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# Effect of Phenformin on Gluconeogenesis in Perfused Rat Liver

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The effect of phenformin on gluconeogenesis from several gluconeogenic substrates in perfused rat liver is reported.

1 mM phenformin inhibited gluconeogenesis from L-lactate and fructose (10 mM). A lag period (20-40 min.) was required before phenformin showed a clear inhibitory effect. The inhibition is more pronounced from 10 mM glycerol and takes place as soon as phenformin is added to the perfusion medium.

Theese results suggest a wide and indirect effect of phenformin on gluconeogenesis, probably related to its inhibitory effect on the electron transport chain.

Phenformin (phenethylbiguanide) is used in the treatment of diabetes mellitus. The mechanism of action of the biguanides has been widely discussed. STEINER and WILLIAMS (17, 18) have suggested that the hypoglycemic effect of the biguanides is related to their effects on aerobic processes. FALCONE *et al.* (3) proposed that biguanides inhibit oxidative phosphorylation. SHÄFER (15, 16) and GUILLORY and SLATER (4) demonstrated that this inhibition occurs at the cytochrome *b* level in the electron transport chain. The decrease of ATP following the inhibition of oxidative phosphorylation by biguanides would lead to a decrease in rates of gluconeogenesis (7, 1). Howeber, MEYER *et al.* (12) claimed to have demonstrated a specific inhibition of gluconeogenesis by biguanides in rat kidney slices.

In this paper, the effects of phenformin on gluconeogenesis from L-lactate, D-fructose and glycerol in isolated perfused rat liver are reported.

## Materials and Methods

Female Wistar rats weighing 150 to 200 g were starved for 48 hr. The perfusion method, based on the methods of MILLER *et al.* (13) and SCHIMASSEK (14), has been described by HEMS *et al.* (5). The perfusate consisted of Krebs-Henseleit physiological saline (8), bovine serum albumin powder fraction V (Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex, England) and washed human red cells. Human blood, stored 30 days at 4° C in citrate-dextrose anticoagulant so-

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lution still possessed the full oxygen-carrying capacity but did not glycolyse.

In all cases, the total volume of the perfusion medium was 150 ml. The following final concentrations were used: albumin, 2.6 % (w/v); haemoglobin, 2.5 % (w/v) and substrates, 10 mM. When phenformin (kindly supplied by Laboratorios Funk, Manlleu, Barcelona, Spain) was to be added, 5 ml of the saline solution was replaced by the same volume of phenformin solution. Two perfusion experiments were conducted simultaneously, one liver serving as a control. 40 min. are needed for liver stabilization, substrates being added thereafter.

Glucose was determined by the glucose oxidase method according to KREBS *et al.* (9, 10). L-lactate was measured with lactate dehydrogenase as described by HOHORST (6) and haemoglobin according to EVELYN and MALLOY (2).

## Results and Discussion

1 mM phenformin strongly inhibited gluconeogenesis from 10 mM L-lactate. When the concentration of phenformin was 0.1 mM only a slight inhibition occured. All the observations are in good agreement. Representative experiments are shown in figure 1. Lactate is not removed following inhibition of glucose synthesis by phenformin.

A lag period of 30 min. was required before 1 mM phenformin showed a clear inhibitory effect. This result is in agreement with the lag period found by TOEWS *et al.* (19) when lactate or pyruvate were used as gluconeogenic substrates.

This suggests that phenformin may accumulate in liver until an inhibitory concentration is reached or, alternatively, that there is an indirect effect produced either by a metabolite of phenformin or as a result of the inhibition of another metabolic process, whose product, i.e. ATP is needed for gluconeogenesis to take place.

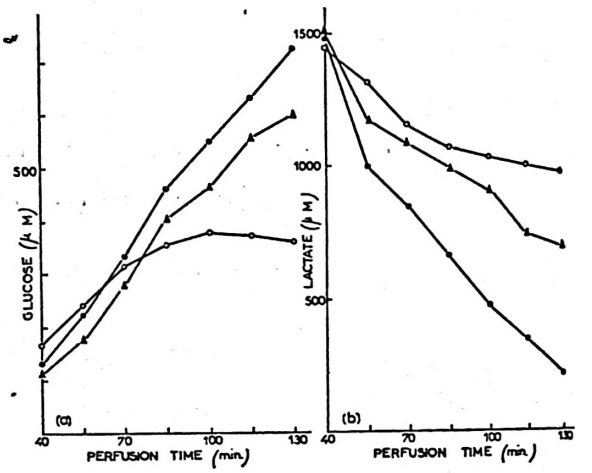


Fig. 1. *Effect of phenformin on gluconeogenesis from 10 mM L-lactate.*

(a) Glucose found in the perfusion medium. (b) Lactate found in the perfusion medium. —●—●— 1500 μmoles of L-lactate added to 150 ml of medium at 38 min. Liver wet weight: 5.5 g. —O—O— 1500 μmoles of L-lactate + 150 μmoles of phenformin added to 150 ml of medium at 38 min. Liver wet weight: 6.0 g. —▲—▲— 1500 μmoles of L-lactate + 15 μmoles of phenformin added to 150 ml of medium at 38 min. Liver wet weight: 5.2 g.

Phenformin (1 mM) also inhibited gluconeogenesis from D-fructose (10 mM) (fig. 2). This inhibition is less pronounced than that found with L-lactate and, again, 30-40 min. of perfusion are needed before a clear effect is observed.

The inhibition found using 10 mM glycerol as substrate is much higher than that obtained when L-lactate or D-fructose were employed (fig. 3). This inhibition takes place as soon as phenformin is added to the perfusion medium.

Following phenformin addition to perfused rat liver, TOEWS *et al.* (19) found marked elevations of pyruvate, phosphoenolpyruvate, 2-phosphoglycerate, and 3-phosphoglycerate and decreased concentrations of glycerolphosphate, fructose-6-P and glucose-6-P when lactate or pyruvate were used as substrates. On the other hand, when alanine was used, none of the glu-

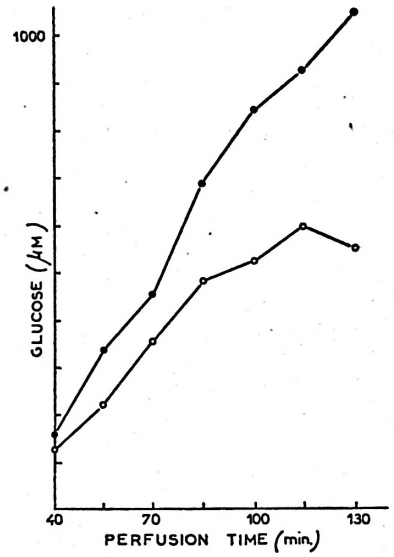


Fig. 2. *Effect of phenformin on gluconeogenesis from 10 mM D-fructose.*

Glucose found in the perfusion medium. —●—●— 1500 μmoles of D-fructose added to 150 ml of medium at 38 min. Liver wet weight: 6.4 g. —O—O— 1500 μmoles of D-fructose + 150 μmoles of phenformin added to 150 ml of medium at 38 min. Liver wet weight: 6.1 g.

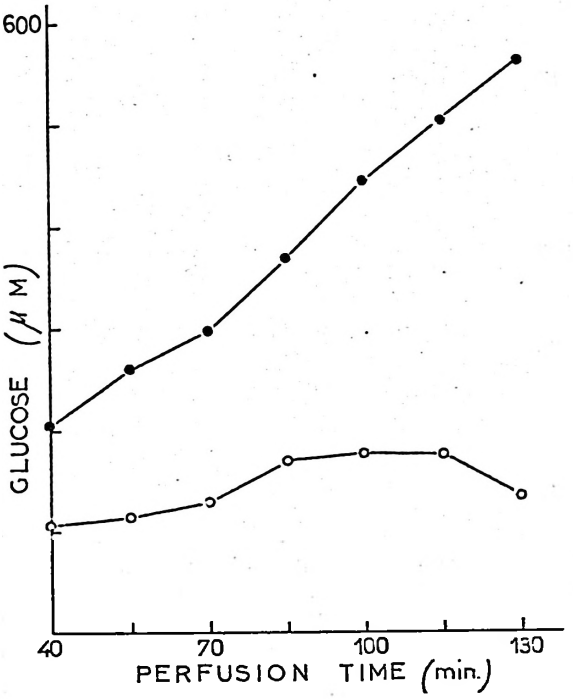


Fig. 3. *Effect of phenformin on gluconeogenesis from glycerol.*

Glucose found in the perfusion medium. —●—●— 1500 μmoles of glycerol added to 150 ml of medium at 38 min. Liver wet weight: 6.3 g. —O—O— 1500 μmoles of glycerol + 150 μmoles of phenformin added to 150 ml of medium at 38 min. Liver wet weight: 6.2 g.

coneogenic intermediates were significantly elevated and α-ketoglutarate concentration decreased, suggesting that the tricarboxylic acid cycle is inhibited between acetyl CoA and citrate. The results obtained in our experiments using glycerol or fructose show that the gluconeogenic flux is also inhibited when substrates directly connected with the last steps of the sequence are used. Again this results suggest a wide and indirect effect of phenformin on gluconeogenesis probably related to its inhibitory effect of the electron transport chain. KREBS (11) found that 1 mM phenformin inhibits the urea synthesis of rat liver slices by about 40 %. Both processes, gluconeogenesis and ureogenesis, require ATP. Consequently, the inhibition of both processes by phenformin is likely to be related to its reported effect on oxidative phosphorylation (3).

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