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THE EFFECTS OF PHENFORMIN ON THE TRANSPORT AND METABOLISM OF SUGARS BY THE RAT SMALL INTESTINE

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Abstract—The effects of 0.25–10 mM phenformin on sugar transport and metabolism have been studied in a preparation for the combined perfusion of the vascular bed and the lumen. At all concentrations the effects of vascular phenformin were more pronounced than those of luminal phenformin. Phenformin inhibited galactose transport across the intestine, the pattern of inhibition depending on whether the phenformin was added to the luminal or vascular compartments. The active accumulation of galactose in the mucosal epithelial cells was also abolished. There was a linear relationship between the percentage reduction in mucosal ATP levels and vascular phenformin concentration. Phenformin reduced the rate of glucose uptake from the lumen, and the proportion of this glucose which reached the vascular effluent. Most of the glucose which did not reach the vascular side could be accounted for by the formation of lactic acid. Vascular phenformin increased glucose uptake from the vascular medium by *ca* 88%, 97% of which could be accounted for by lactate formation. Phenformin was sequestered by the mucosa when added to the vascular, but not the luminal, perfusates. There was very little translocation of intact phenformin across the gut in either the mucosal or serosal directions. It is suggested that the effects of phenformin on the gut mainly derive from an inhibition of mitochondrial oxidative phosphorylation, with a small contribution from a direct effect on the brush border, more pronounced at high phenformin concentrations. The results are consistent with the idea that phenformin delays sugar absorption in man, and that the intestine may be a significant source of lactate production in lactic acidosis.

One of the main sites of action of the oral hypoglycaemic agent phenformin is the small intestine. The evidence for this is derived from numerous studies *in vivo* and *in vitro*. Phenformin improves tolerance to oral glucose in normal and diabetic humans, dogs and rats [1–3]. Glucose absorption is decreased in everted gut sacs prepared from animals pretreated with phenformin *in vivo* [3, 4], and Wick *et al.* [5] showed in rats that after oral administration of [^{14}C]phenformin, much of the label remained in the stomach and intestines. These findings were confirmed by Hall *et al.* [6], and strong binding of phenformin by the gut has also been found during *in vitro* experiments [7, 8]. Further *in vitro* studies using a variety of preparations have shown that phenformin can inhibit the intestinal absorption of glucose, galactose, amino acids and fluid [3, 7–11]. The concentrations of phenformin used in these experiments were mostly in the range 0.5–10 mM, at which there is a decrease in the oxygen uptake and ATP content of the tissue [11]. Thus it has been suggested that impaired intestinal absorption is secondary to the inhibition of ATP formation caused by the high local concentrations of phenformin in the gut. However, Kessler *et al.* [12] reported inhibition of glucose transport into intestinal microvillar membrane vesicles, which are devoid of ATP, by 2.5 mM phenformin, suggesting a direct effect of biguanides on the brush-border glucose carrier.

In an attempt to resolve these differing viewpoints, we have studied the effect of phenformin using a preparation for the combined vascular and luminal perfusion of the rat small intestine. Although vascular perfusion of the intestine is more time-consuming than conventional *in vitro* techniques, it offers considerable advantages since the vascular flow improves tissue oxygenation and prevents substances taken up from the lumen accumulating to unphysiologically high concentrations in the epithelial cells. It has enabled us to study both the transport and metabolism of sugars by the gut, and to examine the effects of phenformin administered either to the basal pole of the epithelial cells via the vascular perfusate, or to the brush-border membrane in the luminal perfusate.

MATERIALS AND METHODS

Lactate dehydrogenase (EC 1.1.1.27), NAD^+, together with the test combinations (GOD–Perid and ATP UV method) used in the determination of glucose and ATP were from Boehringer. Radiochemicals were supplied by Amersham International. Bovine serum albumin (fraction V) was supplied by Sigma. Phenformin was donated by Dr. M. A. Cawthorne of Beecham Pharmaceuticals. Male Wistar rats (240–260 g) were used in all the experiments. They were kept on a 12 hr day/night cycle (light from 7 a.m. to 7 p.m.) and were allowed free access to food (Oxoid, diet 41B) and water, until 2 hr before the perfusion. They were taken to the laboratory at *ca* 11.30 a.m., and all experiments were performed between 1.00 and 3.00 p.m.

The technique for the vascular perfusion of the

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intestine has recently been described [13]. The method is based on that of Hanson and Parsons [14] as modified by Bronk and Ingham [15]. Further modifications were made to minimize vasoconstriction in the vascular bed [13]. The vascular perfusion medium was a modified Krebs–Henseleit bicarbonate medium with the following composition (mM): NaCl, 118; KCl, 4.74; KH_2PO_4, 1.18; $MgSO_4$, 1.18; $CaCl_2$, 1.27; $NaHCO_3$, 24.88, gassed with O_2/CO_2 (19:1), final pH 7.4. This was supplemented with 1.5% w/v dialysed bovine serum albumin (fraction V) and washed bovine erythrocytes (25% haematocrit). The luminal perfusate was unsupplemented Krebs–Henseleit medium.

The vascular perfusate was introduced into the superior mesenteric artery at a rate of 2 ml/min and emerged via a cannula in the hepatic portal vein. It was not recirculated. The luminal perfusate was interrupted with bubbles of O_2/CO_2 (19:1) (segmented circulation) to give increased stirring in the lumen and improved oxygenation of the epithelial tissue [16]. It was recirculated through the lumen at a total flow rate of 10 ml/min (7 ml/min perfusate and 3 ml/min gas). The segment of intestine extended 30–40 cm distally from a point 5 cm below the ligament of Treitz, i.e. comprised the jejunum and upper ileum. Most of the experiments lasted for 30 or 40 min. Radioactive or non-labelled substrates were added to the luminal or vascular perfusates as indicated in the Results. Serial samples of media were deproteinized with ice-cold 0.6 M $HClO_4$ and analysed directly for radioactivity, or after neutralization with 30% K_2CO_3 for metabolites. In some experiments, samples of mucosal tissue were removed by scraping with a glass slide, and also deproteinized with 0.6 M $HClO_4$.

At the end of each experiment, the perfused section of intestine was removed from the animal and its length measured. A standard curve of length vs wet and dry weight was determined to enable the results for glucose fluxes and rates of lactate appearance to be expressed as nmole/min per g dry wt. The regression equation for this was

$$\text{Dry weight (g)} = 0.020 \times \text{length (cm)} - 0.029.$$

Mucosal ATP levels were expressed as concentration (mM) in the tissue water. Glucose was determined by a glucose oxidase method (GOD–Perid) using an autoanalyser. Lactic acid was measured using the automated fluorimetric method of Leese and Bronk [17]. ATP was measured enzymatically. The concentration of phenformin in perchloric acid extracts was determined by a modification of the colorimetric method of Freedman *et al.* [18], incorporating the modifications of Heuclin *et al.* [19]. The results are expressed as means ± S.E.M. with numbers of values (n) in parentheses. The statistical significance of differences between means was determined by Student's t-test.

RESULTS

The effect of phenformin on sugar transport was studied in experiments in which the luminal perfusate contained 5 mM D-[1-^{3}H]galactose, and the appearance of the sugar in the vascular effluent was monitored. D-Galactose was chosen because it is actively transported but not metabolized by the rat small intestine, so enabling the effects of phenformin on transport to be distinguished from those on metabolism. D-Glucose (5 mM) was added to the vascular medium since this enhances galactose transport and better mimics the physiological situation [13].

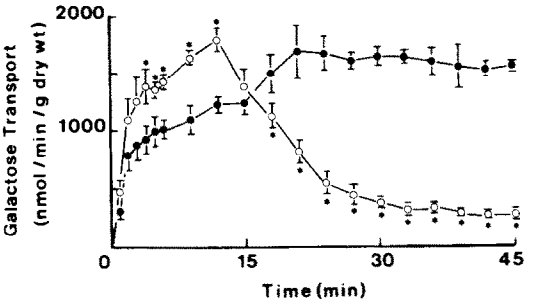

Fig. 1. Effects of 5.0 mM vascular phenformin on the rate of D-galactose appearance in the vascular effluent. Results are means ± S.E.M.; n = number of observations. (●) Control (n = 3), (○) 5.0 mM vascular phenformin (n = 3). The vascular perfusate contained 5 mM D-glucose. D-[1-^{3}H]Galactose (5 mM) was present in the luminal perfusate. *Indicates values significantly different ($P < 0.05$) from the corresponding control.

Vascular phenformin

Experiments were performed with 0.25, 1.0, 5.0 and 10.0 mM phenformin in the vascular perfusate. The pattern of phenformin action was similar at each concentration, and is well-illustrated by the data in Fig. 1 obtained with a phenformin concentration of 5 mM. The rate of D-galactose appearance in the vascular perfusate was greater than the control during the early part of the perfusions, but then fell below that of the control. This inhibition of active D-galactose uptake was reflected in the levels of the sugars in the mucosal tissue after 45 min perfusion (Table 1).

Luminal phenformin

Phenformin was added to the luminal perfusate at concentrations of 1.0, 5.0 and 10.0 mM. The effect of 5 mM phenformin is shown in Fig. 2, the pattern of which was similar to that of 1 mM phenformin. D-Galactose appearance in the vascular perfusate was similar to the control during the first 15 min, but then fell significantly below the control. With 10 mM phenformin (data not shown), the pattern was similar to that observed with vascular phenformin, i.e. D-galactose appearance was stimulated during the early part of the experiment, but then declined after approximately 15 min. Analysis of the mucosal tissue after perfusion (Table 1) showed that galactose accumulation was inhibited by increasing concentrations of luminal phenformin, but not as dramatically as with the equivalent concentrations of vascular phenformin. Only at 10 mM phenformin was the active accumulation of D-galactose abolished (i.e. D-galactose concentration in the lumen > D-galactose concentration in the tissue).

Table 1. Effects of vascular and luminal phenformin on D-galactose uptake from the luminal perfusate and sugar concentrations in intestinal tissue samples taken after 45 min perfusion

Phenformin in vascular perfusate (mM)	Phenformin in luminal perfusate (mM)	n	Average rate of D-galactose uptake from lumen (nmole/min per g dry wt)	Mucosal tissue D-galactose (mM)
0	—	3	1885 ± 94	10.45 ± 1.51
0.25	—	4	1646 ± 172	7.71 ± 0.96
1.00	—	3	1125 ± 57	4.22 ± 0.28*
5.00	—	3	844 ± 83*	3.02 ± 0.06*
10.00	—	5	880 ± 115*	2.70 ± 0.29*
—	0	3	1885 ± 94	10.45 ± 1.51
—	1.0	3	1713 ± 229	9.27 ± 0.69
—	5.0	3	1021 ± 94*	6.40 ± 0.56*
—	10.0	6	1005 ± 161*	4.41 ± 0.40*

D-Glucose (5 mM) was present in the vascular perfusate. The luminal perfusate initially contained 5 mM D-[1-^{3}H]galactose. Results are means ± S.E.M.; n = number of observations.

* Indicate values which are significantly different ($P < 0.05$) from the corresponding control values.

Effects of phenformin on mucosal ATP levels

Mucosal tissue samples from the above experiments, taken after 45 min perfusion, were assayed for their ATP content. At all the concentrations used vascular phenformin gave significant reductions in the ATP content, with a good correlation ($R = 0.88$) between the reduction in ATP levels (expressed as a percentage of the control) and $\log_{10}$ phenformin concentration (Fig. 3). Luminal phenformin had very little effect on the ATP levels, and a significant reduction [from 2.51 ± 0.36 mM ($n = 3$) in the controls to 1.67 ± 0.18 mM ($n = 6$)] was only apparent with 10 mM phenformin.

Effect of phenformin on the transport and metabolism of glucose

Glucose (10 mM) was added to the luminal perfusate after 10 min perfusion with 5 mM glucose present in the vascular perfusate throughout. Phenformin (5 mM) was added to the vascular or luminal perfusates from the start of the experiments.

(a) *Absence of phenformin.* The results of these and other experiments with differing luminal glucose concentrations have been described previously [13]. In the absence of luminal glucose, there was a steady uptake of glucose from the vascular perfusate of 3604 ± 349 nmole/min per g dry wt, which fell after the addition of 10 mM glucose to the lumen to an average rate of 2172 ± 432 nmole/min per g dry wt (Fig. 4). In other words, there was a background of glucose uptake from the vascular side, upon which was superimposed a glucose flux from the lumen. It should be emphasized that a net glucose appearance in the vascular perfusate (i.e. negative values in Fig. 4) was not observed at these initial glucose concentrations. Such an appearance could only be detected if the vascular medium was initially glucose-free [13].

(b) *Vascular phenformin.* With 5 mM phenformin in the vascular perfusate, the average rate of glucose uptake during the period 0–10 min (i.e. prior to glucose addition to the lumen) was 6770 ± 375 nmole/min per g dry wt, which was approxi-

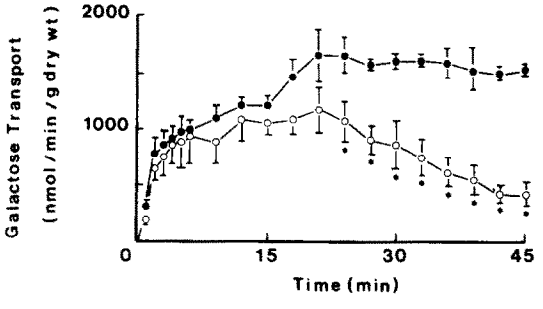

Fig. 2. Effect of 5.0 mM luminal phenformin on the rate of D-galactose appearance in the vascular effluent. (●) Control ($n = 3$). (○) 5.0 mM luminal phenformin ($n = 3$). Remainder of legend as for Fig. 1.

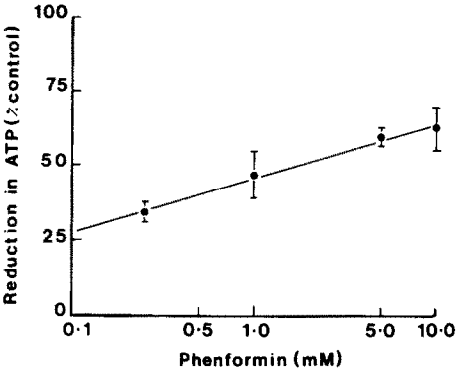

Fig. 3. Reduction of ATP concentration in mucosal tissue samples taken after 45 min perfusion with phenformin present in the vascular perfusate. The reduction in ATP concentration is expressed as a percentage of the concentration in mucosal tissue samples taken after 45 min perfusion with no phenformin present. ATP concentration in control tissue = 2.15 ± 0.36 mM (3). Results are mean ± S.E.M. The vascular perfusate contained 5 mM D-glucose. D-Galactose (5 mM) was present in the luminal perfusate.

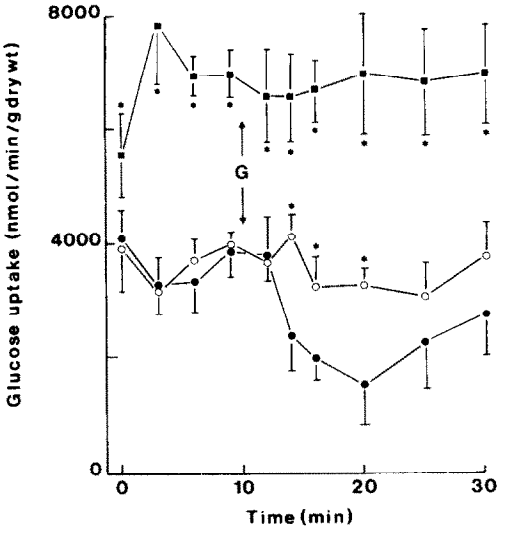

Fig. 4. Effect of 10 mM luminal glucose on the rate of glucose uptake from the vascular perfusate in the presence of phenformin. Glucose (10 mM) was added to the luminal perfusate after 10 min perfusion. Phenformin and 5 mM vascular glucose were present throughout the experiments. **G**, Glucose (10 mM) added to lumen; (●) control, no phenformin ($n = 5$); (■) 5 mM vascular phenformin ($n = 6$); (○) 5 mM luminal phenformin ($n = 4$). All results are means ± S.E.M.; n = number of observations. *Indicates values significantly different ($P < 0.05$) from the corresponding control.

mately 88% higher than in the absence of phenformin, and was maintained for the duration of a normal perfusion. After addition of glucose to the lumen, none of the values for vascular glucose uptake differed significantly from the average preincubation level.

(c) *Luminal phenformin.* In the presence of 5 mM luminal phenformin, the average rate of glucose uptake from the vascular perfusate during the preincubation period (0–10 min) was 3677 nmole/min per g dry wt, which was not significantly different from the rate of uptake observed in perfusions without luminal phenformin. After addition of glucose to the lumen, vascular glucose uptake did not fall significantly from the average preincubation level.

Lactate formation

The lactate output in these three experimental situations is shown in Table 2.

In the absence of phenformin, lactate output to the vascular bed was significantly increased from a steady rate of 3417 ± 156 nmole/min per g dry wt (0–10 min) to 6896 ± 797 nmole/min per g dry wt (10–30 min) after the addition of 10 mM glucose to the lumen. The rate of lactate output to the luminal perfusate was also significantly greater when 10 mM glucose was present in the lumen.

During the preincubation period, lactate output into the vascular perfusate was *ca* 60% greater with vascular phenformin. After addition of glucose to the lumen, vascular lactate output was increased to an average level of 8536 nmole/min per g dry wt, though this was not significantly greater than that observed in the absence of phenformin. The corresponding increases in luminal lactate were all, however, significant.

Lactate outputs into the vascular and luminal fluids with 5 mM phenformin in the lumen were not significantly different from the controls.

These data on the effect of phenformin on the transport and metabolism of glucose are summarized in Table 3 using the assumptions described by Nicholls *et al.* [13]. Both 5 mM vascular and 5 mM luminal phenformin significantly reduced the rate of glucose uptake from the lumen, and the proportion of this glucose uptake which reached the vascular effluent. The effect of vascular phenformin was greater than that of luminal phenformin. In the presence of luminal phenformin, 88% of the glucose taken up from the lumen which did not reach the vascular side could be accounted for by lactate appearance in the luminal and vascular perfusates. The figure for vascular phenformin was 112%, but for the controls only 50%.

Uptake of phenformin by the intestine

Phenformin levels were measured in samples of mucosa after 30 min perfusion with either vascular or luminal phenformin (0.25–10 mM). D-Glucose (5 mM) was present in the vascular perfusate.

(a) *Vascular phenformin.* With perfusate concentrations between 0.25 and 5 mM, the drug was accumulated, or sequestered in some way by the mucosa (Table 4). This was not the case with 10 mM

Table 2. Effect of 10 mM luminal glucose on the output of lactate into the vascular and luminal perfusates in the presence of phenformin

Phenformin (mM)	Luminal perfusate glucose (mM)	n	Average rate of lactate output into vascular perfusate (nmole/min per g dry wt) 0–10 min	Average rate of lactate output into vascular perfusate (nmole/min per g dry wt) 20–30 min	Average rate of lactate output into luminal perfusate (nmole/min per g dry wt) 0–10 min	Average rate of lactate output into luminal perfusate (nmole/min per g dry wt) 10–30 min
None	10	5	3417 ± 156	6895 ± 792	396 ± 36	1161 ± 198
5 mM Vascular	10	6	5442 ± 505*	8536 ± 948	875 ± 109*	3724 ± 963*
5 mM Luminal	10	4	3479 ± 422	7999 ± 958	370 ± 42	990 ± 182

All perfusions were of 30 min duration. Phenformin and 5 mM vascular glucose were present throughout the perfusions. Glucose (10 mM) was added to the luminal perfusate after 10 min perfusion. All results are means ± S.E.M.; n = number of observations.

* Indicates values significantly different ($P < 0.05$) from the corresponding value in the absence of phenformin.

Table 3. Effects of phenformin on the fate of glucose absorbed from the lumen

Phenformin (mM)	n	Average rate of glucose uptake from lumen (nmole/min per dry wt)	Total glucose uptake from lumen (nmole/g dry wt)	Extra glucose appearance in vascular effluent		Extra lactate output to vascular perfusate		Extra lactate output to luminal perfusate	
				Amount (nmole/g dry wt)	%	Amount (nmole/g dry wt)	%	Amount (nmole/g dry wt)	%
None	5	4307 ± 792	86,192 ± 15,780	25,519	29.6	71,089	41.1	15,207	8.9
5 mM Vascular	6	1900 ± 651*	38,018 ± 13,020*	937	2.5	29,998	39.5	54,892	72.2
5 mM Luminal	4	2812 ± 443*	56,246 ± 8854*	5312	9.4	86,974	77.3	12,395	11.0

All perfusions were of 30 min duration. Phenformin and 5 mM vascular glucose were present throughout the perfusion. Glucose (10 mM) was added to the lumen after 10 min perfusion. Values for luminal glucose uptake are shown as means ± S.E.M.; n = number of experiments. %, Percentage of luminal glucose uptake accounted for.

* Indicates values significantly different ($P < 0.05$) from the corresponding value without phenformin.

phenformin, however, since the mucosal tissue level was significantly lower than the perfusate concentration. This was probably due to a large increase in the non-specific permeability of the intestine produced by this high concentration of phenformin (data not shown).

(b) *Luminal phenformin.* When phenformin (1.0–10.0 mM) was presented to the intestine from the lumen, the mucosal tissue concentrations were all significantly lower than those in the luminal perfusate, and there was no evidence of accumulation of phenformin presented by this route. After perfusion with 5 mM vascular phenformin for 30 min, the concentration of phenformin in the lumen was 0.15 mM, and after perfusion of the lumen with 10 mM phenformin for 30 min, the phenformin concentration in the vascular medium was only 0.04 mM, indicating that there was very little translocation of phenformin across the gut in either the mucosal or serosal direction.

DISCUSSION

Phenformin inhibited galactose and glucose transport by the vascularly perfused rat jejunum in agreement with previous reports [3, 4, 7, 8, 11, 20], but the nature of the inhibition depended on the phenformin concentration and whether the drug was added to the luminal or vascular compartments.

Vascular phenformin

At a given concentration, vascular phenformin was a more potent inhibitor than luminal phenformin, probably because it accumulated to a greater extent in the mucosa when supplied from the vascular side. There were two unusual features of the effects of vascular phenformin. Firstly, the transfer of D-galactose from the lumen to the vascular bed was increased above the control in the early part of perfusion (0–12 min), but then fell below the controls during the final 25 min (Fig. 1). Secondly, vascular phenformin almost doubled the rate of glucose uptake from the vascular medium. It is likely that D-glucose uptake from the vascular bed is mediated by the same carrier involved in D-galactose exit from the epithelial cells. If so, a greater uptake of vascular D-glucose in the presence of phenformin could increase the number of spare carrier molecules available to galactose in the cells, and lead to a stimulation of galactose efflux by a counterflow effect. This would continue while the rate of galactose entry into the epithelial cells across the brush border remained high, and would explain the initial stimulation of galactose appearance in the vascular bed induced by phenformin. The subsequent inhibition of galactose transfer may then be related to the reduction in intracellular ATP content (Fig. 4), which would tend to diminish the capacity of the Na^+,K^+ ATPase located in the lateral/basal membrane to maintain the sodium gradient across the epithelial cells which is thought to drive active sugar transport [20]. Uptake of D-galactose at the brush border would then be reduced, so that its appearance in the vascular perfusate would decline as the experiment progressed.

When isolated epithelial cells of the rat small intestine are incubated in the presence of phenformin,

Table 4. Tissue concentrations of phenformin following 30 min perfusion with phenformin in either the vascular or the luminal perfusate

Phenformin in vascular perfusate (mM)	Phenformin in luminal perfusate (mM)	n	Mucosal tissue phenformin (mM)
0.25	—	4	0.56 ± 0.08*
1.00	—	4	1.92 ± 0.28*
5.0	—	6	6.96 ± 0.77*
10.00	—	5	8.34 ± 0.86*
—	1.00	4	0.73 ± 0.09*
—	5.00	4	2.49 ± 0.20*
—	10.00	4	6.89 ± 0.84*

The vascular perfusate contained 5 mM D-glucose in all experiments. Results are means ± S.E.M.; n = number of observations.

* Indicates values which are significantly different ($P < 0.05$) from the corresponding perfusate concentration of phenformin.

respiration is inhibited, and there is a good correlation between the extent of inhibition and $\log_{10}$ phenformin concentration (T. J. Nicholls and H. J. Leese, unpublished results). This is in agreement with results obtained by others, using rings of hamster and rat small intestine [11, 21]. We therefore suggest that the main effects of vascular phenformin are secondary to its inhibition of mitochondrial respiration and oxidative phosphorylation [7, 8, 11, 22–26]. This would cause glycolysis to be stimulated and account for the greater glucose uptake from the vascular perfusate, 97% of which could be accounted for by lactate formation. When phenformin was added to the vascular perfusate, lactate output increased immediately, whereas there was a lag of *ca* 5 min (data not shown) before glucose uptake was stimulated. This suggested that the increased glucose uptake was a response to, rather than the cause of, the greater lactate production. Whatever the mechanism, it seems that the epithelium of the small intestine can increase its supply of a metabolic fuel, glucose, in response to need, and that a positive Pasteur effect exists for glucose supplied via the vascular route [27].

A second effect of vascular phenformin, more pronounced at high phenformin concentrations, may be due to a direct effect on the brush border (see below).

Luminal phenformin

At concentrations of 1.0 and 5.0 mM, luminal phenformin had no effect on the metabolism of the preparation, and there was no accumulation of the drug within the mucosal tissue. It was therefore not surprising that, in contrast to vascular phenformin, 1.0 and 5.0 mM luminal phenformin did not stimulate D-galactose transfer from the lumen to the vascular bed during the early part of the perfusion, but brought about a more straightforward, progressive inhibition as the incubations proceeded. We think the most likely explanation for this is that very little luminal phenformin entered the epithelial cells, but remained tightly bound to the brush border, and inhibited transport by a direct effect on the sugar carrier located in the microvilli, as observed by Kessler *et al.* with isolated brush-border membrane vesicles [12].

At a luminal phenformin concentration of 10 mM, the effects observed began to mimic those obtained with the lower concentrations of vascular phenformin, suggesting that at high luminal concentrations, phenformin could enter the epithelial cells in sufficient quantity to affect metabolic processes.

As with all work on animals, it is difficult to extrapolate these experimental findings to the human situation *in vivo*. Estimation of intestinal phenformin concentrations in man is complicated by the fact that a typical daily dose is taken as four 25 mg tablets or as a single 100 mg timed disintegration capsule [28]. Shortly after a dose, when the luminal concentration of the drug is maximal, sugar absorption could be inhibited by direct effects at the brush-border membrane. Accumulation of phenformin from the vascular bed may be particularly important in the clinical situation where there is repeated treatment over a prolonged period. Thus the phenformin concentration produced in the epithelial cells may be high enough to affect metabolism and hence cause reduced sugar absorption indirectly. It is particularly interesting to note that vascular, but not luminal, phenformin was capable of increasing glucose uptake from, and lactate output to, the vascular bed. This suggests that intestinal effects could contribute to the hypoglycaemic actions of phenformin in the absence of carbohydrate malabsorption [29] or in the post-absorptive state, particularly if the gluconeogenic capacity of the liver is compromised. The human small intestine is 6–7 m long, and could be a significant source of excess lactate production in lactic acidosis.

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