Studies on the Mechanism by Which Exogenous Glucose Is Converted into Liver Glycogen in the Rat

A DIRECT OR AN INDIRECT PATHWAY?

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To quantify the extent to which exogenous glucose is used directly or indirectly for hepatic glycogen synthesis, fasted rats were given [U-14C,3-3H] glucose intragastrically or intravenously ad libitum. In all cases liver glycogen was deposited at high linear rates over a 3-h period. Portal vein glucose levels seldom exceeded 8 mM. At a time when the specific activities of 3H and 14C in circulating glucose were identical with those in the administered material their values in newly synthesized glycogen were reduced by 72–88% and 50–65%, respectively.


Using an improved assay the ability of liver homogenates to phosphorylate glycogen at concentrations of 5–10 mM was found to be far short of what would be needed if glucose were used directly to support hepatic glycogen synthesis in vivo.

These data support the notion that in the rat a major fraction of liver glycogen deposited in response to exogenous carbohydrate is formed by a pathway involving glucose → C3 unit → glycogen, although the site of the initial steps in the sequence is not yet known. The limited capacity of the liver to utilize intact glucose for glycogen synthesis might reside in its limited capacity to phosphorylate the sugar at physiological concentrations.

It has long been accepted that after the ingestion of a carbohydrate load by the mammalian organism a large fraction of the absorbed glucose is taken up by the liver and directly1 converted into glycogen. Yet, paradoxically, it has been shown by a number of laboratories that glucose, even at supraphysiological concentrations and in the presence of insulin, is a poor precursor for glycogen synthesis either in the perfused rat liver or in isolated hepatocytes (1–6). In all of these studies, however, good rates of glycogen synthesis could be obtained when the liver preparations were presented with a mixture of glucose, gluconeogenic precursors, and certain amino acids. The possibility was thus raised that even in vivo the contribution of glucose as a direct precursor of hepatic glycogen might have been overestimated (1–6). It was further suggested that following the absorption of glucose a product of its metabolism (lactate, for example) might serve as the major substrate for glycogen synthesis in the liver, the conversion of triose to glycogen being permissively stimulated by an elevated blood glucose level (6). In keeping with this construct Radziuk (7) concluded from isotopic studies in man that a significant fraction of liver glycogen deposited after glucose loading must have been derived from gluconeogenic substrates. Experiments with rats (8, 9) and mice (10) were qualitatively consistent with such an interpretation.

In the present work we have attempted to provide more quantitative insight into this central issue of mammalian carbohydrate metabolism. Specifically, using the rat as the experimental model we sought answers to the following questions. To what extent is exogenous glucose converted directly or indirectly into liver glycogen when administered to fasted animals via the intragastric or intravenous route? Is carbon flow through the gluconeogenic pathway in liver suppressed in the face of a large glucose load (conventional view) or does it continue for a finite period of time? What are the relative rates of glucose 6-phosphate conversion into glucose and glycogen before and after glucose administration? How does the glucose-phosphorylating capacity of the liver compare with rates of glycogen deposition seen in vivo?

The results outlined below support the concept that with glucose administration, irrespective of the route, the bulk of liver glycogen deposited in liver is synthesized by an indirect mechanism. This requires continued carbon flow in liver from the C3 level to glucose 6-phosphate, with diversion of the latter away from the glucose-6-phosphatase reaction and into the pathway of glycogen synthesis. The low rate of direct conversion of glucose into liver glycogen might be explained by the limited capacity of this tissue to phosphorylate glucose at physiological concentrations.

EXPERIMENTAL PROCEDURES

Animals—Male Sprague-Dawley rats weighing 100–180 g were used. They were maintained on a high sucrose-low fat diet as described previously (11) and were housed in a room with lighting from 3:00 p.m. to 3:00 a.m.

Infusion Studies—Animals were anesthetized with ether and surgically fitted with intragastric femoral artery and/or femoral vein catheters at 1:00 p.m. on day 1. They were then placed in restraining cages with water but no food available and left in a quiet dark room until experiments began at 9:00 a.m. on day 2 (i.e., they were fasted for about 20 h). Glucose, dissolved in water, was infused intragastrically or intravenously at a rate of 20 µl per min. The quantity of...
During the course of experiments arterial blood samples (approximately 200 μl) were taken into heparinized tubes and an equivalent volume of 0.9% NaCl was given intravenously. After centrifugation, plasma was used for analyses. At the desired times the animals were anesthetized with pentobarbital, portal venous blood samples were taken, and livers were quickly removed and frozen in liquid N₂. A batch of the synthetic maintenance diet lacking the sucrose component (and, therefore, devoid of carbohydrate) was obtained from the manufacturer. Glucose was added to a final concentration of 40% by weight and the mixture was pulverized in a mortar. Animals were trained to eat the powdered diet for 4 days. After a 20-h fast they were again presented with the diet, this time containing [U-¹⁴C,³H]glucose of known specific activities and allowed to eat ad libitum. At the desired times blood and liver were taken for analyses.

**Glucose Turnover Studies**—The rate of hepatic glucose production was assessed using the technique of primed continuous infusion with [³H]glucose. In the basal state this was obtained by dividing the counts per min infused by the specific activity (counts per min per μmol) of plasma glucose in the steady state period. With glucose loading, endogenous production was estimated as the difference between the calculated rate of glucose entry into the blood and the rate of exogenous glucose infusion. This assumes that the rate of intestinal absorption of glucose was approximately equal to the intragastric infusion rate. We believe this assumption to be reasonable in view of the known rapidity with which glucose is absorbed by the gut and the fact that no fluid was found to accumulate in the animals' stomachs after the bolus of glucose infusion.

**Analytical Procedures**—Livers were pulverized under liquid N₂ and 0.5 g of the powder was used for the determination of glycogen content (12). From the remainder, glycogen was extracted and purified (13). Plasma glucose and lactate concentrations were determined by standard techniques. To obtain the specific activity of circulating glucose phosphorylating capacity of the tissue (primarily glucokinase). Conventional, this enzyme has been measured spectrophotometrically by following the production of NADPH in an assay mixture containing glucose, ATP, NADP, glucose-6-phosphate dehydrogenase (NADP-specific), and liver extract (15). Serious problems were identified with this method. For example, we found commercial preparations of NADP-specific glucose-6-phosphate dehydrogenase (prepared from yeast by Boehringer Mannheim or Sigma) to be invariably contaminated with a glucokinase-like activity. We, therefore, considered omitting this enzyme from the assay mixture since the activity in liver extracts greatly exceeds that of glucokinase. However, liver extracts also contain 6-phosphogluconate dehydrogenase which catalyzes the further production of NADPH from the 6-phosphogluconate formed in the glucose-6-phosphate dehydrogenase reaction. If for any reason the activity of either dehydrogenase should vary, uncertainty would be introduced into the stoichiometry between moles of NADPH formed and moles of glucose phosphorylated. The arbitrary choice of a value of 1.5 or 1.7 (15, 16) seemed to us unsatisfactory. A further obstacle was that whether the two auxiliary enzymes were omitted or added in excess, reaction rates were often found to accelerate with time, particularly when the quantity of liver extract was increased. This phenomenon was shown to be due, at least in part, to the fact that liver extracts are also rich in the remaining enzymes of the pentose phosphate pathway, with the result that ribulose 5-phosphate produced in the 6-phosphogluconate dehydrogenase reaction is cycled back to glucose 6-phosphate, which in turn generates more NADPH. The net result is that the rate of glucose phosphorylation may be seriously overestimated.

To avoid this problem the conventional procedure was modified so that glucose 6-phosphate production was linked to the reduction of NAD by glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (17). This enzyme utilizes either NAD or NADP as cosubstrate and was shown to be devoid of hexokinase or glucokinase activity. Assays were carried out at 37 °C in a Gilford 240 recording spectrophotometer. The reaction mixture contained in a final volume of 1 ml, 100 mM Tris-HCl, pH 7.4, 7.5 mM MgCl₂, 5 mM ATP, 1 mM NAD, 5.5 units of glucose-6-phosphate dehydrogenase (exogenous enzyme), liver extract (generally 10 μl), and the desired concentration of glucose. The production of NADH was followed at 340 nm. Appropriate controls were performed with the complete mixture in the absence of exogenous enzyme a slow reduction of NAD that was independent of glucose, glucose 6-phosphate, or 6-phosphogluconate was always seen and was subtracted from the rate observed with the complete mixture. In the presence of exogenous enzyme limiting quantities of glucose 6-phosphate yielded stoichiometric amounts of NADH. No response was observed with 6-phosphogluconate. Since each mole of glucose reacting with ATP generated only 1 mol of NADH, a reliable value for the total glucose phosphorylating capacity of the liver could be obtained.

**Materials**—Isotopic substrates were from Amersham Corp. or New England Nuclear. Animal diets were from Teklad Test Diets, Life Science Division, The Mogul Corp., Madison, WI. Glucose-6-phosphate dehydrogenase from *L. mesenteroides* was a product of Boehringer Mannheim. Other materials were of the highest purity commercially available.

**RESULTS**

Fig. 1A depicts the changes in portal vein glucose concentration with time during the various treatment regimens. In

![Fig. 1. Effects of glucose and lactate administration in fasted rats](http://www.jbc.org/)

**Fig. 1.** Effects of glucose and lactate administration in fasted rats. Fasted rats received glucose intragastrically (i.g.), glucose intravenously (i.v.) or sodium lactate intravenously at rates of 167, 334, and 57 mg/100 g body weight/h, respectively. A fourth group of animals was allowed to eat *ad libitum* a solid diet containing 40% by weight of glucose. *P.V.* refers to portal vein. Values are means ± S.E. for 4–10 animals in each group.
animals eating the 40% glucose diet portal glucose rose from its basal value of 5 mM to the region of 6–7 mM at 1 h and remained at this level over the entire 3-h period. Higher concentrations were achieved when glucose was given intragastrically or intravenously (8–9 mM between 1 and 2 h) but these fell to 7 mM during the 3rd hour. With intravenous lactate the glucose concentration changed little over the 1st hour but thereafter began to climb, reaching a value of 8 mM after 3 h. Fig. 1B shows the changes in portal vein lactate concentration. In animals eating the solid diet this rose sharply from a basal value of 2.7 to 8.7 mM at 1 h but fell to 5.3 mM after 3 h. Although the initial increase in lactate was less dramatic with intravenous glucose, concentrations of 6–7 mM were maintained between 1 and 3 h. Lower levels (3–4 mM) were seen in animals receiving glucose intragastrically or lactate intravenously.

The pattern of hepatic glycogen synthesis is shown in Fig. 1C. Similar rates, in the region of 10 mg per g of tissue per h, were observed in all protocols in which the animals received glucose. Lactate infusion supported little glycogen synthesis over the first hour, but did so at an accelerating rate thereafter in a manner that paralleled its effects on the portal glucose concentration. The similarity in the two profiles is reminiscent of the situation in vivo where lactate supported glycogen synthesis only in the presence of glucose (1–6).

To gain insight into the pathway (direct or indirect) by which exogenous glucose was converted into liver glycogen the infusion solution was labeled with [U-14C,3-3H]glucose. The rationale was that if glucose is taken up by the liver and converted directly into glycogen the H/C ratio of the latter should be the same as that in the administered glucose. Conversely, to the extent that glucose is first metabolized to lactate (or other intermediates) and the latter is used for hepatic glycogen synthesis, the H/C ratio in glycogen should be lower than that in the infused glucose (the 3-3H is lost from glucose during glycolysis). As seen from Table I, fasted rats receiving the doubly labeled glucose intragastrically again deposited liver glycogen at good rates. After 1 h

<table>
<thead>
<tr>
<th>Table I</th>
<th>Hepatic glycogen synthesis during intragastric infusion of [U-14C,3-3H]glucose into fasted rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>3, 2, 2 (5)</td>
</tr>
<tr>
<td>Portal plasma glucose (mM)</td>
<td>5.0 ± 0.8, 8.3, 7.8, 6.6 ± 0.4</td>
</tr>
<tr>
<td>Liver glycogen (mg/g wet weight)</td>
<td>0.73 ± 0.3, 8.6, 16.5, 22.1 ± 2.1</td>
</tr>
<tr>
<td>Relative H/C ratio a</td>
<td>104, 104</td>
</tr>
<tr>
<td>Arterial glycogen</td>
<td>100</td>
</tr>
<tr>
<td>Liver glycogen</td>
<td>36, 37, 36 ± 3.9</td>
</tr>
<tr>
<td>Relative specific activity b</td>
<td>92, 93</td>
</tr>
<tr>
<td>Arterial glycogen, 3H</td>
<td>89, 90</td>
</tr>
<tr>
<td>Arterial glycogen, 14C</td>
<td>100</td>
</tr>
<tr>
<td>Liver glycogen, 3H</td>
<td>6, 12, 12 ± 1.2</td>
</tr>
<tr>
<td>Liver glycogen, 14C</td>
<td>17, 33, 32 ± 1.7</td>
</tr>
</tbody>
</table>

a Expressed as (H/C ratio in isolated glucose)/(H/C ratio in infused glucose) × 100.

b Expressed as (specific activity of isolated glucose)/(specific activity of infused glucose) × 100.

c Expressed as glucose units.

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<table>
<thead>
<tr>
<th>Table II</th>
<th>Hepatic glycogen synthesis during intravenous infusion of [U-14C,3-3H]glucose into fasted rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Portal plasma glucose (mM)</td>
<td>10.2, 10.4, 7.7 ± 0.7</td>
</tr>
<tr>
<td>Liver glycogen (mg/g wet weight)</td>
<td>10.8, 17.0, 28.4 ± 1.1</td>
</tr>
<tr>
<td>Relative H/C ratio</td>
<td>50, 54, 55 ± 3.0</td>
</tr>
</tbody>
</table>

a As defined in Table I.

<table>
<thead>
<tr>
<th>Table III</th>
<th>Hepatic glycogen synthesis in fasted rats refed a solid diet containing [U-14C,3-3H]glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Portal plasma glucose (mM)</td>
<td>6.7 ± 0.6, 5.5 ± 0.3, 6.1 ± 0.4</td>
</tr>
<tr>
<td>Liver glycogen (mg/g wet weight)</td>
<td>8.1 ± 1.0, 19.3 ± 2.0, 31.3 ± 1.1</td>
</tr>
<tr>
<td>Relative H/C ratio</td>
<td>97 ± 1.0, 96 ± 1.0, 93 ± 3.0</td>
</tr>
<tr>
<td>Liver glycogen</td>
<td>69 ± 0.0, 61 ± 0.0, 65 ± 2.0</td>
</tr>
</tbody>
</table>

a As defined in Table I.

the H/C ratio and the absolute specific activities of the two isotopes in portal and arterial glucose were essentially equal to those of the infused glucose, indicating that by this time endogenous glucose production had stopped and that all of the circulating glucose was of exogenous origin. By contrast, over the entire 3-h period the H/C ratio of glycogen-glucose never exceeded 37% of that in the infused and the absolute specific activities of 3H and 14C were only 12 and 32%, respectively, of those in the administered glucose.

Qualitatively similar results were obtained when the [U-14C,3-3H]glucose was given intravenously at twice the intragastric rate (Table II) or as a component of the solid diet which the animals were allowed to eat ad libitum (Table III). Again, at a time when circulating glucose had achieved specific activities of 3H and 14C similar to those of the exogenous sugar there was a marked fall in the relative specific activity of both isotopes in liver glycogen (to the region of 28 and 50%, respectively), although in absolute terms the dilutions were less than those found in the experiments of Table I. The same was true of the H/C ratio.

Taken together, the data of Tables I–III indicated that regardless of the route by which the animals received exoge-
ous carbohydrate only 12–28% of the glycogen deposited in liver could have been formed from the direct incorporation of circulating glucose (because of the 72–88% drop in relative specific activity of glycogen-2H). These findings would be consistent with a sequence of events whereby glucose was first metabolized to the triose level (with loss of 3H and retention of 14C), the latter then traversing the gluconeogenic pathway in liver to provide the glucose 6-phosphate that is the proximate substrate for glycogen synthesis. The marked fall in the relative specific activity of glycogen-2C is almost certainly explained as follows: (a) [14C]pyruvate generated from the exogenous glucose would likely be diluted by unlabeled pyruvate derived from endogenous precursors (e.g., amino acids, glycerol); (b) the precursor pool of [14C]pyruvate must react with unlabeled HCO3- in the pyruvate carboxylase reaction to initiate the process of gluconeogenesis. The latter event, followed by rapid interconversion of oxaloacetate and fumarate (a symmetrical molecule) and the subsequent interconversion of oxaloacetate in the P-enolpyruvate carboxykinase reaction, would yield [14C]P-enolpyruvate of lower specific activity than the [14C]pyruvate from which it was derived. Additional dilution would be introduced to the extent that unlabeled acetyl-CoA enters the Krebs cycle (19). Stated in another way, even if lactate derived from the exogenous glucose were the sole source of C3 units for glycogen synthesis, the specific activity of 14C in glycogen-glucose would still be less than that in the infused glucose. Thus, the most reliable index of a pathway involving glucose → C3 unit → glycogen will be the extent to which the 2H specific activity of liver glycogen falls relative to that of the circulating glucose.

More direct evidence that carbon flow from the C3 level to glucose-6-phosphate in liver continued in the face of glucose loading and active glycogen deposition was obtained from the experiment described in Table IV. In this case an intravenous infusion of [U-14C]lactate was combined with an intragastric infusion of either water or unlabeled glucose. Over the first 2 h the rate of glycogen synthesis was low in animals receiving lactate alone (in keeping with the data of Fig. 1). The concurrent infusion of glucose not only increased the mass of glycogen deposited (from 4.0 to 13.1 mg/g wet weight) but also stimulated the incorporation of 14C into glycogen by 2.3-fold (from 2.0 to 5.5% of the administered isotope) such that the relative specific activity of glycogen-glucose was similar whether the [14C]lactate infusion was accompanied by water or unlabeled glucose (10.9 and 9.7, respectively). Thus, the glucose-induced increase in glycogen deposition must have been matched by a glucose-induced acceleration of [14C]lactate incorporation into glycogen. The stimulatory effect of unlabeled glucose on the conversion of labeled lactate into glycogen was less noticeable at the 3-h time point, probably because by this time the rise in the circulating glucose level in animals receiving lactate alone (Fig. 1) had itself begun to stimulate lactate conversion into glycogen. The higher relative specific activity of glucose-14C in the water versus glucose-infused animals at 3 h (16.9 versus 9.7%, respectively) supports this notion. Qualitatively similar results were obtained when [14C]alanine or [14C]glutamine replaced [14C]lactate in these experiments (data not shown).

Radziuk (7) and Shikama and Uii (9) have described the use of [14C]carbonate as a label to detect gluconeogenic activity. We performed experiments similar to theirs while maintaining our standard conditions of glucose infusion. As seen from Table V, in animals receiving tracer [14C]NaHCO3 intravenously and water intragastrically, the liver glycogen level remained low, as did its 14C content. As expected, the simultaneous administration of glucose elevated the blood glucose concentration and promoted brisk glycogen synthesis. Importantly, it also caused a profound stimulation of [14C]HCO3- incorporation into glycogen. Moreover, the specific activity of the glycogen formed was 3-fold higher in the presence of the unlabeled glucose load than in its absence. Taken together with the [14C]lactate experiments of Table IV, these results clearly point to a glucose-induced stimulation of carbon flow from the C3 level to glycogen, in keeping with previous reports (7–9).

The ability of hyperglycemia to suppress hepatic glucose output, a phenomenon clearly established in the dog and man (20, 21), could be inferred from the present experiments because of the similarity in specific activities of the exogenous and circulating glucose (Tables I–III). That this was in fact the case is shown more directly by the data in Fig. 2 and Table VI. In these experiments we measured the rate of endogenous glucose production (presumed to be mainly hepatic in origin) in fasted rats given water or glucose intragastrically. In animals receiving water only or glucose at a rate of 84 mg/100 g body weight/h both the plasma glucose concentration and its specific activity remained stable from the 120-min time point onwards (Fig. 2). Unfortunately, when glucose was infused at the standard intragastric rate of 167 mg/100 g body weight/h (as in the preceding experiments) a sustained steady state could not be achieved because the plasma concentration initially rose to a much higher level and subsequently declined (Fig. 2B). The same trend was seen as regards glucose specific activity (Fig. 2A). Nevertheless, during the 120- to 180-min interval steady state conditions were approximated and this time period was used for calculations. As seen from Table VI, endogenous glucose production in the basal state was 6.17 μmol/min/100 g body weight. This was suppressed by 70% when glucose was given at the lower rate and was essentially abolished with the standard glucose infusion.

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**Table IV**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Intragastric infusion</th>
<th>Liver glycogen</th>
<th>Administered 14C in glycogen (% of infused 14C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (3)</td>
<td>Water</td>
<td>4.0 ± 1.6</td>
<td>2.0 ± 0.8</td>
</tr>
<tr>
<td>3 (4)</td>
<td>Water</td>
<td>6.7 ± 0.5</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>2 (5)</td>
<td>Glucose</td>
<td>13.1 ± 1.6</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>3 (4)</td>
<td>Glucose</td>
<td>17.2 ± 1.4</td>
<td>4.7 ± 0.3</td>
</tr>
</tbody>
</table>

*Expressed as (specific activity of glycogen-glucose)/(2 x specific activity of infused [U-14C]lactate) x 100.

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1 In experiments not shown animals were infused with [1-14C] glucose via the femoral vein at a rate of 40 mg/100 g body weight/h and after 3 h the liver glycogen was purified and hydrolyzed to glucose. The latter was treated enzymatically to release carbon 1 as 14CO2. In the in situ glucose 95% of the 14C could be shown to be present in the C2 position, whereas in the glycogen-glucose this value fell to the region of 40%. Such marked randomization of label between the C1 and other carbon atoms of glycogen-glucose would also be consistent with the initial metabolism of exogenous glucose to the C2 level prior to its conversion into liver glycogen. These findings are in contrast to the studies of Hostetler and Landau (18) who reported that after the administration of [2-14C]glucose to fasted rats about 80% of the label was retained in the 2 position of glucose isolated from liver glycogen.

2 As seen from Table VI, endogenous glucose production in the basal state was 6.17 μmol/min/100 g body weight. This was suppressed by 70% when glucose was given at the lower rate and was essentially abolished with the standard glucose infusion.
Glucose (167 mg/100 g 120-180 mg/g tissue) is a precursor of glycogen, then its metabolism contributes to support a net rate of glycogen synthesis. It can be calculated that if glucose were the direct source of glycogen, the glucose phosphorylating capacity of rat liver homogenates would have to be at least 1.5 mmol/min/g tissue. However, the actual capacity was found to be much lower than this. A reasonable estimate, obtained by averaging the four values for fed and fasted livers measured at 5 and 10 mM glucose, would be in the region of 0.6 mmol/min/g of liver. The fact that much higher rates were seen at a glucose concentration of 100 mM was not surprising in view of the high $K_m$ of glucokinase for this substrate (16). The physiological significance of values obtained at glucose concentrations higher than those seen in vivo suggests that glucose is efficiently converted into glycogen.

From the data presented thus far it is evident that even in the face of exogenous glucose loads the generation of glucose 6-phosphate via the gluconeogenic pathway in liver must have remained active. The further conclusion seems warranted that under conditions of hyperglycemia (and hyperinsulinemia) this glucose 6-phosphate is diverted away from the glucose 6-phosphatase reaction and into the pathway of glycogen synthesis. If this were true, why was glucose itself not efficiently converted into glycogen? We suspected that the answer might lie in a limited ability of the liver to phosphorylate glucose at levels seen in portal blood in most of these experiments. It can be calculated that if glucose were the direct precursor of glycogen, then to support a net rate of glycogen synthesis of 10-15 mg/g of liver/h (as seen in the present and previous studies (6)) the minimum glucose phosphorylation capacity of the liver would have to be at least 1.5 mmol/min/g tissue at portal glucose concentrations of 6-8 mM. This is a minimal estimate since it neglects the flux of glucose 6-phosphate through pathways other than glycogen synthesis during the postprandial phase. As seen from Table VII the actual capacity was found to be much lower than this. A reasonable estimate, obtained by averaging the four values for fed and fasted livers measured at 5 and 10 mM glucose, would be in the region of 0.6 mmol/min/g of liver. The fact that much higher rates were seen at a glucose concentration of 100 mM was not surprising in view of the high $K_m$ of glucokinase for this substrate (16). The physiological significance of values obtained at glucose concentrations higher than those seen in vivo suggests that glucose is efficiently converted into glycogen.

### DISCUSSION

The central role of the mammalian liver in glucose homeostasis was firmly established by the classic studies of Claude Bernard over a century ago. From the later work of Soskin and colleagues (22) and numerous groups since, it has become universally accepted that the liver switches from an organ of net glucose output to one of net glucose uptake in response to a “threshold” concentration of portal venous glucose in the range of 5.5 mM. To place this concept into quantitative perspective, it was concluded as late as 1980 that following the ingestion of a 100-g glucose load in postabsorptive man some 60 g of the sugar is taken up directly by the liver for storage purposes (23).

Viewed against this background the recent demonstration by several laboratories that glucose, even in the presence of insulin, was a poor precursor for glycogen synthesis (or lipogenesis) by rat liver preparations in vitro (1–6) presents an intriguing problem and constitutes what has been termed by Katz and colleagues as the “glucose paradox” (24). One explanation might have been that the so-called “gastric factor,” postulated by DeFronzo et al. (21) to promote the efficient uptake of glucose by the liver in vivo, was missing in the in vivo situation.
vitro studies cited. While such a possibility could not be rigorously excluded, its attractiveness was diminished by the fact that neither we (6) nor others (25) were able to dem-}

strate the presence of such a factor in portal blood from glucose-fed animals. A second possibility was that in an

in vitro setting the liver suffers some serious biochemical im-

pairment in its capacity to convert glucose into glycogen. This

also seemed unlikely since other complex metabolic pathways, e.g., fatty acid oxidation, ketogenesis, fatty acid synthesis, and

gluconeogenesis, are as efficient as the perfused rat liver and isolated hepatocytes as they are in vitro (6, 26, 27). Moreover, good rates of glycogen synthesis could be achieved in vitro provided an appropriate substrate mixture was used. This required, in addition to glucose, a glucosogenic precurs-

or (such as fructose or lactate) and an amino acid (such as alanine or glutamine). Under these conditions it was the glucosogenic precursor, not glucose, that provided the bulk of the glycogen carbon (1–6). A third possibility, favored by us (6) and for which experimental support has recently begun to emerge (7–10), was that even in vivo the conversion of exogenous glucose into liver glycogen might occur largely via an indirect mechanism in which the gluconeogenic pathway plays an important role. We believe the present studies lend strong support to this concept and provide important quantit-}

ative information necessary to assess its validity.

By administering [U-14C,3-3H]glucose to fasted rats and measuring the specific activity of each isotope in the circulat-

ing glucose and in glycogen glucose three important observ-

ations were made. First, whether the glucose was given intra-

gastrically or intravenously liver glycogen was deposited at a linear rate of about 10 mg/g/h (approximately 1 pmol/min/g in terms of glucose units). Since 75–90% of this glycogen appears to have been derived from the gluconeogenic pathway and since there was probably some flow of glucose 6-phosphate through the pentose phosphate pathway (to support lipogenesis which undoubtedly came into play during the anabolic phase) it is evident that most of the carbon flowing from pyruvate to glucose in the basal state must have been diverted into glycogen as a result of glucose loading. The important point is that carbon flux from the C3 level to glucose 6-phosphate in liver seems not to have been diminished during the transition from the fasted to refed state. Rather, the key metabolic adjustment appears to have been in the fate of the glucose 6-

phosphate formed. The biochemical basis underlying this glucose-induced switch in metabolism of glucose 6-phosphate remains to be established. The possibility that glucose acts to “pull” glucose 6-phosphate into glycogen through activation of glycogen synthase, as suggested by Hers (29), constitutes one plausible mechanism. It is also possible that glucose loading somehow brings about the suppression of glucose-6-

phosphatase activity and thereby “pushes” glucose 6-phos-

phate into glycogen. Further studies are needed to clarify this central issue.

A nagging question throughout this work was why glucose itself is not efficiently converted directly into liver glycogen even in vivo. This was particularly troublesome since the reported capacity of rat liver to phosphorylate glucose (15, 16, 30, 31) would appear to be equal to or greater than the rate of glycogen synthesis (in glucose units) that we observed in vivo (1.0–1.5 μmol/min/g of liver). We now believe that these values for glucose phosphorylation are misleading. They were obtained using glucose concentrations of 100 mM, a level vastly in excess of that present in portal blood during glucose ingestion. Moreover, they were derived from the conventional spectrophotometric assay which, as noted, we found to have serious shortcomings. Using a more reliable assay we obtained a value in the region of 0.6 μmol/min/g of liver with glucose concentrations of 5–10 mM. Such rates of glucose phospho-

rylation, which are similar to those calculated by Katz et al. (32) from measurements of detritiation of [2-3H]glucose in hepatocytes from fasted rats, fall short of what would be needed if glucose was used directly to support the high rates
of hepatic glycogen synthesis and lipogenesis that come into play postprandially (minimally 2 µmol/min/g of liver). They would, however, be sufficient to account for the smaller quantities of glycogen that appear to have been derived directly from glucose in the present studies.

Nordlie and co-workers (33, 34) also concluded that the combined activities of hexokinase and glucokinase were insufficient to account for the measured rates of glucose uptake in the perfused rat liver. It was suggested that other glucose phosphorylating mechanisms, possibly certain phosphotransferase activities of glucose-6-phosphatase, function in liver for the efficient uptake of glucose. It should be noted, however, that compared with the situation in vivo the rates of glycogen deposition in these in vitro studies were very low, even in the presence of supraphysiological concentrations of glucose.

In conclusion, there is growing evidence that conventional views on the manner in which the body handles a glucose load require re-evaluation. Certainly, the widely held notion that the bulk of dietary glucose is taken up directly by the liver for storage purposes with concomitant suppression of carbon flow through the gluconeogenic pathway no longer seems tenable. The emerging concept of a glucose flow through the gluconeogenic pathway no longer seems tenable. The emerging concept of a glucose pathway in vivo, though circuitous in nature, is entirely consistent with many in vitro observations (1–6) and, incidentally, with the original formulation of the Cori cycle (35). A key question, yet to be answered, is where the initial metabolism of glucose takes place. Intestine and/or muscle would appear to be likely candidates. A third possibility would be the liver itself if the concept of metabolic zonation of the tissue into glycolytic and gluconeogenic regions (36) can be firmly established.

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