Inhibition by insulin of hepatic glucose production in the normal dog$^{1,2}$

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Glucose-$^{14}C$ was given intravenously in trace amount as an initial dose followed by continuous infusion to tag the circulating glucose of normal unanesthetized dogs in the post-absorptive state. The rate of dilution of this circulating tagged glucose by new ($^{12}C$) glucose produced endogenously was measured. The release to the blood of such new glucose, presumably almost entirely from liver, was reduced by half during the 1st hr of intravenous insulin infusion at 0.1 U/kg per hr or more, provided that enough glucose was also infused to limit hypoglycemia. During the 2nd hr new glucose release was reduced by three-quarters or more. Insulin infusion at lower rates (0.02-0.04 U/kg per hr), along with glucose, produced smaller effects. Glucose alone, infused intravenously in amounts sufficient to raise plasma glucose concentration, and hence presumed to enhance endogenous insulin secretion, reduced new glucose release by half during the 1st hr of infusion at one-half to one and one-half times the resting endogenous glucose production rate. In the 2nd or 3rd hr, with glucose infusion increased to two to five times the resting endogenous glucose production rate, new glucose release was reduced by three-fourths or more.

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 ($^{12}C$) 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-$^{14}C$ 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-$^{14}C$ 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 $^{14}CO_2$ was in place over the head of the dog.

A standard diet previously described (9), containing 58% of its calories as carbohydrate (11), was used to...
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 complete 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-C\textsuperscript{14} (about 30 \( \mu \)c) in trace amount, either randomly labeled or labeled in carbon 6, was administered as a priming dose in about 10 ml saline, iv. A continuous infusion at a measured rate (about 0.5 ml/min) into a saphenous vein, of the same glucose-C\textsuperscript{14} preparation, was begun and continued throughout the entire experiment. The infusion solution was of such glucose-C\textsuperscript{14} content that an amount of C\textsuperscript{14} equal to that contained in the priming dose was delivered in about 125 min. Glucose or insulin and glucose infusions were started about 3 hr after the initial glucose-C\textsuperscript{14} 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 10-90% solution in water into a saphenous vein. Bulk glucose infusions always contained enough glucose-C\textsuperscript{14} to bring the specific activity of the administered glucose near to that already prevailing in the circulating plasma glucose.

The insulin was trypsin-treated (glucagon-low) crystalline 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 (AutoAnalyzer; Technicon Instruments Corp., Chauncey, N. Y.) so that plasma glucose concentration could be controlled by adjustment of the rate of bulk glucose infusion.

Blood determinations were made of plasma glucose concentration, mean glucose-C\textsuperscript{14} production rate in control period, IIC, high carbohydrate diet. I: insulin infusion, U/kg per hr. G: glucose infusion, mg/kg per hr. % R: mean glucose-C\textsuperscript{14} production rate as % of control, R.
Insulin inhibition of hepatic glucose production

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-49, 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-39HC, and B-31HC) 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 22 mg/100 ml) above the pretreatment level. In seven of the experiments (AL-39, B-43, B-47, B-48, AL-39HC, and B-33HC) hepatic glucose-C\textsuperscript{14} production was depressed by 80% or more during a period of the glucose infusion. This occurred 75-135 min after the beginning of glucose infusion, at a time when plasma glucose concentration was elevated 13-65 mg/100 ml (mean 43 mg/100 ml) above the pretreatment.

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The rate of glucose-C\textsuperscript{12} production brought about in the dog that insulin infusion slowed to normal the accelerated rate per hr, iv) the increased glucose-C\textsuperscript{12} production of the continued, this restraint being released when the insulin infusion was terminated. Subsequently, it was found that insulin infusion slowed to normal the accelerated rate of glucose-C\textsuperscript{12} production brought about in the dog by acute phlorizin poisoning. A similar restraint by insulin on the increased glucose production brought about by glucose 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\textsuperscript{12} 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\textsuperscript{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\textsuperscript{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\textsuperscript{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," so measured, was seen to be inhibited by intravenous insulin injection even though hypoglycemia was allowed full development. Several investigators using glucose-C\textsuperscript{14} (24, 31, 33) have reported that glucose-C\textsuperscript{14} 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 precinulin 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-C\textsubscript{14} 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-C\textsubscript{14} load in eviscerated dogs, with body glucose tagged with C\textsubscript{14} 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-C\textsubscript{14} input. However, in sheep, continuous infusion of suitably tagged glucose was shown to inhibit endogenous glucose C\textsubscript{14} 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-C\textsubscript{14} production; however, in the first of these studies (8, 10) little significance was attributed to this effect.

The experiments reported here, also utilizing C\textsubscript{14}-tagged glucose loads to avoid the artifact produced by slow mixing of a glucose-C\textsubscript{14} 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-C\textsubscript{14} 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-C\textsubscript{14} 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-C\textsubscript{12} loss was strongly inhibited with little stimulation of glucose-C\textsubscript{14} 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-C\textsubscript{14} 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-C\textsubscript{14} 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-C\textsubscript{14} 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-C\textsubscript{14} 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-C\textsubscript{12} 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-C\textsubscript{14} 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-C\textsubscript{14} production involves stimulation of the synthesis either of glycogen or of the nonglycogen constituents of liver from hexose-C\textsubscript{19} intermediates. Here, inhibition by insulin of glucose-C\textsubscript{14} 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 glycojenolysis, does involve a decrease in the rate of hexose-C\textsubscript{12} production inside the hepatic cell. In this case the decreased glucose-C\textsubscript{12} 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-C\textsubscript{14} release are discussed fully elsewhere (6).
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REFERENCES