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FROM DIETARY GLUCOSE TO LIVER GLYCOGEN: The Full Circle Round

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CONTENTS

PERSPECTIVES AND SUMMARY 52
HISTORICAL BACKGROUND 53
Physiological Studies 53
Biochemical Studies 54
EMERGENCE OF THE GLUCOSE PARADOX 56
ATTEMPTS TO RESOLVE THE PARADOX 56
Experiments in Animals 57
Experiments in Humans 61
A Noninvasive Approach 62
UNRESOLVED PROBLEMS 63
Why Is Glucose Not Used Efficiently by the Liver? 63
Is Glucose-6-phosphatase Under Metabolic Control? 66
The Fructose-2,6-bisphosphate Paradox 66
Where Does the Glucose-to-Lactate Conversion Take Place? 67
Is There an Advantage to the Indirect Pathway? 68
CAN THE OLDER AND NEWER FINDINGS BE RECONCILED? 68
IN RETROSPECT 69

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PERSPECTIVES AND SUMMARY

The central role of the liver in fuel homeostasis stems largely from two unique properties of this organ. First, it contains all of the enzymes necessary for the synthesis and degradation of glucose, glycogen, and fat. Second, it can switch the direction of carbon flow over key pathways of carbohydrate and lipid metabolism in response to changes in hormonal and nutritional status. In starvation, the fall in circulating insulin levels (with concomitant elevation of the glucagon/insulin ratio) triggers catabolic events. The result is that two essential fuels, glucose and ketone bodies, are released from liver at accelerated rates to support the energy needs of the central nervous system and peripheral tissues. The underlying biochemical mechanisms are now fairly well understood and have been reviewed previously (45).

This article deals with the converse situation. At issue is the question of how liver metabolism reverts from catabolic to anabolic function when a fast is terminated. Until recently the answer was thought to be quite simple and ran along the following lines. Ingestion of carbohydrate after a fast increases the circulating concentrations of glucose and insulin (and, according to one school of thought, of an unidentified "gastric factor"). Such conditions were considered to promote the efficient uptake of glucose by the liver and its subsequent conversion into storage products: glycogen and fat. A corollary was that insufficient secretion of insulin (and/or gastric factor) compromises hepatic glucose uptake and contributes to the glucose intolerance of Type II diabetes.

Findings over the last decade, however, have forced a reevaluation of this widely held view. Available evidence now indicates that glucose is in fact a poor substrate for liver metabolism, regardless of nutritional state. It turns out that in quantitative terms three-carbon intermediates, rather than glucose itself, serve as the proximate precursors not only for fatty acid synthesis but also for glycogen formation in liver during the postprandial phase. Requirement for the putative gastric factor to stimulate hepatic glucose uptake is thus obviated. Insulin, though essential for the induction of anabolic processes in vivo, probably exerts its acute hepatic effects not in direct fashion (as in muscle or adipose tissue) but indirectly by suppressing the secretion of glucagon and counteracting its catabolic action.

In the sections that follow we outline some of the major historical developments in this area. The vast and sometimes conflicting literature involved, coupled with space limitations, dictates a selective approach. Accordingly, emphasis is placed on the emergence of the "glucose paradox" and recent attempts to resolve it.

HISTORICAL BACKGROUND

In the course of investigations into the nature of his newly discovered substance, "matière glycogéne," Claude Bernard came to consider the precursor(s) from which this compound might be derived. In 1877 he remarked, "It is not such a simple matter as it appears. The indisputable fact is that the administration of cane sugar considerably increases the liver glycogen content; but how does the sugar act in this case—as a 'nutritive stimulator,' or as a substance which is directly converted to glycogen? I am inclined to believe, I must confess, that the first suggestion is the more correct" (76). In the century following Bernard's work, the role of the liver in glucose homeostasis in general and the mechanism of hepatic glycogen synthesis in particular have been issues of intense interest to the physiologist and biochemist alike. Despite major advances by both groups and a wealth of accumulated knowledge, confusion and controversy still abound. A brief consideration of historical developments in the physiological and biochemical arenas will serve to illustrate the point.

Physiological Studies

REGULATION OF HEPATIC GLUCOSE BALANCE

Role of the blood glucose concentration In 1938 Soskin and coworkers published their classic paper on the hepatic regulation of the blood sugar concentration (68). They described the "homeostatic mechanism" of the liver as its ability to switch from an organ of net glucose output to one of net glucose uptake in response to a threshold concentration of the blood sugar. Their studies were performed on anesthetized dogs with catheters placed in the hepatic artery, portal vein, and vena cava. Net glucose output was observed in the fasted state. After a large intravenous bolus of glucose, net glucose uptake was seen, followed by a gradual return to a net output by 120 min. Glucose uptake was assumed to result in storage of the sugar as glycogen, although the latter was not measured directly.

Madison and colleagues in the 1960s (using the unstressed porta-caval shunted dog as their model) noted that glucose uptake directly correlated with the glucose load; i.e. the greater the amount of glucose infused the greater was the change in hepatic glucose balance from output to uptake during the infusion. A major fraction of the glucose taken up by the liver was found to be converted into glycogen (40, 41).

Role of insulin At the time of their studies in 1922 Banting, Best, and McLeod were aware that the pancreatectomized dog maintains minimal hepatic glycogen stores despite severe hyperglycemia. One of the earliest effects

of insulin observed by these workers was its promotion of glycogen deposition in liver. According to Soskin's view, the level of circulating insulin would influence the hepatic homeostatic mechanism by modifying the critical level of glycemia above which the liver takes up glucose and below which the organ releases the sugar. The issue was addressed in detail by Madison et al (40, 41), who showed that raising the arterial glucose concentration in diabetic dogs to extremely high values did not result in net hepatic glucose uptake. However, brief treatment of the animal with insulin shifted hepatic glucose balance to a net uptake.

Since the early 1960s numerous reports have documented the fact that in experimental animals insulin promotes glycogen synthesis and activates glycogen synthase in a variety of tissues, including liver. Although the mechanism of insulin's action was not established, these observations seemed to provide a ready explanation for failure of glucose uptake in the livers of diabetic animals: they simply lacked insulin.

Role of the "gas

relative importance of splanchnic and peripheral tissues in the disposal of ingested glucose in humans. They concluded that in the postabsorptive state the bulk of a 100-g oral glucose load was retained within the splanchnic bed (presumably liver) and that only a small fraction was available for utilization by peripheral tissues. Subsequently, using insulin and glucose clamp techniques the same group examined the effects of hyperinsulinemia, hyperglycemia, and the route of glucose administration on total glucose utilization and net splanchnic glucose exchange (15). The major conclusion was that orally consumed glucose caused the release of a gastrointestinal factor that enhances the insulin-mediated uptake of glucose by the liver. Parenthetically, lack of this factor was considered important in the pathogenesis of noninsulin-dependent diabetes mellitus (16).

Biochemical Studies

In 1929 Cori & Cori (12) proposed that glucose carbons could be cycled in mammals through the sequence: liver glycogen → blood glucose → muscle glycogen → blood lactic acid → liver glycogen. Subsequently, they demonstrated that injection of epinephrine into experimental animals resulted in a redistribution of glycogen stores from muscle to liver, presumably via lactate since muscle has a low capacity to generate free glucose (11). It thus became clear that liver glycogen could be synthesized from carbohydrate-derived sources other than glucose itself. The potential significance of this finding was soon overshadowed, however, by the establishment of the fundamental series of reactions involved in the direct conversion of glucose into glycogen: glucose → glucose-6P → glucose-1P → glycogen (71). [It was not until 1957

that Leloir (39) showed the proximate precursor of glycogen to be UDP-glucose, not glucose-1P.] When radioisotopes became available, an explosion of research in carbohydrate metabolism resulted. The fundamental question of whether liver glycogen is formed directly from intact glucose or indirectly from metabolic products of ingested glucose could now be addressed with techniques not available to the early physiologists.

In 1941 Hastings and coworkers administered [1-^{11}C]lactate to fasted rats and noted that although liver glycogen was formed at good rates it contained only 2% of the ^{11}C originally given (9). In similar studies they found that 13% of a labeled bicarbonate injection was incorporated into liver glycogen (74). It was suggested that the test compounds rapidly equilibrate with a "family" of precursors capable of being converted into glycogen.

Two years later Boxer & Stetten (4) approached the problem using a different and quite ingenious technique. They found that isotope from D_2O was incorporated into liver glycogen more efficiently in fasted than in fed rats during unlabeled glucose or lactate administration. Since little deuterium fixation would have been expected if glucose was converted directly[2] into glycogen, it was concluded that liver glycogen must have been derived from "fragments smaller than hexose" rather than from dietary glucose directly. Thus, it is clear that the concept of an indirect mechanism of glycogen synthesis was considered at a very early stage.

It appears, however, that the notion fell into disfavor during the ensuing decade. For example, in 1952 Cook & Lorber (10) administered [1-^{14}C]glucose to fasted rats, isolated liver glycogen, hydrolyzed it to glucose, and examined the isotopic content of its various carbon atoms. Carbon 1 was found to contain 80–90% of the label. The authors reasoned that this was consistent with direct conversion of glucose into liver glycogen (since initial metabolism to the three-carbon level would have resulted in extensive scrambling of the isotope among the other carbons of glycogen-glucose). The same kind of study was performed two years later by Hers (22) with similar results. He concluded that "glucose is built into the glycogen molecule essentially as an intact six carbon unit," a view that was echoed by other groups over the next fifteen years (25, 44, 47, 72).

By the late 1970s, therefore, the combined weight of physiological and biochemical evidence from in vivo studies was widely interpreted as follows. When a fast is terminated the absorption of dietary carbohydrate results in increased portal concentrations of glucose, insulin, and perhaps an unidentified gastric factor. In this setting glucose is efficiently taken up by the liver where it is in large part converted (directly) into glycogen. Though still

[2]The term "directly" refers to the reaction sequence: glucose → glucose-6P → glucose-1P → UDP-glucose → glycogen.

prevalent in current textbooks, this formulation ignored some of the early experiments that were inconsistent with it (see above and 30 and 31). The problem was soon to come into sharper focus.

EMERGENCE OF THE GLUCOSE PARADOX

The last decade has seen a resurgence of interest in the question of how the body handles a glucose load. What prompted this? The answer lies in a series of experiments carried out in the early 1970s with in vitro liver preparations. Key observations were made by Hems and coworkers using the perfused rat liver (21) and by Seglen working with isolated hepatocytes (63). In both systems net glycogen synthesis was minimal when glucose was present as the sole substrate at physiological levels (5–10 mM) and became significant only when the concentration was raised to 30 mM or above. However, good rates of glycogen formation could be obtained if the medium contained, in addition to glucose, gluconeogenic precursors such as lactate, fructose, or glycerol. Though not itself used efficiently, glucose promoted the conversion of gluconeogenic substrates into glycogen. These findings have been extensively confirmed and elaborated upon (5, 18a, 28, 29). Glucose was also shown to be a poor substrate for lipogenesis; here again lactate was far superior (31). Furthermore, with optimal substrate mixtures insulin was neither necessary nor stimulatory for glycogen or fat synthesis. By contrast, glucagon potently suppressed both processes, an effect that could be offset by insulin provided the concentration of the α-cell hormone did not exceed its physiological range.

Thus, glucose and insulin, the two dominant candidates for effecting the catabolic to anabolic switch of liver metabolism during refeeding after a fast, failed to act in this way in an in vitro setting. The ready conversion of glucose into glycogen in vivo and its limited incorporation in vitro has been termed the "glucose paradox" (30, 31).

ATTEMPTS TO RESOLVE THE PARADOX

Why was glucose not used efficiently for anabolic purposes in in vitro liver preparations? One argument might have been that under in vitro conditions liver suffers a serious impairment at some step(s) in the metabolic sequence leading from glucose to glycogen, and presumably also in the pathway from glucose to fatty acids. This seemed unlikely given the fact that both the perfused liver and isolated hepatocyte systems carried out other complex reaction sequences at in vivo rates, e.g. gluconeogenesis, fatty acid oxidation, ketogenesis, and even glycogen synthesis and lipogenesis when the appropriate substrate mixture was provided. A second possibility was that the in vitro

studies were not artifactually flawed but were actually telling us something very important, namely, that even in the intact organism the direct utilization of glucose by liver might be far less efficient than had been generally believed. Clarification of this central issue necessitated a return to the whole-animal model. It also required new experimental designs and reevaluation of some of those used in the past. The central question was this: to what extent is liver glycogen formed directly from dietary glucose or indirectly via the gluconeogenic pathway?

Experiments in Animals

STUDIES WITH ISOTOPES If glycogen deposited in liver after a [^{14}C]glucose load derives exclusively from circulating glucose by the direct pathway, its specific activity should equal that of the plasma glucose. The premise was tested in experiments by Moriwaki & Landau with rats (47) and by Baker with mice (2). Although the results were not definitive, in both series conditions were described in which the specific activity of newly formed glycogen was significantly less than that of the circulating glucose; this indicates that substrates other than the administered glucose contributed to hepatic glycogen synthesis. That the nonglucose precursors of glycogen were of gluconeogenic origin seemed a likely possibility.

The notion that even in the face of exogenous glucose loading continued carbon flow through the gluconeogenic pathway is important to the overall process of hepatic glycogen synthesis might at first sight seem contrary to common sense. Yet the principle has now been firmly established. In experiments by Shikama & Ui (64), and later by Newgard et al (50), fasted rats were given trace quantities of $NaH^{14}CO_3$ together with a load of unlabeled glucose. As expected, hepatic glycogen deposition was brisk. This glycogen was found to be heavily labeled; moreover, its specific activity exceeded that of the circulating glucose. The only reasonable interpretation of the data is that $^{14}CO_2$ was fixed at the pyruvate carboxylase reaction in liver, labeling oxaloacetate and subsequent intermediates in the gluconeogenic sequence. Prior to glucose administration the major product was [^{14}C]glucose, whereas after the glucose load a significant fraction of the intrahepatic [^{14}C]glucose-6-phosphate must have been channeled into the reactions of glycogen synthesis.

It has now been established that carbon flux over the gluconeogenic pathway not only contributes substantially to postprandial glycogen formation in the liver, but is in fact essential for the efficient operation of this process. The experimental approach, adopted independently by Sugden et al (70) and Newgard et al (51), was to administer a glucose load to fasted rats in the absence or presence of 3-mercaptopicolinic acid (3-MP), an inhibitor of the phosphoenolpyruvate carboxykinase (PEPCK) reaction, and to compare rates

of hepatic glycogen synthesis in the two groups. Despite similar levels of glycemia in control and 3-MP–treated animals, glycogen deposition in livers of the latter group was only a small fraction of that in the former. However, this was returned to control rates when, in addition to glucose plus 3-MP, the rats received an infusion of glycerol, which enters the gluconeogenic pathway at the triose phosphate level (distal to the 3-MP block).

Table 1, which summarizes our results, makes an additional point. When the administered glucose was labeled in carbon 1 with ^{14}C, the glucose isolated from liver glycogen of control rats contained only about 50% of its label in this position (most of the remainder was distributed equally among carbons 2, 5, and 6); by contrast, liver glycogen from 3-MP–treated animals retained most of its label in carbon 1, regardless of whether they received unlabeled glycerol along with the isotopic glucose. In the same experiments (51), muscle glycogen in all groups was labeled almost exclusively in carbon 1. Calculations suggested that of the glucose destined for conversion into liver glycogen, minimally two thirds traversed an indirect pathway involving initial metabolism of the hexose to the level of lactate. Muscle glycogen was formed via the direct pathway.[3]

Using similar reasoning, Shulman et al (65, 66) administered [1-^{13}C]-glucose to fasted rats, isolated liver glycogen over a three-hour period, and measured the distribution of ^{13}C in its glucose residues by the technique of nuclear magnetic resonance spectroscopy. Their conclusions were similar to those described above, i.e. a maximum of 27% of glycogen repletion occurred via the direct pathway, with most of the remainder contributed by compounds such as lactate, alanine, and glycerol.

Another approach used to estimate the relative contributions of direct versus indirect mechanisms to liver glycogen synthesis involved the infusion of [3-^{3}H,U-^{14}C]glucose into fasted rats. Operation of the direct route should result in no change in the specific activity of either isotope in glycogen relative to that in the circulating glucose. But to the extent that glucose first traverses the glycolytic sequence (regardless of site) prior to glycogen synthesis, ^{3}H will be lost at the triose level while ^{14}C will be retained (albeit variably diluted by endogenous gluconeogenic precursors). This creates a marked fall in the $^{3}H/^{14}C$ ratio of glycogen relative to that of blood glucose (taken as 1.0). The latter profile was found to predominate, the relative specific activity ratio of liver glycogen falling to the region of 0.4 to 0.5 (50). Subsequently, Scofield et al (62) questioned the appropriateness of the 3-^{3}H label for this

[3]Direct conversion of [1-^{14}C]glucose into glycogen results in no randomization of label. A pathway involving the sequence glucose → lactate → glucose-6P → glycogen will produce [3-^{14}C]lactate from [1-^{14}C]glucose. This lactate will generate phosphoenolpyruvate labeled equally in carbons 2 and 3 (because of equilibration between oxaloacetate, malate, and fumarate), which in turn will give rise to glycogen-glucose carrying ^{14}C mainly on carbons 1, 2, 5, and 6.

Table 1 Effect of mercaptopicolinate on liver glycogen synthesis[a]

Mercaptopicolinate	Intragastric infusion	Intravenous infusion	Liver glycogen mg/g	Liver glycogen %^{14}C in C-1
–	[1-^{14}C]Glucose	—	18.0 ± 2.2	54.6 ± 1.4
+	[1-^{14}C]Glucose	—	2.6 ± 0.7	91.4 ± 1.4
+	[1-^{14}C]Glucose	Glycerol	19.0 ± 1.7	88.8 ± 0.4

[a] Fasted rats received the indicated infusions for 2 hours. Data from Newgard et al (51).

type of experiment since in theory it can be lost through the operation of the pentose phosphate cycle without the intermediacy of triose phosphates or lactate. To circumvent the problem they used [6-^{3}H,U-^{14}C]glucose, which will lose ^{3}H en route to glycogen only if pyruvate is an intermediate. They reported a ^{3}H/^{14}C relative specific activity ratio in liver glycogen of about 0.8. However, we have obtained the same low ratio (approximately 0.5) in parallel experiments using both [3-^{3}H,U-^{14}C] and [6-^{3}H,U-^{14}C]glucose (30), which means the pentose cycle exerts minimal influence in the former case.

While each of the procedures outlined above pointed to a significant if not major contribution of the indirect pathway to hepatic glycogen synthesis, all suffered from a common drawback. This had to do with uncertainties surrounding the extent to which carbon-labeled gluconeogenic intermediates derived from the infused glucose were diluted by unlabeled endogenous precursors. This would have the effect of raising the glycogen ^{3}H/^{14}C ratio in experiments using [3-^{3}H,U-^{14}C] or [6-^{3}H,U-^{14}C]glucose, and increasing the isotopic content on carbon 1 relative to other carbon atoms of glycogen-glucose in experiments with [1-^{14}C] or [1-^{13}C]glucose. In all cases the net result would be an erroneously low estimate of the overall contribution of the indirect pathway.

To deal with this problem yet another strategy was devised. It was based on the use of $^{3}H_2O$, which had previously been employed by Rognstad et al (60) to examine glucose synthesis in isolated hepatocytes and by Postle & Bloxham (54) to measure absolute rates of hepatic glycogen formation in vivo. The rationale was as follows. In the presence of $^{3}H_2O$ the glucose-6-phosphate in liver, regardless of its source, becomes labeled with ^{3}H in carbon 2 because of the high activity of hexose-6-phosphate isomerase (equilibration between the hydrogen at this position and the protons of water is essentially complete). When glucose-6-phosphate is formed from pyruvate, tritium from $^{3}H_2O$ is also incorporated into carbons 1, 3, 4, 5, and 6 in such a manner that, at least in carbons 1, 5, and 6, its specific activity closely approximates that of the cell water. Thus, by isolating liver glycogen and measuring the positional distribution of ^{3}H among the carbon atoms of its glucose residues (positions 2 and 6 are convenient and sufficient for this purpose), the fraction derived from

pyruvate is given by the percentage of ^3H on carbon 6 divided by two times the percentage of ^3H on carbon 2 (the factor of two in the denominator is required because there are two hydrogens on carbon 6). In a sense the method is analogous to the use of ^3H_2O to measure absolute rates of lipogenesis since in neither case are the results influenced by changes in specific activity of isotopic carbon precursors.

These principles were applied to previously fasted rats and mice given ^3H_2O intravenously and allowed to eat a regular chow diet ad libitum (34). It was calculated that in both species about 75% of the glycogen deposited in liver over a 2–3-hour period was derived from pyruvate (Table 2). Even when the diet contained 58% sucrose, some 50% of liver glycogen was still formed from the level of pyruvate, with much of the remainder arising from triose-phosphates generated in liver from the fructose moiety of sucrose.[4] In other words, on both diets probably no more than 25% of liver glycogen came directly from glucose. The indirect pathway also predominated in glucose-infused animals and, predictably, was essentially abolished when 3-MP was used to block the PEPCK step.

BALANCE STUDIES Measurement of glucose balance across the liver is not a simple task, particularly in small animals, since it generally requires sampling of blood together with blood flow determinations at three sites (hepatic artery, portal vein, and hepatic vein). The problem is compounded if one wishes to calculate the percentage of the glucose load taken up when glucose is given

Table 2 Labeling of liver glycogen after administration of ^3H_2O[a]

Species	Refeeding regimen	Total ^3H (in %) in glycogen-glucose at C-2	C-6	Glycogen from pyruvate (in %)
Mouse	Chow diet	18.5	26.4	71.7
"	Sucrose diet	19.5	19.4	49.6
Rat	Chow diet	18.5	28.1	76.0
"	Sucrose diet	18.4	17.8	49.0
"	Glucose	21.0	26.0	61.9
"	Glucose + mercaptopicolinate	65.0	4.8	3.7

[a] Fasted rats and mice were injected with ^3H_2O and refed as indicated. Liver glycogen was analyzed after 3 hr (mice) or 70 min (rats). Data from Kuwajima et al (34) and McGarry et al (46).

[4]In liver the initial metabolism of fructose involves its conversion into fructose-1P followed by cleavage to dihydroxyacetone-P and glyceraldehyde. It thus traverses the triose stage before the formation of glucose-6P.

orally or intragastrically, as opposed to intravenously, since in the former case accurate rates for glucose entry into the blood are seldom known. It is not surprising therefore that conflicting results have been obtained. For example, as already noted, in the early work of Soskin et al (68) and Madison et al (40, 41) significant rates of hepatic glucose uptake were reported in dogs receiving large intravenous glucose loads. On the other hand, Landau et al (38) observed little or no net uptake of glucose by the liver in dogs maintained on a normal diet, although uptake was seen in animals conditioned to a diet rich (80%) in carbohydrate. More recently, Davis et al (14) showed that in dogs given one mixed meal per day there was no net hepatic uptake of glucose.[5] Similar conclusions can be drawn from the experiment of Rémésy et al (59) and Niewoehner et al (51a) in rats.

Thus, the bulk of the evidence would suggest that, at least in animals kept on normal diets, fasting followed by administration of carbohydrate in amounts giving rise to normal postprandial glucose levels results in only modest direct utilization of the sugar by liver.

Experiments in Humans

Attempts to elucidate the mechanism of hepatic glycogen repletion in humans have for obvious reasons been restricted both in number and scope. Nevertheless, the emerging picture points to a situation strikingly similar to that in lower mammals. Probably the most detailed studies have been those of Radziuk et al (55, 56, 58). By administering to postabsorptive man 100 g of [1-^{14}C]glucose orally together with a tracer infusion of [3-^{3}H]glucose intravenously, they were able to measure both the peripheral appearance rate of ingested glucose and total glucose entry into the blood. It was calculated that no more than 10% of the ingested load could have been taken up on its first pass through the liver (58), which implies that the position of the liver between the portal and peripheral circulation does not necessarily mean that it acts as a filter for newly absorbed glucose.

Although a first-pass uptake of 8–10% of ingested glucose would be compatible with a total uptake of 40–50 g over a 4-hr period, the data allowed no conclusions on what fraction might be converted into glycogen. This was addressed using a more sophisticated approach. Overnight-fasted volunteers ingested 100 g of glucose with simultaneous infusion of multiple isotopic substrates designed to monitor such parameters as glucose absorption, total glucose turnover, gluconeogenic carbon flow, and hepatic glycogen formation by the direct and indirect pathways. Liver glycogen was analyzed on the basis of the amount and labeling patterns of the glucose "flushed" into the

[5]Although net hepatic glucose uptake could be enhanced by intraportal delivery of the sugar (48), the concentration of glucose sensed by the liver under these circumstances was not reported.

blood after a stepped infusion of glucagon (55, 56). The data indicated that of the approximately 40 g of glycogen deposited in liver over a 4-hr period at most 10 g could have been formed directly from glucose. Of the remainder, a major fraction, if not all, was synthesized from gluconeogenic precursors.

These findings from isotope studies did not support the earlier notion that in humans the bulk of ingested glucose is efficiently taken up by the liver (presumably in large part for storage purposes) under the influence of insulin plus an unidentified gastric factor (see above). In this regard two points warrant emphasis. First, no information on the possible nature of the putative gastric factor has been forthcoming; indeed, its existence has been seriously questioned (3, 7, 51a). Second, experiments similar to those that led to the initial postulate (i.e. measurements of splanchnic glucose balance in humans undergoing hepatic vein catheterization) have since been repeated by L. D. Katz et al (33), but under more physiological conditions. This time a very different conclusion was reached. The data clearly indicated that in healthy volunteers given 92 g of glucose after an overnight fast, well over two thirds of the load escaped splanchnic removal. Peripheral tissues (primarily muscle), not the liver, appeared to play the dominant role in glucose disposal. Similar findings had been reported by Maehlum et al (42). Thus, in keeping with the suggestions by others (3, 7, 51a), the need for an unidentified factor to facilitate glucose uptake by the liver became redundant.

A Noninvasive Approach

Further exploration of the problem in man would be greatly facilitated if intrahepatic glucose metabolism could be monitored using noninvasive techniques. One such approach has recently been developed in the rat (19, 20) and used in preliminary fashion in man (43). It is based on the fact that in liver UDP-glucose is a precursor both of glycogen and UDP-glucuronic acid. The latter reacts with a wide range of compounds, including various xenobiotics such as the commonly used drugs acetaminophen and diflunisal, to form water-soluble glucuronides that are readily excreted in the urine. The procedure entails administration of the drug together with strategically labeled glucose, followed by isolation of the excreted glucuronide. The glucuronide is then reduced to form the glucoside, which in turn is hydrolyzed, with the release of free glucose. Positional isotopic analysis of this glucose should in principle define the label distribution in the glucose moiety of UDP-glucose feeding the glycogen synthetic pathway.

From this information insight into the relative contributions of direct versus indirect pathways to glycogen formation can be obtained as in the more conventional procedures where glycogen itself is isolated and analyzed. But one caveat must be expressed. The validity of the method hinges on the assumption that glycogen and the glucuronide are synthesized from the same

pool of UDP-glucose, or at least from separate pools that are in isotopic equilibrium. This has yet to be firmly established; indeed, the premise has been questioned (20). Also, the method will still be subject to the limitation of uncertainty surrounding the degree of dilution of labeled three-carbon precursors of UDP-glucose by endogenous substrates (see above). Nevertheless, the approach has exciting potential.

To summarize, views on whole-body glucose metabolism and the mechanism of liver glycogen repletion during the fasted-to-fed transition have shifted considerably in the past five years or so. Available evidence now indicates that in both experimental animals and in humans only a small fraction of dietary glucose is initially metabolized in the liver. As to the mechanism of hepatic glycogen repletion, there can be little doubt that the gluconeogenic pathway predominates, at least during the first few hours postprandially.[6] Almost certainly, glucose-derived lactate, rather than the hexose itself, serves as the proximate precursor of glycogen.[7] A substantial contribution will also be made by other components such as fructose and glycerol (via triose phosphates) and amino acids (via oxaloacetate), depending upon the dietary mix. The conventional and revised schemes are depicted in Figure 1.

UNRESOLVED PROBLEMS

Despite recent progress the glucose paradox cannot yet be considered solved in all aspects. Some outstanding problems are outlined below.

Why Is Glucose Not Used Efficiently by the Liver?

We suggested that the answer might lie, at least in part, in a limited capacity of liver to phosphorylate glucose at physiological concentrations of the hexose (50). There has, however, been disagreement concerning the most appropriate method for measuring the activity of glucokinase (the major enzyme for glucose phosphorylation) in liver homogenates (13, 36, 50, 69). The most commonly used procedure monitors the formation of NADH or NADPH spectrophotometrically when liver extracts are incubated with glucose, ATP, Mg^{2+}, NAD^+ or $NADP^+$, and glucose-6-phosphate dehydrogenase (NAD^+

[6]As liver glycogen stores approach repletion, the relative contribution of the direct pathway probably exceeds that of the indirect mechanism (20). However, at this stage the absolute rate of glycogen synthesis will be low compared with that immediately following refeeding.

[7]It follows that any dietary component capable of suppressing the conversion of lactate into glucose-6P in liver could interfere with hepatic glycogen repletion when a fast is terminated. This has recently been established in the case of ethanol (75), a finding of obvious interest in the field of nutrition.

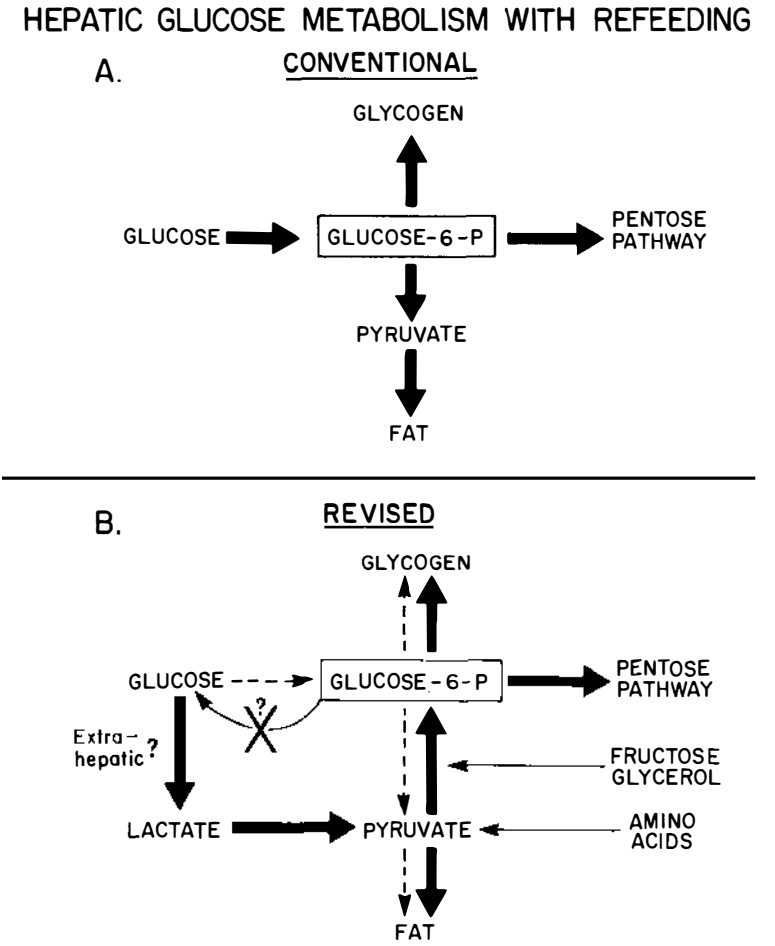

Figure 1 Schematic representation of hepatic glycogen and fat synthesis in the fasted-to-fed transition. *(Panel A)* Conventional view showing direct utilization of glucose for anabolic processes. *(Panel B)* Revised formulation emphasizing (*a*) the intermediacy of lactate on the pathway from glucose to glycogen (and fat); (*b*) continued traffic over the gluconeogenic reaction sequence in the face of glucose loading; and (*c*) the contribution of other nutrients to glycogen (and fat) synthesis. Question marks denote uncertainty about the site(s) of the glucose → lactate conversion and our speculation that glucose-6-phosphatase is suppressed in the postprandial phase.

or $NADP^+$ linked). Isotopic assays have also been described (36, 69). A potential problem with the spectrophotometric approach has to do with uncertainties surrounding the stoichiometry between glucose-6P and NAD(P)H generation, because of secondary reactions catalyzed by other enzymes in liver extracts (50). Also, as noted by Davidson & Arion (13), inclusion of KCl and dithiothreitol in the assay mixture appears to activate glucokinase.

Table 3 Glucose-phosphorylating capacity of liver[a]

Species	Dietary status	Glucose (mM)	Glucose phosphorylation (μmol/min/g)
Rat	Fed	5	0.95
		7	1.44
		10	1.88
Rat	Fasted 18 hr	5	0.86
		7	1.19
		10	1.55
Mouse	Fed	5	0.72
		7	1.10
		10	1.55
Mouse	Fasted 18 hr	5	0.72
		7	1.00
		10	1.49

[a] The high-speed supernatant fraction from liver homogenates was assayed for total glucose phosphorylation capacity (hexokinase plus glucokinase) at 37°C using a spectrophotometric assay described in (36) and modified in (37).

Recently, conditions have been established that provide reproducible values for glucokinase activity (identical whether NAD^+ or $NADP^+$ was used as electron acceptor) equal to those obtained using the procedure of Davidson & Arion (13) or tracer methods (P. A. Wals and J. Katz, unpublished). Similar findings by Kuwajima et al (37) are reported in Table 3. Although all values are somewhat higher than those recorded with the original assay (36), they still fall short of what would be needed to support hepatic glycogen synthesis directly from glucose in vivo. This would require a minimal rate of glucose phosphorylation (at a substrate concentration of 7–8 mM) of 2 and 3 μmol/min per g liver for rat and mouse, respectively (36). From Table 3 the average value for measurements at 5, 7, and 10 mM glucose turns out to be 1.3 and 1.1 μmol/min per g liver for rat and mouse, respectively. Even if these activities were applicable to the intact cell (which we consider unlikely), the rate of glucose phosphorylation would still not be equatable with the rate of net glucose uptake. The point is well illustrated by studies with hepatocytes incubated with [2-^{3}H]glucose in which substantial glucose phosphorylation could be demonstrated with little or no glycogen deposition or net uptake of glucose (8, 32). This was due to activity of glucose-6-phosphatase that cycled glucose-6P back to glucose. Such recycling is known to occur to some extent in vivo, even under conditions favoring glycogen deposition (50; see below).

On balance, we continue to feel that the glucose phosphorylating capacity of liver is insufficient to support glycogen synthesis directly from glucose.

Is Glucose-6-phosphatase Under Metabolic Control?

It is well established that in the fasted state glucose-6-phosphate formed from gluconeogenic precursors in liver has essentially only one fate, i.e. hydrolysis through the action of glucose-6-phosphatase with the release of free glucose to maintain euglycemia. Implicit in the formulation of Figure 1*b* is the assumption that even with an influx of exogenous glucose, carbon flow through the gluconeogenic pathway of liver must remain active, but in this case the glucose-6-phosphate formed is largely diverted away from the glucose-6-phosphatase reaction and into the sequence leading to glycogen formation.

What triggers this metabolic switch? One mechanism, postulated by Hers (23), might be that an increase in the blood glucose concentration brings about a series of events (described earlier) culminating in activation of glycogen synthase and a "pulling" of glucose-6-phosphate into glycogen formation. The resultant fall in glucose-6-phosphate concentration was considered sufficient to account for a diminished flux through glucose-6-phosphatase with concomitant suppression of hepatic glucose output (23). Observations made during the first hour of glucose infusion into fasted rats are consistent with this construct, i.e. the association of active glycogen synthesis with a fall in hepatic UDP-glucose and glucose-6-phosphate concentrations (49). However, in the second hour of those studies glucose-6-phosphate levels rebounded to pre-infusion values, yet carbon flux through glucose-6-phosphatase was suppressed by some 70–80%. UDP-glucose levels remained low. The data suggest that immediately after glucose infusion the "pull" mechanism predominated, but thereafter this was accompanied by inhibition of glucose-6-phosphatase, with the result that glucose-6-phosphate was both "pulled" and "pushed" into glycogen. The operation of the latter system had been predicted on the basis of computer modeling studies (17). Although the putative inhibitor has yet to be identified, it is attractive to speculate that it might operate at the level of the translocase responsible for transporting glucose-6-phosphate across the endoplasmic reticulum membrane to the site of the phosphohydrolase that resides on the lumenal side (1).

The Fructose-2,6-bisphosphate Paradox

An important new development in the regulation of hepatic glucose metabolism has been the discovery of fructose-2,6-bisphosphate (Fru-2,6-P_2) and its role as a potent stimulator of phosphofructokinase and an inhibitor of fructose-1,6-bisphosphatase (24, 52, 73). In keeping with these properties, the concentration of Fru-2,6-P_2 in liver is low in starvation and greatly elevated in the fully fed state. The liver Fru-2,6-P_2 concentration was found to remain at low basal levels for two to three hours after fasted rats were infused with glucose or allowed to refeed on a regular chow diet (8a, 35). Only later, when

glycogen stores were largely replete, did Fru-2,6-P_2 begin to climb toward its ad libitum fed level. This was consistent with active carbon flow from pyruvate to glycogen via fructose-1,6-bisphosphatase, an essential component of the indirect pathway, during the early refeeding period.

A different picture was seen, however, when the animals were given a diet rich in sucrose (34). In this case the hepatic Fru-2,6-P_2 content rose 20-fold to fed levels within one hour of refeeding, yet isotope studies showed that at this time glycogen was being formed primarily from three-carbon compounds, i.e. flux through fructose-1,6-bisphosphatase must have been active. No explanation for the conundrum has yet been found. One might be that in the sucrose-refed animals some other factor(s) overrode the inhibitory effect of Fru-2,6-P_2 on liver fructose-1,6-bisphosphatase, thus allowing unrestricted gluconeogenic carbon traffic. Alternatively, if Fru-2,6-P_2 were heterogeneously distributed throughout the liver lobule [in line with the "metabolic zonation" theory of liver metabolism favored by Jungermann et al (27)], it could be that cells rich in Fru-2,6-P_2 are active in glycolysis while a separate population of cells, presumably deficient in the regulator, are responsible for gluconeogenesis and glycogen synthesis. Against this scenario, however, is the recent finding that Fru-2,6-P_2 appears to be homogeneously distributed throughout the liver lobule (Y. M. Pulalin, P. A. Wals, and J. Katz, unpublished; see also below).

Where Does the Glucose-to-Lactate Conversion Take Place?

A second question mark in Figure 1*b* is assigned to the site of the initial conversion of glucose into lactate. Although muscle would seem a logical candidate, it has been reported (26, 57) that after glucose loading in humans there was little net output of lactate across forearm muscle; most of the lactate appeared to be of splanchnic origin. Yet to be established, however, is whether sampling across the forearm adequately reflects events occurring in the whole-body muscle mass. From studies in the pig, Radziuk calculated that about one third of the lactate taken up by liver during intraduodenal glucose administration is formed in the intestine (56); he speculated that the remainder might be contributed by red blood cells and the central nervous system.

The problem is compounded by studies in dogs by Davis et al (14), who reported that liver simultaneously produces glucose and lactate even after feeding. This led Pilkis et al (53) and Soley et al (67) to suggest that lactate formation and glycogen synthesis proceed simultaneously in different zones of the liver, a hypothesis in keeping with the metabolic zonation model of liver metabolism (27). The notion would be that glucose is metabolized to lactate in the perivenous cells (glycolytic); the lactate is then swept out of the liver and, after mixing with circulating lactate, is taken up by periportal cells (gluconeogenic) for conversion into glycogen. Though it has attractive fea-

tures, the hypothesis lacks direct experimental support and is not entirely consistent with other enzymatic and histological studies of glucose and glycogen metabolism in different regions of the liver (6, 61). Even more important, as noted earlier, balance studies have shown that uptake of glucose across the liver is much less than glycogen formation on a mass basis. Thus there is not enough glucose available to produce the requisite amount of lactate to account for the glycogen deposited.

Is There an Advantage to the Indirect Pathway?

Compared with the direct mechanism the indirect pathway for hepatic glycogen synthesis is circuitous and wasteful of energy. At present an obvious teleological explanation for why it should operate is not available. One possibility might be that after a fast it is more important for muscle than for liver to reestablish a carbohydrate economy and restore glycogen reserves in readiness for fight or flight. If so, it would seem prudent that liver not avidly extract incoming glucose, but rather allow the major fraction to pass through for use by the central nervous system, the muscle bed, and other peripheral tissues. Presumably peripheral metabolism of glucose produces lactate (possibly after saturation of the glycogen-synthesizing sequence), which, together with other gluconeogenic precursors, could then be readily cleared with high efficiency by the liver. Whether these precursors are released back to the blood as glucose, stored in the liver as glycogen, or converted into fat would then depend on the prevailing blood glucose and insulin concentrations, which in turn would be dictated by the magnitude of the glucose load. Regardless of its *raison d'être,* a better understanding of the dynamics of the indirect pathway is important in elucidating the pathophysiology of glucose-intolerant states.

CAN THE OLDER AND NEWER FINDINGS BE RECONCILED?

Although several areas of conflict between the earlier and more recent findings can now be discerned, the discrepancies are probably more apparent than real. For example, there is no doubt that in their pioneering studies Soskin et al and later Madison and colleagues (see above) observed substantial rates of hepatic glucose uptake in healthy dogs. Careful inspection of the data, however, reveals that glucose uptake sufficient to support good rates of glycogen deposition was achievable only at the cost of marked hyperglycemia. We now know that animals (and probably man) consuming mixed meals actively synthesize liver glycogen at a time when plasma glucose levels seldom exceed 7–8 mM and there is little or no net hepatic glucose uptake.

A similar explanation probably underlies the conclusion arrived at by Hers (after administering a bolus of [1-^{14}C]glucose to fasted rats and finding little randomization of label in liver glycogen) that the glucose → glycogen conversion occurs directly. Again, very high glucose loads were used, resulting in blood concentrations of >15 mM after 15–30 min. Under these conditions a major contribution of the direct pathway becomes evident, but only because glucose is now artificially "forced" through the sluggish glucokinase reaction and subsequently into glycogen (51). Moreover, as noted elsewhere (30), such bolus-type experiments underestimate the contribution of the indirect pathway because of the different kinetics of dilution and utilization of [^{14}C]glucose and [^{14}C]lactate derived from it.

Finally, the question must be asked as to why in the intact organism insulin appears to be essential for glycogen deposition in liver, whereas in an in vitro setting (given optimal substrate mixtures) it is not. Relevant here is the fact that acute, large, and reproducible metabolic effects of insulin, when present as the sole hormone, are readily demonstrated in tissues such as muscle and fat. This has never been the case for liver, where the most striking responses to insulin have been seen only against a backdrop of counterregulatory hormone action. For these reasons we favor the view that the essentiality of insulin for hepatic glycogen synthesis in vivo stems from three indirect actions of the β-cell hormone: first, insulin suppresses pancreatic glucagon secretion; second, insulin serves to offset the potent glycogenolytic effect of glucagon (and probably catecholamines) on the liver; and third, by stimulating glucose uptake in peripheral tissues insulin might be expected to facilitate the production of lactate and thus provide a major substrate for liver glycogen (and fat) synthesis. None of these functions would be necessary in isolated liver preparations.

IN RETROSPECT

We have attempted to summarize some of the constantly changing views on how liver glycogen stores are repleted when a fast is terminated. If, as the weight of evidence now indicates, the indirect pathway proves to be predominant then it seems that we will have come, in the words of the poet, the full circle round. This applies at two levels, metabolically and conceptually: metabolically, because the central component inherent in the revised formulation is none other than the Cori cycle, first postulated sixty years ago; conceptually, because the story began with an even earlier hint from Claude Bernard that blood glucose might not be used directly for liver glycogen synthesis. Decades later there emerged strong pointers to the involvement of "fragments" in the glucose → liver glycogen conversion, a notion that

subsequently gave way to the opposing and long-standing view of a direct pathway, only to be resurrected and placed on a more solid biochemical footing in the last five years or so.

Is there anything new under the sun?

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