SPECIES DIFFERENCES IN THE INTESTINAL RESPONSE TO SULPHATE IONS

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1. Introduction

Considerable interest has been shown in recent years in the effect of the ionic composition of the surrounding medium on the absorption of non-electrolytes by the intestinal mucosa *in vitro.* Although changes in the cationic composition of the medium have been studied in great detail, the effect of the anionic content has been relatively neglected. Bihler and Crane [1] showed several years ago that when hamster intestinal slices were incubated in a chloride-free, sulphate-substituted medium, monosaccharide transport was reduced but by no means abolished, despite the fact that sulphate was apparently unable to penetrate the intestinal epithelial cells. In view of the fact that monosaccharide and amino-acid transport in the intestinal mucosa is coupled to the movement of sodium ions into the cell [2], a re-assessment of these findings and correlation with sodium-pump activity, on which the maintenance of non-electrolyte transport depends, seemed desirable. Furthermore, active sulphate transport has been postulated in certain epithelial tissues, such as the stomach [3, 4] and the kidney [5], and in addition, sulphate has been shown to be transferred preferentially from the mucosal to the serosal fluid across the jejunal mucosa of the tortoise [6]. Hence a simultaneous investigation of sulphate and non-electrolyte movements in the intestine would appear useful. The results reported in the present paper show that although the initial uptake of L-phenylalanine by excised rings of rat and guinea-pig intestine is only slightly reduced when measured in sulphate buffer, the permeability of the two intestines to sulphate ions is rather different. Both species appear to possess a Na^+-dependent transport mechanism for sulphate, but the capacity of that of the rat intestine is markedly inferior to that of the guinea-pig. As a corollary to these results, it is suggested that the rat intestine must possess a markedly stronger sodium pump in the luminal membrane of the epithelial cell than that of the guinea-pig.

2. Methods

Rats and guinea-pigs bred in the Institute were used for all experiments. The methods used for the determination of intestinal transport consisted of the measurement of the uptake of radioactive substrate by excised rings of ileum or the entry of radioactive substrate into the tissue of everted ileal sacs. Incubations of 5 min duration in the labelled medium were employed to determine the intital velocity of uptake by intestinal rings, whereas 60-min incubations were used to ascertain the equilibrium position, as described previously [7]. The sacs were always incubated for 30 min, the radioactive substrate being added solely to the mucosal solution, after which the tissue was divided into mucosal and muscular moieties for separate analysis. The radioactivity in the tissue was determined accroding to our usual method [7, 8] and compared with the specific activity of the incubation medium. Results concerning sulphate uptake are expressed as the ratio (in per cent) of the concentration of radio-sulphate in the tissue water to its concentration in the incubation medium, whereas amino-acid uptake is expressed as μmoles of substrate absorbed by 100 mg tissue (wet weight). The amount of tissue water was determined by desiccating the

Table 1
Uptake of L-phenylalanine by intestinal rings incubated in chloride or sulphate media.

	L-Phenylalanine uptake (μmoles/100 mg)		"t"
	Chloride medium	Sulphate medium	
Initial uptake:			
Rat	0.37 ± 0.019	0.27 ± 0.018	11.72 (6)
Guinea pig	0.37 ± 0.025	0.30 ± 0.017	7.44 (6)
Equilibrium uptake:			
Rat	1.50 ± 0.048	0.82 ± 0.099	7.47 (4)
Guinea pig	1.42 ± 0.128	1.36 ± 0.142	0.53 (6)

Inital uptake determined after 5-min incubations and equilibrium uptake after 60-min incubations. Medium concentration of L-phenylalanine was 5 mM. Values of "t" for paired data from the same animals are given in the final column, the number of observations being shown in parentheses.

tissue for not less than 15 hr at 100° C. Results are expressed throughout ± S.E.M. with the number of separate observations in parentheses.

The incubation media employed were based on Krebs bicarbonate buffer, pH 7.4, prepared in the usual manner [9]. 0.2% Glucose was added to all solutions. In the sulphate medium, all chloride ions were replaced by equimolar quantities of sulphate, and calcium was omitted. When a medium free of sulphate was required, $MgSO_4$ was replaced by $MgCl_2$. A potassium buffer was prepared by replacing all Na^+ ions by equimolar quantities of K^+ ions.

3. Results

The initial uptake of 5 mM [U-^{14}C] L-phenylalanine by both rat and guinea-pig intestinal rings was slightly but significantly depressed when the chloride ions of the incubation medium were replaced by sulphate (table 1). The equilibrium concentration was considerable lowered in the case of the rat, but not in the guinea-pig. It may be observed that the ratio of equilibrium concentrations of phenylalanine in the two species following incubation in the sulphate buffer is similar to the ratio of the uptakes of radiosulphate from the same solution (table 2, column 1, final two rows). This indicates that the sulphate entry does represent a limiting factor in phenylalanine uptake in the rat.

To study the uptake of radiosulphate, three sets of conditions were employed. The tissues were incubated either in the equimolar sulphate solution, labelled with $^{35}SO_4^{--}$, or in a solution containing exactly 5mM labelled sulphate ions, or finally in a sulphate-free solution to which only a tracer dose of rdiosulphate was added. In addition, this final experiment was repeated in Na^+-free, K^+-substituted buffer. The results of these experiments are shown in table 2. It can be seen that the uptake of sulphate by guinea-pig intestine is uniformly greater than by rat intestine, provided sodium ions are present in the incubation medium. When a tracer dose of sulphate is used, a distinct accumulation of sulphate in rings of guinea-pig intestine appears to occur. To verify that this was not an artefact due to isotopic exchange with endeogenous sulphate, an additional experiment was performed using 1×10^{-4}M and 1×10^{-5}M sulphate as carrier. The uptakes by guinea-pig rings from these solutions were 256.1 ± 17.9% (3) and 519.5 ± 89.5% (3) respectively. In addition, this result provides evidence for the saturable nature of the sulphate transport system. Although these results might indicate that one membrane of the mucosal cell is permeable to sulphate whereas the other is impermeable, in analogy with findings in the frog stomach [4], this hypothesis is not borne out by the results using everted sacs, where it can be seen that the radiosulphate passes into the muscular moiety of the tissue, where its concentration is more than half that of the mucosa. The dependence of the uptake on the presence of sodium ions indicated that a mediated pathway for sulphate transfer by the

Table 2
Uptake of $^{35}SO_4$ by excised rings and everted sacs of rat or guinea-pig intestine.

Experimental conditions	Percentage of tissue water occupied		
	Excised rings	Sacs (mucosa)	Sacs (muscle)
Tracer dose of $^{35}SO_4$ in NaCl buffer			
Rat	54.0 ± 2.3 (7)	69.3 ± 13.8 (8)	32.6 ± 7.3 (8)
Guinea pig	270.8 ± 21.7 (11)	141.8 ± 27.2 (10)	79.2 ± 6.9 (10)
Tracer dose of $^{35}SO_4$ in KCl buffer			
Rat	24.6 ± 1.9 (4)	47.6 ± 4.3 (4)	4.5 ± 0.3 (4)
Guinea pig	19.5 ± 3.1 (4)	9.6 ± 2.2 (5)	2.9 ± 1.2 (5)
5 mM $Na_2{}^{35}SO_4$ in chloride buffer			
Rat	21.6 ± 2.5 (6)	8.6 ± 2.0 (4)	5.9 ± 2.0 (4)
Guinea pig	39.5 ± 2.3 (4)	22.6 ± 1.1 (4)	14.0 ± 1.3 (4)
Sulphate buffer labelled with $^{35}SO_4$			
Rat	27.3 ± 3.4 (4)	12.1 ± 3.2 (4)	6.1 ± 1.7 (4)
Guinea pig	33.0 ± 2.2 (4)	10.2 ± 1.2 (4)	6.1 ± 0.5 (4)

Sacs were incubated for 30 min and rings for 60 min. All results are expressed as percentages of the external sulphate concentrations which are to be found in the tissue water at the end of the incubation.

intestine may be involved. Equally, sulphate entry into the rat intestine appears to be a mediated process, since it is slightly dependent on Na^+ ions (the high value of apparent uptake bythe mucosa of sacs incubated in K^+-buffer is almost certainly sue to contamination with radioactive medium, since high-K^+-buffers have a deleterious effect on rat tissue [8] with the result that the mucosa is difficult to wash free of adsorbed solution). Deyrup [10] has also reported Na^+-dependent sulphate transfer across rat ileal sacs. Active accumulation, however, does not seem to occur in this species, although values for the uptake of 1×10^{-4}M and 1×10^{-5}M sulphate by rat rings were 58.1 ± 7.2% (4) and 124.7 ± 9.2% (4) respectively, indicating that at least equilibration can be obtained under favourable circumstances.

Since anion transport in the intestine is generally considered to be secondary to cation transport [11], it might be expected that anion entry would be reduced when Na^+ ions were replaced by K^+ ions, which are not preferentially transported across the intestinal cells. However, experiments with labelled chloride have shown that chloride entry is slightly but insignificantly reduced in K^+-buffer, the values for the per-cent filling of guinea-pig slices after one hour's incubation in 5mM $Na^{36}Cl$ in sulphate buffer being 97.3 ± 12.1 (6) and 78.4 ± 2.0 (4) in sodium and potassium buffers respectively. Note too that 5mM chloride equilibrates with the tissue water, whereas 5mM sulphate does not, a results which suggests limited permeability characteristics for sulphate, even in the guinea-pig intestine. From these various results, sodium-dependent sulphate transport in the guine-pig ileum and to a lesser extent in the rat appears to be assured.

The foregoing results tend to show that the intestine of the guinea-pig is considerably more permeable to sulphate than is the intestine of the rat. This is further supported by the fact that the intestine of the guinea-pig gains water when incubated in sulphate medium, whereas the rat intestine is unaffected. Values of the water content of intestinal

slices after 60-min incubation in the two media are as follows: Rat: chloride, 80.6 ± 0.16% (24); sulphate, 81.1 ± 0.37% (24); Guinea-pig: chloride, 82.3 ± 0.18% (32); sulphate, 84.5 ± 0.23% (32).

4. Discussion

The most intriguing aspact of the present results concerns their theoretical implications. As stated above, under normal conditions, the sodium ion that accompanies the entry of sugar or amino-acid into the epithelial cell is also accompanied by the passive entry of an anion. Hoever, the permeability of the rat intestinal epithelium to sulphate appears to be considerably less than that of the guinea-pig, yet the reduction of the initial uptake of amino-acid in sulphate medium is of the same order of magnitude in both species. In order to maintain the electroneutrality of the entry mechanism, this finding must indicate that the sodium ion that enters the cell under these conditions must be immediately expelled again by an electrogenic sodium pump situated in the same luminal membrane which extrudes the sodium in the same direction from which it has come. This proposition agrees with results obtained on measuring diffusion potentials across the intestinal mucosae of these two species [12]: an electrogenic sodium pump directed towards the lumen had to be postulated in the luminal membrane of the rat intestine, whereas it was weak or absent from the epithelium of the guinea-pig intestine. In view of the relatively high permeability of the guinea-pig luminal membrane to sulphate, it would not be necessary to propose the existence of such a pump in this species to explain the results concerning the uptake of phenylalanine in sulphate buffer.

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