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Inhibition of Intestinal Sugar Transport by Phenformin

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The effect of phenformin on the absorption of D-glucose and D-galactose by hamster and rat intestine, was studied. Phenformin did not affect D-glucose absorption by rat intestine, but it inhibited at 10^{-3} to 10^{-2} M the absorption of D-glucose and D-galactose by hamster intestine. The inhibition was higher when D-glucose was tested.

Phenformin also inhibited active accumulation of these sugars by rings of hamster small intestine, *in vitro;* this effect was greater when D-glucose was utilized. The drug inhibits the oxygen uptake in the tissue in the absence or in the presence of added substrate.

Phenformin, as previously suggested, does not seem to act as a specific inhibitor on D-glucose transport, but most likely by its inhibitory effect on mitochondrial respiration.

It is commonly accepted that the antidiabetic effect of biguanides differs qualitatively from the effect of the sulfonylureas (6). Early studies led to the hypothesis that biguanides lowers the blood sugar level by direct effects on peripheral glucose metabolism (22, 23), but further papers showed that their action can best be explained by an inhibitory effect on intestinal absorption (13, 15).

Phenformin (phenethylbiguanide) inhibits sugar and amino acid transport *in vitro* (1, 7, 8). Its inhibitory action on intestinal absorption of glucose by dog (10) and rat (4) has been studied *in vivo*, utilizing hipertonic solution (10 and 54 %) that damage the intestinal epithelium (16). PEREIRA *et al.* (17) studied the effect of phenformin on normal subjects and suggested that the absorption of glucose was not altered by it. In the present paper a comparative study of phenformin effect on intestinal absorption of D-glucose and D-galactose by hamster and rat was made using *in vivo* and *in vitro* techniques.

Materials and Methods

Golden hamsters *(Mesocricetus auratus)*, 80-110 g body weight, and Wistar rats, 200-250 g body weight, fasted for 24 hours, were used.

The *in vivo* experiments were carried out utilizing the tehcnique of successive periods of absorption according to SOLS and PONZ (20). In each animal five periods were performed in the same jejunal loop. The results are expressed in μmoles of sugar absorbed per cm intestine.

Everted intestinal rings were obtained by the CRANE and MANDELSTAM technique (9), and were placed in 4 ml of Krebs-Henseleit bicarbonate buffer (14), with the appropiate added substrate. The medium was hubbled with carbogen during the whole incubation period. Data are processed as previously described (5).

Oxygen consumption was measured by the Warburg direct method (24) in an O_2 atmosphere, at 37° C, 80 oscillations/min. and 3 cm amplitude. In these experiments Tris-HCl buffer instead of bicarbonate buffer was used.

D-galactose was determined by the SOMOGYI method (21) and D-glucose by the glucose-oxidase method using the Biochemica Test Combination (Boehringer, Mannheim).

Results

Effect on the intestinal absorption of D-glucose and D-galactose in vivo. Phenformin at 10^{-2} M concentration did not affect the absorption of 2.77 mM D-glucose by rat intestine when the drug was present in the second and fourth absorption period.

However the phenformin at 10^{-3} to 10^{-2} M concentrations inhibited the intestinal absorption of 2 mM D-glucose in hamster. This inhibition accumulated with each successive absorption (fig. 1). At short periods (5 minutes), no effects were noticeable.

Since D-glucose is metabolised during experimentation (2) D-galactose was used. Phenformin also inhibited the intestinal absorption of this sugar, but, to a lesser degree (fig. 2).

Action on the active transport of D-glucose and D-galactose in vitro. Incubation time was 25 minutes for the experiments with intestinal rings of hamster. The inhibitory action of phenformin in the incubation solution on the active transport of D-glucose 1 mM was mani-

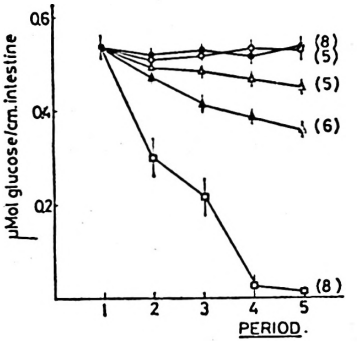

Fig. 1. *Effect of phenformin on the intestinal absorption of D-glucose 2 mM* in vivo, *in hamster.*
Phenformin 10^{-4} M (○), 10^{-3} M (△), 5.10^{-3} M (▲) and 10^{-2} M (□) is present in the second and fourth period. Absorption time, 20 min. Results are given as μM glucose/cm intestine and are accompanied ± SEM. The number of animals is given in bracket.

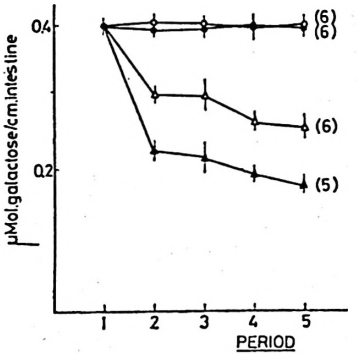

Fig. 2. *Effect of phenformin on the intestinal absorption of D-galactose 2 mM,* in vivo *in hamster.*
Phenformin 10^{-4} M (○), 5.10^{-3} M (△) and 10^{-2} M (▲) in the second and fourth absorption. Absorption time, 20 minutes. Results are given as means ± SEM. Number of animals in brackets.

fest. Concentrations ranging from 10^{-2} to 10^{-4} M notably inhibited the accumulation of glucose. The tissue sugar level after incubation falls from 8.90 mM in the control experiments to 6.45 mM, 3.32 mM and 1.25 mM when the phenformin concentration in the medium is 10^{-4}, 10^{-3} and 10^{-2} M respectively. In other words, inhibitions in tissue concentrations of 27, 62 and 85 % were observed (fig. 3). When D-galactose was used, the phenformin effect was significative only at a 10^{-2} M concentration, producing an inhibition of 20 per cent.

Effect of phenformin on the consumption of oxygen. Phenformin added to the incubation medium inhibited oxygen consumption by hamster intestinal rings (fig. 4). Measurements were made after 10, 20, 30, 40 and 60 minutes. The inhibition ranged from 15 % to 20 % with 10^{-3} M phenformin concentration. This effect was produced both in the presence and in the absence of a metabolizable exogen substrate, such is glucose.

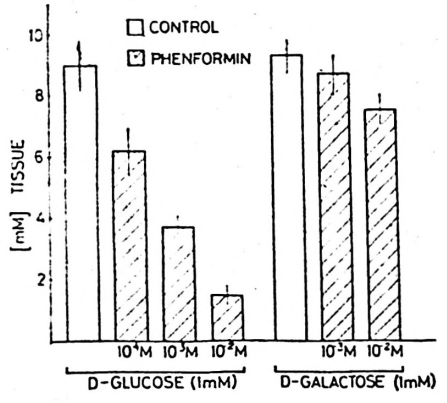

Fig. 3. *Comparative effect of phenformin on* In vitro *intestinal transport of D-glucose and D-galactose in hamster.*
Intestinal rings were incubated for 25 minutes with the appropriate substrates and phenformin was added as indicated in the graph. Results are given as means ± SEM. The number of data in each experimental condition is twelve.

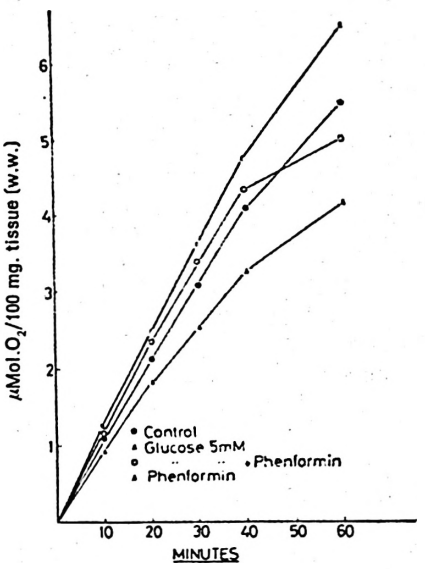

Fig. 4. *Effect of phenformin on the oxygen consumption by rings of hamster small intestine.*
The points are means ± SEM and are the average of ten experiments. Phenformin concentration was 10^{-3} M.

Discussion

The results show that phenformin inhibits the active transport of sugars by hamster intestine *in vivo* and *in vitro*. This inhibition is stronger for D-glucose than for D-galactose.

Rat intestine is specially resistant to phenformin *in vivo* technique, since high concentrations (10^{-2} M) did not affect the intestinal absorption of D-glucose. Oral administration of biguanides to rats, however, resulted in a subsequent decreased transport of D-glucose *in vitro* (15). These differences could be attributed to the techniques used (12). It has also been observed that phenformin affects the gluconeogenesis and the ATP level in guinea-pig liver but not in rat liver (9). The results from *in vitro* experiments in addition to earlier observations (8, 18) suggest that biguanides affect active energyrequiring

transport systems in general. The drug possibly acts by entering the absorbing cells and inhibiting mitochondrial respiration, as suggested by DAVIDOFF (11).

Phenformin inhibition is higher for glucose than for galactose transport. BERGER *et al.* (3) have shown an increase in portal lactate concentration after an oral load of D-glucose in patients pretreated with dimethylbiguanide. As CASPARY *et al.* (7) confirm *in vitro* this increase in lactate production by the intestinal tissue, probably the phenformin increases the anaerobic glycolysis by the inhibition of mitochondrial respiration (11). According to these findings the intracellular glucose accumulated in the tissue decreases by stimulation of anaerobic glycolisis, but this effect is not possible when the poorly metabolizable sugar, D-galactose is used.

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