Efficient Hepatic Glycogen Synthesis in Refeeding Rats Requires Continued Carbon Flow through the Gluconeogenic Pathway*

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Intragastric infusion of [1-14C]glucose into awake, fasted rats at rates that produced physiological increases in the circulating glucose concentration resulted in active glycogen deposition in liver. However, degradation of this glycogen revealed extensive randomization of the label among the carbon atoms of glucose. By contrast, muscle glycogen-glucose was labeled primarily in C-1. Treatment of rats with 3-mercaptopicolinic acid, a potent inhibitor of phosphoenolpyruvate carboxykinase, prior to [1-14C]glucose infusion reduced hepatic glycogen synthesis by 85%; this glycogen contained most of its label in C-1 of glucose. The additional infusion of unlabeled glycerol, which enters the gluconeogenic pathway distal to the 3-mercaptopicolinic acid block, reinstated hepatic glycogen synthesis, but again the label was associated almost exclusively with C-1. In all animals treated with 3-mercaptopicolinic acid, plasma lactate concentrations rose markedly, as did the rate of hepatic lipogenesis.

When [1-14C]glucose was infused into pentobarbital-treated rats or administered to awake animals as a large intragastric bolus, the degree of isotopic randomization in liver glycogen-glucose was considerably reduced when compared with that seen in the awake, infused state. This finding raises certain problems. For example, it requires that carbon flow through the gluconeogenic pathway of liver remain active for several hours into the postprandial period, a view that is also contrary to current belief. Moreover, it runs counter to several early studies in the literature in which the administration of specifically labeled glucose to rats, followed by degradation of liver glycogen and positional isotopic analysis of the glucose carbon atoms, led to the conclusion that the glucose to glycogen conversion occurred almost exclusively by the direct pathway (5–9).

The present investigation had three goals. First, using a different experimental approach from that described previously (1), we wished to confirm that, when administered to fasted rats under conditions leading to physiological increments in its circulating level, glucose is converted into liver glycogen largely by an indirect pathway. This was done by infusing glucose labeled with 14C solely in the 1 position and demonstrating a substantial randomization of the label among the carbon atoms of glycogen-glucose in liver. Second, we wished to prove the essentiality of an active gluconeogenic pathway for efficient hepatic glycogen synthesis from exogenous glucose. This was accomplished by using a selective inhibitor of gluconeogenesis, namely, 3-mercaptopicolinate. Finally, we wished to determine whether the apparent discrepancies between the older (5–9) and newer (1–4) observations could be explained on the basis of differences in experimental design. The results indicate that conclusions on directness vs. indirectness of the glucose to liver glycogen conversion hinge in large part on the manner in which the exogenous glucose load is administered. The choice of awake or anesthetized animals also appears to be important. Under physiological conditions the indirect pathway seems to predominate.

Recent studies from this and other laboratories have cast doubt on the long-standing concept that upon termination of a fast, dietary glucose is converted into liver glycogen primarily via a direct pathway involving the sequence glucose → lactate → glucose-6-P → glycogen, whereas muscle glycogen is formed by the conventional, direct pathway: glucose → glucose-6-P → glycogen. They also establish that a predominantly direct mechanism can be induced in liver, but only under artificial conditions, e.g. chemical blockade of the gluconeogenic sequence, pentobarbital anesthesia, or the administration of massive glucose loads that lead to severe hyperglycemia.

EXPERIMENTAL PROCEDURES

Animals—Male Sprague-Dawley rats weighing 100–160 g were used. They were maintained on a high sucrose, low fat diet, as described previously (10) with lighting from 3:00 p.m. to 3:00 a.m. at 1:00 p.m. on day 1 the animals were fitted with intragastric, intravenous, and arterial catheters, placed in restraining cages and left overnight with water but no food available. Experiments began at 9:00 a.m. on day 2, i.e. after a 20-h fast.

Infusion Studies—In most experiments a solution of [1-14C]glucose (containing 4 μCi/ml and sufficient glucose to deliver 40, 84, or 126 mg/100 g of body weight/h) was infused into awake animals intragastrically or intravenously at a rate of 20 μl/min. In other studies the labeled glucose was given intragastrically as a 1.5-ml bolus containing 400 mg/100 g of body weight. To determine the effect of anesthesia on the labeling pattern of liver glycogen, some animals received sodium pentobarbital (7.5 mg/100 g of body weight) prior to the infusion of [14C]glucose. When used, 3-mercaptopicolinic acid (15

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mg/100 of body weight) was given intragastrically as a suspension in 1.5 ml of 0.5% tragacanth (11) immediately prior to the glucose infusion. Glycerol was given intravenously at a rate of 50 or 100 mg/100 g of body weight/h.

Arterial blood samples were taken at intervals for analysis of plasma glucose and lactate concentrations by conventional enzymatic techniques. At the desired time, awake animals were anesthetized by the intravenous administration of sodium pentobarbital and livers were quickly frozen in liquid N₂. Leg muscle was treated similarly.

**Lipogenesis**—To assess the rate of hepatic lipogenesis in vivo under different infusion protocols, animals received H₂O (25 mCi/100 g of body weight in 0.25 ml of 0.9% NaCl) intravenously at the 45-min time point. Thirty minutes later an arterial blood sample was taken and the liver was frozen as described above.

**Analytical Procedures**—Tissues were analyzed for glycogen content by the method of Chan and Exton (12) and glycogen was purified as described previously (1). To determine the per cent of total ¹⁴C in carbon 1 of glycogen-glucose the following procedure was adopted. Five milligrams of purified glycogen were hydrolyzed completely to glucose by incubation for 90 min at 37°C with 0.5 units of amyloglucosidase dissolved in 2 ml of sodium acetate buffer, pH 4.85. Fifty microliters of the hydrolysate was then placed in a 25-ml Erlenmeyer flask containing 0.9 ml of a "mixture" composed of 0.1 M Tris-HCl, pH 7.4, 7.5 mM MgCl₂, 5 mM ATP, 2 mM phosphoenolpyruvate, and 2 mM NaHCO₃. Next was added 0.04 ml of a second mixture containing in a total volume of 1.8 ml of a 100,000 x g supernatant from a 10% rat liver homogenate prepared in 100 mM KCl, 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, and 10 mM mercaptot ethanol (source of t-thioglycolic acid), 28 units of glucose-6-P dehydrogenase, 1.2 units of 6-P gluconate dehydrogenase, 14 units of hexokinase, and 20 units of pyruvate kinase. The flasks were quickly sealed with rubber stoppers, fitted with plastic cups containing 0.3 ml of hyamine hydroxide (1 M in methanol), and shaken at 37°C for 1 h. Reactions were terminated by injection of 0.4 ml of 8% HClO₄ and, after further shaking at room temperature for 45 min, the cups were removed for measurement of trapped ¹⁴CO₂ by liquid scintillation counting. A standard of tracer [¹⁴C]glucose plus 5 mg of pure unlabeled glycogen was run together with each test sample and generally yielded 98-99% recovery of ¹⁴C as ¹⁴CO₂.

Frozen livers taken from animals receiving H₂O were weighed, pulverized, and mixed with a solution of alcoholic KOH (1.94 g of KOH in 100 ml of 90% ethanol) in a ratio of 15.5 ml/g of tissue. After heating on a steam bath for 2 h in a loosely stoppered flask, the mixture was adjusted to its original volume with 50% ethanol (v/v). A measured aliquot was then processed as described previously (13) for the extraction and counting of radioactive fatty acids. From a count on the plasma obtained from each donor animal the specific activity of the body water pool could be determined, allowing calculation of the rate of hepatic fatty acid synthesis (14).

**Materials**—3-Mercaptopicolinic acid was kindly provided by N. W. DiTullio of Merck, Sharp and Dohme. The sources of other materials have been given previously (1).

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**RESULTS**

**Labeling Pattern of Glycogen Formed from Exogenous [¹⁴C]Glucose**—The rationale for the first series of experiments was as follows. If [¹⁴C]glucose is administered to fasted rats, the glycogen deposited in tissues should contain label only in carbon 1 of its glycogen residues if a direct synthetic pathway were operative. By contrast, if the glucose first traverses the glycolytic pathway (regardless of site) prior to glycogen formation the label in glycogen-glucose should be randomized between carbon 1 and other positions of the molecule. As seen from the data of Table I, after a 2-h infusion of [¹⁴C]glucose either intravenously or intra-gastrically, the plasma glucose concentration had risen only to the 5-7 mM range, but significant quantities of glycogen were deposited in liver. However, unlike the infused glucose, which contained essentially all of its ¹⁴C in carbon 1, that derived from liver glycogen contained only about 50% of its label in this position. Such extensive randomization of the label suggested that liver glycogen was synthesized in large part via an indirect pathway. A different picture was seen in muscle where the glycogen-glucose remained most of its ¹⁴C in C-1, indicating that in this tissue the direct pathway predominated.

**Effect of Inhibition of Gluconeogenesis on Hepatic Glycogen Synthesis**—The labeling pattern of liver glycogen noted in Table I might have been explained in either of two ways. One scenario would be that the [¹⁴C]glucose was taken up directly by the liver and reversibly metabolized to the triose-P level prior to its conversion into glycogen. Such a mechanism would result in the randomization of label between C-1 and C-6 of the glycogen-glucose and would not require the operation of gluconeogenic reactions below the triose-P level. On the other hand, if, as previously suggested (1), the glucose was first metabolized to the level of lactate (site unspecified) and the latter was converted into glycogen, ¹⁴C would appear not only in C-1 and C-6, but also in C-2, C-3, and C-5 of the glycogen (because of additional randomization of label at the level of malate ↔ fumarate). This would require that carbon flow through the entire gluconeogenic sequence in liver remain active during the fasted to refed transition. To discriminate between these possibilities we made use of the compound, 3-mercaptopicolinic acid, a potent inhibitor of phosphoenolpyruvate carboxykinase and thus of gluconeogenesis (15). It was reasoned that if the gluconeogenic pathway is essential for efficient hepatic glycogen synthesis from glucose, administration of MP¹ prior to [¹⁴C]glucose infusion should suppress glycogen deposition in liver; but, glycogenesis should be reinstated during the simultaneous infusion of glycerol, which enters the gluconeogenic sequence distal to the MP block. In addition, MP should reduce the degree of label randomization in the glycogen formed.

As seen from Fig. 1, infusion of fasted rats with glucose alone at a rate of 84 mg/100 g of body weight/h raised its circulating concentration to the region of 6-7 mM (panel A) but had no effect on the plasma lactate concentration (panel B). In keeping with the report of DiTullio et al. (11), MP alone resulted in profound hypoglycemia and a significant elevation of plasma lactate, which rose from 1 to 3 mM during the first 30 min and reached 6 mM by the 2-h time point. When glucose was given together with MP, hypoglycemia was prevented and, although slightly lower at 30 min, the plasma [glucose] achieved was similar at later time points

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¹ The abbreviation used is: MP, 3-mercaptopicolinate.
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FIG. 1. Effects of 3-mercaptopicolinate in fasted rats. Fasted rats were treated with tragacanth alone or with 3-mercaptopicolinate suspended in tragacanth as described under “Experimental Procedures.” They were then infused with glucose (Gluc) intragastrically and glycerol (Glyc) intravenously as indicated. Numbers in parentheses refer to infusion rates in milligrams/100 g of body weight/h. Values are means ± S.E. for four to six animals in each group.

FIG. 2. Effects of 3-mercaptopicolinate in fasted rats. Experiments were identical with those of Fig. 1 except that the infusion rates of glucose and glycerol were increased to 126 and 100 mg/100 g of body weight/h, respectively. The data for animals treated with MP alone are taken from Fig. 1. Values are means ± S.E. for four to six animals in each group.

to that seen when glucose was given in the absence of the inhibitor. Under these conditions plasma [lactate] rose more slowly than in the presence of MP alone and plateaued at about 2.7 mM by 60 min (see below). The additional infusion of glycerol at a rate of 50 mg/100 g of body weight/h, which raised plasma [glycerol] from 1.0 to 1.5 mM (data not shown), had little further effect on the circulating levels of glucose and lactate. Qualitatively similar results were obtained when the infusion rates of glucose and glycerol were increased to 126 and 100 mg/100 g of body weight/h, respectively (Fig. 2). In this case plasma glucose levels were predictably somewhat higher than in the equivalent experiments of Fig. 1 and, when given exogenously, the concentration of glycerol in plasma plateaued at about 3 mM by the 30-min time point (data not shown).

The effects of these manipulations on the pattern of liver glycogen synthesis are shown in Table II. Liver glycogen content after infusion of [1-14C]glucose at 84 and 126 mg/100 g of body weight/h rose to 12.4 and 18 mg/g, respectively, but in both cases almost 50% of its 14C resided in carbon atoms other than the C-1 of glucose. By contrast, glycogenesis in rats treated with MP prior to glucose infusion was reduced by some 85%, and what glycogen was synthesized contained most of its 14C in C-1 of glucose. Infusion of unlabeled glycerol together with [1-14C]glucose into MP-treated animals restored glycogen synthesis to rates seen with glucose alone. In this case the specific activity of the glycogen was much lower (data not shown), since it derived in large part from the unlabeled glycerol; again the label was primarily in C-1. The restoration of glycogenesis by glycerol eliminated the possibility of adverse effects of MP at steps distal to phosphoenolpyruvate carboxykinase.

The glycogen samples from the experiments of Table II are presently undergoing more detailed analysis. Preliminary results indicate that in those cases where major randomization of label had occurred (i.e. in animals receiving [1-14C]glucose alone) some 30-35% of the 14C was associated with the lower half of the glucose molecule. In one sample subjected to complete degradation the per cent of total label present in C-1 of glucose was 35%, and in another 33%, so that the glycogen was essentially uniformly labeled.

The glycogen samples from the experiments of Table II were subjected to further degradation by enzymes in a rotating vessel. In a typical experiment, the glycogen samples were incubated for 30 min at 37°C with a suspension of rat liver hexokinase or glucokinase by 3-mercaptopicolinate.

| Mercapto- | Intragastric | Intravenous | Liver glycogen |
|-----------|--------------|-------------|----------------|
| polinate  | infusion     | infusion    | mg/g, wet wt* | % 14C in C-1* |
| +         | [1-14C]Glucose (84) | 12.4 ± 1.1 | 54.7 ± 3.5 |
| −         | [1-14C]Glucose (84) | 1.9 ± 0.3  | 84.0 ± 3.6 |
| +         | [1-14C]Glucose (84) | 9.3 ± 2.3  | ND*         |
| −         | [1-14C]Glucose (126) | 18.0 ± 2.2 | 54.6 ± 1.4 |
| +         | [1-14C]Glucose (126) | 2.6 ± 0.7  | 91.4 ± 1.4 |
| +         | [1-14C]Glucose (100) | 19.0 ± 1.7 | 88.8 ± 0.4 |

*Liver glycogen prior to infusion was <1 mg/g, wet weight.
*Infused [1-14C]glucose contained 94-95% of its label in C-1.
*ND, not determined.

Neither we nor others (17) have found any evidence for inhibition of rat liver hexokinase or glucokinase by 3-mercaptopicolinate.
Effects of Experimental Conditions on the Mechanism of Hepatic Glycogen Synthesis—At first sight, the above findings would appear to be in direct conflict with earlier studies in the literature from which it was concluded that glucose given to fasted rats is converted into liver glycogen primarily via the conventional, direct pathway (5–9). In seeking to rationalize the discrepancy we noted certain differences in experimental approach between the older and present work. For example, Hers (5) administered [1-14C]glucose as a large intragastric bolus (about 350 mg/100 g of body weight) to fasted rats and found little randomization of the label either in liver or muscle glycogen. We suspected that the difference between his findings and ours as regards liver glycogen might have stemmed from differences in the blood glucose concentration achieved in the two studies. Support for this notion is provided by the data in Fig. 3. As seen from Fig. 3A, when we administered [1-14C]glucose as a single intragastric bolus of 400 mg/100 g of body weight the plasma glucose concentration rose to 15 mM over the first 30 min, thereafter declining to basal levels. A similar profile was seen for plasma [lactate] (panel B). Liver glycogen was deposited in significant quantities after 1 h but tended to fall during the second hour (panel C). Retention of label in C-1 of glycogen-glucose averaged 72 and 61%, respectively, at 1 and 2 h (panel D). The former value markedly exceeded those observed during the constant infusion of [1-14C]glucose (Tables I–III), but approached those observed by Hers (5). As there was considerable variability in the final liver glycogen levels and their labeling patterns (Fig. 3, C and D) it was of interest to examine the correlation between the two parameters in the 2-h samples. The higher the glycogen content of the liver, the less label was retained in C-1 (r = −0.78). We interpret the data as follows. The intense, early glycolysis caused by the glucose bolus would be expected to force glucose through the relatively sluggish, high K_m glucokinase reaction of liver and into glycogen via the direct pathway. The randomization at 2 h was probably a function of glycogen breakdown and glycogen synthesis occurring at varying rates in the different animals. Thus, to the extent that glycogen synthesis continued in the 1–2 h interval, the indirect pathway would likely have predominated (because of the low blood glucose concentration), resulting in randomization of label. On the other hand, active glycogenolysis during this period would result in the purification of glycogen laid down mainly in the first hour, and thus largely nonrandomized.

The explanation advanced above might also explain the findings of Taylor and Langdon (6), Marks and Feigelson (7), and Moriwaki and Landau (8), all of whom administered labeled glucose as a bolus and showed that it reached liver glycogen primarily by the direct pathway. It would not, however, account for the observations of Hostetler and Landau (9), who reported little randomization of label in liver glycogen following the intravenous infusion of [2-14C]glucose into fasted rats at a rate of only 30 mg/100 g of body weight/h.

Since the animals used in those studies were under pentobarbital anesthesia, whereas ours were awake, we felt it important to examine the effects of this drug on the system. Experiments similar to those described in Table 1 were performed with awake and pentobarbital-treated animals. As seen from Table III, regardless of the route of [1-14C]glucose infusion the plasma glucose concentration remained in the 5–7 mM range over the entire experimental period. In awake animals some 50% of the label in liver glycogen resided in carbon atoms other than C-1. In anesthetized animals, however, the retention of label in C-1 of glycogen was clearly increased, indicating a greater contribution of the direct pathway under these circumstances. The basis for this effect of pentobarbital remains to be elucidated.

Effect of Mercaptopicolinate on Hepatic Lipogenesis in Vivo—If, under our experimental conditions a glucose → lactate → glycogen sequence represents the major route for hepatic glycogen synthesis in the early postprandial phase, the data of Figs 1B and 2B might be considered paradoxical. In other words, since fasted rats treated with MP alone developed a striking hyperlactatemia, why were lactate concentrations less with the additional infusion of glucose? We felt that the answer might lie in the relative rates of hepatic lipogenesis since, as shown previously (18), lactate is a particularly efficient substrate for this process. Support for this formulation is provided by the data in Table IV. Infusion of glucose into otherwise untreated animals produced only a 50% increase in the fasting rate of hepatic lipogenesis during the time interval studied. This modest response likely stemmed from the fact that lactate, whose circulating level failed to rise under these conditions (Fig. 2), continued to traverse the gluconeogenic pathway efficiently despite the presumed stimulation of insulin secretion. By contrast, treatment with mercaptopicolinate alone increased lipogenesis to three times the basal rate in the face of severe hypoglycemia (Fig. 2) and (presumably) hypoinsulinemia. The driving force in this case was undoubtedly the marked elevation in the circulating concentration of lactate (Fig. 2), which is known to support high rates of fatty acid synthesis in rat hepatocytes even in the absence of insulin (18). The highest rate of lipogenesis, however, was seen in those animals receiving both mercaptopicolinate and glucose. Almost certainly, this resulted from a combination of increased lactate availability (Fig. 2) coupled with a glucose-induced suppression in the secretion of glucagon, a known antagonist of hepatic lipogenesis (18). Such conditions would be expected to facilitate the flow of lactate through the lipogenic pathway, thus attenuating its accumulation in the blood (Fig. 2).

**DISCUSSION**

Earlier reports (cited in Refs. 1 and 18) have documented that glucose, at physiological concentrations, is poorly utilized for anabolic purposes by rat liver in vitro. These findings,
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TABLE III
Effect of pentobarbital on the labeling pattern of liver glycogen in rats given [1-14C]glucose

| Route of [1-14C]glucose infusion | Pentobarbital* | Plasma glucose (mm) at 0 min | Liver glycogen at 2 h mg/g | % 14C in C-1 |
|---------------------------------|---------------|------------------------------|--------------------------|----------------|
| Intragastric (6)                | -             | 5.0 ± 0.1                    | 9.5 ± 2.4                | 49 ± 1.0       |
| Intragastric (6)                | +             | 4.7 ± 0.1                    | 10.6 ± 2.7               | 64 ± 1.8       |
| Intravenous (4)                 | -             | 4.8 ± 0.2                    | 9.1 ± 0.4                | 56 ± 1.3       |
| Intravenous (4)                 | +             | 5.3 ± 0.7                    | 15.5 ± 1.7               | 70 ± 3.2       |

* Pentobarbital given intravenously at zero time in a dose of 7.5 mg/100 g of body weight.

† Liver glycogen at zero time was <1 mg/g, wet weight.

‡ Infused [1-14C]glucose contained 93–95% of its label in C-1.

TABLE IV
Effect of 3-mercaptoisocitrate on hepatic lipogenesis in vivo

| Mercaptopicolinate | Intragastric infusion | Hepatic lipogenesis μmol H2O → fatty acids/g |
|--------------------|-----------------------|---------------------------------------------|
| -                  | Water                 | 1.36 ± 0.13                                 |
| +                  | Water                 | 1.92 ± 0.19                                 |
| +                  | Glucose               | 3.96 ± 0.58                                 |
| +                  | Glucose               | 5.31 ± 0.78                                 |

*p < 0.05 compared with water-infused controls.

coupled with the well known fact that refueling fasted animals a carbohydrate containing diet leads to rapid activation of hepatic glycogen synthesis and lipogenesis, gave rise to what has been termed “the glucose paradox” (1, 18, 19). A plausible solution to the problem emerged when fasted rats were given [U-14C,3-3H]glucose and the labeling pattern of liver glycogen subsequently examined (1). The disproportionate loss of 3H relative to 14C in glycogen suggested that the efficiency with which dietary glucose is directly converted into liver glycogen in vivo which had been greatly overestimated. In keeping with previous work by Shikama and Ui (20), it was proposed that the bulk of liver glycogen deposited postprandially was formed by an indirect mechanism in which a C6 compound, rather than glucose itself, served as the proximate precursor (1). The studies of Radziuk (2) in man and, in some respects, those of Baker (21) in mice were consistent with this notion.

The present studies, in which the labeling pattern of liver glycogen was analyzed after the administration of [1-14C]glucose, provide further support for the operation of an indirect pathway. In addition, they establish three points that had not hitherto been rigorously proved. First, uninterrupted carbon flow through the gluconeogenic reactions of liver is essential for efficient glycogen deposition in this tissue during the fasted to refed transition. Second, the glucose-derived intermediary functioning as substrate for synthesis of liver glycogen enters the gluconeogenic pathway at a point proximal to phosphoenolpyruvate carboxykinase. Lactate, or a closely related compound, remains a logical candidate, although its site(s) of formation is still not clear. Third, when gluconeogenic flux is blocked, the less active direct pathway of hepatic glycogen synthesis becomes primary.

In quantitative terms it appears that under our experimental conditions the major fraction (at least two-thirds) of the glycogen deposited in liver when a fast is terminated is formed via the indirect pathway. By contrast, glycogen synthesis in muscle, a relatively nongluconeogenic tissue, occurs mainly, if not exclusively, through the direct mechanism. Such a conclusion contrasts with the traditional view which holds that in both of these tissues the synthesis of glycogen occurs directly from glucose. We believe that the discrepancy stems in large part from differences in experimental design. If, as was done in the earlier studies, we administered labeled glucose as a large intragastric bolus (equivalent to 280 g/70 kg in man), we found the direct pathway for hepatic glycogen synthesis predominant. However, this was at the expense of a profound postprandial hyperglycemia which presumably allowed glucose to traverse the low affinity glucokinase reaction of liver at higher than normal rates. Pentobarbital anesthesia also increased the fractional contribution of the direct pathway, although the underlying mechanism is unclear. The question is thus raised: which experimental conditions most closely approach the physiological situation? We favor the constant intragastric infusion of glucose into awake animals, for the following reasons. First, the levels of circulating glucose achieved by this method were similar to those seen in fasted rats allowed to eat ad libitum (1, 18). These values seldom exceeded 7–9 mm, a concentration range in which the activity of rat liver glucokinase is insufficient to support the observed rates of hepatic glycogen synthesis (1). Second, the source of dietary carbohydrate for most mammals is not glucose, but starch, which, because of the time required for intestinal hydrolysis, will likely result in even lower portal blood glucose concentrations than those reached when glucose is administered. Third, the labeling pattern of liver glycogen was similar when fasted rats were given [U-14C,3-3H]glucose by constant infusion or in solid diet eaten ad libitum (1),

3 This estimate was derived as follows. Let X and (1 – X) be the fractional contributions of the indirect and direct pathways, respectively. Let X be the per cent of total 14C residing in C-1 of [1-14C]glucose and glycogen-glucose be 100 and Y, respectively. Assume that the indirect pathway results in equal labeling of C-1, C-2, C-3, and C-4 of glycogen-glucose, with negligible labeling of C-5 and C-4. Assume further that the phosphoenolpyruvate derived from the [1-14C]glucose by this indirect mechanism is not diluted from endogenous sources (an extreme and unlikely case). Then Y = 100 (1 – X) + 25X. Therefore, X = (100 – ) / 25. Since the average value for Y in animals receiving [1-14C]glucose alone was about 50 (Tables I–III), the calculated value for X is about 2/3. Obviously, this is a minimum value and will underestimate the contribution of the indirect pathway to the extent that unlabeled materials feed into the labeled phosphoenolpyruvate pool.
suggesting that the former technique mimicked physiological eating patterns.

If carbon flow from pyruvate to glucose-6-P in liver remains active in the immediate postprandial period some intriguing questions are raised. For example, it is known that under these conditions, glucose-6-P is diverted away from the glucose-6-phosphatase reaction and into the pathway of glycogen synthesis (22). What effects this crucial metabolic switch is not known, although we suspect that suppression of the microsomal glucose-6-phosphatase system is an important mechanism (22). Also uncertain is how events at the fructose-6-P/fructose 1,6-bisphosphate step are directionally controlled when fasting is terminated. If, as is generally believed, the concentration of hepatic fructose 2,6-bisphosphate, which activates phosphofructokinase and inhibits fructose-1,6-bisphosphatase (23, 24), rises acutely with elevation of glucose and insulin levels (24–26), how is carbon flow from fructose 1,6-bisphosphate to glucose-6-P maintained in the postprandial state? Answers to these questions are now being sought.

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Note Added In Proof—Since this article was submitted, the publication by Sugden et al. ((1983) Biochem. Int. 7, 329–337) was called to our attention. Of interest is the fact that these authors also demonstrated the inhibition of hepatic glycogen synthesis and stimulation of lipogenesis by 3-mercaptopicolinate when fasted rats were refed with glucose.

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