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Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange

(liver/glucose uptake/gut factors)

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ABSTRACT **The effects of hyperinsulinemia, hyperglycemia, and the route of glucose administration on total glucose utilization and on net splanchnic glucose exchange were studied in 20 normal volunteers with the hepatic venous catheter technique. Euglycemic hyperinsulinemia [induced by a priming plus continuous infusion of insulin resulting in plasma insulin levels of 400–1200 μunits (international)/ml and a variable glucose infusion] caused a 5- to 6-fold increase above basal in total glucose turnover. However, net splanchnic glucose uptake (0.5 ± 0.2 mg/kg per min) accounted for only 4–5% of total glucose utilization. When hyperglycemia (223 ± 1 mg/dl) was induced in addition to hyperinsulinemia by the intravenous infusion of glucose, splanchnic glucose uptake increased 100% to 1.0–1.1 mg/kg per min but was still responsible for only 10–14% of total glucose utilization. In other studies hyperglycemia (223 ± 2 mg/dl) was maintained constant by a variable intravenous infusion of glucose for 4 hr and oral glucose (1.2 gm/kg) was administered at 1 hr. After the oral glucose, net splanchnic glucose uptake increased to values 6-fold higher than with intravenous glucose despite unchanged plasma glucose levels and plasma insulin concentrations well below those observed in the studies with euglycemic hyperinsulinemia. The results indicate that hyperinsulinemia or hyperglycemia induced by intravenous infusion of glucose or insulin causes minimal net uptake of glucose by the splanchnic bed despite marked stimulation of total glucose turnover. In contrast, administration of glucose by the oral route has a marked stimulatory effect on net splanchnic glucose uptake. These findings suggest that orally consumed glucose causes the release of a gastrointestinal factor that enhances insulin-mediated glucose uptake by the liver.**

The liver has long been recognized to play a central role in blood glucose homeostasis (1–4). However, the factors that regulate hepatic glucose uptake still remain controversial. The pioneering studies of Soskin and Levine (5) suggested that hyperglycemia per se was responsible for the switch of the liver from a glucose-producing to a glucose-assimilating organ. However, subsequent *in vivo* studies by Madison (3) and Felig and Wahren (2, 4) indicated that this switch is dependent upon the presence of hyperinsulinemia. More recently Bergman (6) has reemphasized the importance of hyperglycemia in the regulation of hepatic glucose uptake and has suggested that concommitant hyperinsulinemia enhances its effect. What role, if any, the route of glucose administration (i.e., oral versus intravenous) has in determining hepatic glucose uptake has not been established. The present study was consequently undertaken to evaluate the relative effects of hyperglycemia, hyperinsulinemia, and the route of glucose administration on net splanchnic glucose uptake.

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MATERIALS AND METHODS

Subjects. Twenty male subjects (74 ± 4 kg in body weight), within 20% of ideal body weight (mean = 101 ± 2%) (based on Metropolitan Life Insurance Tables, 1959) and ranging in age from 21 to 31 yr (mean = 26 ± 1 yr), were studied. All subjects were consuming a weight-maintaining diet containing at least 200 g of carbohydrate per day for 3 days prior to study. No subjects were taking any medications. None had a family history of diabetes mellitus. The purpose, nature, and potential risks of the study were explained to all subjects and written consent was obtained prior to their participation. The protocol was reviewed and approved by the Ethical Committee of the Karolinska Institute and the Committee on Human Investigation at the Yale University School of Medicine.

All subjects were studied in the postabsorptive state at 8 a.m. after a 12–14 hr overnight fast. Prior to each study, a catheter was inserted into an antecubital vein under local anesthesia for the administration of insulin or glucose. Catheters were also inserted percutaneously into a brachial artery and a hepatic vein as described (4).

Euglycemic Hyperinsulinemia. The effect on splanchnic glucose balance of hyperinsulinemia at a basal plasma glucose concentration was determined by the "insulin clamp" technique in six subjects (7). After a control period, we administered a priming plus continuous infusion of crystalline porcine insulin (Eli Lilly Co., Indianapolis, IN) to obtain steady state hyperinsulinemia (7). The priming dose was administered in a logarithmically decreasing manner for 10 min at which time the continuous insulin infusion was begun. The total amount of insulin infused during the priming period was twice that infused during subsequent 10-min intervals. The continuous infusion dose (200 mU/m^2 surface area per min in three subjects and 400 mU/m^2 per min in three subjects) was maintained for 110 min (U = international units). We chose these infusion rates to achieve plasma insulin concentrations (approximately 400 μU/ml and 1200 μU/ml, respectively) that are equal to or in excess of maximal portal insulin levels observed during hyperglycemia (8). The plasma glucose level was maintained at basal preinfusion levels by determination of the arterial plasma glucose concentration every 5 min and the periodic adjustment of a variable infusion of a 20% glucose solution. The adjustment of the glucose infusion rate is based on a servo-control negative feedback principle (7). Under these steady-state conditions of constant euglycemia, all of the glucose infused (M) is taken up by tissue cells and thus serves as a measure of the total amount of glucose metabolized by the body (7, 9).

Intravenous Glucose. In eight subjects the effect on splanchnic glucose uptake of hyperglycemia and endogenous hyperinsulinemia induced by intravenous administration of

Abbreviation: U, international unit.

glucose was determined by the "hyperglycemic clamp" technique (10). Plasma glucose concentration was increased to 125 mg/100 ml above the basal level by the intravenous administration of a priming infusion of glucose given in a logarithmically decreasing manner during 14 min. The plasma glucose concentration was subsequently maintained at 125 mg/100 ml above the basal level by the periodic adjustment of a variable intravenous infusion of a 20% glucose solution (10). Under these steady-state conditions of constant hyperglycemia, the infusion rate of glucose, corrected for urinary losses of glucose, must equal the amount of glucose taken up by all the cells of the body.

Combined Intravenous Glucose and Intravenous Insulin. In two subjects the plasma glucose concentration was acutely raised and maintained at 125 mg/100 ml above the basal level by a priming and variable glucose infusion as described above. At the same time a prime-continuous infusion of insulin (40 mU/m^2 surface area per min) was simultaneously administered as described above in the euglycemic hyperinsulinemia ("insulin clamp") protocol. We chose this infusion rate to raise the plasma insulin concentration by an additional 100 μU/ml above that provided by the hyperglycemic stimulus alone (7, 9).

Combined Intravenous and Oral Glucose Administration. In five subjects the plasma glucose concentration was acutely raised and maintained at 125 mg/100 ml above basal by a priming and variable intravenous infusion of glucose. After 60 min of sustained hyperglycemia, each subject ingested 1.2 g of glucose per kg of body weight for 10 min. The variable intravenous glucose infusion was appropriately adjusted to maintain the plasma glucose concentration constant at 125 mg/100 ml above the basal level for an additional 180 min after oral glucose. Because the total duration of the intravenous glucose infusion in this study was 240 min, three subjects were restudied after a three-week interval with prolonged intravenous glucose alone. During this repeat study the plasma glucose concentration was acutely raised and maintained at 125 mg/100 ml above the basal level for 4 hr with intravenous glucose. No oral glucose was administered in the repeat study.

Analytical Procedures. Plasma glucose was determined during the studies by using the glucose oxidase method (Glucostat, Beckman Instruments Corp., Fullerton, CA). Methods for the determination of blood glucose, plasma immunoreactive insulin (11), and hepatic blood flow (2, 4) have been described.

Calculations. During the insulin or glucose infusion studies, the mean glucose infusion rate (M) was determined by calculating the mean value observed from 20 to 120 min (7, 9). To calculate the "steady state" plasma glucose and insulin concentrations during the insulin infusion, we employed the mean of the values from 20 to 120 min (7, 9).

Net splanchnic glucose uptake in the studies involving intravenous infusion of glucose or insulin was calculated as the product of the arterio-hepatic venous difference for blood glucose and the hepatic blood flow (determined at 10-min intervals). Because orally administered glucose is absorbed via the portal vein (12) (which could not be catheterized in this study), net splanchnic glucose uptake was calculated as the sum of the orally administered glucose load and the product of the arterio-hepatic venous difference and hepatic blood flow. The assumption was made that the entire glucose load is absorbed during the 3-hr period after oral glucose administration. Studies in both man (13) and dog (14) have indicated that this is a valid assumption. All data are presented as the mean ± SEM. Statistical comparisons were performed with either the paired or unpaired t test analysis (15) as indicated in the text.

RESULTS

Euglycemic Hyperinsulinemia. The fasting plasma insulin concentration increased more than 40-fold during the 200-μU per m^2/min insulin infusion and over 100-fold during the 400-μU per m^2/min insulin infusion (Table 1). The stability of the plasma insulin concentration is indicated by the coefficients of variation, which were 11 ± 4% and 17 ± 2%, respectively, during the two different insulin infusion studies.

The fasting plasma glucose concentration of 94 ± 2 mg/dl was maintained constant during the period of hyperinsulinemia with a coefficient of variation of 4.0 ± 0.4%. The amounts of glucose infused to maintain euglycemia during the insulin infusion studies (9.5–11.5 mg/kg per min) indicated a 5- to 6-fold increment in total glucose utilization above basal levels.

During the insulin infusion, splanchnic glucose balance reverted from a basal net output to a net uptake. However, this value represented only 4–5% of the total glucose taken up by all tissues of the body.

Intravenous Glucose. The plasma glucose concentration was increased and maintained at 120–125 mg/dl above the fasting level during the glucose infusion (Table 1). The constancy of the plasma glucose concentration during the hyperglycemic plateau is indicated by the coefficient of variation of 3.3 ± 0.3%. The fasting plasma insulin concentration increased 5-fold during the glucose infusion.

The amount of glucose infused to maintain the hyperglycemic plateau indicated a 3- to 4-fold increment in total glucose utilization above basal rates. During this same time period net splanchnic glucose uptake represented only 14 ± 4% of the total glucose taken up by body tissues. The values for net splanchnic glucose uptake were, however, 2-fold greater than those observed with euglycemic hyperinsulinemia ($P < 0.05$).

Combined Intravenous Glucose and Intravenous Insulin. During the combined glucose and insulin infusion the fasting

Table 1. Plasma insulin and glucose concentrations, glucose infusion rates, and splanchnic glucose exchange during intravenous insulin or glucose infusion*

Intravenous infusion	Plasma insulin, μU/ml		Plasma glucose, mg/dl		Glucose, mg/kg per min			Total glucose infusion taken up by splanchnic bed,†‡ %
	Basal	Response†	Basal	Steady-state†	Basal splanchnic production‡	Infusion rate†	Net splanchnic uptake†‡	
Insulin, 200 mU/m^2 per min	11 ± 1	428 ± 37	93 ± 2	92 ± 2	1.8 ± 0.2	9.5 ± 0.8	0.5 ± 0.2	5 ± 2
Insulin, 400 mU/m^2 per min	9 ± 1	1189 ± 14	95 ± 2	97 ± 2	2.2 ± 0.1	11.5 ± 1.6	0.5 ± 0.2	4 ± 3
Glucose	11 ± 2	55 ± 5	101 ± 2	223 ± 2	1.9 ± 0.2	7.0 ± 1.3	1.0 ± 0.2	14 ± 4
Glucose and insulin	11 ± 3	191 ± 11	96 ± 1	223 ± 1	1.7 ± 0.3	12.1 ± 3.8	1.1 ± 0.6	9 ± 4

* Mean values ± SEM are shown.
† Mean value during the 20–120 min after starting the insulin, glucose, or the combined glucose-insulin infusion.
‡ Calculated as the product of the hepatic blood flow and the arteriohepatic venous blood glucose concentration difference.

Table 2. Plasma insulin and glucose concentrations and splanchnic glucose exchange during combined intravenous–oral glucose administration and during prolonged intravenous glucose infusion*

Infusion	Plasma insulin, μU/ml			Plasma glucose, mg/dl		Glucose, mg/kg per min†		
	Basal plasma	Mean plasma response 0–60 min	Mean plasma response 60–240 min	Basal	Steady-state	Basal splanchnic production	Net splanchnic uptake 20–60 min	Net splanchnic uptake 60–240 min
Combined intravenous–oral glucose	12 ± 1	44 ± 3	153 ± 7‡	101 ± 3	224 ± 3	2.00 ± 0.25	1.0 ± 0.1	5.9 ± 0.6‡
Prolonged (4 hr) intravenous glucose	11 ± 1	40 ± 8	101 ± 23	100 ± 3	221 ± 1	2.23 ± 0.25	1.1 ± 0.1	1.3 ± 0.2

* Mean values ± SEM are shown.
† Calculated as the product of the hepatic blood flow and the arteriohepatic venous blood glucose concentration difference.
‡ P value < 0.01 compared to control study without oral glucose (paired t test).

plasma insulin concentration increased 18-fold (Table 1). The plasma glucose concentration was increased and maintained at 220–225 mg/dl with a coefficient of variation of 3.3 ± 0.4%.

The amount of glucose infused to maintain the hyperglycemic plateau represented an 8- to 9-fold increase in glucose turnover above basal rates. Net splanchnic glucose uptake accounted for less than 10% of the total amount of glucose metabolized.

Combined Intravenous-Oral Glucose Administration. During the first 60 min of intravenous glucose infusion alone, the plasma glucose was maintained at a hyperglycemic plateau of 224 ± 3 mg/dl with a coefficient of variation of 4.7 ± 0.6% (Table 2). Net splanchnic glucose uptake (20–60 min) was 1.0 ± 0.1 mg/kg per min. After the ingestion of oral glucose (60–240 min), net splanchnic glucose uptake increased 6-fold to 5.9 ± 0.6 ($P < 0.001$). When the same subjects were restudied on another day and the plasma glucose concentration was maintained at a similar hyperglycemic plateau (221 ± 2 mg/dl) with intravenous glucose alone for 240 min, the splanchnic glucose uptake remained stable at 1.1–1.3 mg/kg per min which was only 22% of that observed after oral glucose ($P < 0.001$).

The fasting plasma insulin concentration increased approximately 4-fold during the first 60 min of administration of intravenous glucose alone (Fig. 1). After the ingestion of oral glucose at 60 min, there was a further increase in the plasma insulin response, despite unchanged plasma glucose levels, reaching a mean value of 153 ± 7 μU/ml (60–240 min). The mean plasma insulin response (101 ± 23 μU/ml) from 60–240 min with prolonged intravenous glucose was only 66% of the value reached after oral glucose ($P < 0.01$).

DISCUSSION

The regulation of hepatic glucose metabolism has been the focus of much attention in recent years. Studies in man and animals employing isotope dilution techniques (16) as well as the hepatic venous catheter technique (3, 4) have demonstrated that physiologic increases in circulating insulin result in a prompt inhibition of hepatic glucose production. The quantitative importance of the liver in the uptake of an oral glucose load has also been documented. Comparison of oral and intravenous glucose tolerance curves in rats (17) and man (13) suggested that approximately 60% of an orally administered glucose load was retained in the liver. These indirect results were subsequently confirmed by direct measurements across the splanchnic bed (2). The importance of insulin in the hepatic uptake of an oral glucose load has recently been underscored by the observation that splanchnic retention of an orally ingested glucose load is reduced in maturity-onset diabetes (18).

The present studies have examined the relative contributions of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose disposal. When the plasma insulin concentration was raised to levels ranging from 400 to 1200 μU/ml while maintaining euglycemia, glucose balance across the splanchnic bed changed from a basal output to a net uptake. However, despite an increase in glucose turnover to values 5- to 6-fold above basal levels, net splanchnic glucose uptake accounted for only 5% of the total glucose taken up by all tissues of the body. These data thus indicate that compared to extra-hepatic glucose uptake, euglycemic hyperinsulinemia results in only minimal stimulation of net uptake of glucose by the liver. It should be noted that studies in the intact human being have indicated that portal insulin levels during glucose-stimulated insulin secretion generally remain below 500 μU/ml (8). Thus it is likely that the hepatic insulin levels achieved with

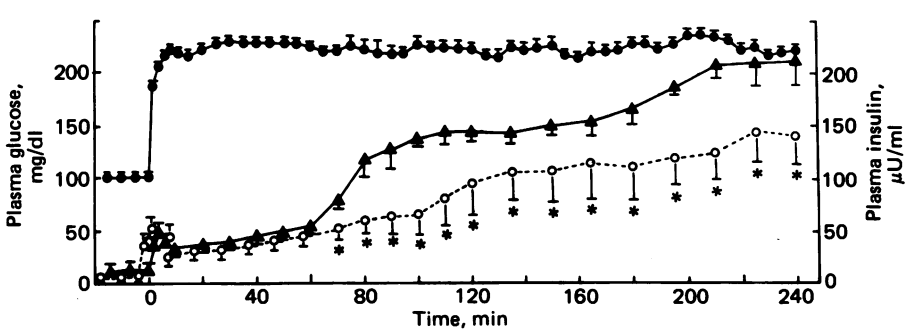

FIG. 1. Plasma glucose (●) and plasma insulin (▲) concentrations during the combined administration of intravenous and oral glucose. Glucose was infused intravenously at varying rates for 4 hr so that the plasma glucose concentration was maintained at 125 mg/dl above basal levels. Oral glucose was administered at 60 min. The dashed line represents the plasma insulin response (○) in control studies in the same subjects in which plasma glucose concentration was maintained at the same hyperglycemic plateau for 4 hr with intravenous glucose only. The plasma glucose concentration (not shown) during the control studies was similar to that observed during combined intravenous-oral glucose administration. (* = $P < 0.02$)

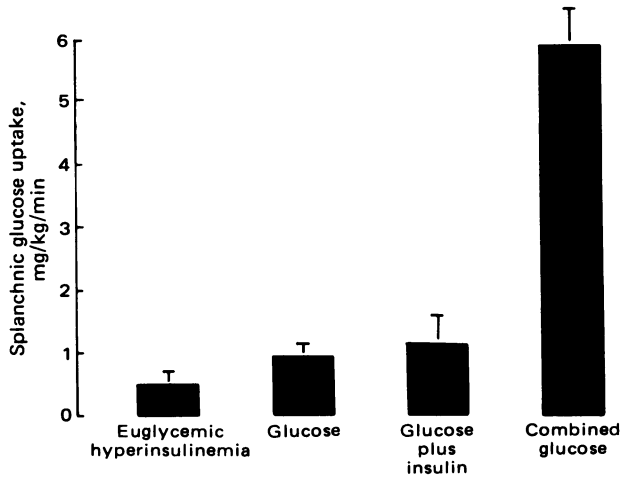

FIG. 2. Net splanchnic glucose uptake during euglycemic hyperinsulinemia (intravenous insulin), intravenous glucose, combined intravenous glucose plus intravenous insulin, and combined intravenous plus oral glucose administration.

intravenous insulin in these studies are comparable to or greater than that observed with maximal physiologic, endogenous hyperinsulinemia.

With regard to the role of hyperglycemia on hepatic glucose metabolism, Soskin and Levine (5) were the first to suggest that an elevation in plasma glucose concentration was the primary signal for hepatic glucose uptake. A similar conclusion has recently been reached by Bergman (6) employing the *in vivo* perfused liver of weanling pups. In contrast, Madison (3) was unable to demonstrate significant hepatic glucose uptake in alloxan-diabetic dogs despite plasma glucose levels in excess of 900 mg/dl. Administration of insulin to the same diabetic dogs prior to glucose loading markedly enhanced the hepatic uptake of glucose to levels observed in normal (nondiabetic) dogs. Similar results have been reported by Issekutz *et al.* in diabetic dogs (19). In human diabetics, intensification of fasting hyperglycemia by administration of glucose to insulin-withdrawn diabetics failed to inhibit net splanchnic glucose production (20). In the present studies, hyperglycemia induced by intravenous glucose resulted in net splanchnic glucose uptake, which was 2-fold greater than that observed with euglycemic hyperinsulinemia (Table 1 and Fig. 2). These observations indicate that in the presence of hyperinsulinemia, hyperglycemia stimulates a greater net hepatic uptake of glucose than that attributable to the rise in plasma insulin.

It should be noted that whereas hyperglycemia induced by intravenous glucose increased total glucose turnover 3- to 4-fold above basal levels (Table 2), net splanchnic glucose uptake accounted for only 14% of total glucose metabolism. It is of interest that the peripheral plasma insulin concentration in these studies (55 ± 5 μU/ml) was similar to that observed after the oral administration of 100 g of glucose to normal subjects (2, 13). Yet in the latter circumstance, net splanchnic glucose uptake accounts for approximately 60% of the total glucose metabolized (2, 18). Even when peripheral insulin levels were raised an additional 135 μU/ml by the combined intravenous infusion of glucose and insulin, splanchnic glucose uptake accounted for only 9% of total glucose metabolism (Table 1). These findings, coupled with previous observations on the quantitatively major role of the liver in the metabolism of orally administered glucose (2, 13), thus suggest that in addition to hyperinsulinemia or hyperglycemia, the route of glucose administration (oral versus intravenous) is an important factor in the stimulation of hepatic uptake of glucose.

To evaluate further the importance of the oral route in hepatic uptake of administered glucose, we examined the response to sustained hyperglycemia involving intravenous glucose alone for 60 min followed by oral glucose administration. After the administration of oral glucose at 60 min, net splanchnic glucose uptake increased to 6-fold greater than that observed with intravenous glucose despite the fact that the plasma glucose concentration was maintained at the same hyperglycemic plateau (Fig. 2). Although the insulin response when oral glucose was added reached levels of 212 ± 20 μU/ml, the studies with intravenous insulin or glucose (Table 1) indicate that neither hyperinsulinemia nor hyperglycemia can account for the marked stimulation of net splanchnic glucose uptake that followed oral glucose ingestion. Because the steady-state portal-peripheral insulin ratio is generally 3:1 (8), portal insulin levels may be estimated to have reached concentrations of 600–700 μU/ml, which is well below that observed in the high dose "insulin clamp" studies (Table 1). To eliminate the possible effect of prolonged hyperinsulinemia and hyperglycemia on splanchnic glucose uptake, the same subjects were restudied and the hyperglycemic plateau was maintained for 4 hr with intravenous glucose alone. Despite the persistance of hyperglycemia and hyperinsulinemia, splanchnic glucose uptake was only one-fifth that observed with combined intravenous and oral glucose. The data thus are compatible with the conclusion that stimulation of hepatic glucose uptake after oral glucose administration is due at least in part, to the route of glucose administration.

With respect to the mechanism whereby oral glucose enhances splanchnic glucose uptake, changes in portal vein glucose concentration, some unique aspect of the portal regions of the liver tubules, or release of some gastrointestinal hormone are possible considerations. Concerning portal glucose levels, studies in dogs with implanted portal vein catheters to whom oral glucose is given in doses comparable to those employed in the present study have revealed portal vein glucose concentrations of 200–250 mg/dl (14). Data on portal vein glucose levels in humans after an oral glucose load are not available. However, assuming a portal venous flow of 1200 ml/min (2, 18), the mean rise in portal vein glucose concentration would be approximately 140 mg/dl if the glucose load were absorbed in 60 min, and 70 mg/dl if, as is more likely (13), the glucose load were absorbed in 2 hr. As shown during the intravenous glucose infusions, an increase in peripheral (and presumably portal) glucose levels of 120–125 mg/dl caused minimal stimulation of splanchnic uptake when the glucose was administered by the intravenous route. Regardless of the portal glucose concentration, the possibility must also be considered that the portal regions of the liver tubule are so constituted that more effective glucose uptake occurs when glucose enters via this route. However, studies in dogs have shown that oral glucose tolerance is significantly greater than intravenous glucose tolerance, regardless of whether the glucose is administered via the portal or a peripheral vein (21). Thus, neither portal hyperglycemia nor some unique aspect of the portal region of the liver tubule is likely to account for the increased stimulation of hepatic glucose uptake when glucose is administered via the oral route.

With regard to the possible stimulation of a gastrointestinal hormone that enhances hepatic uptake of glucose, it is now well established that oral glucose administration results in a greater insulin secretory response than that observed with intravenous glucose (22). This effect of oral glucose is further confirmed in the present study by the rise in plasma insulin after oral glucose administration despite unchanged plasma glucose levels (Fig. 1). Recent studies indicate that gastric inhibitory polypeptide is the gastrointestinal hormone that is released by oral glucose ingestion and enhances the beta cell response to hyperglycemia

(23). The present findings suggest that oral glucose administration may also stimulate the release of a gastrointestinal factor(s) that enhances insulin-stimulated glucose uptake by the liver. In earlier studies comparing the responses to intraduodenal and intravenous glucose in normal dogs and dogs with protacaval anastomosis, Lickley *et al.* (21) also suggested that normal disposal of oral glucose may depend on an effect of the gut upon the liver by which net uptake of glucose is stimulated. Whether a known gastrointestinal hormone (e.g., gastric inhibitory polypeptide, gastrin, secretin, pancreozymin, or cholecystokinin) or some as yet unidentified gastrointestinal factor is responsible for the enhancement of splanchnic glucose uptake induced by oral glucose in the present study remains to be established.

The current observations indicating the importance of the route of glucose administration on hepatic glucose metabolism may also have relevance to *in vitro* studies with insulin. A discrepancy between the relatively meager *in vitro* effect of insulin in stimulating net glucose uptake by liver compared to the *in vitro* effects of insulin on glucose uptake by muscle and adipose tissue has long been recognized (24). The relatively minor effect of insulin in stimulating glucose uptake by the isolated liver may in part reflect the lack of a gut-derived factor that enhances insulin action on the liver when glucose is ingested orally in an *in vivo* condition.

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