Interaction of insulin and glucose in the control of hepatic glucose balance

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The role of insulin in regulating hepatic carbohydrate metabolism has been intensively studied (19, 26, 29). A controversy exists as to the importance of the portal vein insulin concentration in switching the liver from net glucose production to net glucose uptake after carbohydrate ingestion or glucose infusion. Several investigators, most notably Madison (19) and Felig and Wahren, (12), have taken the view that insulin is the primary signal for hepatic glucose uptake after feeding. They have based their opinions on demonstrations of the direct effect of insulin in diminishing glucose production in the porta caval shunt dog preparation (20) as well as on measurements using isotope-dilution techniques which show diminished glucose production in the unanesthetized dog after insulin administration (32). They have also cited the inability of the diabetic liver to take up more glucose than it produces except at extremely elevated portal glucose concentrations (21, 37).

We, along with others (7, 29), have recently questioned whether insulin plays the dominant role in regulating hepatic glucose uptake during glucose loading. Recent studies in our laboratory have led us to adopt the classical view of Soskin et al. (31) that glucose, rather than insulin, is the major stimulus to hepatic glucose uptake during the intravenous infusion of glucose. Using the isolated canine liver preparation, cross-perfused with blood, we were able to investigate effects of elevating the portal vein glucose concentration without changing the portal vein insulin level. We found the net hepatic glucose balance to be extremely sensitive to glucose levels in portal vein plasma. Raising the portal plasma glucose concentration from 100 mg/100 ml to values above 137 mg/100 ml switched the liver from net glucose production to net uptake, and the uptake increased in proportion to the glucose load, in the absence of changes in portal vein insulin levels. Glucose uptake increased an average of 2.1 mg/min for every 10 mg/min increase in glucose flux through the liver (7).

Although glucose itself may be a major stimulus to the change in hepatic glucose balance observed during glucose infusion, the question remains open whether insulin has a quantitatively significant role in the uptake process. In these studies, we have examined the effects of insulin on glucose balance across the isolated perfused canine liver and have found that insulin, at concentrations in the physiological range, modulates the effect of glucose on the liver.

METHODS

The techniques used in these studies were previously described in detail (7), and we will therefore summarize them only briefly.

Perfusion Technique

Seventeen perfusion experiments were performed (Table 1). The pilot liver technique of Urquhart and Keller (35) was used, with modifications previously published (7). The pilot perfusion technique is briefly described as follows: the circulation of the liver of a small, weaned beagle puppy is surgically isolated, and the organ is cross-perfused with blood from a large, anesthetized adult mongrel dog (source dog). Arterial blood enters the hepatic artery, and systemic venous blood enters the portal vein. The total hepatic venous effluent from the perfused liver is returned to the venous circulation of the source animal. During the surgical extirpation procedure, the perfused liver is never without blood flow, because the hepatic arterial and portal venous inflow vessels are cannulated at different times. Once cross-circulation of the puppy liver is complete, the liver is carefully removed from the puppy and is floated in a dish of Ringer solution with 0.5% albumin, maintained at 38°C. Bile is continuously collected from a small catheter fixed in
the bile duct. Portal venous (F_PV) and hepatic venous (F_HV) blood flow rates are obtained automatically (35), and hepatic arterial blood flow (F_HA) is calculated as F_HA = F_HV - F_PV.

The pilot perfusion technique is designed to allow the infusion of substances of interest into the portal inflow blood of the perfused liver. Concentrations of test substances can be varied widely, and in the case of glucose levels in portal blood can be changed from fasting values well beyond the upper limits that occur physiologically after feeding. These changes are accomplished without causing significant alterations in the composition of source dog systemic blood (2, 7, 34). To assure that the composition of the incoming blood from the source dog is unaffected by the performance of the pilot liver, the pilot livers are small and the source dogs are large. We have demonstrated that if the weight ratio (source dog/liver donor dog) is 10 or greater, increments in the portal vein plasma glucose concentration of the pilot liver, the pilot livers are small and the source dogs are large* We have demonstrated that if the weight ratio (source dog/liver donor dog) is 10 or greater, increments in the portal blood concentration of the systemic blood of the source dog vary from 9.5 to 20.4 with a mean of 14.3 (Table 1).

Calculations

NHGB. Net hepatic glucose balance, with the dimensions of milligrams times minute\(^{-1}\) per 100 g liver is defined as

### Table 1. Animal and organ data for 17 perfusion experiments

<table>
<thead>
<tr>
<th>Type of Expt</th>
<th>Expt No.</th>
<th>Source dog wt, kg</th>
<th>Liver donor dog wt, kg</th>
<th>Weight ratio (source dog/liver donor)</th>
<th>Liver wt, g</th>
<th>Hepatic blood flow, ml/min</th>
<th>Blood flow per g wet wt, ml/min(^{*} g^{-2})</th>
<th>Average bile flow rate, ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>500(\mu)U/ml Increment in insulin in portal vein</td>
<td>47</td>
<td>31.6</td>
<td>1.80</td>
<td>17.6</td>
<td>93.1</td>
<td>115</td>
<td>1.24</td>
<td>1.0</td>
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<td></td>
<td>48</td>
<td>27.7</td>
<td>1.36</td>
<td>20.4</td>
<td>88.5</td>
<td>98</td>
<td>1.11</td>
<td>1.5</td>
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<td></td>
<td>49</td>
<td>38.2</td>
<td>1.82</td>
<td>16.0</td>
<td>86.3</td>
<td>120</td>
<td>1.39</td>
<td>1.3</td>
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<tr>
<td></td>
<td>50</td>
<td>29.1</td>
<td>1.82</td>
<td>16.0</td>
<td>82.7</td>
<td>121</td>
<td>1.46</td>
<td>1.3</td>
</tr>
<tr>
<td>200 (\mu)U/ml Portal vein insulin increment</td>
<td>51</td>
<td>27.3</td>
<td>2.04</td>
<td>13.4</td>
<td>88.0</td>
<td>110</td>
<td>1.25</td>
<td>1.2</td>
</tr>
<tr>
<td>140-mg/100 ml Glucose + 500-(\mu)U/ml insulin increments in portal vein</td>
<td>35</td>
<td>24.6</td>
<td>2.05</td>
<td>12.0</td>
<td>98.0</td>
<td>50</td>
<td>0.92</td>
<td>1.2</td>
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<tr>
<td></td>
<td>36</td>
<td>26.4</td>
<td>2.04</td>
<td>12.9</td>
<td>93.0</td>
<td>100</td>
<td>1.07</td>
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<td>2.26</td>
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<td>2.27</td>
<td>13.6</td>
<td>102.4</td>
<td>94</td>
<td>0.92</td>
<td>0.6</td>
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<td>2.27</td>
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<td>40</td>
<td>30.9</td>
<td>2.72</td>
<td>11.4</td>
<td>93.4</td>
<td>93</td>
<td>0.94</td>
<td>1.3</td>
</tr>
<tr>
<td>140-mg/100 ml Glucose + 200-(\mu)U/ml insulin (preinfused) increment in portal vein</td>
<td>35</td>
<td>32.3</td>
<td>3.41</td>
<td>9.5</td>
<td>170.0</td>
<td>108</td>
<td>0.64</td>
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<td>19.2</td>
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<td>37</td>
<td>27.2</td>
<td>2.72</td>
<td>10.0</td>
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<td></td>
<td>38</td>
<td>29.1</td>
<td>1.82</td>
<td>16.0</td>
<td>76.0</td>
<td>101</td>
<td>1.33</td>
<td>0.5</td>
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<tr>
<td></td>
<td>39</td>
<td>32.3</td>
<td>2.28</td>
<td>14.2</td>
<td>140.5</td>
<td>120</td>
<td>0.85</td>
<td>1.8</td>
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<tr>
<td></td>
<td>40</td>
<td>36.4</td>
<td>2.27</td>
<td>16.0</td>
<td>100.0</td>
<td>117</td>
<td>1.17</td>
<td>0.7</td>
</tr>
<tr>
<td>Average ± SEM</td>
<td></td>
<td>14.3 ± 0.80</td>
<td>105 ± 6.4</td>
<td>107 ± 2.4</td>
<td>1.08 ± 0.06</td>
<td>1.17 ± 0.15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Protocols

Three distinct types of experiments were performed. In all types sampling was begun 1 h after the liver was placed in the bath. Control samples for the determination of net hepatic glucose balance (NHGB) were collected every 5 min for 30 min. In the first type of experiment (experiments 47−51) the control period was followed by an infusion of insulin sufficient to raise the portal plasma insulin level by 200 or 500 \(\mu\)U/ml, and sampling was continued every 5 min for 2 h. The infusion was then shut off, and sampling was continued for 30 min more. In the second type of experiment (experiments 35−40) following the 30-min control period an infusion of glucose plus insulin was begun into the portal vein, and rapid blood sampling was started. Samples were collected every minute for 5 min, then every 2.5 min for 55 min, and finally every 5 min until 30 min later. The infusion was ended at 2.5 h. In the third type of experiment (experiments 53−58), the sampling schedule was identical to that of experiments 35−40. The insulin infusion, however, was started immediately after the liver was placed in the bath, 1 h before the beginning of control sampling.

At the end of all experiments, glucagon at a concentration of 1 ng/ml was infused for 10 min, and one final NHGB determination was scheduled at the end of the glucagon infusion.

### Animals

Source dogs were adult mongrels of either sex. Liver donors were pure-bred beagle puppies, male or female, obtained from Marshall Laboratories, North Rose, N.Y. The animals were offered a fixed amount of food once per day and given ample time to eat it. The source dogs ate Purina dog chow and the puppies ate Purina puppy chow. All dogs were kept in cages lighted from 6 A.M. to 6 P.M. and were allowed water ad libitum. Only animals which seemed to be in good health and were eating well were used for experiments. Food (but not water) was removed at 3 P.M. the day before the experiment; surgery was begun at 8 A.M. the day of the experiment, and the perfusion was usually established by noon.
follows:

\[
\text{NHGB} = (F_{PV}G_{PV} + F_{HV}G_{HV} - F_{TR}G_{HV} + \text{INF}) \times \frac{100}{\text{liver weight (g)}}
\]  

where \( F \) represents total blood flow rate (ml/min), \( G \) is whole-blood glucose concentration (mg/ml), \( PV, HA, \) and \( HV \) are hepatic vein, hepatic artery, and portal vein, respectively, and \( \text{INF} \) represents the rate of infusion of glucose into the portal vein in milligrams per minute. It should be noted that \( \text{NHGB} \) is defined as in our previous publication (7), except that the sign is reversed; i.e., uptake positive, production negative. In this system, any single calculation of \( \text{NHGB} \) has a measurement error (expressed as SD) of 4.01 mg⋅min\(^{-1}\)/100 g liver (2). Therefore, two \( \text{NHGB} \) determinations separated by a net difference of 8 mg⋅min\(^{-1}\)/100 g liver cannot be explained by measurement error alone (\( P < .05 \)).

\( G_{PV\ total} \): When glucose is infused into the portal vein, it increases the prevailing portal vein glucose concentration, \( G_{PV} \), to result in the final portal vein glucose concentration \( G_{PV\ total} \):

\[
G_{PV\ total} = G_{PV} + \frac{G_s f}{F_{PV}(1 - h)}
\]  

in which \( G_s \) is the concentration of glucose in the infusion solution (mg/ml), \( f \) is the infusate flow rate (ml/min), \( h \) is the hematocrit, and \( G_{PV} \) and \( F_{PV} \) are defined as before. In accord with the slow rate of entry of glucose into canine erythrocytes (38), preliminary experiments have demonstrated that the infused glucose does not enter red cells to any appreciable extent during the transit of blood through the liver.

\( I_{PV} \): The increment in insulin in the portal vein, \( I_{PV} \) (\( \mu U/ml \)), was calculated according to equation 3:

\[
I_{PV} = \frac{I_s f}{F_{PV}(1 - h)}
\]  

in which \( I_s \) is the concentration of insulin in the infusate (\( \mu U/ml \)).

Materials

Anesthetics. The liver donors were anesthetized with one intraperitoneal injection (39 mg/kg) of sodium pentobarbital (Abbott Laboratories, North Chicago). The source dogs were injected intravenously with sodium pentothal (2.5% solution, Abbott Laboratories), and pentothal was continuously infused at a lower concentration (0.2%).

Heparin (Abbott Laboratories). Large initial doses of heparin were administered to both dogs before cross-perfusion was begun (liver donor: 5,000 U iv; source dog: 25,000 U iv), and a supplementary dose of 3,000 U was injected intravenously into the source dog every hour.

Insulin. Glucagon-free insulin (Eli Lilly and Company, Indianapolis, lot P/I 46309) was dissolved at acid pH (1 mg/ml), buffered to pH 7.4, rediluted in saline, and infused into the portal vein when appropriate.

Glucose. Dextrose (50%) was diluted in saline and infused into the portal vein.

Glucose was determined on an AutoAnalyzer by the method of Hoffman (17). To minimize the effects of drift, standards were run frequently, and the values of \( G_{PV}, G_{HV}, \) and \( G_{HA} \) for a single determination of \( \text{NHGB} \) were analyzed in sequence. Insulin was measured by radioimmunoassay by the method of Herbert et al. (16).

Samples were preserved for assay as previously described (7).

Statistics

Significance of the differences between means was evaluated by the Student t test. Slopes were compared by evaluating the difference between slopes relative to the standard error of the difference, which is known to have a Student t distribution.

RESULTS

Blood Flow and Bile Production by Perfused Livers

The results from all 17 perfusion experiments are shown in Table 1. The value for hepatic blood flow is similar to the value of 1.21 mg⋅min\(^{-1}\)/g tissue reported by Shoemaker (28, 29) for the dog liver in vivo. (Liver weight assumed to be 3.9% of body weight (24.).)

Effect of Insulin on Hepatic Response to Glucose

We have previously reported the dynamic changes in net glucose balance across the perfused liver which occur in response to stepwise increases in the portal vein plasma glucose concentration (7). For reference, these data are reproduced in Fig. 1A. During the initial period of observation, 0 30 min, the livers were producing glucose at an average rate of 4.7 mg⋅min\(^{-1}\)/100 g. Increasing the portal vein glucose concentration by 140 mg/100 ml switched the liver to net glucose uptake, which reached a maximum value of 27 mg⋅min\(^{-1}\)/100 g within 2 min and decreased to a steady value of uptake by 10 min, which was then maintained until the infusion was terminated at 150 min. The average value of \( \text{NHGB} \) was 10.3 ± 1.6 (SE) mg⋅min\(^{-1}\)/100 g during the last hour of the glucose infusion.

In a series of six perfusion experiments we investigated whether insulin had any effect on the response of the liver to glucose. In the experiments plotted in Fig. 1B, insulin and glucose were infused simultaneously into the portal vein plasma of the perfused liver. The increment in \( G_{PV} \) was 140 mg/100 ml. Blackard and Nelson (6) have measured the portal vein plasma insulin concentration in human subjects given 25-g injections of glucose. They found that the average portal vein insulin concentration reached a peak value of 300 \( \mu U/ml \) within 5 min and then fell to a reasonably constant plateau level of about 200 \( \mu U/ml \) 5 min later. Based upon the studies of Blackard and Nelson, we increased the insulin concentration in the portal vein (\( I_{PV} \)) by 500 \( \mu U/ml \). It can be seen that a reasonably steady value for glucose uptake was reached during the 2nd h of infusion (90–150 min). We have therefore compared the total amount of glucose uptake by these six livers during the
INSULIN AND GLUCOSE BALANCE

FIG. 1. A: effect of glucose on net balance of glucose across liver (data from ref. 7). Glucose was infused into portal vein of perfused liver from 30 to 150 min, raising portal vein glucose concentration by 140 mg/100 ml. Glucagon was infused into portal vein from 180 to 200 min. Lower 2 panels show concentrations of insulin and glucose in arterial plasma of source dogs. Top panel: light lines represent SE about control period and the 2nd h of infusion with the uptake of those livers represented in Fig. 1A (Table 2). Neither the control nor the steady-state values for NHGB were significantly different. One reason the average steady-state uptake by the group of livers given insulin appeared to be lower was that the insulin group had a higher control production rate. The net change in NHGB was slightly greater for the livers to which insulin was given, but there was no significant difference \( (P > .05) \). Shown in the lower panels of Fig. 1, A and B, are the concentrations of glucose and insulin in the portal blood of the perfused livers, upstream from the site of infusion. Figure 1B shows that in the second group of livers the glucose level in the portal blood \( (G_P) \) rose slightly, from 96 to 105 mg/100 during the glucose infusion, and the portal plasma insulin rose significantly, from a preinfusion value of 10 to 55 \( \mu U/ml \) at 90 min. In the experiments in which only glucose was infused, neither \( G_P \) nor \( I_P \) changed significantly.

Effect of Insulin Alone on NHGB

In four experiments, insulin was infused into the portal vein of the liver for 120 min at a rate calculated to raise \( I_P \) by 500 \( \mu U/ml \) (Fig. 2, Table 3). During the 30-min control period and for the first 30 min of the infusion the livers produced glucose. During the 2-h insulin infusion, production declined. Table 3 compares the glucose production of these four livers with that of seven livers to which no insulin was given during six consecutive 30-min periods. During the first period (31–60 min) after the infusion was begun, the glucose production with and without insulin was the same. During the final period, from 152 to 180 min, an apparent fall in the mean value of glucose production was observed, but there was no significant change in the value of NHGB between the first and final periods \( (t_0 = 1.53, df = 8, .05 < P < 0.1) \). The number of experiments in the insulin group was small, and the measurement error incurred in determining NHGB was large \( (SD \text{ of measurement} = 120.3 \text{ mg} \cdot \text{30 min}^{-1}/100 \text{ g}) \).

The failure of NHGB to change during insulin infusion takes on added significance when the time course of the perfusate glucose concentration is considered (Fig. 2, lower panel, and Table 3). It is clear from the data that the rate of insulin infusion into the perfused livers was great enough to lower the systemic blood glucose concentration of the source dogs, and therefore lower significantly the levels of \( G_P \) for those livers given insulin. Thus, the rate of insulin infusion was large enough that the assumption that the cross-perfusion technique allowed “open-loop testing” of the liver did not hold. During the insulin infusions, the average source dog systemic venous glucose concentration \( (G_P) \) fell very significantly \( (t_0 = 5.09, df = 3, P < .01) \) during the final observation period (Table 3). This fall in the blood glucose concentration should have been reflected in an increase in glucose output from the perfused liver, either because of a direct effect of the lowered glucose concentration on the livers \( (14) \) or because of an increase in the concentrations of...
TABLE 2. Effects of glucose and glucose plus insulin on control and steady-state values of NHGB

<table>
<thead>
<tr>
<th>Exp No.</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (0-30 min)</td>
<td>Steady State (60-150 min)</td>
<td>ΔNHGB (B-A)</td>
</tr>
<tr>
<td></td>
<td>NHGB</td>
<td>Gpv</td>
<td>Gpv increased by</td>
</tr>
<tr>
<td></td>
<td>(mg·30 min⁻¹·100 g⁻¹)</td>
<td>No infusion</td>
<td>140 mg/100 ml</td>
</tr>
<tr>
<td>21</td>
<td>-78</td>
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<td>420</td>
</tr>
<tr>
<td>24</td>
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<td>177</td>
<td>468</td>
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<tr>
<td>Mean ± SE</td>
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<td>311 ± 24d</td>
<td>401 ± 39g</td>
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D E F

<table>
<thead>
<tr>
<th>Exp No.</th>
<th>Control NHGB</th>
<th>Steady state NHGB</th>
<th>ΔNHGB (E-D)</th>
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<tbody>
<tr>
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<td>534</td>
<td>663</td>
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<td>40</td>
<td>-171</td>
<td>267</td>
<td>438</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>-223 ± 47b</td>
<td>286 ± 77d</td>
<td>510 ± 63f</td>
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</table>

* Negative NHGB = net glucose output.  
  a A vs. D, NS.  
  b Gpv = glucose concentration in portal vein.  
  c B vs. E, NS.  
  d Ipv = insulin concentration in portal vein.  
  e Cvs. F, NS.

---

glucagon or epinephrine in the plasma of the source dog secondary to the insulin-induced hypoglycemia (13).

Thus, after the infusion of insulin into the perfused liver, no change in the value of NHGB was observed, despite source dog hypoglycemia, which by itself would have been expected to increase rather than decrease NHGB. These results strongly suggest that insulin had the effect of inhibiting glucose output by the perfused livers, but in contrast to the inhibitory effect of glucose itself on glucose output, which occurs very quickly (7), the insulin effect developed slowly.

Because of the hypoglycemic effect on the source dog caused by increments in Ipv of 500 μU/ml, lower doses of insulin were given, sufficient to induce only a 200-μU/ml increment in Ipv. Figure 3 shows the results from one experiment in which insulin was administered at this lower rate to the liver. During a 2-h infusion of the hormone a progressive decline in NHGB occurred. However, this amount of insulin did not cause a decline in the blood glucose concentration, which was, on the average, higher during the final hour of insulin infusion than before the infusion was begun.

**Effect of Insulin Preinfusion on Hepatic Response to Glucose**

In six experiments, insulin was infused into the portal vein of the perfused liver, at a concentration of 200 μU/ml, for a total of 270 min, beginning 90 min before the start of “control sampling” (Fig. 4, Table 4). In our previous studies (7) and in the experiments discussed above, the livers invariably produced glucose during the 0- to 30-min control period. In the 13 perfusion experiments listed in Table 2, and drawn in Fig 1, the total glucose production during the 30-min control period averaged 178 ± 31 mg·30 min⁻¹·100 g. In the experiments shown in Fig. 4, the control NHGB was not different from 0 (t₀ = 1.16, df = 5, P > 0.1). The average glucose production rate for the livers preinfused with insulin was significantly lower than the net glucose production previously seen (t₀ = 3.47, df = 17, P < .005). Thus, these experiments demonstrate a highly significant effect of insulin in diminishing the normally positive fasting rate of glucose production to 0.

The dynamic response of the liver, preinfused with insulin, to a 140-mg/100 ml increment in Gpv is shown in Fig. 4. The temporal pattern of NHGB was similar to that seen for glucose alone. During the 2nd h of glucose infusion, glucose uptake reached a steady-state average value which was significantly greater than when insulin was not infused (Table 2, Fig. 1A) (P < .01).

Because insulin lowered the rate of glucose production during the 0- to 30-min control period, we tested whether the average net change in NHGB (steady-state control) was increased by insulin. The value for ΔNHGB increased significantly with insulin (column 1, Table 4) (t₀ = 2.723, df = 10, P < .025).

**Effect of Insulin on Relationship Between Gpv total and NHGB**

In a previous study, we showed that a linear relationship exists between the steady-state value of NHGB and Gpv total, described by equation 4:

\[
NHGB = -11.3 + 0.083 \text{Gpv total}
\]
TABLE 3. NHGB of perfused livers with and without infusion of insulin

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Control</th>
<th>Insulin Infusion: Increment = 500 μU/ml</th>
<th>No Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-30 min</td>
<td>NHGB: mg/100 ml</td>
<td>NHGB: mg/100 ml</td>
<td>NHGB: mg/100 ml</td>
</tr>
<tr>
<td>No insulin, n = 7</td>
<td>162 ± 23</td>
<td>111 ± 33</td>
<td>84 ± 42</td>
</tr>
<tr>
<td>(data from ref. 7)</td>
<td>93 ± 4</td>
<td>91 ± 3</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>31-60 min</td>
<td>96 ± 60</td>
<td>108 ± 75</td>
<td></td>
</tr>
<tr>
<td>61-90 min</td>
<td>87 ± 3</td>
<td>91 ± 3</td>
<td></td>
</tr>
<tr>
<td>91-120 min</td>
<td>87 ± 3</td>
<td>91 ± 3</td>
<td></td>
</tr>
<tr>
<td>121-150 min</td>
<td>86 ± 2</td>
<td>84 ± 2</td>
<td></td>
</tr>
<tr>
<td>151-180 min</td>
<td>86 ± 2</td>
<td>84 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Insulin increment 500 μU/ml, n = 4

<table>
<thead>
<tr>
<th>NHGB: mg/100 ml</th>
<th>NHGB: mg/100 ml</th>
<th>NHGB: mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 ± 50</td>
<td>105 ± 50</td>
<td>54 ± 37</td>
</tr>
<tr>
<td>102 ± 3</td>
<td>92 ± 1</td>
<td>72 ± 4</td>
</tr>
<tr>
<td>51 ± 37</td>
<td>65 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 3. One experiment (experiment 51) of type shown in Fig. 2, except that portal vein insulin concentration was raised by 200 μU/ml. Shaded bars in top panel represent average NHGB for successive 30-min periods. Large variation seen in NHGB due to measurement error.

FIG. 4. Effect of an insulin infusion on response ofperfused liver to glucose. Insulin was infused into portal vein from 60 min before beginning of sampling until after sampling was ended at 190 min. Glucose was infused from 30 to 150 min only. Portal plasma glucose increased by 140 mg/100 ml. Portal plasma insulin increased by 200 μU/ml. Insulin levels in plasma of source dogs were not determined.

where $G_{PV}$ total is in units of milligrams per 100 ml and NHGB is in milligrams times minute⁻¹ per 100 g. This line was defined by the data from 20 experiments similar to those reproduced in Fig. 1A, in which the effect of various increments in $G_{PV}$ on steady-state NHGB was examined.

Shown in Fig. 5 are the control and steady-state values of NHGB for the six experiments with insulin preinfusion (Table 4), plotted as functions of $G_{PV}$ total. The points are fitted with the best regression line:

$$NHGB = -13.0 + 0.12 G_{PV} \text{ total}$$

with the variables defined as in equation 4. The increase in the slope of the line relating NHGB and $G_{PV}$ from +0.083 without insulin preinfusion to +0.12 with insulin preinfusion is highly significant ($P < .01$) and reflects the much increased sensitivity of the liver to glucose when preinfused with insulin ($I_{PV} = 200 \mu U/ml$). Figure 5 also predicts that insulin preinfusion, besides increasing the change in NHGB for a given change in $G_{PV}$ total, will lower the glucose concentration at which the liver switches from net glucose production to net glucose uptake. Without insulin, the zero-balance concentration is 137 mg/100 ml; with insulin preinfusion it is lowered to 107 mg/100 ml.

In a previous report (7) we calculated from the slope of the dashed line reproduced in Fig. 5 that during one pass of blood the liver compensated 21% for an increase in $G_{PV}$, by glucose autoregulation alone. From the slope of the solid line in Fig. 5, it is possible to estimate the effect that insulin preinfusion has on the percent compensation of NHGB to an increment in $G_{PV}$: assuming the total hepatic blood flow of a 100-g liver to be 108 mg/min (Table 1), and a portal flow to be 64% of total blood flow (7), a 100-mg/100 ml increment in $G_{PV}$ represents an increase in glucose flux into a 100-g liver of

$$0.64 \times 108 \times (1 - 0.4) \times (1 \text{ mg/ml}) = 41.5 \text{ mg/min}.$$

From the slope of the solid line in Fig. 5, $\Delta NHGB/\Delta G_{PV} = 0.122$ or 12.2 mg min⁻¹/100 g for a 100 mg/100 ml increment. Thus, with insulin preinfusion, the percent compensation of the liver for an increase in the portal vein glucose concentration was 12.2/41.5 = 29%.

DISCUSSION

These studies have shown that insulin has demonstrable effects on basal glucose production by the liver of fasted animals. The effect occurred only slowly, and we found no
by glucose uptake was 5.5 mg glucose concentration of 200 mg/lOO ml, with no insulin, net the liver proved to be considerably more sensitive to the glucose increment. For example (Fig. 5), with a portal vein before the increase in the portal vein glucose concentration, however, when the liver was preinfused with insulin for 2 h...

TABLE 4. Effects of insulin (preinfused) and glucose plus insulin on control and steady state values of NHGB

<table>
<thead>
<tr>
<th>Expt No.</th>
<th>G</th>
<th>H</th>
<th>I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (60-30 min)</td>
<td>Steady State (90-50 min)</td>
<td>NHGB (I-G)</td>
</tr>
<tr>
<td>53</td>
<td>-21</td>
<td>444</td>
<td>465</td>
</tr>
<tr>
<td>54</td>
<td>-33</td>
<td>696</td>
<td>790</td>
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<tr>
<td>55</td>
<td>-40</td>
<td>597</td>
<td>607</td>
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<tr>
<td>56</td>
<td>-51</td>
<td>297</td>
<td>648</td>
</tr>
<tr>
<td>57</td>
<td>-18</td>
<td>552</td>
<td>570</td>
</tr>
<tr>
<td>58</td>
<td>-49</td>
<td>918</td>
<td>837</td>
</tr>
</tbody>
</table>

Mean ± SE: 

-12 ± 10* 624 ± 687 636 ± 541

Values from expts in which no insulin was given: 

- 140 ± 37* 311 ± 142* 151 ± 35* 

Gpv increased by 140 mg/100 ml (during the period 31-50 min); Ipv increased by 200 μU/ml (during the period -90 to +190 min).  

- Values from expts from Table 2, A) (From Table 2, B) (From Table 2, C)

- r = -0.92 rYHGB = -13.02 ± 1.22 Gpv rOrAL

- t = 3.7222 P < .01.  

- Gpv vs. H, t = 2.723 P < .025.

- A vs. G, t = 2.653 P < .025.

- Fig. 5. Dependence of glucose balance across liver on portal glucose concentration with (solid line) and without (dashed line) insulin preinfusion. Solid line is best linear regression data for each subject. Dashed line is from experiments similar to those shown in Fig. 4, in which effects of different increments of portal vein glucose concentrations on NHGB were examined.

Evidence that insulin might cause a rapid shift from net glucose production to net glucose uptake similar to the rapid switch in glucose balance provoked by glucose itself (7).

In these studies, besides confirming the effect of insulin alone on the liver, we were able to define the dynamic interactions between glucose and insulin as they affect net hepatic glucose balance. When glucose and insulin were administered together at the same time (Fig. 1B, Table 2), the magnitude and the temporal patterns of changes in NHGB were the same as observed when glucose was given alone. However, when the liver was preinfused with insulin for 2 h before the increase in the portal vein glucose concentration, the liver proved to be considerably more sensitive to the glucose increment. For example (Fig. 5), with a portal vein glucose concentration of 200 mg/100 ml with no insulin, net glucose uptake was 3.5 mg·min⁻¹/100 g liver, whereas with insulin preinfusion uptake was 11.4 mg·min⁻¹/100 g. Table 4 and a comparison of the slopes of the lines in Fig. 5 show that the increased uptake was not simply due to the insulin-induced diminution in hepatic glucose production, but was an independent effect of the infused hormone. As a result of the two effects of insulin on the liver, the glucose concentration at which NHGB = 0 (uptake = production) decreased, a change which was predicted some years ago by Leonards et al. (18).

It has been difficult to define the role of insulin in regulating hepatic carbohydrate metabolism from studies of perfused livers (15, 23, 25, 26). Immediately following surgical extirpation of the rat liver for perfusion, a period of abnormally high glucose output has frequently been observed (8), and insulin has been shown to diminish the excessive glycolysis seen (9). Recently, however, it has been determined that the elevated glucose output is related to abnormally low red cell content of the perfusing fluids, and when rat livers have been perfused with media composed of 90% blood, glucose production has been low (26) or 0 (25). With a 90% blood perfusate, insulin was observed to have no effect on hepatic glucose output (25). Also, in studies in perfused rat liver it has not been possible to demonstrate enhancement of glucose uptake by insulin when the inflow glucose concentration was elevated (15, 25).

In these studies, we were able to demonstrate for the first time in a perfused liver system both insulin-induced depression of fasting glucose production (in a liver perfused with whole blood) and augmentation of glucose uptake during glucose loading by insulin. These results, while contrary to the findings of others using perfused rat liver (25), are in agreement with in vivo results. Steele et al. (32), using isotope-dilution techniques, and Madison and his colleagues (21), who measured glucose balance directly across the liver of dogs with porta caval shunts, demonstrated clearly that insulin alone could depress fasting hepatic glucose production to 0, providing the portal vein glucose concentration did not fall. Also, while it has not been possible in vivo to control independently the portal vein concentrations of glucose and insulin, Bishop et al. (4, 5) demonstrated that insulin could augment glycogen deposition in the liver of the unanesthetized dog during the infusion of glucose.

Because net hepatic balance of glucose was measured in our studies, and not the rates of glucose production and utilization via distinct biochemical pathways, we may only speculate as to the biochemical mechanisms which would explain the dynamic responses of the hepatic glucose balance to glucose and insulin. The rapid effect of glucose on NHGB (7) might be explained as a mass action effect of glucose 6-phosphate on glycogen synthesis. However, enhanced glucose 6-phosphate levels are not invariably seen during enhanced glycolysis synthesis (33). A second, equally likely, possibility is the direct activation of glycogen synthetase (UDP-glucose pyrophosphorylase, glycogen synthetase) by glucose. De Wulf and Hers (11) have shown that glucose markedly stimulates synthetase in vitro, and Buschiazzo et al. (10) have demonstrated in perfused rat liver a rapid glucose-mediated conversion of synthetase D (inactive in vivo (22)) to synthetase I in the absence of elevated levels of insulin (11).

The slow effect of insulin alone in diminishing hepatic glucose production is probably due to insulin-mediated diminution in glycolysis and gluconeogenesis, possibly because of lowered intracellular cyclic 3',5'-AMP (39).
The slow onset of this effect indicates that it may be at the level of de novo synthesis of ribonucleic acid. Similarly, the effect of insulin in augmenting the effect of glucose on glucose uptake may involve protein synthesis. Whereas insulin could increase the synthesis of glycogen synthetase, the effect is not seen without the glucose-induced conversion of synthetase D to synthetase I. The time course of the insulin effect seen in our experiments is slower than the 7- to 13-min conversion of synthetase D to synthetase I reported by Bishop et al. (4), but it is similar to the time course reported by Steiner and King (33) for the de novo synthesis of the liver synthetase enzyme.

The reasons for the difference between the function of the cross-perfused dog liver used in these experiments and the isolated, buffer-perfused rat liver preparation are not known. It is possible, however, that the presence of hepatic arterial inflow or perfusion with whole blood oxygenated by the lungs of the source animal may prevent the anoxia which has been observed with the rat liver (9). Mondon and Burton (25) and Mortimore (26) have suggested that the failure of the rat liver to simulate the function of the liver in vivo may be due to the lack of autonomic innervation of the perfused organ. The fact that our perfused dog livers do, in fact, function in a manner similar to the dog liver in vivo has been observed with the rat liver (9). Mondon and Burton (25) and Mortimore (26) have suggested that the arterial inflow or perfusion with whole blood oxygenated by the lungs of the source animal may prevent the anoxia which is known. It is possible, however, that the presence of hepatic synthetase enzyme.

By Steiner and King (33) for the de novo synthesis of the liver synthetase enzyme.

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REFERENCES