and serosal		Final concentration (mM)			
			Mucosal		Serosal	
	Ionized	Non-ionized	Ionized	Non-ionized	Ionized	Non-ionized
6·24	19·2	0·8	16·5	1·9	17·5	3·1
6·67	19·7	0·3	17·6	0·9	20·9	2·4
6·86	19·8	0·2	17·1	0·8	25·0	2·3
6·94	19·8	0·2	16·6	0·6	27·4	1·8
7·25	19·9	0·1	16·5	0·3	31·9	1·0
7·44	19·9	0·1	17·6	0·1	26·3	0·5
7·64	20·0	0·0	19·2	0·1	21·7	0·4
7·87	20·0	0·0	19·3	0·0	18·0	0·2

Effect of pH on the movement of propionate and fluid

The changes in propionate concentration depend upon two factors, propionate movement and fluid movement, and these parameters are given in Table 3 for the same experiments included in Table 1. Over the range of pH used in these experiments propionate moved from the mucosal to the serosal sides, i.e. towards the side with lower pH. A maximum transfer was obtained between 7·25 and 7·44 and there was a marked decrease on either side of this.

A different pattern was found for the effects of pH on fluid transfer. The fluid movement was in the same direction as that of propionate in all cases, so that the higher concentration of propionate on the serosal side could not be explained by loss of fluid from the sac, and in fact the fluid movement tended to mask the propionate movement if this is judged by concentration changes. There was a significant reduction in fluid movement below an initial pH of 7·25, but an increase in pH above this did not significantly alter the transfer of fluid.

The differences in the effects of pH on the transfers of propionate and fluid are shown more clearly by consideration of the concentration transferred. The effects of pH on the mucosal and serosal concentrations transferred are shown in Table 4. The mucosal concentration transferred decreased from a high level at an initial pH of 6·24 to a value at the highest pH tested which was not much greater than the initial propionate concentration (20 mM).

The serosal concentration transferred followed more closely the pattern found for propionate transfer. A maximum occurred at an initial pH of 7·25, when the serosal concentration transferred was equal to the mucosal concentration transferred. As the pH was increased the serosal concentration transferred decreased in parallel with the fall in the mucosal concentration transferred. A decrease in initial pH below 7·25 led to a significant decrease in the serosal concentration transferred, so that as the pH was decreased the mucosal and serosal concentrations transferred diverge. At the extreme values of initial pH used in these experiments the serosal concentrations transferred are not significantly different from the concentration of propionate present initially in the saline.

Effect of different initial mucosal and serosal pH

According to the hypothesis of Hogben *et al.* (1959) the transfer of a weak acid across the intestine is dependent upon the existence of a difference in pH across the intestinal wall. In order to test this hypothesis further a series of experiments was carried out in which solutions of different pH were used in the mucosal and serosal fluids. In this way the

TABLE 3. Effect of initial pH on fluid and propionate transfer in the experiments given in Table 1. Values given are means ± S.E. of mean

Initial pH	Fluid (ml./100 mg dry weight gut)		Propionate (μmoles/100 mg dry weight gut)	
	Mucosal transfer	Serosal transfer	Mucosal transfer	Serosal transfer
6·24	0·27 ± 0·02	0·08 ± 0·01	15 ± 2	2 ± 1
6·67	0·34 ± 0·02	0·14 ± 0·01	18 ± 2	5 ± 1
6·86	0·45 ± 0·04	0·19 ± 0·04	21 ± 2	8 ± 1
6·94	0·48 ± 0·05	0·24 ± 0·04	24 ± 4	9 ± 1
7·25	0·79 ± 0·06	0·47 ± 0·05	33 ± 2	21 ± 2
7·44	0·73 ± 0·07	0·45 ± 0·05	31 ± 2	16 ± 2
7·64	0·75 ± 0·06	0·47 ± 0·06	19 ± 1	11 ± 1
7·87	0·70 ± 0·14	0·44 ± 0·02	18 ± 3	7 ± 3

TABLE 4. Effect of initial pH on propionate concentration transferred in the experiments given in Table 1. Values given are means ± S.E. of mean

Initial pH	Mucosal concentration transferred (mM)	Serosal concentration transferred (mM)	Initial pH	Mucosal concentration transferred (mM)	Serosal concentration transferred (mM)
6·24	55·9 ± 5·8	23·4 ± 5·3	7·25	40·9 ± 2·2	43·9 ± 0·8
6·67	52·2 ± 3·7	33·8 ± 2·8	7·44	42·0 ± 2·5	36·4 ± 5·5
6·86	47·6 ± 2·9	43·3 ± 5·8	7·64	25·6 ± 1·5	24·8 ± 0·7
6·94	50·8 ± 2·2	37·6 ± 1·2	7·87	25·2 ± 2·4	16·8 ± 1·8

TABLE 5. Effect of different initial pH in mucosal and serosal fluids on propionate and fluid transfer. The conditions were as in Table 1 except for the initial pH of the solutions. Transfers are expressed per 100 mg dry weight, and all values are means ± S.E. of mean

Group	No. of expts.	Initial pH		Final pH		Final propionate concentration (mM)		Mucosal transfer		Mucosal concentration transferred (mM)
		Mucosal	Serosal	Mucosal	Serosal	Mucosal	Serosal	Propionate (μmoles)	Fluid (ml.)	
1	6	6·67	6·67	6·16 ± 0·02	5·81 ± 0·01	18·5 ± 0·1	23·3 ± 0·2	18 ± 2	0·34 ± 0·02	52·2 ± 3·7
2	5	6·67	7·54	6·22 ± 0·02	6·32 ± 0·02	17·5 ± 0·3	26·0 ± 0·7	27 ± 1	0·46 ± 0·03	58·2 ± 1·2
3	6	7·54	6·67	7·18 ± 0·02	6·51 ± 0·03	17·3 ± 0·4	23·6 ± 0·8	25 ± 2	0·74 ± 0·06	33·8 ± 0·6
4	5	7·54	7·54	7·25 ± 0·03	6·70 ± 0·02	19·0 ± 0·2	24·3 ± 0·7	19 ± 2	0·79 ± 0·04	24·3 ± 4·0
5	5	6·24	6·24	5·81 ± 0·03	5·63 ± 0·02	18·4 ± 0·2	20·6 ± 0·6	15 ± 2	0·27 ± 0·02	55·9 ± 5·8
6	6	6·24	7·87	5·93 ± 0·02	6·63 ± 0·02	17·4 ± 0·1	26·7 ± 0·4	24 ± 2	0·38 ± 0·03	64·3 ± 2·0
7	5	7·87	6·24	7·47 ± 0·02	6·69 ± 0·01	19·3 ± 0·1	17·9 ± 0·7	18 ± 2	0·70 ± 0·08	25·8 ± 1·0
8	5	7·87	7·87	7·49 ± 0·03	6·91 ± 0·03	19·3 ± 0·1	18·2 ± 0·5	18 ± 3	0·70 ± 0·14	25·2 ± 2·4

magnitude and polarity of the final pH difference existing across the intestine could be changed. The results of these experiments are given in Table 5 and for comparison the results of experiments in which the same medium was used on both sides of the gut are included. Table 5 contains the results of two sets of experiments, in the first (groups 1–4) the pH values chosen were 6·67 and 7·54 and in the second set (groups 5–8) a wider range of pH was used, 6·24 and 7·87. In all groups there was movement of both propionate and fluid from the mucosal to the serosal side. In groups 2 and 6 the final serosal pH was greater than the final mucosal, giving a pH difference which was favourable to a greater concentration of propionate in the serosal fluid according to the hypothesis of Hogben *et al.* (1959). The stimulation of propionate movement found under these conditions was not a specific effect and was accompanied by increased fluid transfer, with the result that the concentration transferred was not significantly increased above that found when the lower pH was used on both sides of the intestine (groups 1 and 5). In groups 7 and 8 the final mucosal concentration was greater than the final serosal concentration,

TABLE 6. Effect of pH on serosal loss of propionate. Experimental conditions as in Table 1 except that the serosal fluid contained initially labelled propionate, while the propionate in the mucosal fluid was unlabelled. The 2 ml. of serosal fluid contained initially 100 nc. The serosal loss is the disappearance of activity from the serosal fluid. The final pH values given were not measured in these experiments, and are taken from Tables 1 and 5, where the conditions were identical except for the radioactivity

Initial pH		Final pH		Serosal loss (nc/g wet weight)
Mucosal	Serosal	Mucosal	Serosal	
6·24	7·87	5·93 ± 0·02	6·63 ± 0·02	25 ± 1
7·25	7·25	6·67 ± 0·03	6·38 ± 0·01	23 ± 0·3
7·87	6·24	7·47 ± 0·02	6·69 ± 0·01	27 ± 1

and this is in agreement with the polarity of the final pH difference according to the theory of non-ionic diffusion. The reversal of final concentration gradient in these two groups, however, could be explained by the relatively large fluid movement from mucosal to serosal side, and did not reflect a substantial change in the magnitude or direction of propionate movement.

Effect of pH on serosal loss of propionate

In all the above experiments there was a net transfer of propionate from the mucosal to the serosal fluid. Experiments were carried out in which [^{14}C]-labelled propionate was used to determine the effect of pH on the movement of propionate out of the serosal fluid. In these experiments equal concentrations of propionate were initially present in the mucosal and serosal fluids, but the serosal fluid contained a small amount of

labelled propionate. The amount of activity in the serosal fluid was determined at the end of the incubation, and the disappearance is referred to in Table 6 as serosal loss. Only small differences in serosal loss were observed between the experiments and these were not correlated with the size or polarity of the pH gradient.

TABLE 7. Effect of initial pH of mucosal and serosal fluids on metabolism of glucose by sacs of rat everted small intestine. The conditions were as set out in the heading in Table 1. The glucose metabolized is the amount of glucose disappearing from the whole system during the course of the experiment. Values are means ± S.E. of mean

Initial pH of mucosal and serosal fluids	No. of expts.	Glucose metabolized (μmoles/g wet weight)
6·67	5	22 ± 7
6·94	5	48 ± 6
7·25	5	97 ± 2
7·64	5	128 ± 1
7·84	6	138 ± 4

TABLE 8. Effect of conditions affecting the electrical potential on propionate transfer. Sacs of everted intestine made from the middle fifth of the combined jejunum and ileum containing initially 1 ml. saline with 20 mM propionate were incubated for 1 hr in 15 ml. of the same saline with the additions to the mucosal and serosal solutions shown below. For discussion of effect of these conditions on electrical potential see Text. The values given are the means of five experiments ± S.E. of mean

Mucosal fluid	Serosal fluid	Mucosal propionate transfer (μmoles)
—	—	27 ± 3
28 mM glucose	28 mM glucose	73 ± 5
28 mM galactose	28 mM galactose	11 ± 1
5×10^{-4} M phlorrhizin	224 mM glucose	80 ± 3

Effect of pH on glucose metabolism

Smyth & Taylor (1958) found that transfer of volatile fatty acids was stimulated in the presence of glucose, and it was therefore relevant to ask to what extent the effect of pH on propionate transfer could be explained by an indirect effect on glucose metabolism. A series of experiments was carried out in which glucose metabolism was measured when sacs were incubated in saline with the pH range shown in Table 1. The results of these experiments are shown in Table 7. Glucose metabolism increased almost linearly with pH over the range studied, and no maximum rate of metabolism was found, to correspond with the maximum rate of propionate transfer.

Effect of electrical potential on propionate transfer

Barry, Dikstein, Matthews, Smyth & Wright (1964) have shown that the transfer mechanism for hexoses is associated with an electrical potential.

This raises the question whether the stimulation of fatty-acid transfer by glucose might be due to the potential generated by the hexose pump facilitating the transfer of the anion.

Experiments were performed with sacs prepared from the middle fifth of the intestine to allow direct comparison with the work of Barry *et al.* (1964). In these experiments the electrical potential was not measured but conditions were created which Barry *et al.* (1964) have shown would affect the electrical potential.

The results of these experiments are shown in Table 8. Glucose and galactose present in the mucosal fluid have obviously very different effects on the transfer of propionate. Glucose stimulated propionate transfer, while galactose actually inhibited it. Further, glucose present in the serosal fluid with phlorrhizin in the mucosal fluid caused as much stimulation of propionate transfer as glucose present in the mucosal fluid.

DISCUSSION

A weak electrolyte, such as propionic acid, exists in two forms in the experimental conditions described, a larger ionized fraction and a smaller non-ionized fraction. The simplest type of transfer would be diffusion of each of these forms and it must be assumed that two routes may be available for this, (*a*) the main surface of the lipid membrane, which is permeable to lipid-soluble substances, and (*b*) aqueous pores which offer a route for smaller hydrophilic compounds and ions. The results presented here cannot be accounted for by diffusion only, either through pores or through a lipid membrane, as both ionized and non-ionized fractions show movement at least in some experimental conditions against a concentration gradient. Some other factors must be involved and four possibilities must be considered: (1) non-ionic diffusion, (2) the effect of an electrical potential on ion movements, (3) solvent drag which may affect the fraction going through the aqueous pores, (4) some kind of specific transport mechanism.

Non-ionic diffusion

Hogben *et al.* (1959) have suggested that the unequal distribution of volatile fatty acids across the gut wall found by Smyth & Taylor (1958) was due to a much greater permeability of the gut wall to the non-ionized form of the acid than to its anion combined with a difference in pH on the two sides of the gut. This hypothesis has been widely applied by Hogben, Brodie and co-workers to explain the distribution of drugs across biological membranes (see Schanker, 1962, for review). This concept is in fact not new, and was first put forward in quantitative terms by Jacobs (1940), who derived an expression to give the concentration ratio of a weak acid on two sides of a membrane which is permeable to the non-ionized form

only. At equilibrium the concentrations C_1 and C_2 of a weak acid in two solutions of different pH (pH_1 and pH_2) separated by a membrane which is permeable only to the non-ionized form of the acid is given by the equation

$$\frac{C_1}{C_2} = \frac{1+10^{(pH_1-pK_a)}}{1+10^{(pH_2-pK_a)}} \quad (1)$$

An identical expression was used by Shore, Brodie & Hogben (1957) to account for the movement of drugs across the stomach, and similar equations have also been used to calculate intracellular pH (see Caldwell, 1956). This hypothesis is usually referred to as the theory of non-ionic diffusion.

This theory might explain the distribution of fatty acids found by Smyth & Taylor (1958) if the pH of the mucosal fluid was lower than that of the serosal fluid, and Hogben (1960) suggested that this pH gradient may arise as a result of ion movements, described by Wilson (1954). In calculating the pH of the fluids from the bicarbonate concentrations, Wilson (1954) assumed that the fluid was in equilibrium with 5 % CO_2. This assumption does not appear to be justified in the experiments described here. When the final serosal fluid was gassed with 5 % CO_2 the pH rose, indicating that the P_{CO_2} of the serosal fluid was greater than that of the gas phase with which it was initially in equilibrium. In all the experiments where the initial pH was the same on the two sides of the gut, the final serosal pH was lower than the final mucosal pH. However, in all cases, except at the highest pH tested, the final serosal propionate concentration was greater than the final mucosal concentration. Thus the pH gradient was in the opposite direction to that required to explain the final distribution. The experiments in which the final pH gradient was altered did produce conditions in which the final propionate concentration difference was in qualitative agreement with the final pH gradient according to the theory of non-ionic diffusion. This concentration difference could also be explained by the effect of fluid transfer superimposed on a mucosal to serosal net movement of propionate, which was independent of the magnitude or polarity of the pH gradient. In the experiments where [^{14}C]propionate was used, the loss of propionate from the sac, which might be expected to contribute to any passive movement of propionate, was apparently not affected by the pH gradient across the intestine. Similarly, the stimulating effect of glucose on propionate movement could not be explained by the production of a more favourable pH gradient since the gradient produced in the presence of glucose was less favourable than that found in its absence.

In applying this hypothesis to the absorption of drugs from the intestine, Hogben *et al.* (1959) themselves reported observations not explicable in

terms of the theory of non-ionic diffusion. For example, salicylic acid ($pK_a = 3$) was absorbed rapidly from a solution in which it was highly dissociated, and in other cases the distributions at equilibrium were not those predictable from the final pH values. These inconsistencies led Hogben *et al.* (1959) to suggest that the absorption of weak electrolytes from the intestine is determined not by the pH in the bulk phase of the solution bathing the intestinal mucosa but by the pH of a microclimate close to the epithelial cell. If the pH of the microclimate is lower than that of the bulk phase, the concentration of non-ionized acid at the membrane surface will be increased, and hence the rate of absorption will also increase. However, it is difficult to see how this microclimate could explain the discrepancy between the observed equilibrium concentration ratios and those calculated from eqn. (1). The condition for equilibrium between the bulk phases is that the concentration of non-ionized acid is the same in the two phases. This condition is unaltered by interposing a microclimate as an additional phase, although it is conceivable that the rate of achieving equilibrium might be altered.

The distribution of propionate according to the theory of non-ionic diffusion could also be modified by some degree of permeability of the intestine to the ionized form. This possibility was considered by Hogben *et al.* (1959), who showed that it was possible to calculate the permeability of the gut to the ionized form relative to that of the non-ionized form by a modification of eqn. (1),

$$\frac{C_1}{C_2} = \frac{1+10^{(pH_1-pK_a)}}{1+10^{(pH_2-pK_a)}} \cdot \frac{10^{(pH_2-pK_a)}+R}{10^{(pH_1-pK_a)}+R}, \qquad (2)$$

where R is the ratio of the permeabilities of the membrane to the non-ionized and ionized forms of the compound. Values of R could fall between zero and infinity to cover the complete range of permeability ratios. In fact substitution in eqn. (2) of the values given in Table 1 give values for R which are negative in most cases, indicating that the results cannot be explained by differences in the permeability of the gut to the ionized and non-ionized forms. It therefore appears that neither the original theory of non-ionic diffusion nor any of the modifications suggested can explain the distribution of propionate across the gut.

The effect of an electrical potential

The possibility must be examined that propionate moves partly as the ionized form but that this movement is affected by the electrical potential. Barry *et al.* (1964) showed that a potential of 1·5 mV is present across the gut in the absence of glucose, the serosal side being positive to the mucosal. On addition of glucose or galactose, the potential increased to about 12 mV,

and this effect was abolished by phlorrhizin in the mucosal fluid. Phlorrhizin, however, creates a further change in the experimental conditions as it also greatly reduces fluid transfer. This complication can be avoided by use of a high concentration of glucose in the serosal fluid and phlorrhizin in the mucosal fluid, and in these conditions Barry *et al.* (1964) showed that the potential was reduced by about 6 mV, while fluid transfer was unaffected. In the experiments reported here (Table 8) the potentials have not been measured, but conditions were created similar to those of Barry *et al.* (1964). The results of these experiments appear to rule out the possibility of propionate transfer depending on the electrical potential. Glucose and galactose are known to cause the same electrical potential, but glucose stimulates propionate transfer, while galactose inhibits it. Furthermore, in the experiments with phlorrhizin the propionate transfer was not affected although the potential was reduced by about half.

Solvent drag

Yet another factor which must be considered in propionate movement is solvent drag, i.e. movement of propionate in the fluid stream which is known to exist in the gut. This fluid stream can play a part in the movement of any solute provided it is small enough to pass through the pores, and Hakim & Lifson (1964) have shown that this mechanism can account for the movement of small solute molecules against a concentration difference. This possibility can be assessed by consideration of the ratios of propionate to fluid transfer, the concentrations transferred. Hakim & Lifson (1964) have shown that the maximum concentration transferred which can be achieved is equal to the concentration in the mucosal fluid and it does not seem possible for any combination of simple diffusion and solvent drag to produce both mucosal and serosal concentrations transferred which are greater than the initial concentration of propionate in the saline. At some stage there would have to be a concentrating mechanism for the solute in the gut wall. Table 4 shows that only at the extreme pH values used could solvent drag account for propionate transfer.

Specific transfer mechanism

This leaves the possibility of some specific transfer mechanism for the volatile fatty acids. The effect of glucose on propionate transfer shown in Table 8 may be regarded as evidence for this. Glucose has been shown by Fisher (1954) and Smyth & Taylor (1954) to increase fluid transfer in the intestine, by Newey & Smyth (1962) to increase glycine transfer, by Newey, Sanford & Smyth (1965) to increase galactose transfer, and by Sanford, Smyth & Watling (1965) to increase proline transfer. These authors have suggested that a number of transfer systems in the rat intestine can

derive energy for transfer by endogenous metabolism, but this is not sufficient to maintain transfer at maximum rate. For this reason, the presence of glucose causes a stimulation. Newey & Smyth (1964) have shown that galactose can inhibit amino acid transfer, and have suggested that this is in competition for energy between different transfer systems. Whether inhibition of propionate transfer produced by galactose has the same explanation must be regarded as speculative, as other interactions between different transfer systems are conceivable.

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