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The use of isolated cells to assess the contribution of the mucosal epithelium to the metabolism of the intestinal wall

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SUMMARY We have used suspensions of isolated cells to study the metabolism of the mucosal epithelium and to compare its activity with that of other tissues in the intestinal wall of guinea pig. Only 4% of the total glycolytic activity of the intestinal wall could be attributed to the mucosal epithelium. In contrast, about 76% of the activity was located in the intestinal muscle and the remaining 20% in the intervening tissue.

Clearly the view that the major proportion of the glycolytic activity of the small intestine resides in the mucosal epithelium does not apply to the guinea pig. In the light of our results, it would be prudent to re-examine any conclusions about the distribution of metabolic activity throughout the gut wall if the supporting evidence has been drawn from experiments with mucosal homogenates.

The metabolism of the small intestinal mucosa has usually been studied using homogenates of mucosal scrapings (Dickens and Weil-Malherbe, 1941; Srivastava and Hübscher, 1966; Clark and Sherratt, 1967) and it has become customary to assume that the values obtained are due to activities of the mucosal epithelium. This assumption is not justified, however, as the epithelium may represent only a relatively small proportion of the mucosal scrape.

Recently, methods have been developed for isolating intact and viable epithelial cells from the small intestines of experimental animals (Evans, 1969; Kimmich, 1970; Iemhoff, Van Den Berg, De Pijper, and Hülsmann, 1970; Evans, Wrigglesworth, Burdett, and Pover, 1971). We have been using such preparations to study the metabolism of the mucosal epithelium when it is uncontaminated by other gut tissue.

Materials and Methods

ANIMALS AND DIETS

Female albino guinea pigs of 600 to 800 g were used. They were fed a diet of SG1 pellets (Pilsbury's Ltd, Birmingham), hay, water, and cabbage.

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PREPARATION OF CELL SUSPENSIONS FROM THE INTESTINAL WALL

Epithelial cells

These cells were isolated after incubation of the intestine with a buffered EDTA solution as described by Evans *et al* (1971), and suspended in a solution of 200 mM mannitol, 76 mM Na_2HPO_4, 19 mM KH_2PO_4, *p*H 7·4 (Fig. 1a).

Subepithelial mucosal tissue

After the mucosal epithelium had been removed with EDTA, the remaining mucosal tissue, referred to in this paper as 'subepithelial mucosal tissue', was extruded from the intestine by gently scraping a wooden spatula along the outside of the segment. The tissue was suspended in the phosphate-buffered mannitol solution and was dispersed by gentle withdrawal into a 20 ml syringe fitted with a needle of bore size 2 mm (Fig. 1b).

Muscle

The remaining muscular layer (Fig. 1c) was cut into small rings about 3 mm thick and suspended in the buffered mannitol solution.

PREPARATION OF MUCOSAL SCRAPES

The mucosa was scraped from segments of intact,

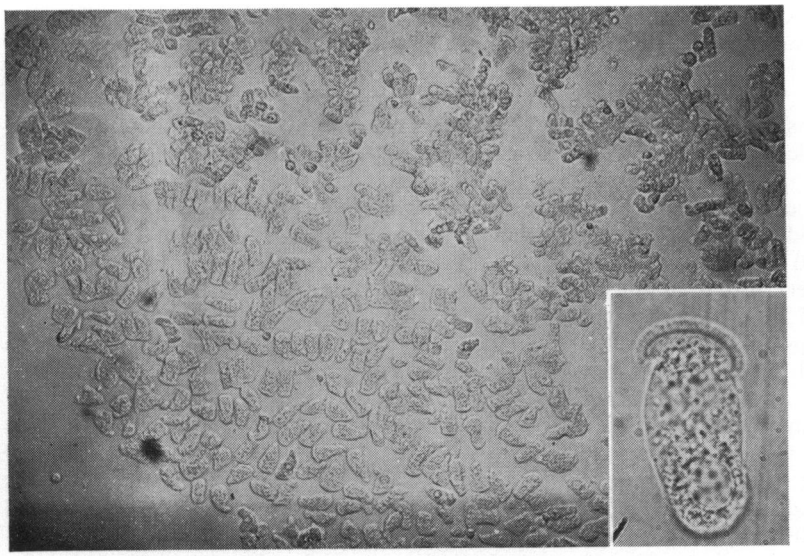

Fig. 1a

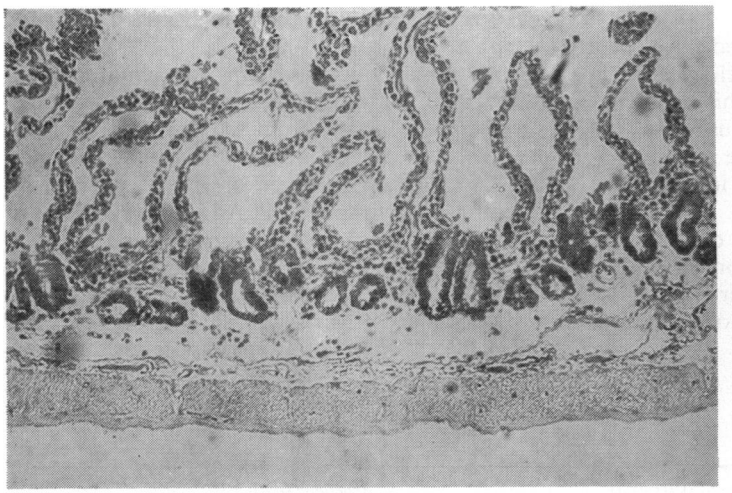

Fig. 1b

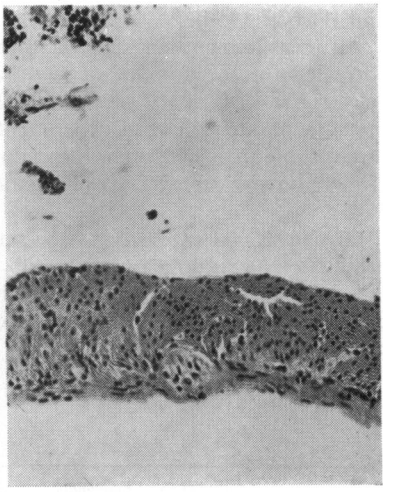

Fig. 1c

Fig. 1 *Photomicrographs of tissue preparations from the intestinal wall.*

a *Suspension of epithelial cells in the phosphate-buffered mannitol solution one to four hours after isolation from the small intestine* (× 150). *The isolated cells retained a columnar shape and nuclei and brush borders could be easily identified* (*inset*, × 400).

b *Section of intestinal wall* (× 60) *after removal of mucosal epithelial cells. The villous substructure remained intact and there was no significant loss of subepithelial cells. Tissue samples were fixed in formol-saline and embedded in paraffin wax. Sections were stained with aqueous solutions of eosin and haematoxylin.*

c *Section of intestinal wall* (× 120) *after removal of the sub-epithelial mucosal tissue* (*see Methods*). *Only the muscle layers remained. Sections were prepared and stained as in Figure* 1b

washed intestine and treated thereafter in the same way as described above for the subepithelial mucosal tissue.

MEASUREMENT OF GLYCOLYSIS

The rate of production of lactic acid was used as a measure of glycolytic activity. Samples containing about 80 mg cell protein were incubated for one hour at 37°C with or without the addition of sugars. The cell suspensions were incubated in equilibrium with air and all solutions used were pregassed for 10 minutes with O_2:CO_2 (95%:5%). This procedure was sufficient to keep the suspensions oxygenated for several hours. To prevent cell damage (see Evans *et al*, 1971) samples were not agitated continually during incubation but were intermittently shaken gently by hand. The reaction was stopped by the addition of fluoride and the suspension rapidly homogenized with a syringe and needle. The homogenate was deproteinized with 0·1 ml perchloric acid (final concentration 5%) and after standing for 60 minutes at 4°C the protein precipitated was sedimented by centrifugation. The lactic acid content of 0·1 ml samples of the supernatant was determined enzymatically by the method of Hohorst, Kreutz, and Bücher (1959).

All measurements of lactic acid production were corrected for the amount of lactic acid present in cells at the start of the incubation. This was determined separately in each experiment using at least three samples of cells. The procedure was as described above except that there was no incubation at 37°C.

Glycolysis was expressed as the rate of lactic acid production (mμmoles lactic acid produced per milligram cell protein per hour) either from endogenous substrates or in the presence of exogenous glucose or fructose.

MEASUREMENT OF OXYGEN UPTAKE

The rate of oxygen uptake by 2 ml samples of the cell suspensions (5 to 30 mg cell protein) was measured at 37°C using a Clark oxygen electrode (Yellow Springs Instrument Co, Ohio, USA). The current output from the electrode was calibrated using soiutions of known oxygen content and results expressed as microlites of oxygen taken up per milligram cell protein per hour (Q_{O_2}).

MEASUREMENT OF PROTEIN

Protein was measured using a modified biuret method (Hübscher, West, and Brindley, 1965). In respiration experiments the samples of cell suspensions which had been used for the measurement of oxygen uptake were homogenized and duplicate samples of the homogenate taken for protein determination. In glycolysis experiments protein was measured in at least four samples of cell material and the results used to calculate the amount of cell protein in the wet weight of those samples taken for lactic acid measurement.

Results

AEROBIC GLYCOLYSIS IN DIFFERENT TISSUES OF THE INTESTINAL WALL

When the mucosal epithelium was removed with EDTA and the subepithelial mucosal tissue was extruded mechanically from the residual intestinal muscle, the three preparations were found to contain 45%, 20%, and 35% respectively of the total protein present in the intestinal wall. All three preparations produced lactic acid both from endogenous sources and, at a higher rate, when glucose (25 mM) was added (Table I). The glycolytic rate in the presence of exogenous glucose was obviously the lowest in the epithelial cell suspensions and in terms of total

Tissue	*Total Protein from three Animals (mg)*	*Endogenous Lactic Acid Content (mμmoles/mg cell protein)*	*Aerobic Glycolysis*			*Anaerobic Glycolysis*		
			Rate of Lactic Acid Production (mμmoles/mg cell protein/hour)		*Percentage Total Lactic Acid Production by Gut Wall in the Presence of 25 mM Glucose*	*Rate of Lactic Acid Production (mμmoles/mg cell protein/hour)*		*Percentage of Total Lactic Acid Production by Gut Wall in the Presence of 25 mM Glucose*
			Endogenous (No Added Glucose)	*In the Presence of 25 mM Glucose*		*Endogenous (No Added Glucose)*	*In the Presence of 25 mM Glucose*	
Epithelial cells	1228 ± 25	4·6 ± 0·27	6·5 ± 0·45	13·7 ± 0·53	3·96	7·3 ± 0·32	11·7 ± 1·99	4·69
Sub-epithelial mucosal tissue	554 ± 10	46·8 ± 2·6	62·5 ± 2·48	138·7 ± 5·37	19·69	63·6 ± 1·98	112·5 ± 4·90	20·36
Muscle	1074 ± 37	97·2 ± 3·52	89·8 ± 4·93	277·4 ± 4·89	76·35	103·4 ± 4·36	213·6 ± 4·75	74·95

Table I *Glycolytic activity of different tissues from the intestinal wall*[1]

[1]Values are means ± SEM and are calculated from the results of three experiments in each of which cells from three animals were pooled and triplicate sample :were incubated under each set of conditions.

activity of the intestinal wall represented approximately 4%. The subepithelial mucosal tissue contributed a further 20% and the intestinal muscle the remaining 76%.

These results contrasted markedly with previously published values which showed that the intestinal mucosa had considerably more glycolytic activity than the muscle from which it had been scraped (Wilson, 1954; Sherratt, 1968). As the glycolytic rate for our muscle preparations was similar to other values reported for guinea pigs (Sherratt, 1968) we had to consider the possibility that the incubation with EDTA was damaging the mucosal epithelium and the tissue immediately underlying it, thereby reducing the capacity of these tissues for glycolysis. This possibility was discounted by the results in Table II, in which the composite values for glycolysis in epithelial cells plus subepithelial mucosal tissue are compared with those obtained using the conventional mucosal scrape technique, where the gut is not exposed to EDTA at all. The two preparations gave almost identical values.

A consideration of the results in Table II emphasizes the hazard in ascribing results obtained with mucosal scrapes to the activity of the mucosal epithelium alone; the subepithelial mucosal tissue, which must form a significant part of all mucosal scrapes, contributed 31% of the protein and 81% of the glycolytic activity in these experiments.

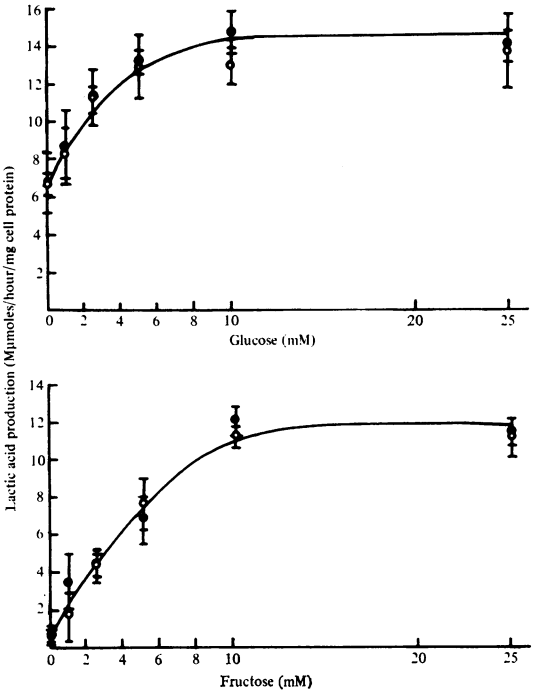

Fig. 2 *Lactic acid production by epithelial cells: the response to the addition of glucose or fructose.*

The values plotted are means ±SEM for five samples of cell suspension and have been corrected for endogenous lactic acid content (see Table I). ○—○ *suspensions of intact cells,* ●—● *suspensions of cells that had been mildly stressed to increase membrane permeability.*

EFFECTS OF GLUCOSE AND FRUCTOSE ON THE RATE OF AEROBIC GLYCOLYSIS

The glycolytic activity of isolated epithelial cells was stimulated by glucose or fructose as shown in Fig. 2, with maximum values produced when the sugar concentration was greater than 10 mM. It was for this reason that glucose-stimulated glycolysis was measured in the presence of 25 mM glucose.

Identical results were obtained when the rate of glycolysis in suspensions of intact cells was compared with that in cells that had been mildly stressed by withdrawal through a syringe needle of 0·7 mm bore (Evans *et al*, 1971) to increase their membrane permeability (Fig. 2). The exposure to EDTA had not therefore so affected the epithelial cell plasma membrane that the pasage of sugars through it became rate limiting to glycolysis.

Tissue	*Total Protein from Three Animals (mg)*	*Endogenous Lactic Acid Content (mμmoles/mg cell protein)*	*Rate of Aerobic Lactic Acid Production (mμmoles/mg cell protein/hour)*	
			Endogenous (No Added Glucose)	*In the Presence of 25 mM Glucose*
Mucosal scrapings	1809 ± 96	14·9 ± 0·37	18·9 ± 0·46	53·2 ± 2·10
Epithelial cells + subepithelial mucosal tissue	1782 ± 23	18·3 ± 0·76	23·9 ± 0·69	52·6 ± 2·35

Table II *Comparison of results for the glycolytic activity of the intestinal mucosa obtained with mucosal scrapings and isolated epithelial cells plus subepithelial mucosal tissue*[1]

[1]Values are means ± SEM and are calculated from the results of three experiments in each of which triplicate samples were incubated under each set of conditions.
The data for epithelial cells plus subepithelial mucosal tissue were calculated from the results in Table I and are not significantly different from the values obtained with mucosal scrapings ($P > 0.10$).

Sodium iodoacetate (10^{-4}M) and sodium fluoride (10^{-4}M) completely inhibited lactic acid production from endogenous and exogenous substrates.

COMPARISON OF THE AEROBIC GLYCOLYSIS RATE IN MUCOSAL EPITHELIAL CELLS FROM DIFFERENT REGIONS OF THE SMALL INTESTINE

In our experiments the suspensions of isolated epithelial cells normally consisted of a mixture of cells from the whole length of the small intestine. It was possible that this mixing obscured any variation in glycolytic activity down the small intestine and that in some regions the contribution of the mucosal epithelium to the total activity of the gut wall was more nearly that reported by other workers. However, when the intestine was divided into four segments of equal length from which the mucosal epithelial cells were isolated separately, we found no significant difference between the rates of glycolysis in the four samples.

ANAEROBIC GLYCOLYSIS IN DIFFERENT TISSUES OF THE INTESTINAL WALL

When glucose-stimulated glycolysis was measured under anaerobic conditions, ie, in an atmosphere of high-purity nitrogen, the relative contribution of each of the three preparations from the whole intestinal wall was comparable with that obtained aerobically (Table I). Thus the mucosal epithelium was again the minor contributor, with values not significantly different from those obtained with aerobic incubation, ie, no Pasteur effect.

RESPIRATORY ACTIVITY

The rate of oxygen uptake (Q_{O_2}) and the length of time for which the initial rate was maintained were not significantly different in mucosal epithelial cells isolated from various segments of small intestine. We did not therefore overlook regional differences in respiratory activity by the normal practice of pooling cells from the entire length of the small intestine.

The respiratory rates for the three layers of the intestinal wall are summarized in Figure 3. In contrast to the glycolysis results, here the mucosal epithelium had the highest activity and the muscle the lowest. An additional difference was the failure of glucose and fructose to stimulate the respiratory rate of any of the three types of preparation. If the sugars were added to epithelial cells that had been mildly stressed there was still no increase in their rate of respiration so once again membrane permeability was not limiting metabolism.

The Q_{O_2} values for stressed cells were always lower than those for intact preparations (Evans *et al*, 1971) which indicate a greater susceptibility to damage than was shown by the glycolysis data.

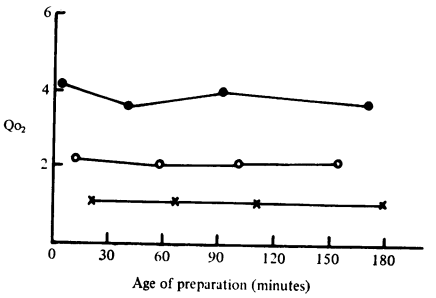

Fig. 3 *Oxygen uptake by different tissues from the intestinal wall.*

The respiratory activity of tissue samples is expressed by a Q_{O_2} value (μlitres O_2 taken up/milligram cell protein/hour).

●—● *epithelial cells,* ○—○ *subepithelial mucosal tissue,* ×—× *muscle.*

Discussion

A great deal of the published work on the metabolism of the intestinal absorptive cell has been based on heterogeneous preparations such as mucosal scrapes (Dickens and Weil-Malherbe, 1941; Srivastava and Hübscher, 1966; Clark and Sherratt, 1967). This paper describes work on the metabolism of a relatively homogeneous preparation of the mucosal epithelium from guinea pig. It reflects our view that much previously published work may be in error because the contribution to the results of tissues other than the mucosal epithelium has either been ignored or assumed to be insignificant.

The results in Tables I and II form the basis of this opinion. When the mucosal epithelium was removed from the intestine by incubation with EDTA, the rate of aerobic glycolysis was found to be low. The remaining intestinal wall was subdivided into subepithelial mucosal tissue and muscle, both of which showed a higher rate of glycolysis per milligram protein than the epithelium, and a much greater contribution to the total glycolytic activity of the intestinal wall. Other authors have come to a quite different conclusion using guinea pig (Sherratt, 1968) and other experimental animals (Wilson, 1954; Srivastava and Hübscher, 1966; Sherratt, 1968), namely, that most of the glycolytic activity of the gut wall is to be found in the mucosa, with less in the muscle. A comparison of the results in Table I with data from the more conventional mucosal scrape (Table II), precluded damage to the mucosal epithelium by EDTA as the reason for its low glycolytic rate. This observation has the twofold advantage of increasing our confidence in the value of isolated epithelial cells for the study of glycolysis in particular

and of cell metabolism in general. Isolated epithelial cells were able to increase their rate of glycolysis with increasing sugar concentration (Fig. 2) and to respond to inhibitors such as sodium fluoride and iodoacetate. Glycolysis was unimpaired when cells were mildly stressed, and the fact that the addition of sugars to these preparations gave the same degree of stimulation to lactic acid production as in unstressed cell suspensions encouraged our belief that EDTA incubation had not radically altered the permeability of the plasma membrane to sugars. Taken collectively, these results indicate that the rate of glycolysis was not artificially low and that the intact epithelial cell, of the guinea pig at least, produces little lactic acid in comparison with the underlying tissues. The high values reported previously in the literature may in part result from the use of homogenized preparations in which intracellular control mechanisms that depend on compartmentation become deranged.

Additional evidence that the epithelium was not so damaged by the isolation procedure as to suppress its metabolism was obtained with the oxygen uptake measurements. Here the mucosal epithelium had the highest rate of the three preparations from the gut wall. Exogenous sugars did not increase the Q_{O_2} value and their failure to do so was not due to decreased membrane permeability.

The low rate of glycolysis in the epithelial cells coupled with the oxygen uptake data are additional evidence (MS in preparation) that the aerobic metabolism of the guinea pig absorptive cell depends on substrates other than carbohydrates. It also suggests (Table III) that the mucosal epithelium in this animal at least does not have the metabolic characteristics of tumours (Dickens and Šimer, 1930), ie, a low respiratory quotient coupled with a high rate of glycolysis.

However, the small intestinal mucosa taken as a whole, ie, the epithelial cells plus subepithelial mucosal tissue, did not show a Pasteur effect and in this respect it appears to be different from other normal tissues (Dickens and Weil-Malherbe, 1941).

The suspensions of epithelial cells were always slightly contaminated with cells from other parts of the gut wall (Evans *et al*, 1971). It is possible, therefore, that the rate of glycolysis for the mucosal epithelium alone may be even lower, and the rate of oxygen uptake rather higher, than the values recorded here.

Inasmuch as the isolated cells represent both an intact and a relatively pure preparation of mucosal epithelium, we think they could be used to reassess the value of previous findings, especially where these were based on results from homogenized mucosal scrapings.

Tissue	*Protein* (%)	*Glycolysis* (%)	*Respiration* (%)
Epithelial cells	45	4	71
Subepithelial mucosal tissue	20	20	12
Muscle	35	76	17

Table III *The distribution of metabolic activities in different tissues of guinea pig small intestine*[1]

[1]The protein and glycolysis values were derived from the data in Table I and the respiration values from Figure 3. In each calculation the activity of the whole intestinal wall was taken as 100%.The glycolysis measurements selected for comparison were those obtained after aerobic incubation in the presence of 25 mM glucose.

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