J. Physiol. (1958) 141, 73–80

INTESTINAL TRANSFER OF SHORT-CHAIN FATTY ACIDS *IN VITRO*

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(*Received* 14 *October* 1957)

Interest in the absorption of short-chain fatty acids from the alimentary tract has been confined mainly to studies on ruminants. Very little information exists on absorption of short-chain fatty acids in other species. Höber (1945) included acetate in a list showing the relative rates of absorption of various anions from the intestine, but this was based on very early experiments (Höber, 1899) involving freezing-point measurements only. Deuel, Hallman & Reifman (1941) studied the absorption of short-chain fatty acids from rat intestine and gave the rates of absorption for all members of the series from acetic to tridecylic acid. No suggestion was made by these authors that any active process was involved in absorption of short-chain fatty acids, and Höber (1945), in considering the fatty acids along with other anions, thought that diffusibility was probably the predominant factor in determining the absorption rate. Recently Smyth & Taylor (1954, 1957*a*), using an *in vitro* intestinal preparation of rat intestine, found that the fluid transferred by the intestine contained short-chain fatty acids in a concentration higher than that in the lumen of the intestine, and in this respect fatty acids resembled glucose and L-amino acids, substances which there is reason to believe are transferred by an active process. One of the criteria of an active process is the ability to move substances against a concentration gradient; and it was therefore decided to test the capacity of the intestine to transfer short-chain fatty acids against a concentration gradient. A preliminary account of this work has been given by Smyth & Taylor (1957*b*).

METHODS

The intestinal preparation used was the sac of everted small intestine of the rat described by Wilson & Wiseman (1954). The procedure was, however, modified by using sacs of intestine 30 cm long instead of the very short sacs used by those authors. These sacs, prepared from the upper part of the small intestine, were suspended in 50 ml. bicarbonate saline (Krebs & Henseleit, 1932) and were shaken in conical flasks at a rate of 90 c/min. Except where otherwise stated, the bicarbonate saline contained 500 mg glucose/100 ml. The gas phase was 5% carbon dioxide and 95% oxygen except in the anaerobic experiments where 5% carbon dioxide and 95% nitrogen was used.

Each sac was filled with about 3 ml. bicarbonate saline which, relative to the size of the sac, is a much smaller volume than that used by Wilson & Wiseman (1954) and consequently produced no distension or turgidity. From some points of view distension may be advantageous, for example it may possibly improve the conditions for oxygen uptake by increasing the surface and diminishing the thickness of the intestinal mucosa. On the other hand, the increased pressure inside the sac will tend to oppose transfer of water from mucosal to serosal fluid, and we believe that the different degrees of distension of the sacs explain some of the differences between our results and those of Wilson & Wiseman (1954).

In describing the results the fluid inside the sac is referred to as the serosal fluid and the fluid in which the sac is suspended as the mucosal fluid. The piece of intestine used for the sac was weighed before (W_1) and after (W_2) filling with bicarbonate saline, and also at the end of the experiment (W_3). It was then opened and the serosal fluid drained out and the sac weighed again (W_4). The tissue was finally dried at 110° during the night and weighed (W_5). The initial serosal volume is obtained from $W_2 - W_1$ and the final volume from $W_3 - W_4$. The difference between initial and final volumes gives the volume of water transferred. (This cannot be directly determined from $W_3 - W_2$ because a certain amount of water is taken up by the tissue.)

The fatty acids were used as the sodium salts and were present initially in equal concentrations in the mucosal and serosal fluids. The final concentrations were determined by steam distillation in a Markham still of the filtrate obtained by deproteinizing with copper sulphate and sodium tungstate. For the subsequent titration 0·033 N-NaOH was used, with phenol red as indicator. From the concentrations of fatty acids and the initial and final volumes of the serosal solutions the initial and final amounts of fatty acid in the serosal fluid were calculated, and from these the amount of fatty acid transferred. A positive value for transfer means an increase in fatty acid on the serosal side and a negative value a decrease.

The units chosen for expressing the results deserve some consideration. In previous work on transfer and metabolism *in vitro* the Q notation has been frequently used. While this notation is well established in dealing with oxygen consumption, it has some serious disadvantages in expressing utilization and transfer, the chief of these being the use of the symbol μl. In the Q notation 1 μl. means $10^{-6} \times \frac{1}{22 \cdot 4}$ mole irrespective of whether the substance is a solid, liquid or gas. In expressing transfer of fluids, however, the symbol μl. is now generally taken to mean 10^{-3} ml. We feel that two different uses of the symbol μl. can give rise to confusion, and we therefore propose to keep the symbol μl. (10^{-3} ml.) for volumes of fluid, and to express fatty acids as m-moles, μmoles or n-moles (10^{-9} mole). The concentration of fatty acid has been expressed as μmoles/ml. When the rate of transfer is expressed in μmoles/mg dry weight of tissue the number is inconveniently small, and it is therefore given as n-moles/mg dry weight of tissue/hr.

RESULTS

Movement of volatile fatty acids against a concentration gradient

In these experiments glucose was present initially in a concentration of 500 mg/100 ml. both in the mucosal and serosal fluid, and the fatty acid was also present initially in equal concentrations in the two fluids. The sacs were shaken under aerobic conditions for 90 min. The results are shown in Table 1. From this it is seen that there is a very considerable movement of fatty acid from the mucosal fluid to the serosal fluid. This results in the serosal fluid acquiring a higher concentration of fatty acid than the mucosal fluid, and hence the transfer of fatty acid must be against this concentration gradient. The movement of fatty acid is accompanied by movement of water, but it should be noted that the water movement is in the opposite direction to that which

would be required to produce the observed differences in concentration of fatty acid in the mucosal and serosal fluids. Control experiments were carried out in which no added fatty acid was present, and in these cases no significant amounts of fatty acid were detected at the end of the experimental period.

Experiments have also been carried out with smaller concentrations of fatty acid than those given in Table 1. An 0·01 M solution is about the smallest concentration which, after deproteinization, gives a solution which can be conveniently estimated. Concentration against a gradient was observed with such solutions. This is of considerable interest as 0·01 M is in the range of concentrations of fatty acid found by Elsden, Hitchcock, Marshall & Phillipson (1946) to exist in the small intestine of the rat under physiological conditions.

TABLE 1. Transfer of fatty acids against a concentration gradient by sacs of everted small intestine in aerobic conditions in the presence of glucose. Positive values for fatty acid and water transfer indicate movement to the serosal side

	Fatty acid concentration			Rate of transfer of	
Fatty acid	Initial, mucosal and serosal (μmole/ml.)	Final, mucosal (μmole/ml.)	Final, serosal (μmole/ml.)	Fatty acid (n-mole/mg dry wt./hr)	Fluid (μl./mg dry wt./hr)
Acetic	22·6	20·6	28·6	278	8·54
	21·0	18·7	26·3	203	6·40
	20·4	18·1	29·3	228	6·25
	20·4	18·1	34·0	282	6·25
Propionic	19·7	18·4	29·9	237	5·82
	20·4	19·2	31·8	289	6·66
	20·9	19·1	33·7	273	5·89
	20·9	19·4	31·4	292	7·12
Butyric	18·4	15·6	35·8	314	6·01
	19·5	17·8	31·6	250	5·25
	18·6	15·2	33·2	321	7·07
	18·6	15·5	36·1	297	5·76

The figures for transfer expressed in n-moles may appear very small, and it is worth considering what contribution this amount of fatty acid could make to the total metabolic need. If the figures from the first line of Table 1 are taken, the transfer of fatty acid of 278 n-moles/mg dry weight/hr is equivalent to 5·62 mg/hr for 30 cm of intestine of a rat weighing 220 g. Since less than one third of the intestine has been used, and as the ileum may transfer acetate at a lower rate than the jejunum, the figure of 12 mg/hr might be taken for the whole intestine. The heat of combustion of acetic acid is 209 kcal/mole and therefore 12 mg acetic acid will yield 0·042 kcal. The rate of metabolism of the rat is given by Carr & Krantz (1942) as 0·183 g oxygen/100 g body weight, and a rat of 220 g will have a metabolic rate of 0·282 l. oxygen/hr, or about 1·4 kcal/hr. Thus, the acetate transferred could contribute about 3% of the total metabolic requirements. The amounts are thus not insignificant.

Under physiological conditions the mechanism would not have to transfer against a concentration gradient, and the amount moved could be considerably greater. Furthermore, as Elsden *et al.* (1946) have shown, the small intestine in the rat contains much less volatile fatty acid than the large intestine, and hence absorption from the small intestine may represent a relatively small part of the total volatile fatty acid used.

Effect of anaerobic conditions

The results of experiments carried out under anaerobic conditions are shown in Table 2. From this it is seen that in anaerobic conditions there is no transfer of fatty acid. The serosal concentration actually falls, and this could be due to diffusion of fatty acid into the tissue. Since the serosal volume is much smaller than the mucosal, only a small movement of fatty acid is required on the

TABLE 2. Transfer of fatty acids by sacs of everted small intestine in anaerobic conditions. Positive values for fatty acid and water transfer indicate movement to the serosal side

	Fatty acid concentration			Rate of transfer of	
Fatty acid	Initial, mucosal and serosal (μmole/ml.)	Final, mucosal (μmole/ml.)	Final, serosal (μmole/ml.)	Fatty acid (n-mole/mg) dry wt./hr)	Fluid (μl./mg dry wt./hr)
Acetic	22·3	22·4	20·5	− 3·2	0·40
	22·3	22·6	18·3	− 6·7	0·87
Propionic	19·4	19·2	14·2	−25·6	1·16
	19·4	18·9	11·9	−37·0	0·80
Butyric	18·3	17·7	12·5	−16·1	1·37
	18·3	18·7	13·5	−29·0	0·33

serosal side to produce a change in concentration. In anaerobic conditions the rate of water movement is greatly reduced but not completely abolished. This is in agreement with the results of Smyth & Taylor (1957 *a*) with a different type of *in vitro* preparation. Wilson & Wiseman (1954) found no transfer of water in everted sacs under anaerobic conditions, and usually even some reductions in volume of the sac. We believe the difference is due to the much greater distension of the sacs in their experiments, so that a considerable hydrostatic pressure existed to force water out of the sac.

Effect of glucose on fatty acid transfer

Glucose has been shown by Fisher (1955) to be essential for water transfer in the intestine *in vitro*, and it was of interest to see whether it is necessary for fatty acid transfer. A series of experiments was therefore carried out, in conditions similar to those of the experiments in Table 1, except that glucose was absent from both the mucosal and serosal fluids. The results are shown in Table 3. It is evident that the absence of glucose caused reduction in transfer of both fatty acids and of water. It does not, however, abolish the transfer, and a

considerable concentration gradient can still be built up. It will be noted that some water movement still takes place. Fisher (1955) and Smyth & Taylor (1957*a*) found an insignificant amount of water movement in the absence of glucose in *in vitro* intestinal preparations. We think this difference in water transfer is explained by the superiority of the everted sac of intestine over other *in vitro* preparations. In the everted sac conditions are such as greatly to favour the oxygenation of the intestinal mucosa and it is the general experience in our laboratory that the everted sac is more robust and has better powers of transfer of water and other materials than any other form of *in vitro* preparation. It seems likely that in these conditions the substrates present in the mucosa, without addition of glucose, are sufficient to maintain a small amount of water movement. Wilson (1956) has also recently obtained some water movement in sacs of everted intestine in the absence of glucose.

TABLE 3. Transfer of fatty acids by sacs of everted small intestine in aerobic conditions in the absence of glucose. Positive values for fatty acid and water transfer indicate movement to the serosal side

	Fatty acid concentration			Rate of transfer of	
Fatty acid	Initial, mucosal and serosal (μmole/ml.)	Final, mucosal (μmole/ml.)	Final, serosal (μmole/ml.)	Fatty acid (n-mole/mg dry wt./hr)	Fluid (μl./mg dry wt./hr)
Acetic	21·5	19·7	22·2	34·8	1·38
	21·5	19·5	22·3	46·0	1·85
	20·2	19·2	20·3	16·5	0·76
	20·2	18·8	24·0	58·1	1·38
Propionic	23·0	19·0	24·8	22·3	0·55
	23·0	19·8	23·0	45·6	2·07
	20·3	18·6	27·2	68·2	1·16
	20·3	19·4	25·7	78·1	1·72
Butyric	19·1	17·5	24·0	54·5	1·01
	19·1	17·7	23·8	80·4	2·02
	23·3	23·2	25·6	45·5	1·17
	23·3	22·6	26·0	25·9	0·25

Effect of inhibitors

The inhibitors tested were phlorrhizin and 2:4-dinitrophenol. The inhibitor was present initially in equal concentration in the mucosal and serosal fluids, and otherwise the experimental conditions were similar to those given in Table 1. The results are seen in Tables 4 and 5. The concentration of phlorrhizin used, 20 mg/100 ml., has been shown by Smyth & Taylor (1955*a*, *b*) to cause very considerable inhibition of transfer of both glucose and water in everted sacs of intestine, and it is found here to cause a similar inhibition of water transfer. While the transfer of fatty acid is also considerably reduced, it is seen that movement of fatty acid against a concentration gradient can still take place, and it is possible that the reduction in transfer of fatty acids is due at least partly to the reduction of water transport.

In the case of 2:4-dinitrophenol (10^{-42}M) there is also some reduction in water transfer, again in agreement with the results of Smyth & Taylor (1955*a*). In this case, however, while there is about the same degree of reduction in water transfer as occurs with phlorrhizin there is a much greater reduction in transfer of fatty acids, and in no case is there any evidence of movement against a concentration gradient. It would thus seem that 2:4-dinitrophenol has a definite effect on fatty acid movement apart from its effect on water transport.

TABLE 4. Effect of phlorrhizin on fatty acid transfer by the intestine under aerobic conditions in the presence of glucose. The phlorrhizin was present in all cases in an initial concentration of 20 mg/100 ml. Positive values for fatty acid and water transfer indicate movement to the serosal side

	Fatty acid concentration			Rate of transfer of	
Fatty acid	Initial, mucosal and serosal (μmole/ml.)	Final, mucosal (μmole/ml.)	Final, serosal (μmole/ml.)	Fatty acid (n-mole/mg dry wt./hr	Fluid (μl./mg dry wt./hr)
Acetic	20·3	19·5	24·3	75	2·08
	20·6	19·2	25·6	141	4·00
Propionic	20·4	19·1	34·2	171	2·12
	16·1	14·7	24·2	146	3·35
Butyric	18·4	16·7	30·6	128	1·97
	20·0	19·0	26·8	89	1·34

TABLE 5. Effect of 2:4-dinitrophenol on the transfer of fatty acids by the intestine under aerobic conditions in the presence of glucose. The inhibitor was present in all cases in a concentration of 10^{-4}M. Positive values of fatty acid and water transfer indicate movement to the serosal side

	Fatty acid concentration			Rate of transfer of	
Fatty acid	Initial, mucosal and serosal (μmole/ml.)	Final, mucosal (μmole/ml.)	Final, serosal (μmole/ml.)	Fatty acid (n-mole/mg dry wt./hr)	Fluid (μl./mg dry wt./hr)
Acetic	20·0	20·0	16·8	12·5	2·09
	20·3	20·4	20·5	25·0	1·17
Propionic	19·6	19·2	17·3	8·5	1·31
	16·1	15·8	13·1	19·6	3·03
Butyric	19·5	19·2	17·4	16·5	1·73
	20·0	19·8	17·3	41·1	3·64

DISCUSSION

The experiments described show that acetic, butyric and propionic acids are transferred by rat intestine *in vitro* against a concentration gradient. This transfer against a concentration gradient is completely abolished by anaerobic conditions and by the presence of 2:4-dinitrophenol. It is also greatly reduced by the absence of glucose and by the presence of phlorrhizin. These results suggest that the movement of the lower fatty acids depends on factors other than diffusion, and also that it is related to the metabolic activity of the intestine. The transfer could therefore be said to involve an active process.

Whether this is an active process specifically related to fatty acid transfer is much less certain. Smyth & Taylor (1957*a*) have discussed a possible mechanism which could account for accelerated transfer of fatty acids by the intestine, and even transfer against a gradient, without requiring a specific active transfer of fatty acids. Sodium is present in the fluid transferred through the intestinal wall in the same concentration as in the mucosal fluid. It is possible that the movement of anions is linked to movement of sodium, and there may be competition between the various anions present in the intestinal lumen. For some reason, possibly related to their greater lipoid solubility, fatty acids may pass through the intestinal wall more readily than chloride, and such a process could result in building up a concentration gradient of fatty acids against which the movement takes place. This view is consistent with the findings of Wilson (1954) and of Smyth & Taylor (1957*a*) that chloride moves through the intestine at a slower rate than sodium. In the case of some non-electrolytes Höber (1945) has concluded that lipoid solubility may be an important factor in absorption rate, and a similar principle could apply to electrolytes also. It is thus possible to conceive of an active process which might be responsible for fatty acid transfer, but which might not be specifically related to fatty acids.

On the other hand, a consideration of the effect of the inhibitors suggests that fatty acid transfer cannot be entirely explained as a result of sodium or water transfer. The effects of the two inhibitors used differ in some striking ways. Thus while phlorrhizin in the concentration used inhibited both fatty acid transfer and water transfer to a considerable extent, it did not abolish the establishment of a higher concentration of fatty acid on the serosal side than on the mucosal side. For these reasons we might think that the inhibiting effect of phlorrhizin on fatty acid transfer is not a specific one, but is possibly due to reduction of fatty acid movement secondary to the reduction of the water or sodium movement. On the other hand, dinitrophenol exerted a greater inhibiting action on fatty acid transfer than on water transfer, and also abolished completely the establishment of a concentration gradient. Since the two inhibitors had about the same effect on water transfer, this would suggest that there is some process related to fatty acid transfer affected particularly by dinitrophenol. There is clearly a resemblance between fatty acid transfer and amino acid transfer, as Friedlander & Quastel (1955) have shown that the transfer of some L-amino acids *in vitro* is markedly inhibited by dinitrophenol but only slightly by phlorrhizin. It seems safe to conclude that the transfer of fatty acids by the intestine is brought about by processes dependent on the metabolic activities of the intestine and that these processes are not identical with, although they may be related to, those concerned with the transfer of water and sodium.

Elsden *et al.* (1946) found from 0·04 to 0·10% acetic acid in the small

intestine of the rat. The concentrations we have used are of the same order of magnitude, and it is therefore likely that the transfer mechanism we have described plays a physiological role in absorption of short-chain fatty acids.

SUMMARY

1. Experiments are described in which transfer of short-chain fatty acids by an *in vitro* intestinal preparation is described.

2. The transfer takes place against a concentration gradient, is abolished by anaerobic conditions and reduced by absence of glucose. The mechanism is also affected by phlorrhizin and by dinitrophenol.

3. Possible explanations for the transfer of fatty acids against a concentration gradient are discussed.

We are indebted to Mrs M. R. Snowden for technical assistance. The expenses of the work were defrayed by grants from the Medical Research Council and Medical Research Fund of the University of Sheffield. Dr C. B. Taylor was in receipt of a Medical Research Council training grant.

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