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EFFECTS OF PHLORIZIN ON GLUCOSE TRANSPORT INTO BLOOD AND LYMPH

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SUMMARY

Phlorizin was administered by oral and intraperitoneal routes to rats prepared with cannulated mesenteric lymph ducts. Oral phlorizin (fed with glucose by stomach-tube) depressed the uptake of fed glucose into both blood and lymph. No inhibition of water transport occurred while glucose transport was decreased, yet very high levels of oral phlorizin (220 μmoles) would block water transport to lymph from the intestine. Intraperitoneally injected phlorizin did not show an effect on glucose transported by lymph or blood, but did promote glycosuria. Typical findings are presented in the text.

INTRODUCTION

The effect of protein inhibitors on lymph protein concentration[1] and absorption of amino acids[2] into mesenteric lymph system has been followed. The inhibitory effect of phlorizin on intestinal glucose absorption has been known for many years; most recent studies have been on *in vitro* effects[3–5]. Brush border membrane fragments have been isolated and phlorizin was shown to have a much greater affinity than the natural substrate, glucose, for the carrier[6].

The studies of phlorizin effects on glucose absorption directly from the small intestine in the animal have been limited to *in situ* studies on an anesthesized animal[7]. We utilized rats with cannulated mesenteric lymph ducts to investigate the effects of phlorizin administered orally and intraperitoneally on glucose and water absorption from the intestine. This provided a means to follow [^{14}C]glucose transported directly from the intestinal lumen and also to follow the effects on blood and urine glucose levels simultaneously.

METHODS AND MATERIALS

Surgical preparation

Male rats of Sprague–Dawley origin (supplied by Simonsen Laboratories, White Bear, Minn.) weighing 225–275 g and maintained on Purina Laboratory Chow were

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used. The mesenteric lymph duct was cannulated in a manner similar to that described by BOLLMAN *et al.*[8]. After surgery, the rats were placed in a restraining cage and fasted for 24 h before carrying out an experiment. Water was made available to the animals *ad libitum*.

Phlorizin studies

Phlorizin was purchased from Mann Research Laboratories (New York, N.Y.). In the orally administered phlorizin experiments, 250 mg D-glucose *plus* 5 μC [^{14}C]glucose (in a total volume of 2 ml) was fed by stomach-tube to each rat. Phlorizin was added to this solution in amounts ranging, from 0.094 to 94.0 mg or 0.22 to 220 μmoles. The higher phlorizin concentrations were administered as a suspension due to solubility characteristics of phlorizin.

The effects of intraperitoneally injected phlorizin on glucose absorption into mesenteric lymph was studied at a dosage of 400 mg/kg body weight. The phlorizin was injected as a suspension in 2 ml of saline. In all phlorizin experiments, glucose concentrations and radioactivity were followed in mesenteric lymph, tail blood, and urine before and after feeding the test meal.

Glucose assay

Glucose was assayed enzymatically on protein-free solutions using the Glucostat system purchased from Worthington Biochemical Corp. (Freehold, N.J.). Radioactivity measurements were made using a liquid scintillation spectrophotometer[2]. Radioactivity was found to be more than 98 % in the glucose R_F fraction by using paper chromatography.

RESULTS AND DISCUSSION

Glucose distribution (blood and lymph)

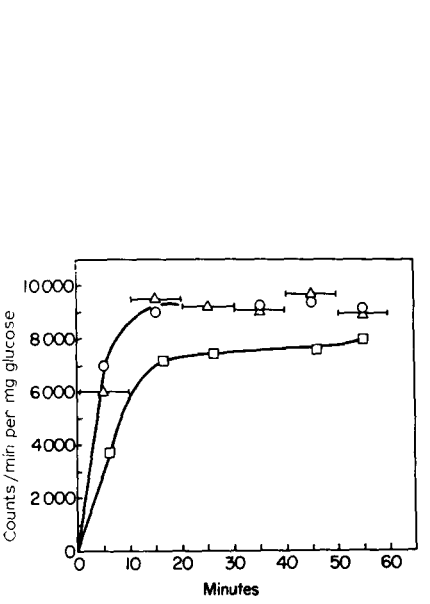
In order to determine the relationship between glucose in the mesenteric lymph and hepatic portal blood draining the intestine, 250 mg unlabeled D-glucose fortified with 5 μC [^{14}C]glucose was injected directly into the upper jejunum in an anesthesized rat with its mesenteric lymph duct cannulated. Blood samples (0.2 ml) were taken from the hepatic portal vein and femoral vein. There was an equal distribution of radioactivity between the mesenteric lymph and hepatic blood, but peripheral blood from the femoral vein remained at a lower specific activity level (Fig. 1).

The specific activity of glucose rises more rapidly in mesenteric lymph and hepatic portal blood than in the peripheral blood. This is due to body fluids draining the intestine obtaining the labeled glucose directly from the intestinal lumen. It appears that mesenteric lymph is in equilibrium with small metabolites, such as glucose, found in the hepatic portal blood and therefore offers a means of obtaining information on absorption of these metabolites from the small intestine.

Orally administered phlorizin

Fig. 2A reveals the effect of 0.22–220 μmoles of orally administered phlorizin (a total of 0.095–95 mg) on transport of D-glucose into mesenteric lymph. Points directly on the ordinate represent 24-h fasting levels which ranged between 90 and 110 mg/100 ml. In control experiments, without phlorizin, the glucose level increased

nearly 3-fold in 20 min, *i.e.* from 100 to 200 mg/100 ml after feeding the test dose of glucose. The lymph glucose level then decreased rapidly reaching near fasting in 70 min. Adding phlorizin in increasing amounts, 0.095–95.0 mg, inhibited glucose entry into lymph and decreased the total glucose load transported, since lower glucose concentrations were evident during the absorption phase. Phlorizin at a level of 95 mg completely blocked the entrance of glucose into the lymph from the intestinal lumen.



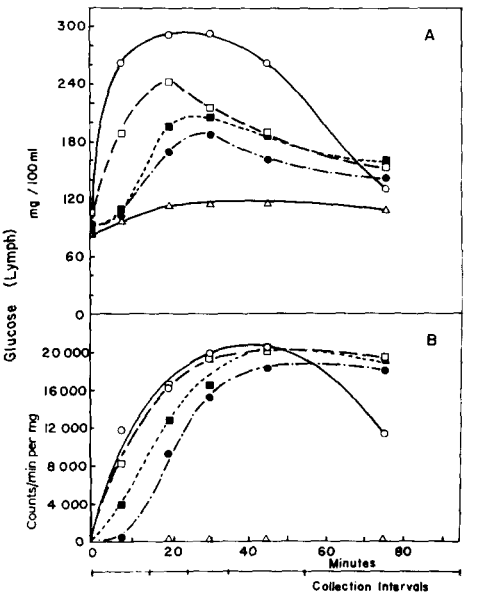


Fig. 1. Distribution of [^{14}C]glucose between mesenteric lymph (△), hepatic portal blood (○), and tail blood (□) when 5 μC [^{14}C]glucose was injected directly into the upper jejunum in anesthesized rat. The horizontal bars represent 0.2-ml collection times for mesenteric lymph. See text for further details.

Fig. 2.A. Glucose concentration in mesenteric lymph after feeding 250 mg D-glucose *plus* 5 μC [^{14}C]glucose with varied amounts of phlorizin (by stomach-tube). Phlorizin amounts present were: none (○), 0.22 μmole (□), 2.2 μmoles (■), 22 μmoles (●), and 220 μmoles (△). The 24-h fasting values are shown as the points on the ordinate. B. Specific activity for [^{14}C]glucose in mesenteric lymph after feeding, 250 mg D-glucose *plus* 5 μC [^{14}C]glucose with varied amounts of phlorizin.

The change in glucose specific activity in this same set of experiments is shown in Fig. 2B. At lower phlorizin levels, 0.22–22 μmoles, the peak in specific activity appeared to be delayed 10–20 min from the control peak, but the specific activity appeared to be the same. However, with 220 μmoles phlorizin, complete inhibition of glucose absorption occurred with no radioactivity appearing in lymph. The animal also had diarrhea with glucose and radioactivity appearing in the feces.

The delay of entry of [^{14}C]glucose into mesenteric lymph under the influence of phlorizin could be due to blocking of glucose transport sites in the upper part of the small intestine. As increasing concentrations of phlorizin are added more glucose transport sites are blocked farther down the gastrointestinal tract. Thus, the delay would probably be due to the increased lumenal distance from the stomach that glucose must 'travel' to be absorbed. With an increase in the number of transport sites blocked, the total load or concentration of glucose leaving the intestine would also be

decreased. When 220 μmoles of phlorizin were added, all the transport sites apparently were blocked, with the result that no glucose crossed the mucosal cells, but simply passed down the gastrointestinal tract into the feces.

From these experiments, the possible number of glucose binding sites in the brush border of the mucosal cells could be calculated if it is assumed that there is a 1:1 stoichiometry between phlorizin and the receptor site. The critical levels of phlorizin lie between 22 and 220 μmoles, where a complete block of glucose transport occurred. A total of 220 μmoles phlorizin would equal $1.2 \cdot 10^{20}$ molecules of the inhibitor. Thus, we could assume that the entire glucose transporting surface of the gastrointestinal tract contains approx. $1 \cdot 10^{20}$ glucose receptor sites. However, this is probably the upper limit estimate since the brush border contains the enzyme β-glucosidase which hydrolyses the glucose moiety from phlorizin to produce the aglycone phloretin and glucose[9]. Phloretin apparently enters the mucosal cell where it has a secondary effect of inhibiting metabolism of the cell as shown by *in vitro* studies[5]. However, phlorizin is the primary structure which inhibits glucose binding to brush border sites since phloretin has little or no effect on transport of glucose[4].

Peripheral blood, taken from the tail, was also analyzed for glucose and radioactivity after oral phlorizin administration. The increase in blood glucose following such feeding of 250 mg D-glucose *plus* varying concentrations of phlorizin is shown in Fig. 3A. The 24-h fasting value, given by points on the ordinate, ranged between 65 and 75 mg/100 ml. In general, blood glucose levels followed those of the mesenteric lymph (Fig. 2A) in each case, except that the increase in tail blood glucose was not as great as in mesenteric lymph. The effect of increasing levels of phlorizin was to decrease the transport of dietary glucose into the peripheral blood which was reflected by a decreased peak concentration in the blood.

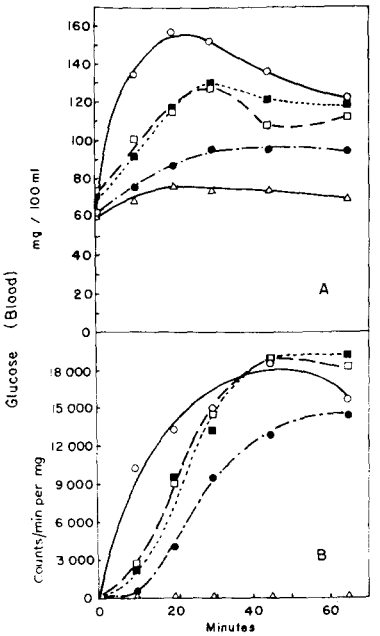
The specific activity of peripheral blood glucose likewise was affected (Fig. 3B). In controls without phlorizin, the specific activity rose rapidly to a peak 30 min after feeding the radioactive glucose. Increasing phlorizin concentrations decreased the transport of glucose into the blood, but the lower levels of phlorizin, 0.22 and 2.2 μmoles, presented specific activities similar to the control within 40 min after the oral meal. A phlorizin level of 22 μmoles showed a somewhat lower specific activity while 220 μmoles of the inhibitor completely blocked entrance of dietary glucose into the blood.

The effects of phlorizin on mesenteric lymph glucose concentrations are reflected to a lesser degree in peripheral blood where radioactive dietary glucose has undergone a dilution with body carbohydrate. It should be mentioned that while oral phlorizin had a great effect on the transport of glucose across the mucosal cell, none of the animals had glucosuria ,which is consistent with the fact that phlorizin cannot be absorbed across the mucosal cell[6, 10].

Intraperitoneally administered phlorizin

Since oral phlorizin affects only the intestinal transport of glucose, it was of interest to see if intraperitoneal injection would also have an effect on glucose entry into mesenteric lymph. The effect of intraperitoneally injected phlorizin on total glucose load transported by mesentric lymph is shown in Fig. 4. Upon feeding 250 mg D-glucose (stomach-tube) the mesenteric lymph glucose rose from a 24-h fasting value of 90 mg/100 ml to a peak of nearly 300 mg/100 ml within 30 min. Blood glucose (tail)

simultaneously rose from the fasting value of 65 mg/100 ml to 155 mg/100 ml. 5 h later, when lymph and blood glucose had returned to the fasting levels, the same animal was injected with phlorizin at a dosage of 400 mg/kg body weight. 30 min later, 250 mg D-glucose was again fed (stomach-tube) with the result that lymph glucose rose sharply



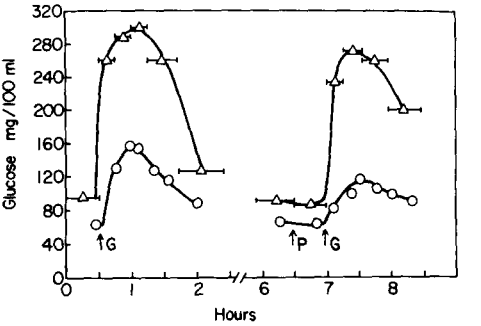


Fig. 3.A. Glucose concentration in tail blood after feeding 250 mg D-glucose *plus* 5 μC [^{14}C]glucose with varied amounts of phlorizin. Phlorizin amounts present were none (○), 0.22 μmole (□), 2.2 μmoles (■); 22 μmoles (●), and 220 μmoles (△). B. Specific activity curves for [^{14}C]glucose in tail blood after feeding D-glucose with varied amounts of phlorizin.

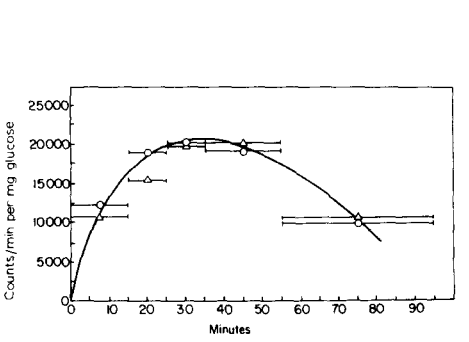
Fig. 4. Effect of intraperitoneally injected phlorizin (400 mg/kg body weight) on transport of D-glucose into mesenteric lymph (△) and tail blood (○). Rats were fasted for 24 h and fed 250 mg D-glucose by stomach tube; several hours later, phlorizin (P) was injected intraperitoneally followed by the same test meal of glucose (G) 0.5 h later. Horizontal bars represent the lymph collection times.

from its fasting value to a peak of 280 mg/100 ml within 30 min. Glucosuria was evident within the first 30 min after phlorizin injection and continued at high levels (greater than 8 g/100 ml) for several hours.

Specific activity of [^{14}C]glucose in mesenteric lymph was also followed after injection of phlorizin intraperitoneally (Fig. 5). In the control animal, the test meal was fed (stomach-tube) 30 min after intraperitoneal injection of 2 ml saline, while in the experimental animal, 30 min after injection of phlorizin (intraperitoneally) at a dosage of 400 mg/kg body weight. The test meal consisted of 250 mg D-glucose *plus* 5 μC [^{14}C]glucose in a total volume of 2 ml. The data reveal that under these conditions intraperitoneal phlorizin had no effect on glucose transport into mesenteric lymph from the intestinal lumen. Concomitant with this, glucosuria was present in these animals at 8 g/100 ml or greater. Rats with saline injected intraperitoneally had no glucose in the urine.

It is apparent the intraperitoneal phlorizin does not have an effect on the total

load of glucose carried by lymph, but does have a marked effect on the ability of the kidney to reabsorb glucose. The reason for the difference between intraperitoneally injected and oral phlorizin may be due to the ability of the intact phlorizin molecule to reach the glucose receptor site. It may be postulated that intraperitoneally injected



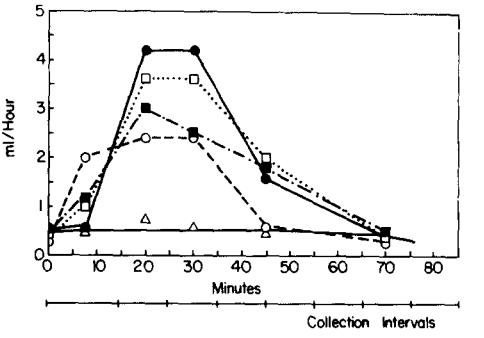


Fig. 5. Specific activity of [^{14}C]glucose in mesenteric lymph. 2 ml of saline (○) or phlorizin (△) at a dosage of 400 mg/kg body weight were injected intraperitoneally to animals 30 min before feeding by stomach tube 250 mg D-glucose *plus* 5 μC [^{14}C]glucose at time 0.

Fig. 6. Effect of phlorizin on mesenteric lymph flow. Rats were fed by stomach tube 250 mg D-glucose as a 2-ml solution which also contained varied amounts of phlorizin; none (○), 0.2 μmole (□), 2.2 μmoles (■), 22 μmoles (●), and 220 μmoles (△) of phlorizin.

phlorizin might be found in the glomerular filtrate and thus will be able to bind the glucose receptor sites in the proximal convoluted tubule, where most of the glucose absorption is thought to occur. In the intestine, however, phlorizin would have to cross several cell membranes to reach the lumen and its brush border where the glucose receptor sites are located. Since the mucosal cell contains β-glucosidase activity, it would hydrolyze phlorizin which has little or no effect on glucose transport[9]. Thus, the availability of the intact phlorizin molecule to the glucose receptor site seems to be the primary reason for inhibition or lack of inhibition of glucose transport.

Effect of phlorizin on lymph flow

Phlorizin injected intraperitoneally (400 mg/kg body weight) did not have an effect on water transport from the intestinal lumen into the lymph. Likewise, oral phlorizin, at lower concentrations, did not have an effect on water transport as shown in Fig. 6. The lymph flow, after 24 h of fasting (water made available *ad libitum*) ranged between 0.3 and 0.6 ml/h. In each experiment, 250 mg D-glucose, taken up to a volume of 2 ml with distilled water, was fed by stomach-tube to the animals with varying concentrations of phlorizin added to the 2-ml solution. In the control (no phlorizin), the lymph flow rose sharply during the first 15 min to 2 ml/h and then leveled off for the next 30 min before dropping to the fasting level. The addition of phlorizin, 0.22–22 μmoles, delayed somewhat the rapid rise in lymph volume in the first 15 min, however, the amount of fluid transported was not inhibited but actually exceeded the control value. Phlorizin levels of 220 μmoles failed to increase the lymph flow which remained at the fasting rate for the entire length of the experiment.

It is apparent from these studies that oral administration of phlorizin can inhibit glucose transport at lower concentrations while having little or no effect on the

total water load passing into the lymph. Only at very high levels of phlorizin did inhibition of water transport across the intestine become apparent. The effect of phlorizin *in vitro* in everted intestinal sacs revealed that it could inhibit transport of glucose, water, and also at higher concentrations inhibit metabolism of the mucosal cell as revealed by a decrease in O_2 uptake[11]. PARSONS *et al.*[11] found that inhibition of metabolism also inhibited water uptake in the intestinal sacs. In our studies, no inhibition of water transport occurred during the time when glucose transport was markedly decreased. However, water transport from the intestine was apparently blocked at phlorizin levels of 220 μmoles, since the rate of flow was not increased in response to the water fed with phlorizin. Thus, water transfer into the lymph does not depend on glucose transfer, but may depend on glucose metabolism in the mucosal cell. We have no direct proof that mucosal cell metabolism is inhibited at high phlorizin concentration, but water transport is an ATP-requiring mechanism and may reveal blockage of normal metabolism.

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