Inhibition by insulin of hepatic glucose production in the normal dog1,2

R. STEELE, J. S. BISHOP, A. DUNN,3 N. ALTZSULER, I. RATHGEB, AND R. C. DE BODO
Biology Department, Brookhaven National Laboratory, Upton, and Department of Pharmacology, New York University School of Medicine, New York, New York

THE EFFECT OF INSULIN on the rate of glucose production by the liver has an extensive background. Since there is ambiguity in the expression “rate of glucose production,” it is necessary to define briefly what is meant by this expression in the present report.

When glucose production is measured by catheterization across the hepatic circulation, the figure obtained is net glucose production, i.e., glucose production minus glucose uptake, and may assume a negative value under some conditions. When glucose production is measured by isotope dilution, as in the present study, the figure obtained represents new (C12) glucose release to the circulating blood, presumably almost entirely from the liver; theoretically this might become zero but cannot become negative.

In some of the experiments reported here, the immediate effect of rather small amounts of infused insulin on the rate of glucose-C14 production has been measured in trained, unanesthetized, normal dogs. In these experiments glucose was also infused to prevent, or limit, hypoglycemia. In other experiments glucose was infused alone, to create mild hyperglycemia and so to increase the rate of endogenous insulin secretion. Trace amounts of glucose-C14 were employed to measure glucose production and uptake.

MATERIALS AND METHODS

All experiments were carried out on trained, normal mongrel dogs (15–24 kg), without general anesthesia, at 17–20 hr after the last feeding. Many of the experiments were done in such a way that liver biopsy samples, required for another purpose, could be taken; in these instances the dogs were in standing position in a harness. Otherwise the dogs were in reclining position. In all instances the respiratory mask (41) to carry away exhaled C14O2 was in place over the head of the dog.

A standard diet previously described (9), containing 58% of its calories as carbohydrate (12), was used to
itself, was infused at a measured rate in
given along with insulin to limit hypoglycemia or by
sions always contained enough glucose-Cl4 to bring the
tion in water into a saphenous vein. Bulk glucose infu-
size and turnover rate. Glucose in bulk amount, whether
of sampling for determining control body glucose pool
initial glucose-Cl4 injection, subsequent to a a-hr period
and glucose infusions were started about 3 hr after the
infusion salution was of such glucose-C4 content that
measured rate (about 0.5 ml/min) into a saphenous
vein, of the same glucose-Cl4 preparation, was begun

An indwelling polyethylene catheter in a jugular vein.

Blood samples were withdrawn at intervals through
an indwelling polyethylene catheter in a jugular vein.
The samples were collected in heparinized syringes and
centrifuged immediately to obtain plasma for Somogyi
filtrate (35) preparation. When necessary, whole-blood
samples, taken by the same catheter, were monitored
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Maintain the dogs routinely. The high-carbohydrate
diet fed for a number of days prior to some experiments
contained 78% of its calories as carbohydrate, 18% as
protein, and 4% as fat (12). Both diets were made com-
plete with regard to content of vitamins, unsaturated
fatty acids, essential amino acids, and inorganic salts
and were consumed readily by the animals.

At the beginning of an experiment D-glucose-C4
(about 30 μc) in trace amount, either randomly labeled
or labeled in carbon 6, was administered as a priming
dose in about 0.5 ml saline, iv. A continuous infusion at a
measured rate in 10 ml saline, iv. A continuous infusion at a
measured rate (about 0.5 ml/min) into a saphenous vein,
of the same glucose-C4 preparation, was begun
and continued throughout the entire experiment. The
infusion solution was of such glucose-C4 content that
an amount of C4 equal to that contained in the priming
dose was delivered in about 12½ min. Glucose or insulin
and glucose infusions were started about 3 hr after the
initial glucose-C4 injection, subsequent to a 2-hr period
of sampling for determining control body glucose pool
size and turnover rate. Glucose in bulk amount, whether
given along with insulin to limit hypoglycemia or by
itself, was infused at a measured rate in 10–20% solu-
tion in water into a saphenous vein. Bulk glucose infu-
sions always contained enough glucose-C4 to bring the
specific activity of the administered glucose near to that
already prevailing in the circulating plasma glucose.

The insulin was tryptic-treated (glucagon-low) crystal-
line zinc insulin freshly dissolved in saline brought to
pH 3 with HCl. It was infused into a cephalic vein.

Blood samples were withdrawn at intervals through
an indwelling polyethylene catheter in a jugular vein.
The samples were collected in heparinized syringes and
centrifuged immediately to obtain plasma for Somogyi
filtrate (35) preparation. When necessary, whole-blood
samples, taken by the same catheter, were monitored
(41, 42). Procedures.

Precisely measured 100-mg amounts of carrier glucose-
C4 were added to separate aliquots of the Somogyi
filtrates, and the glucose was isolated as the glucoso-
triazole derivative, as previously described (38), for
liquid-scintillation counting of C4.

Calculations for body glucose pool size, glucose pro-
duction, and glucose utilization were made as previously
described (37, 41, 43), using C4 priming dose, C4
infusion rate, and observed plasma glucose concentra-

<table>
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<th>EXPT</th>
<th>CONTROL PERIOD</th>
<th>INSULIN AND GLUCOSE INFUSION</th>
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<td></td>
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<td>R (mg/kg/hr)</td>
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<tr>
<td></td>
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<td>2</td>
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<tr>
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FIG. 1. Inhibition by infused insulin of endogenous glucose-C4 production when hypoglycemia is limited by glucose infusion. Mean plasma glucose concentrations are given for control periods and individually observed values of plasma glucose concentration at times indicated are given for infusion periods. Values omitted due to lack of space are as follows: B-49 insulin infusion, 49–61 min: 0.04; 160–170 min: 0.13; B-90 glucose infusion, 0–5 min: 0.5–10 min: 163, 60–76 min: 61; B-71 glucose infusion, 0.5 min: 0; 5–10 min: 180; 185–201 min: 406; 201–207 min: 163; B-58 glucose infusion, 0–5 min: 0; 5–10 min: 67; 10–20 min: 144; B-58 glucose infusion, 0.5 min: 0; 5–10 min: 266; 10–15 min: 351; 33–44 min: 436. PGC: plasma glucose concentration, mg/100 ml. R: glucose-C4 production rate in control period. HIC: high carbohydrate diet. I: insulin infusion, U/kg per hr. G: glucose infusion, mg/kg per hr. % R: mean glucose-C4 production rate as % of control, R.
The plasma glucose concentration during the periods of insulin infusion was prevented from falling to low levels by infusion of varying amounts of glucose as shown in Fig. 1. The highest amount of glucose infused was about four times the amount which the animal produced in the control period.

The effect of insulin to decrease glucose production was accompanied in all instances (except in exp. B-45) by an increase in over-all glucose uptake. For representative data see the accompanying paper (6), where uptakes for all experiments of Fig. 1 and 2 except exp. B-40, AL-39, AL-39HC, and AL-39HC are given.

Maintenance of the animals on a high-carbohydrate diet was not necessary to elicit the effect of insulin infusion on glucose-C\textsuperscript{14} production. The last two experiments shown in Fig. 1 were done using dogs which had been maintained prior to the experiment for 5 and 10 days, respectively, on the high-carbohydrate diet. These are not sufficient to allow judgment as to whether glucose-C\textsuperscript{14} production is more sensitive to insulin infusion in dogs kept on a high-carbohydrate diet.

Glucose infusion. In Fig. 2 are shown the results of nine experiments in which glucose was infused at increasing rates; the last four of these were done using animals maintained on the high-carbohydrate diet prior to the experiment for 10, 7, 22, and 8 days, respectively. When glucose infusion was begun at a rate corresponding to one-half to one and one-half times the pretreatment glucose production rate, hepatic glucose-C\textsuperscript{14} production was depressed by 40% or more after about 30 min in all of the nine experiments; in five of the experiments (AL-39, B-43, AL-39, B-31, and B-33) this extent of depression of glucose-C\textsuperscript{14} production was visible in the first half-hour of glucose infusion, when plasma glucose concentration was elevated by only 4-44 mg/100 ml (mean 17 mg/100 ml) above the pretreatment level. In seven of the experiments (AL-39, B-43, B-47, B-48, AL-39, and B-33) hepatic glucose-C\textsuperscript{14} production was depressed by 80% or more during a period of the glucose infusion. This occurred 7½-13½ min after the beginning of glucose infusion, at a time when plasma glucose concentration was elevated 13-65 mg/100 ml (mean 45 mg/100 ml) above the pretreat-

### Table: INHIBITION OF HEPATIC GLUCOSE PRODUCTION

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<tr>
<th>EVPT</th>
<th>PGC mg/100 ml</th>
<th>% R</th>
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<td>326</td>
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<td>44</td>
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<td>B-32</td>
<td>117</td>
<td>133</td>
<td>% R</td>
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<td>61</td>
<td>51</td>
</tr>
<tr>
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<td>169</td>
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<td>% R</td>
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<td>B-33 (HC)</td>
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<td>G</td>
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<td>153</td>
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### Fig. 2. Inhibition of endogenous glucose-C\textsuperscript{14} production by continuous intravenous infusion of glucose. Abbreviations as in Fig. 1.
when glucose infusion was proceeding at 1.6–5.0 times (mean 3.4 times) the pretreatment rate of endogenous glucose production. On cessation of glucose infusion, plasma glucose concentrations quickly fell to around the preinsulin level (4 exp.) or somewhat below (3 exp.) and hepatic glucose-C\(^{14}\) production increased (except for exp. B-32) above the last value observed during glucose infusion. The depressing effect of the glucose infusion on endogenous glucose-C\(^{14}\) production was seen in all animals, whether kept on the regular or the high-carbohydrate diet prior to the experiment.

Effect of high-carbohydrate diet on rate of glucose turnover in the postabsorptive state. In the 12 experiments performed on animals kept on the mixed diet, glucose production and uptake during the control period ranged from 190 to 247 mg/kg per hr with a mean value of 184 ± 10 (4 exp) mg/kg per hr, as seen in Fig. 1 and 2. This is a somewhat higher value than has been observed in a large series of animals lying at rest. Most of the animals of the current series were standing in a harness during the control period. In the six experiments performed on the animals on the high-carbohydrate diet, pretreatment glucose production and uptake ranged from 187 to 306 mg/kg per hr with a mean value of 247 ± 19 mg/kg per hr, as seen in Fig. 1 and 2. The increased glucose turnover of the animals on the high-carbohydrate diet was not associated with a corresponding increase in resting plasma glucose concentration. The animals fed the mixed diet had a mean plasma glucose concentration of 101 mg/100 ml; the animals fed the high-carbohydrate diet, 104 mg/100 ml.

DISCUSSION

The use of glucose-C\(^{14}\) to measure the effect of insulin on new (C\(^{14}\)) glucose production has given rise to conflicting observations. In the human subject (24, 31, 33), in the dog (16, 30), and occasionally in the rabbit (4, 5), insulin has been said to lower glucose-C\(^{14}\) production. Other experiments in the dog (22, 34, 43) and rat (4) have led to statements that insulin does not lower glucose-C\(^{14}\) production. In our own earlier studies in dogs the first effect of insulin on glucose production which was reported (43) was a transient and not very consistent depression of glucose-C\(^{14}\) production immediately following a single intravenous injection of insulin (0.25 \(0.10\) U/kg). Thereafter glucose production became nearly normal as the blood glucose concentration continued to fall, and after about 20 min rose far above normal as the blood glucose concentration began to rise again. Later (8, 10, 15, 17) it was observed that on prolonged continuous infusion of insulin (0.7–1.7 U/kg per hr, iv) the increased glucose-C\(^{14}\) production of the dog in response to the insulin-induced hypoglycemia was restrained as long as the insulin infusion was continued, this restraint being released when the insulin infusion was terminated. Subsequently (18) it was found that insulin infusion slowed to normal the accelerated rate of glucose-C\(^{14}\) production brought about in the dog by acute phlorizin poisoning. A similar restraint by insulin on the increased glucose production brought about by glucagon was also reported at that time, but this effect has since proven to be impossible to reproduce consistently. Finally it was reported (11, 12) that, in dogs maintained on a high-carbohydrate diet, insulin infusion accompanied by glucose infusion to prevent hypoglycemia caused a diminution of the rate of glucose-C\(^{14}\) production; preliminary observations were also reported indicating that a similar effect could be obtained in dogs maintained on a normal mixed diet.

The experiments involving insulin infusion which are reported in this communication establish firmly that an increase in the amount of insulin which is introduced into the circulating blood of the dog in the postabsorptive state to above the amount normally being delivered from endogenous sources causes a decrease in the resting rate of glucose C\(^{12}\) production by the liver, provided that glucose is also infused to prevent the development of hypoglycemia. The effect is seen whether the dog has been maintained on a high-carbohydrate diet or on a standard diet prior to the experiment. Not enough experiments were done using the high-carbohydrate diet to add to or detract from the observations of Leonards et al. (26), who reported a greater reduction in net glucose output (rather than glucose-C\(^{12}\) production) during insulin infusion in dogs kept on a high-carbohydrate diet.

It is of interest that the inhibition by insulin of net glucose production in the dog, as measured by transhepatic catheterization, is also observable only when hypoglycemia is controlled. When hypoglycemia was allowed to develop fully after insulin, a number of investigators (19, 34, 42) found no decrease in net glucose production. Leonards et al., using unanesthetized dogs kept on a high-carbohydrate diet (26), and Madison and colleagues (27), using anesthetized dogs with previously established portacaval shunts, observed decreases in net glucose production only when hypoglycemia was limited to about 20 mg/100 ml or less below the preinsulin level. Madison and associates, but not Leonards and co-workers, were able to accomplish this by giving insulin intravenously at a low rate without an accompanying glucose infusion. Only on rare occasions in our own experiments has glucose C\(^{12}\) production in the unanesthetized dog been seen to be significantly inhibited during insulin infusion at a low rate in the absence of glucose infusion.

In the human the long-standing evidence (3, 7) regarding insulin inhibition of net glucose production, as measured by transhepatic catheterization, is less secure because portal vein glucose concentrations were not observed and any possible action of insulin to increase glucose uptake by the tissues drained by the portal vein would have contributed to the arteriovenous glucose differences observed. Net splanchnic glucose production, as measured, was seen to be inhibited by intravenous insulin injection even though hypoglycemia was allowed full development. Several investigators using glucose-C\(^{14}\) (24, 31, 33) have reported that glucose-C\(^{12}\) production in the normal human subject
was inhibited after the subcutaneous injection of insulin, and remained inhibited for a long period even though the blood sugar concentration was allowed to fall as much as 40–50 mg/100 ml below the preinsulin level. This seeming difference in the response of the human, if it is real, requires elucidation.

Intravenous glucose administration in a single large dose was claimed (30, 32) to stop glucose-C14 production in the dog immediately, whereas other investigators (49) failed to obtain this effect. The positive findings were made doubtful by the observation (39) that injection of a large glucose-C14 load in eviscerated dogs, with body glucose tagged with C14 and with plasma glucose concentration maintained by a continuous infusion of glucose (instead of by the liver), gives a similar false impression of cessation of glucose-C14 input. However, in sheep, continuous infusion of suitably tagged glucose was shown to inhibit endogenous glucose C14 production very markedly (11).

In our own earlier studies (8, 10, 12) two of three animals were observed to respond to a suitably tagged glucose load by a decrease in glucose-C14 production; however, in the first of these studies (8, 10) little significance was attributed to this effect.

The experiments reported here, also utilizing C14-tagged glucose loads to avoid the artifact produced by slow mixing of a glucose-C14 load with part of the tagged body glucose pool, establish firmly that prolonged infusion of glucose alone in gradually increasing amounts causes a decrease in the rate of glucose-C14 production by the liver.

Net glucose production in the normal dog, as measured by transhepatic catheterization, was shown to be decreased by oral glucose administration in 1937 by Cherry and Crandall (13). A number of investigators (14, 25, 36) have since demonstrated that intravenous glucose infusion decreases net hepatic glucose production.

It is presumed that infused glucose decreases glucose-C14 production by causing an increase in the rate of endogenous insulin secretion, and that this sequence of events reflects one of the parts played by insulin in ordinary circumstances, i.e., after ingestion of a carbohydrate-containing meal. Landau and co-workers (25), in discussing their finding that a high-carbohydrate diet accentuates the inhibition by infused glucose of net hepatic glucose output, mention two possible reasons. One is a greater increase in the secretion of insulin in response to hyperglycemia and the other is an inherently greater increase in glucose uptake by liver at elevated glucose concentrations due to alterations brought about by the diet in the levels of hepatic enzymes concerned with glucose utilization. In the course of the present work, also, the impression has been gained that plasma glucose concentrations in dogs fed the high-carbohydrate diet do not rise as high at a given rate of glucose infusion as in dogs fed the standard diet. A greater increase in the secretion of insulin in response to hyperglycemia is the acceptable explanation for this difference in view of observations of glucose uptake by liver presented elsewhere (6). Thus in the early periods of glucose infusion in the present experiments glycogen-C12 loss was strongly inhibited with little stimulation of glucose-C14 incorporation into glycogen. This early relationship was evident also in dogs infused with insulin at plasma glucose concentration which were maintained at the normal level. Since increased glucose uptake by liver at this time should have resulted in a concomitant increase in glucose-C14 incorporation into glycogen it is suggested that, in these early periods increased glucose uptake by the liver did not play an important part in decreasing net hepatic glucose output or glucose-C12 release in either the high-carbohydrate or normally fed animals. In later periods of glucose infusion, when greatly increased glucose uptake by the liver became apparent (6), an inherently greater capacity of the livers of the dogs fed the high-carbohydrate diet to take up more glucose under the influence of insulin may also have played a part in limiting the rise in plasma glucose concentration and in decreasing glucose-C14 production. Not enough animals on the high-carbohydrate diet were studied to make a valid comparison with normal dogs in this respect.

In any event the present experiments demonstrate that maintenance of the animal on a high-carbohydrate diet prior to the experiment is not a necessary condition for the demonstration of decreased glucose-C14 release by liver in response either to insulin infusion at normal plasma glucose levels or to glucose infusion with elevated plasma glucose levels.

The simplest explanation for the depressing effect of insulin on hepatic glucose-C14 production is that insulin acts directly on the hepatic cells to produce the effect. This view is encouraged by other findings in this same series of experiments, as reported elsewhere (6), that incorporation of plasma glucose-C14 units directly into hepatic cell glycogen is enhanced by insulin. Recent reports indicate that insulin added to the perfusing medium affects the metabolism of perfused liver in vitro (20) and influences glucose balance across the perfused liver (23, 28). In the past an effect of insulin, added in vitro, on isolated hepatic tissue has been very difficult to establish.

One of the ways in which insulin might decrease glucose-C14 production involves stimulation of the synthesis either of glycogen or of the nonglycogen constituents of liver from hexose-C13 intermediates. Here, inhibition by insulin of glucose-C14 production could be erroneously interpreted in the sense that it actually results from a stimulatory effect of insulin exerted in another direction. However, another possible explanation for a part of the effect of insulin on the liver, i.e., inhibition of glycogenolysis, does involve a decrease in the rate of hexose-C12 production inside the hepatic cell. In this case the decreased glucose-C12 release from the liver would be a straightforward indication of a mechanism involved in insulin action. These possibilities and the relationship of increased hepatic glucose uptake to glucose-C14 release are discussed fully elsewhere (6).
We express our appreciation to Dr. O. K. Behrens, E. Lilly and Company, for tryptein-treated insulin, low in glucagon content.

REFERENCES


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