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Research Article

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Differential Effects of Insulin on Splanchnic and Peripheral Glucose Disposal after an Intravenous Glucose Load in Man

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ABSTRACT The present study was designed to investigate the mechanisms by which insulin regulates the disposal of an intravenous glucose load in man. A combined tracer-hepatic vein catheter technique was used to quantitate directly the components of net splanchnic glucose balance (NSGB), i.e., splanchnic glucose uptake and hepatic glucose output, and peripheral (extrasplanchnic) glucose uptake. Four different protocols were performed: (*a*) intravenous infusion of glucose alone (6.5 mg kg^{-1} min^{-1}) for 90 min (control group); (*b*) glucose plus somatostatin (0.6 mg/h) and glucagon (0.8 ng kg^{-1} min^{-1}); (*c*) glucose plus somatostatin, glucagon, and insulin (0.15 mU kg^{-1} min^{-1}); and (*d*) glucose plus somatostatin, glucagon, and insulin (0.4 mU kg^{-1} min^{-1}). In groups 2–4, arterial blood glucose was raised to comparable levels to those of controls (≃170 mg/dl) by a variable glucose infusion. In the control group, plasma insulin levels reached 40 μU/ml at 90 min. NSGB switched from a net output of 1.71±0.13 to a net uptake of 1.5–1.6 mg kg^{-1} min^{-1} due to a 90–95% suppression of hepatic glucose output ($P < 0.01$) and a 105–130% elevation of splanchnic glucose uptake (from 0.78±0.13 to 1.6–1.8 mg kg^{-1} min^{-1}; $P < 0.01$). Peripheral glucose uptake rose by 150–160% ($P < 0.01$). In group 2, plasma insulin fell to <5 μU/ml. Net splanchnic glucose output initially rose twofold but later returned to basal values. This response was entirely accounted for by similar changes in hepatic glucose output since splanchnic glucose uptake remained totally unchanged in spite of hyperglycemia. In contrast, peripheral glucose uptake rose consistently by 100% ($P < 0.01$) despite insulin deficiency. In an additional group of experiments, glucose metabolism by the forearm muscle tissue was quantitated during identical conditions to those of group 2 (hyperglycemia plus insulin deficiency). Both the arterial-deep venous blood glucose difference and forearm glucose uptake increased markedly by 300–400% ($P < 0.05 - <0.01$). In group 3, plasma insulin was maintained at near-basal, peripheral levels (12–14 μU/ml). Hepatic glucose output decreased slightly by 35–40% ($P < 0.05$) while splanchnic glucose uptake remained unchanged. Consequently, the net glucose overproduction seen in group 2 was totally prevented although NSGB still remained as a net output. In group 4, peripheral insulin levels were similar to those of the control group (35–40 μU/ml). The suppression of hepatic glucose output was more pronounced (60–65%) and splanchnic glucose uptake rose consistently by 65% ($P < 0.01$). Consequently, NSGB did not remain as a net output but eventually switched to a small uptake (0.3 mg kg^{-1} min^{-1}). Peripheral glucose uptake rose to the same extent as in controls.

It is concluded that: (*a*) the suppressive effect of hyperglycemia on hepatic glucose output is strictly dependent on the degree of hepatic insulinization; (*b*) insulin plays an essential role in promoting splanchnic glucose uptake after an intravenous glucose load whereas hyperglycemia per se is totally unable to activate this process; (*c*) peripheral glucose uptake is markedly stimulated by hyperglycemia even in the face of insulin deficiency. Direct evidence also demonstrates that the skeletal muscle is involved in this response. Our data, thus, indicate that insulin rather than hyperglycemia regulates splanchnic glucose disposal in man. On the other hand, hyperglycemia per se appears to be an important regulator of glucose disposal by peripheral tissues.

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INTRODUCTION

Although the importance of insulin in the regulation of glucose metabolism is well recognized, only in recent years some of the effects of this hormone have

been precisely quantitated by inducing selective insulin deficiency with somatostatin. In particular, studies by Cherrington et al. (1) have firmly established the essential role of insulin in the maintenance of glucose homeostasis in the postabsorptive state. In contrast, the role of insulin in the disposal of a glucose load has been more difficult to quantitate in part because hyperglycemia per se exerts important regulatory influences that mimic those of insulin. In particular, it has been shown both in dogs (2) and in man (3, 4) that hyperglycemia suppresses hepatic glucose output. Circumstantial evidence also suggests that a minimum amount of insulin is required for hyperglycemia to exert this effect (5, 6). The precise relation between the degree of insulinization and the response of hepatic glucose output to a glucose load has not, however, been investigated in man.

Even less information is available regarding the role of insulin in the regulation of glucose utilization following a glucose load in man. Previous tracer studies have documented the importance of the extra insulin evoked by glucose administration in promoting overall glucose uptake (7). However, due to the method used, those studies have provided no information as to the consequences of insulin deficiency on splanchnic as compared with peripheral glucose utilization. Since it is now well established that both sites are involved in the disposal of a glucose load in man (8, 9), such data would be particularly useful not only to appreciate thoroughly the physiologic role of insulin in the disposal of a glucose load but also to elucidate the mechanisms leading to glucose intolerance in the diabetic state and the relative contribution of splanchnic vs. peripheral tissues.

The current study was consequently undertaken to determine the influence of the degree of insulinization on the glucoregulatory response to an intravenous glucose load in normal man. For this purpose, glucose was administered either alone or in conjunction with somatostatin, glucagon, and variable replacement amounts of insulin so as to create a spectrum of splanchnic and peripheral insulinizations ranging from severe insulin deficiency to normal insulin levels. A combined tracer-hepatic vein catheter technique was used to quantitate the influence of the degree of insulinization on each of the components of the glucoregulatory process, that is, endogenous glucose output, splanchnic glucose uptake, and peripheral glucose uptake. Furthermore, since the combined technique measures glucose uptake by all extrasplanchnic tissues and does not distinguish between insulin-dependent and -independent tissues, an additional group of studies was performed to examine specifically the consequences of insulin deficiency on glucose disposal by the forearm muscle tissue.

METHODS

Subjects. 25 male subjects aged 22–36 yr were studied. All were within 15% of their ideal body weight (Metropolitan Life Insurance Tables, 1959) and exhibited normal glucose tolerance after a 75-g oral glucose test (10). None had a history of liver disease or were taking any drugs. The group ($n = 20$) that participated in the hepatic vein catheterization studies consisted of asymptomatic patients admitted to the Institute of Medical Pathology for right cardiac catheterization for diagnostic purposes. The indication to the diagnostic catheterization was formulated on the basis of both physical examination and noninvasive diagnostic procedures that were suggestive of: atrial septal defects ($n = 5$), isolated mitral valve disease ($n = 9$), and isolated aortic valve disease ($n = 6$). Only those patients who were thought to have mild cardiovascular abnormalities but required cardiac catheterization were selected for this study. None of them had a positive history of congestive heart failure or arrythmias. The nature, purpose, and possible risks of the additional procedures were fully explained to each subject before his written consent to participate was obtained prior to cardiac catheterization.

Procedures. All subjects were studied in the postabsorptive state after a 15–17-h overnight fast. Teflon catheters were inserted percutaneously in a brachial or radial artery for blood sampling and in an antecubital vein for infusion of [3-^{3}H]glucose, unlabeled glucose, indocyanine green dye, and hormones. A Cournand catheter (7F) was introduced percutaneously in a contralateral antecubital vein and advanced under fluoroscopic control into the right heart for diagnostic catheterization. After the diagnostic procedure was completed, the catheter was advanced into a right-sided main hepatic vein and its tip placed 2–3 cm from the wedge position. Patency of the hepatic vein catheter was maintained by a continuous infusion of saline containing no anticoagulant. At the beginning of each experiment ($t = -90$ min), a priming dose of [3-^{3}H]glucose (Amersham, Buckinghamshire, England) and indocyanine green dye (Cardio-Green, Hynson, Westcott and Dunning, Baltimore, MD) was injected rapidly followed by a continuous infusion at a rate of 0.5 μCi/min and 0.5 mg/min, respectively, for the remainder of the study.

After a 90-min equilibration period, four groups of experiments were performed. In the first ($n = 5$), glucose alone was infused intravenously at a rate of 6.5 mg kg^{-1} min^{-1} for 90 min (control group). In the second group ($n = 5$), glucose was infused together with somatostatin (0.6 mg/h) and glucagon (0.8 ng kg^{-1} min^{-1}). The replacement dose of glucagon was chosen so as to maintain peripheral glucagon levels at/or slightly above base line. Insulin was not replaced in this group of studies in order to induce a state of acute, severe insulin deficiency at both splanchnic and peripheral level. In the third ($n = 4$) and fourth ($n = 6$) group of experiments, insulin was added in doses of 0.15 and 0.4 mU kg^{-1} min^{-1}, respectively. The dose of 0.15 mU kg^{-1} min^{-1} was chosen so as to replace the basal insulin levels in the peripheral circulation. The rate of 0.4 mU kg^{-1} min^{-1} was used to supply the liver with basal amounts of insulin and at the same time to simulate the increment of insulin concentration occurring in the periphery in the control group during the infusion of glucose alone (vide infra). The two doses of insulin (0.15 and 0.4 mU kg^{-1} min^{-1}) will be referred to as low and high replacement doses, respectively. Furthermore, while in the control group the glucose infusion rate was fixed, in the remaining three groups it was variable (1.7±0.3, 3.1±0.3, and 4.2±0.4 mg kg^{-1} min^{-1}, respectively) so as to raise blood

glucose concentration to comparable levels to those of the control group. This was done in order to be able to evaluate the net effects of insulin on glucose metabolism, independent of the mass effect of glucose per se. Cold glucose was added to the tritiated glucose solution to a final concentration of 0.5 mg/ml. The hormonal infusate was prepared in sterile saline containing human albumin (250 mg/dl) to prevent adsorption to tubing. Blood samples were withdrawn simultaneously from the arterial and the hepatic vein catheters for chemical analyses and hepatic blood flow measurements in the basal state and at 15-min intervals thereafter.

Analytical methods. Blood glucose concentration was measured by the glucose oxidase method (11). The methods used for the determination of plasma immunoreactive insulin and glucagon (using antibody 30K) have been previously described (12). The plasma concentration of indocyanine green was determined in a Beckman spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). For the assay of [3-^{3}H]glucose radioactivity, blood samples were deproteinized with $Ba(OH)_2$-$ZnSO_4$ and the supernatant was evaporated to dryness at 60°C to remove tritiated water. The dry residue was dissolved in 1 ml of water and counted with 10 ml of Insta-Gel (Packard Instruments, Inc., Downers Grove, IL) in a liquid scintillation system. The counting error was always <0.6% ($\pm 2\sigma$).

Calculations. Hepatic blood flow was estimated according to the method of Leevy et al. (13). Net splanchnic glucose balance was calculated by multiplying the hepatic venous-arterial (HV-A) blood glucose difference by the estimated hepatic blood flow (EHBF). Splanchnic glucose uptake and hepatic glucose output were calculated using the following equations:

$$\text{Splanchnic glucose uptake} = \frac{\text{(HV-A) blood [3-}^3\text{H]glucose}}{\text{arterial blood [3-}^3\text{H]glucose}} \times \text{EHBF} \times \text{arterial blood glucose concentration.} \quad (1)$$

$$\text{Hepatic glucose output} = \text{net splanchnic balance} + \text{splanchnic uptake.} \quad (2)$$

Glucose uptake by peripheral (extra-splanchnic) tissues was estimated according to the formula:

$$\text{Peripheral glucose uptake} = \text{systemic glucose delivery} - pVdg/dt$$

where the systemic glucose delivery = net splanchnic glucose balance + exogenously infused glucose; p is the rapidly mixing compartment of the glucose space, assumed to be 0.70 (14); V is the distribution volume of glucose, assumed to be 20% of body weight (15); g is the blood glucose concentration. The values of g and dg/dt were calculated from their polynomial functions fitted by the method of least squares.

Forearm studies. Five studies were performed in normal, nonobese male subjects in the postabsorptive state. A Teflon catheter was introduced into a large antecubital vein and threaded as deeply as possible into the forearm tissue toward the wrist. In these conditions the effluent venous blood drained predominantly muscle tissue. A second Teflon catheter was inserted percutaneously into the ipsilateral brachial artery for blood sampling and for infusion of indocyanine green dye to measure forearm blood flow. Simultaneous blood samples were collected from the arterial and venous catheters at 15-min intervals in the basal state. Then, a combined infusion of glucose (1.7 mg kg^{-1} min^{-1}), somatostatin (0.6 mg/h), and glucagon (0.8 ng kg^{-1} min^{-1}) was delivered through an indwelling catheter placed in a contralateral antecubital vein and blood samples were collected at 30-min intervals for 90 min. 5 min before each blood collection, a sphygmomanometer cuff placed around the wrist was inflated 100 mm Hg above the arterial blood pressure in order to exclude the hand from the circulation. Soon after blood collection, indocyanine green was infused through the arterial catheter, while keeping inflated the cuff around the wrist. After 4–5 min, a venous blood sample was taken to measure the plasma concentration of the dye. The infusion of indocyanine green was then stopped and the arterial catheter maintained patent in the intervals between samplings by intermittent flushing with saline. Plasma flow to the forearm was estimated by dividing the infusion rate of the dye by its concentration in the venous plasma and converted to blood flow according to the hematocrit. No correction for recirculation of dye was made since the concentration of recirculated dye (as determined in the contralateral vein blood in the basal state) was found to be negligible. Glucose uptake by deep forearm tissue (mainly muscle) was determined by multiplying the A-V blood glucose difference by the forearm blood flow and expressed as micrograms per 100 ml tissue per minute. The forearm volume was measured by water displacement. The theory and the assumptions inherent in the current approach for quantitating forearm tissue metabolism have been discussed in detail in previous papers (16, 17).

All calculations were performed on a Wang 2200 computer (Wang Laboratories, Inc., Lowell, MA). Statistical analyses were performed with the one- or two-way analysis of variance and the Dunnett's test (18). Data are presented as the mean±SEM.

RESULTS

Infusion of glucose alone. The glucoregulatory response to the infusion of glucose alone is illustrated in Fig. 1. Arterial blood glucose concentration rose progressively reaching 175±9 mg/dl at 90 min. Net splanchnic glucose output (1.71±0.13 mg kg^{-1} min^{-1}, basal) fell rapidly, switching to a net uptake of 1.6–1.7 mg kg^{-1} min^{-1} ($P < 0.01$) between 45–90 min. The mechanism responsible for the reversal of net splanchnic glucose balance is twofold: (*a*) hepatic glucose output fell from the basal value of 2.53±0.23 mg kg^{-1} min^{-1} to levels of 0.11–0.25 mg kg^{-1} min^{-1} (45–90 min), corresponding to a 90–95% suppression ($P < 0.01$); and (*b*) splanchnic glucose uptake (0.78±0.13 mg kg^{-1} min^{-1}, basal) rose consistently, stabilizing at levels 105–130% above base line between 30–90 min ($P < 0.05 - <0.01$). Glucose uptake by peripheral tissues (1.71±0.13 mg kg^{-1} min^{-1}, basal) increased progressively during the glucose infusion reaching levels 150% above base line at the end of the study period ($P < 0.01$).

As shown in Table I, the estimated hepatic blood flow was not significantly affected by the infusion of glucose alone. Arterial insulin levels increased three–fourfold after the infusion of glucose, while arterial plasma glucagon fell by 25–30% below base line ($P < 0.05$) (Table I).

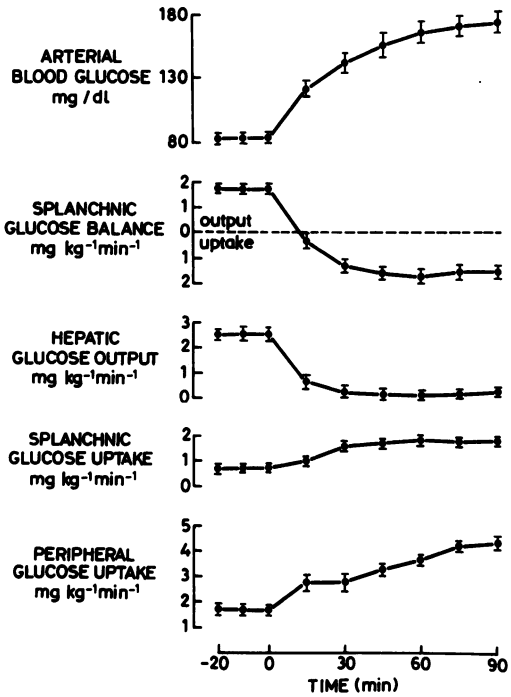

FIGURE 1 Changes in arterial blood glucose concentration, net splanchnic glucose balance, hepatic glucose output, splanchnic glucose uptake, and peripheral glucose uptake during infusion of glucose (6.5 mg kg^{-1} min^{-1}) alone.

Infusion of glucose, somatostatin, and glucagon (combined splanchnic and peripheral insulin deficiency). As shown in Table I, the addition of somatostatin to the glucose infusion caused the arterial insulin concentration to fall to values < 5 μU/ml. On the other hand, the replacement dose of glucagon maintained the arterial concentration of this hormone at, or slightly above base line. Considering that the portal-peripheral gradient for glucagon is only 1.5:1 (19) and that plasma glucagon fell by 25–30% during the infusion of glucose alone, it may be reasonably expected that in this group of experiments the liver was exposed to nearly the same amount of glucagon as in controls. The estimated hepatic blood flow decreased by 20–25% ($P < 0.05$), an expected consequence of somatostatin infusion.

The changes in blood glucose and glucose kinetics are illustrated in Fig. 2. Blood glucose concentration rose to values comparable to those observed in the control group, in spite of the much lower dose of glucose used (1.7 mg kg^{-1} min^{-1}). Net splanchnic glucose output increased significantly between 30–75 min ($P < 0.05$–<0.01) and then returned to values not statistically different from base line at 90 min. The mechanisms responsible for this opposite response of the splanchnic balance to that seen in controls (where net glucose output switched to a net uptake) are evident when the two components of the splanchnic balance are examined individually. Hepatic glucose output increased initially ($P < 0.05$–<0.01 between 30 and 60 min) but later returned to base line (P = NS at 75 and 90 min); a virtual identical profile to that observed for splanchnic glucose balance. On the other hand, splanchnic glucose uptake remained totally unchanged in the face of hyperglycemia. Peripheral glucose uptake increased to a comparable extent to that seen in the control group in the first 60 min of the glucose infusion and then declined to values significantly lower than those observed in controls ($P < 0.01$).

TABLE I

Changes in Plasma Insulin (IRI), Plasma Glucagon (IRG), and Estimated Hepatic Blood Flow (EHBF) during Infusion of Glucose Alone (I), Glucose Plus Somatostatin and Glucagon (II), Glucose Plus Somatostatin, Glucagon and Low Dose Insulin (III), and Glucose Plus Somatostatin, Glucagon, and High Dose Insulin (IV)

	Group	0	15 min	30 min	45 min	60 min	75 min	90 min
IRI, μU/ml	I	10±3*	20±6	25±7	28±8	35±13	37±13	40±12
	II	9±7	4±0.4	3±0.5	3±0.8	4±0.9	3±0.5	3±0.4
	III	12±2	14±3	13±3	14±4	14±3	13±3	13±3
	IV	11±2	31±4	31±3	34±5	35±6	35±6	37±6
IRG, pg/ml	I	118±15	98±12	81±8	75±7	72±5	72±5	75±6
	II	122±17	129±23	131±27	139±28	129±24	130±26	120±28
	III	120±6	121±13	129±18	132±26	130±20	128±16	115±12
	IV	124±21	151±20	138±20	145±17	147±18	123±23	135±16
EHBF, ml/min	I	1,107±60	1,170±59	1,277±195	1,224±160	1,169±93	1,138±89	1,230±145
	II	1,409±232	1,108±217	1,004±148	1,142±219	1,134±198	1,220±197	1,094±143
	III	1,384±153	1,069±137	1,000±107	1,084±116	1,026±52	1,051±82	1,100±161
	IV	1,294±123	968±92	963±95	946±97	892±81	874±73	891±58

* Data presented as mean±SE.

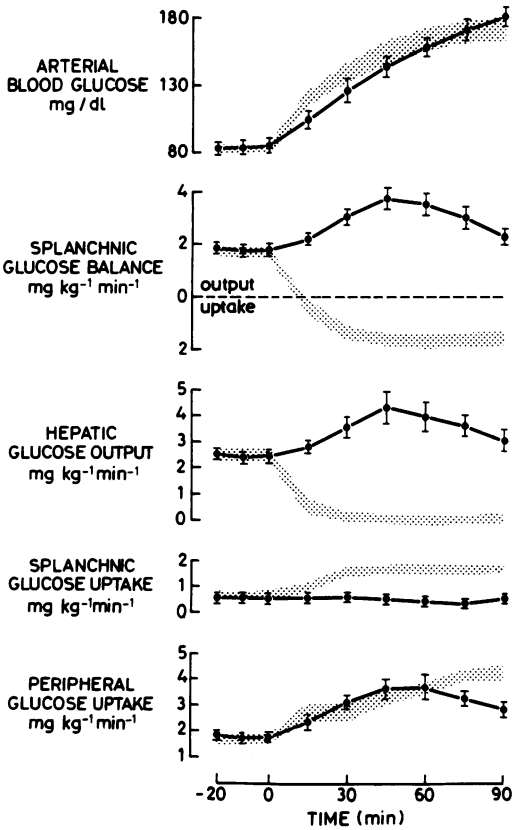

FIGURE 2 Changes in arterial blood glucose concentration, net splanchnic glucose balance, hepatic glucose output, splanchnic glucose uptake, and peripheral glucose uptake during combined infusion of glucose (1.7±0.3 mg kg^{-1} min^{-1}), somatostatin (0.6 mg h^{-1}), and glucagon (0.8 ng kg^{-1} min^{-1}). The shaded area denotes the response to glucose alone.

Infusion of glucose, somatostatin, glucagon, and low dose insulin (under-basal hepatic insulinization, basal peripheral insulin levels). As shown in Table I, the replacement dose of insulin used in this group (0.15 mU kg^{-1} min^{-1}) maintained the insulin concentration in the periphery at values only slightly above those existing in the basal state. Thus, it may be reasonably assumed that some degree of hepatic underinsulinization was present as compared with the basal state, due to the well known 2.5–3:1 portal-peripheral gradient for insulin (19). On the other hand, the arterial concentration of glucagon remained at nearly basal values as in the previous group of experiments. The estimated hepatic blood flow declined significantly below base line by 15–25% ($P < 0.05$).

The response of blood glucose and glucose kinetics is illustrated in Fig. 3. The increment in arterial glucose concentration was similar to that of the control study in spite of the lower infusion rate of exogenous

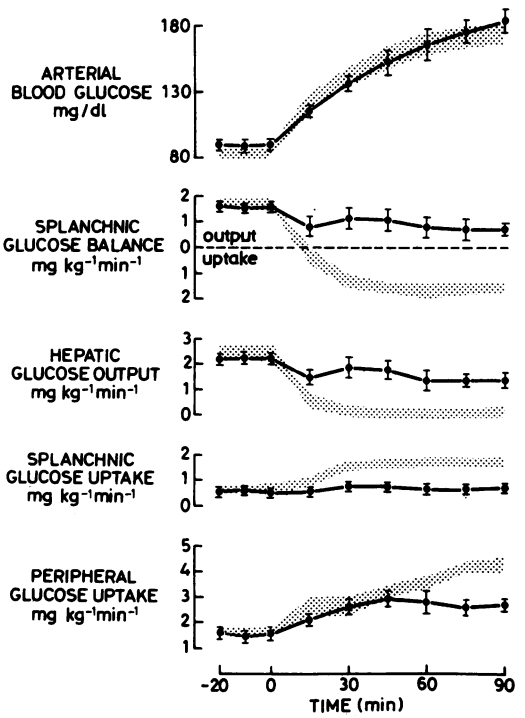

FIGURE 3 Changes in arterial blood glucose concentration, splanchnic glucose balance, hepatic glucose output, splanchnic glucose uptake, and peripheral glucose uptake during combined infusion of glucose (3.1±0.3 mg kg^{-1} min^{-1}), somatostatin (0.6 mg h^{-1}), glucagon (0.8 ng kg^{-1} min^{-1}), and low dose insulin (0.15 mU kg^{-1} min^{-1}). The shaded area denotes the response to glucose alone.

glucose (3.1 mg kg^{-1} min^{-1}). Net splanchnic glucose output decreased by 30–55% ($P < 0.05$) and thus did not switch to a net uptake. Noteworthy is, however, the fact that the splanchnic glucose overproduction seen in the previous group (Fig. 2) did not occur at any time during the entire study period. Similarly, hepatic glucose output did not rise above base line, but rather decreased significantly ($P < 0.05$), although the magnitude of its suppression (35–40%) was considerably smaller than that observed in the control study (90–95%; $P < 0.025$). Furthermore, splanchnic glucose uptake remained virtually unchanged throughout the experimental period. Glucose uptake by peripheral tissues increased to a similar extent to that seen in controls in the first 60 min. Afterwards, it tended to stabilize at values 30% below those reached in the control group ($P < 0.05$ and <0.01 at 75 and 90 min, respectively).

Infusion of glucose, somatostatin, glucagon, and high dose insulin (basal hepatic insulinization, normal peripheral insulinization). The dose of insulin used in this group of experiments (0.4 mU kg^{-1} min^{-1}) produced peripheral insulin levels comparable to those

of the control study (Table I). Furthermore, because plasma insulin concentration in the periphery was three times above base line, it may be expected that the liver was exposed to basal amounts of insulin also during the infusion period. As observed in the previous two groups, plasma glucagon concentration was maintained at nearly basal values. The estimated hepatic blood flow decreased significantly by 25–35% ($P < 0.01$).

The response of blood glucose and glucose kinetics is illustrated in Fig. 4. Arterial blood glucose rose to comparable values to those reached in the control experiments despite the lower rate of glucose infusion (4.2 mg kg^{-1} min^{-1}). Net splanchnic glucose output fell consistently and eventually switched to a small net uptake (0.3–0.35 mg kg^{-1} min^{-1}) in the last 30 min of the infusion period. However, a marked difference was still present as compared with the control group between 30–90 min ($P < 0.01$). The failure of the splanchnic balance to switch to the considerable net uptake seen in controls was due to incomplete suppression of hepatic glucose output as well as to the smaller elevation of splanchnic glucose uptake. Hepatic glucose output fell by 60–65%, thus remaining at levels significantly higher than those of the control group ($P < 0.05$). Splanchnic glucose uptake did rise in this group of experiments by 55–65% between 45 and 90 min ($P < 0.05$–<0.01). This increment, however, was significantly smaller when compared with that reached with the infusion of glucose alone ($P < 0.01$). In contrast, peripheral glucose uptake rose to a comparable extent to that seen in the control study throughout the entire study period.

Forearm studies. The conditions of this group of studies were the same as those of the experiments illustrated in Fig. 2 (hyperglycemia plus insulin deficiency). Plasma insulin levels (not shown) fell from 10±2 μU/ml to 3–5 μU/ml during the infusion period. Plasma glucagon (110±9 pg/ml, basal) remained nearly unchanged. As shown in Fig. 5, arterial blood glucose rose to similar values to those reached in the other groups of experiments. The arterial-deep venous blood glucose difference (3.28±.33 mg/dl, basal) increased fivefold in response to hyperglycemia, despite insulin deficiency ($P < 0.05$–<0.01). A similar profile was observed for glucose uptake by the forearm tissue, which rose from the basal value of 88 μg/100 ml min^{-1} to the peak value of 480 μg/100 ml min^{-1} at 60 min ($P < 0.05$ between 30 and 90 min). The estimated fore-

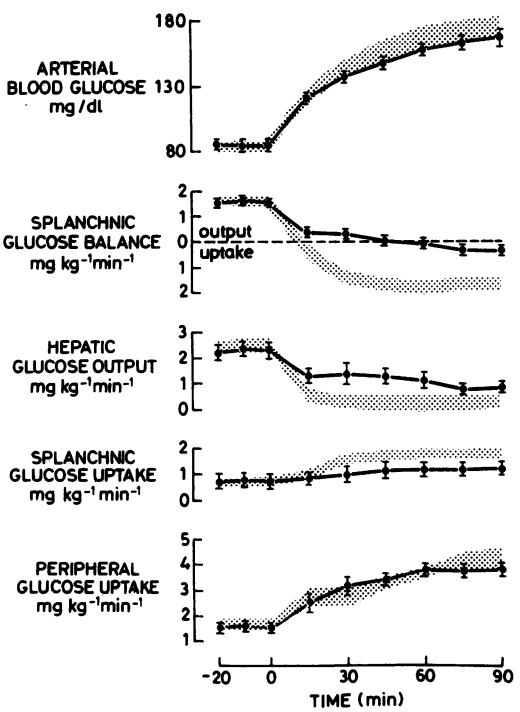

FIGURE 4 Changes in arterial blood glucose concentration, splanchnic glucose balance, hepatic glucose output, splanchnic glucose uptake, and peripheral glucose uptake during combined infusion of glucose (4.2±0.4 mg kg^{-1} min^{-1}), somatostatin (0.6 mg h^{-1}), glucagon (0.8 ng kg^{-1} min^{-1}), and high dose insulin (0.4 mU kg^{-1} min^{-1}). The shaded area denotes the response to glucose alone.

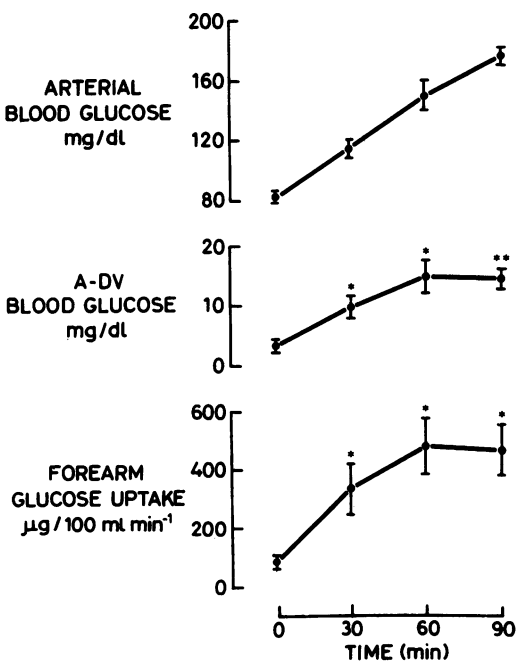

FIGURE 5 Changes in arterial blood glucose concentration, forearm arterial-deep venous blood glucose difference, and forearm glucose uptake during combined infusion of glucose (1.7 mg kg^{-1} min^{-1}), somatostatin (0.6 mg h^{-1}), and glucagon (0.8 ng kg^{-1} min^{-1}).

arm blood flow was 2.77±0.24 ml/100 ml min^{-1} in the basal state and 3.22±0.32, 3.19±0.38, and 3.27±0.38 ml/100 ml min^{-1} at 30, 60, and 90 min, respectively (P = NS).

DISCUSSION

In this study we examined the role of insulin in the response of splanchnic and peripheral glucose metabolism to an intravenous glucose load in man. Different degrees of insulinization were induced by infusing glucose, either alone or combined with somatostatin and variable replacement amounts of insulin. In order to evaluate the net effect of insulin independent of the mass action of glucose concentration per se, similar glycemic increments were reproduced in the various experimental groups by varying the infusion rate of exogenous glucose. In addition, a fixed replacement dose of glucagon was used in somatostatin-treated subjects so as to simulate the glucagon response observed in the control group.

The response of the control subjects to the infusion of glucose alone (Fig. 1) has been described in detail in a previous paper (9). Schematically, three mechanisms become operative to minimize the hyperglycemia consequent to the glucose load: (*a*) suppression of hepatic glucose output; (*b*) activation of splanchnic glucose uptake; and (*c*) activation of peripheral glucose uptake. The 90–95% suppression of hepatic glucose output seen in the current study is in close agreement with previous studies in normal humans (7, 20). Regarding the disposal of the glucose load, of particular interest is the observation that glucose uptake by the splanchnic area rose to values of 1.7–1.8 mg kg^{-1} min^{-1}, corresponding to approximately one-quarter of the exogenous glucose load (6.5 mg kg^{-1} min^{-1}). This accounts for the need to quantitate selectively this component of the glucoregulatory process (combined tracer-hepatic technique) in order to be able to appreciate thoroughly the role of insulin in the disposal of an intravenous glucose load.

When glucose was infused with somatostatin and no replacement dose of insulin (severe hepatic insulin deficiency), net splanchnic glucose balance exhibited an opposite response in that a net glucose overproduction occurred, although transiently, rather than a reversal to a net uptake (Fig. 2). This response may be entirely accounted for by the changes in endogenous glucose output inasmuch as splanchnic glucose uptake remained totally unaltered. In keeping with previous studies in dogs (6), the current data thus indicate that in a setting of severe insulin deficiency, hyperglycemia in itself is unable to suppress acutely endogenous glucose output, although it probably plays an important role in preventing sustained hepatic glucose overproduction. This view would not be in contrast with previous observations that hyperglycemia per se may induce an absolute inhibition of hepatic glucose output since this effect has been previously shown only in situations when near-basal amounts of insulin were supplied exogenously (2, 3, 7). Of particular interest in this context is also the observation by Wahren et al. (5) that glucose administration is unable to inhibit net splanchnic glucose output in insulin-withdrawn diabetic humans.

Particularly important is the current finding that during insulin deficiency the splanchnic area is unable to utilize glucose to a greater extent after an intravenous glucose load than in the postabsorptive state, despite hyperglycemia. This observation has three important implications: (*a*) insulin plays an essential role in promoting splanchnic glucose uptake after an intravenous glucose load; (*b*) hyperglycemia per se is incapable of activating splanchnic glucose disposal; and (*c*) the failure of splanchnic glucose uptake to increase is a contributory mechanism to the glucose intolerance associated with insulin deficiency.

Most of previous studies aimed at evaluating the relative roles of insulin and glucose in hepatic glucose uptake have used various in vitro preparations and have provided extremely contradictory results (e.g., 21–30). In contrast, studies performed in vivo on the diabetic dog (31) as well as in diabetic humans (5) have clearly documented the failure of the diabetic liver to switch to a net uptake during glucose infusion. Our data are consistent with those in vivo observations and indicate that the activation of splanchnic glucose uptake during glucose loading is an exquisite insulin-dependent process and not a passive consequence of hyperglycemia. The possibility that the reduced hepatic blood flow by somatostatin may have played a role by diminishing the rate of splanchnic glucose delivery is extremely unlikely for two reasons: (*a*) the reduction was small and might have produced only a 20–25% decrease in the splanchnic delivery of glucose; and (*b*) during somatostatin infusion splanchnic glucose uptake remained unchanged throughout the experimental period while in the control study, the reversal of splanchnic glucose balance already occurred at 30 min when the blood glucose concentration and, consequently, splanchnic glucose delivery had not yet increased markedly.

The conclusion that splanchnic glucose under-utilization is a contributory factor to glucose intolerance may not be readily apparent because blood glucose concentration rose to values similar to those seen in controls. It must be stressed, however, that in this group the infusion rate of exogenous glucose was only 1.7 instead of 6.5 mg kg^{-1} min^{-1} (controls). This difference (4.8 mg kg^{-1} min^{-1}) may be accounted for by

the splanchnic bed by ~25% inasmuch as splanchnic glucose uptake remained at values of 0.5–0.6 mg kg^{-1} min^{-1}, whereas in controls it rose up to 1.8 mg kg^{-1} min^{-1}. The contribution of splanchnic glucose underutilization to glucose intolerance was clearly smaller than that provided by the lack of suppression of hepatic glucose output. It may be speculated, however, that the role played by the splanchnic area could be even greater after an oral glucose load because in that circumstance a larger proportion of the load is utilized by the splanchnic tissues as compared with the intravenous load (32).

In spite of insulin deficiency, a striking increment in peripheral glucose uptake was observed after glucose infusion, although this response was not sustained as in the control group but tended to wane after 60 min (Fig. 2). This finding in itself may not be particularly impressive in view of the fact that the tracer-catheter technique measures glucose uptake by a variety of peripheral tissues including those insulin independent. An additional group of studies was, consequently, performed to determine whether the observed elevation of peripheral glucose uptake during insulin deficiency involved specifically the forearm muscle tissue. The data shown in Fig. 5 demonstrate that hyperglycemia is able to stimulate glucose uptake by the forearm to a considerable extent, even in the face of insulin deficiency. This observation is consistent with previous data, published in abstract form, indicating that hyperglycemia does stimulate glucose uptake by the forearm in insulin-dependent diabetic humans (33). It is also noteworthy in this context that earlier studies have demonstrated that increments in plasma insulin concentration up to 20–40 μU/ml, as occur in our control subjects, have little or no effect on forearm glucose uptake (17, 34). Taken together, the data from the experiments of Figs. 2 and 5 along with previous observations (17, 33, 34) lead to two important conclusions: (*a*) insulin deficiency produces differential effects on glucose utilization by splanchnic as compared with peripheral tissues (including the skeletal muscle); and (*b*) blood glucose concentration rather than insulin appears to be the primary regulator of muscle glucose uptake in man.

When the low replacement dose of insulin was used (0.15 mU kg^{-1} min^{-1}), the arterial insulin concentration was maintained at levels slightly above base line. Therefore, a near-basal insulinization was present in the periphery while the amount of insulin reaching the liver was in all likelihood less than that required to maintain a basal degree of insulinization. In spite of this, the response of the splanchnic area to the glucose load (Fig. 3) was substantially different from that observed when insulin was not replaced at all (Fig. 2). In fact, not only was glucose overproduction totally prevented by the small amount of insulin used, but even a significant suppression of hepatic glucose output took place, although to a considerably lesser extent than in controls (35–40% vs. 90–95%; $P < 0.01$). This finding substantiates the concept that a minimum degree of hepatic insulinization must be present for the inhibitory effect of hyperglycemia on hepatic glucose output to occur. It is also true, on the other hand, that glucose is not a very potent inhibitor of hepatic glucose output in circumstances of under-replacement of the basal insulin delivery to the liver.

The response of splanchnic glucose uptake to the glucose load was not altered by the low dose insulin infusion (Fig. 3). Splanchnic glucose uptake remained virtually unchanged, despite hyperglycemia, as also observed in the experiments illustrated in Fig. 2. Similarly, the pattern of the response of peripheral glucose uptake was comparable to that seen when insulin deficiency was present in the periphery (Fig. 2). Taken together, the data from the experiments of Figs. 2 and 3 demonstrate that small differences in the degree of insulin deficiency have a drastic impact on the response of hepatic glucose output to hyperglycemia but leave practically unmodified the response of glucose uptake by both splanchnic and peripheral tissues. This conclusion is consistent with recent studies by Rizza et al. (35) indicating that in circumstances of normoglycemia hepatic glucose output is more sensitive to changes in insulin concentration than is total-body glucose utilization.

The current data also permit some considerations as to the mechanisms underlying the glucose intolerance associated with insulin deficiency. In particular, they indicate that glucose intolerance may be in part alleviated by the presence of small amounts of insulin (10–15 μU/ml). This conclusion is based on the fact that similar glycemic increments to those reached in circumstances of more severe insulin deficiency (<5 μU/ml; Fig. 2) were obtained, although the infusion rate of exogenous glucose was almost doubled (3.1 instead of 1.7 mg kg^{-1} min^{-1}). It should be also noted that the only contributory mechanism to the improved glucose tolerance was provided by the different response of hepatic glucose output. The lack of a significant increment in splanchnic glucose uptake clearly indicates that this component does not cooperate to improve glucose intolerance, but, rather it now represents a more important contributory factor as compared to the situation when a more severe insulin deficiency was present (Fig. 2).

The addition of insulin at the dose of 0.4 mU kg^{-1} min^{-1} produced arterial insulin levels comparable to those reached in the control group. Furthermore, during the infusion period the insulin concentration was three–fourfold above base line and, therefore, ade-

quate to provide the liver with basal amounts of insulin in view of the 2.5–3:1 portal-peripheral gradient existing in the basal state (19). As shown in Fig. 4, the suppression of endogenous glucose output observed in these experiments (60–65%) was more pronounced than in the previous groups, although still incomplete as compared with that seen in controls ($P < 0.05$). It is clear from these data that the magnitude of the suppression of hepatic glucose output in response to hyperglycemia is strictly dependent on the degree of hepatic insulinization and that only when uninhibited insulin secretion is free to occur, maximal suppression is achieved.

Of particular interest is the observation that splanchnic glucose uptake rose consistently in this series of experiments, although the response seen in controls was not fully restored. An important consequence of this partial elevation of splanchnic glucose uptake associated with the more pronounced suppression of hepatic glucose output was that net splanchnic glucose balance did not remain as a net output as in the previous groups (Figs. 2 and 3) and eventually switched to a small net uptake. This finding may have practical implications as to the treatment of diabetic patients with artificial devices that deliver insulin continuously. Based on our data, it might be anticipated that only when the insulin infusion rate is adjusted to produce plasma insulin levels in the range of 30–40 μU/ml, the splanchnic area ceases to be a site of net glucose production and begins to utilize in part an incoming glucose load.

With respect to peripheral glucose uptake in the experiments involving the high dose insulin infusion, the magnitude of the response to the glucose load was restored to that seen in the control group, an expected finding in view of the similar plasma insulin concentration in the two groups.

The current approach is based on the assumption that somatostatin exerts no influence on the glucoregulatory system other than that mediated by suppression of insulin secretion. Supporting this assumption is the fact that in previous studies simultaneous infusions of somatostatin and basal amounts of insulin and glucagon were without effect both on glucose turnover (1, 6) and, specifically, on hepatic glucose balance (2). Similarly, in the current experiments involving somatostatin and high dose insulin (Fig. 4), the degree of peripheral insulinization was comparable to that of the control group and this was associated with restoration of peripheral glucose uptake to control values. This finding is also consistent with previous in vitro observations that somatostatin fails to alter glucose uptake by adipose or muscle tissue (36). Finally, somatostatin is known to inhibit the secretion of a number of other hormones, including growth hormone, thyroid-stimulating hormone, prolactin, and various gastro-intestinal hormones (37). It can be reasonably excluded, however, that these hormones may have influenced the results of the current study since they are incapable of producing acutely the changes in glucose metabolism described here. In conclusion, although the experimental condition created by somatostatin cannot be strictly regarded as physiological due to the multiple effects of the peptide, the available evidence supports the validity of the current approach to examining the glucoregulatory role of insulin in the disposal of an intravenous glucose load.

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REFERENCES

1. Cherrington, A. D., J. L. Chiasson, J. E. Liljenquist, A. S. Jennings, U. Keller, and W. W. Lacy. 1976. The role of insulin and glucagon in the regulation of basal glucose production in the postabsorptive dog. *J. Clin. Invest.* **58:** 1407–1418.
2. Shulman, G. I., J. E. Liljenquist, P. E. Williams, W. W. Lacy, and A. D. Cherrington. 1978. Glucose disposal during insulinopenia in somatostatin-treated dogs. The roles of glucose and glucagon. *J. Clin. Invest.* **62:** 487–491.
3. Saccà, L., R. Hendler, and R. S. Sherwin. 1978. Hyperglycemia inhibits glucose production in man independent of changes in glucoregulatory hormones. *J. Clin. Endocrinol. Metab.* **47:** 1160–1163.
4. Liljenquist, J. E., G. L. Mueller, A. D. Cherrington, J. M. Perry, and D. Rabinowitz. 1979. Hyperglycemia per se (insulin and glucagon withdrawn) can inhibit hepatic glucose production in man. *J. Clin. Endocrincol. Metab.* **48:** 171–175.
5. Wahren, J., P. Felig, E. Cerasi, and R. Luft. 1972. Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. *J. Clin. Invest.* **51:** 1870–1878.
6. Cherrington, A. D., W. W. Lacy, and J. L. Chiasson. 1978. Effect of glucagon on glucose production during insulin deficiency in the dog. *J. Clin. Invest.* **62:** 664–677.
7. Saccà, L., D. Vitale, M. Cicala, B. Trimarco, and B. Ungaro. 1981. The glucoregulatory response to intravenous glucose infusion in normal man: roles of insulin and glucose. *Metab. Clin. Exp.* **30:** 457–461.
8. Felig, P., J. Wahren, and R. Hendler. 1975. Influence of oral glucose ingestion on splanchnic glucose and gluconeogenic substrate metabolism in man. *Diabetes.* **24:** 468–475.
9. Saccà, L., C. Vigorito, M. Cicala, B. Ungaro, and R. S. Sherwin. 1982. Mechanisms of epinephrine-induced glucose intolerance in normal humans. Role of the splanchnic bed. *J. Clin. Invest.* **69:** 284–293.
10. National Diabetes Data Group. 1979. Classification and

diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes.* **28:** 1039–1057.
11. Huggett, A. S. G., and D. A. Nixon. 1957. Use of glucose oxidase, peroxidase and o-dianisidine in determination of blood and urinary glucose. *Lancet.* **II:** 368–370.
12. Saccà, L., B. Trimarco, G. Perez, and F. Rengo. 1977. Studies on the mechanism underlying the influence of alanine infusion on glucose dynamics in the dog. *Diabetes.* **26:** 262–270.
13. Leevy, C. M., C. L. Mendenhall, W. Lesko, and M. M. Howard. 1962. Estimation of hepatic blood flow with indocyanine green. *J. Clin. Invest.* **41:** 1169–1179.
14. Steele, R., H. Rostami, and N. Altszuler. 1974. A two compartment calculator for the dog glucose pool in non-steady state. *Fed. Proc.* **33:** 1869–1876.
15. Insel, P. A., J. E. Liljenquist, J. D. Tobin, R. S. Sherwin, P. Watkins, R. Andres, and M. Berman. 1975. Insulin control of glucose metabolism in man. *J. Clin. Invest.* **55:** 1057–1066.
16. Andres, R., K. L. Zierler, H. M. Anderson, W. N. Stainsby, G. Cader, A. S. Ghrayyib, and J. L. Lilienthal, Jr. 1954. Measurement of blood flow and volume in the forearm of man; with notes on the theory of indicator-dilution and on production of turbulence, hemolysis, and vasodolatation by intra-vascular injection. *J. Clin. Invest.* **33:** 482–504.
17. Zierler, K. L., and D. Rabinowitz. 1962. Role of insulin and growth hormone, based on studies of forearm metabolism in man. *Medicine (Baltimore).* **42:** 385–402.
18. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Co., Inc., New York. 99–160.
19. Felig. P., R. Gusberg, R. Hendler, F. E. Gump, and J. M. Kinney. 1974. Concentrations of glucagon and the insulin:glucagon ratio in the portal and peripheral circulation. *Proc. Soc. Exp. Biol. Med.* **147:** 88–90.
20. Wolfe, R. R., J. K. Allsop, and J. F. Burke. 1979. Glucose metabolism in man: response to intravenous glucose infusion. *Metab. Clin. Exp.* **28:** 210–220.
21. Glinsman, W. H., E. P. Hern, and A. Lynch. 1969. Intrinsic regulation of glucose output by rat liver. *Am. J. Physiol.* **216:** 698–703.
22. Buschiazzo, H., J. H. Exton, and C. R. Park. 1970. Effects of glucose on glycogen synthetase, phosphorylase, and glycogen deposition in the perfused rat liver. *Proc. Natl. Acad. Sci. U. S. A.* **65:** 383–387.
23. Hems, D. A., P. D. Whitton, and E. A. Taylor. 1972. Glycogen synthesis in the perfused liver of the starved rat. *Biochem. J.* **129:** 529–538.
24. Miller, T. B., Jr., and J. Larner. 1973. Mechanism of control of hepatic glycogenesis by insulin. *J. Biol. Chem.* **248:** 3483–3488.
25. Seglen, P. O. 1974. Autoregulation of glycolysis, respiration, gluconeogenesis and glycogen synthesis in isolated parenchymal rat liver cells under aerobic and anaerobic conditions. *Biochim. Biophys. Acta.* **338:** 317–336.
26. Bucolo, R. J., R. N. Bergman, D. J. Marsh, and F. E. Yates. 1974. Dynamics of glucose autoregulation in the isolated, blood perfused canine liver. *Am. J. Physiol.* **227:** 209–217.
27. Witters, L. A., and J. Avruch. 1978. Insulin regulation of hepatic glycogen synthase and phosphorylase. *Biochemistry.* **17:** 406–410.
28. Katz, J., S. Golden, and P. A. Wals. 1979. Glycogen synthesis by rat hepatocytes. *Biochem. J.* **180:** 389–402.
29. Baldwin, Jr., D., S. Terris, and D. F. Steiner. 1980. Characterization of insulin-like actions of anti-insulin receptor. *J. Biol. Chem.* **255:** 4028–4034.
30. Boyd, M. E., E. B. Albright, D. W. Foster, and J. D. McGarry. 1981. In vitro reversal of the fasting state of liver metabolism in the rat. Reevaluation of the roles of insulin and glucose. *J. Clin. Invest.* **68:** 142–152.
31. Madison, L. L. 1969. Role of insulin in the hepatic handling of glucose. *Arch. Intern. Med.* **123:** 284–292.
32. DeFronzo, R. A., E. Ferrannini, R. Hendler, J. Wahren, and P. Felig. 1978. Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. *Proc. Natl. Acad. Sci. U. S. A.* **75:** 5173–5177.
33. Fineberg, S. E., S. H. Schneider, and D. Follas. 1979. Does hyperglycemia affect basal or insulin-stimulated forearm metabolism? *Diabetes.* **28:** 443(Abstr.).
34. Pozefsky, T., P. Felig, J. D. Tobin, J. S. Soeldner, G. F. Cahill, Jr. 1969. Amino acid balance across tissues of the forearm in postabsorptive man. Effect of insulin at two dose levels. *J. Clin. Invest.* **48:** 2273–2282.
35. Rizza, R. A., L. J. Mandarino, and J. E. Gerich. 1981. Dose-response characteristics for the effects of insulin on production and utilization of glucose in man. *Am. J. Physiol.* **240**(Endocrinol. Metab.): E630–E639.
36. Cherrington, A. D., M. D. Caldwell, M. R. Dietz, J. H. Exton, and O. B. Crofford. 1977. The effect of somatostatin on glucose uptake and production by rat tissues in vitro. *Diabetes.* **26:** 740–748.
37. Porte, D., Jr., and J. B. Halter. 1981. The endocrine pancreas and diabetes mellitus. *In* Textbook of Endocrinology. R. H. Williams, editor. W. B. Saunders Company, Philadelphia. 716–843.