Perspectives

The Glucose Paradox

Is Glucose a Substrate for Liver Metabolism?

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iver contains the full complement of enzymes for the synthesis as well as the catabolism of glucose, glycogen, and fat, and in many species, including the pig, chicken, and man, it is the predominant if not the sole site of triglyceride synthesis. Because the ingestion of a carbohydrate diet after a fast is followed promptly by replenishment of liver glycogen and activation of hepatic lipogenesis, it has become commonly accepted that glucose is a major hepatic substrate and serves as a direct¹ precursor for glycogen and fat. However, over the last decade, evidence has mounted that under physiological conditions glucose utilization by liver is rather limited and that it is in fact a poor precursor for glycogen and fatty acids (much inferior to gluconeogenic substrates such as fructose,² glycerol, or lactate). This finding, at first sight at variance with common sense and established views, has been designated the "glucose paradox" (Riesenfeld et al. [1]; Boyd et al. [2]). Our purpose here is to call attention to the problem and to present the case that longstanding concepts in this area are in need of reevaluation.

An exhaustive quotation of the voluminous literature in the field will not be attempted since much of this has already been covered in the excellent reviews of Stalmans (3) and Hers (4, 5). Rather, we have adopted a selective but, we believe, representative approach to the published work, both pro and con of our interpretations.

Hepatic utilization of glucose

The central role of the liver in glucose homeostasis has been recognized since the time of Claude Bernard, over 100 years ago. In the 1930's, Soskin and co-workers showed that when

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1. As used here, the term "direct" implies the uptake of glucose by the liver followed by a sequential series of reactions involving: glucose \rightarrow glucose-6-phosphate \rightarrow glucose-1-phosphate \rightarrow UDP-glucose \rightarrow glycogen, or glucose \rightarrow glucose-6-phosphate \rightarrow pyruvate \rightarrow fat. 2. In liver, fructose is metabolized by phosphorylation to fructose-1P, which is split into dihydroxyacetone-P and glyceraldehyde.

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© The American Society for Clinical Investigation, Inc. 0021-9738/84/12/1901/09 \$1.00 Volume 74, December 1984, 1901-1909 dog liver was perfused in situ with a large glucose load, it switched from glucose production to glucose uptake (see reference 6 for review). These and numerous subsequent studies formed the basis of the notion that liver regulates blood glucose levels by releasing glucose (through glycogenolysis or gluconeogenesis) in the postabsorptive and fasted states, and by removing glucose (through conversion into glycogen and other products) after a carbohydrate meal. This concept is prevalent in textbooks and recent reviews (3-5).

The published experimental data on rates of glucose uptake in vivo are ambiguous. In the studies of Soskin and other earlier investigators, significant glucose uptake was seen only at very high glucose loads and at unphysiological blood glucose levels; there was little if any response to insulin. A major difficulty in such experiments is the sampling of portal blood. Madison and co-workers (7) circumvented this obstacle by using dogs with portacaval shunts. These animals survive only when kept on a high carbohydrate, low protein diet. In a series of experiments, they demonstrated the cessation of glucose production at a plasma glucose level of 6.5 mM and an enhancement of glucose uptake with insulin. Landau et al. (8) reexamined this problem with dogs kept on a high carbohydrate (80%), low protein (16%) diet or on a "normal" diet (45% carbohydrate, 45% protein). Arterial, hepatic vein, and portal vein blood was sampled. They found that in dogs kept on "normal" diets, there was little or no uptake of glucose even with massive glucose infusions. The production of glucose ceased at arterial concentrations of 200 mg/100 ml and there was no effect of insulin. On the other hand, with dogs kept on a high carbohydrate diet, the threshold for the hepatic glucose balance was 120 mg/ml, and this was lowered with insulin (9). The only study in rats where portal blood was sampled was that by Rémésy et al. (10), who conditioned the animals to a diet containing 42 or 79% carbohydrate and measured hepatic substrate balances during food consumption after a 16-h fast. At the time of sampling, portal vein glucose concentration was 8.8 mM in the rats eating the 42% carbohydrate diet but net hepatic glucose output was still positive. Although animals consuming the high carbohydrate diet exhibited hepatic glucose uptake, this was at the expense of a much higher portal vein glucose concentration (13.6 mM). Absolute glucose balance could not be quantified because blood flow was not measured.

In the great majority of studies with perfused livers, there was little or no glucose uptake at substrate concentrations below 15 or 20 mM and no response to insulin. However, some reports indicated that when the perfusion media contained washed erythrocytes, glucose uptake could be demonstrated (11, 12). Recently, Storer et al. (13) showed that in a liver preparation perfused with undiluted defibrinated rat blood, there was a threshold for hepatic glucose at 6.2 mM and a large uptake of the sugar at 12 mM. This was increased some 20% with insulin, up to a utilization of the order of 1.3 μ mol/ min per g, and most of this was accounted for as glycogen. Dilution of blood to a hematocrit of 19% markedly depressed glucose uptake and abolished the insulin effect (14). These interesting observations require confirmation. Perfusion with whole blood is difficult due to hemoconstriction factors which have to be removed before perfusion (13, 14) and has rarely been used. In addition, measurement of glucose removal from the perfusate will overestimate direct hepatic uptake to the extent that erythrocyte glycolysis is operative.

In a variety of experiments with isolated hepatocytes, virtually no glucose utilization was observed at concentrations < 12 mM; significant rates were achieved only at levels of 30 mM or above, and in no instance did insulin have a significant effect (2, 15–18). This has also been the finding of the great majority of studies with the perfused liver preparation.³

Measurements of splanchnic glucose uptake after oral glucose ingestion in man are of limited value since they require assumptions on the rate of glucose entry into the blood and ignore intestinal glucose metabolism. Moreover, they have produced conflicting results, even from workers at the same institution. Thus, Felig et al. (21) concluded that in postabsorptive man the bulk of a 100 g oral glucose load is trapped in the splanchnic bed with only a minor fraction escaping for use by nonhepatic tissues. Yet, from the same type of experiment, Katz et al. (22) deduced that the initial metabolism of ingested glucose takes place primarily in skeletal muscle. The earlier claim that glucose, which is given intragastrically but not that delivered intravenously, is taken up efficiently by the liver (21) could not be confirmed.

Maehlum et al. (23) measured arteriovenous (splanchnic) glucose differences after an intravenous glucose infusion of 0.5 g/kg per h to fasted, resting and exercising volunteers. Despite arterial glucose levels of 12 mM, arterio-venous differences across the splanchnic bed were almost zero, indicating negligible hepatic uptake of the sugar. The most extensive study in man, using doubly labeled tracers, was that by Radziuk et al. (24). They found that a 96-g glucose load depressed endogenous hepatic glucose production by \sim 70%, but that at least 90% of

the absorbed glucose passed through the liver to appear in the systemic pool.

In summary, there is no question that in vivo a glucose load will depress and perhaps even block glucose output. Also, when blood glucose concentrations are experimentally raised to a range of 12–15 mM, the liver will respond with substantial glucose uptake. It should be remembered that free glucose is a rare dietary component, and even with high starch diets, portal glucose concentrations will seldom reach 12 mM. In rats and dogs, the threshold for glucose uptake is lowered to the physiological range when the animals are conditioned to a high glucose diet. The available evidence shows clearly that in humans on a Western-type diet, the uptake of glucose by the liver is quite low, and the administered glucose is metabolized predominantly in the periphery.

Glycogen synthesis

In most studies with isolated hepatocytes and perfused liver preparations, net glycogen synthesis was minimal when glucose was the sole substrate at concentrations < 12-15 mM and became significant only when glucose levels were raised to 30 mM or above. Working with liver slices in 1942, Hastings and Buchanan observed that media high in potassium stimulated the incorporation of [¹⁴C]glucose into glycogen (25). More recently, Hue et al. (16) found with isolated hepatocytes, which were incubated in a medium 140 mM in sodium and 5 mM in potassium, virtually no glycogen synthesis from 15 mM glucose and a rate of only 0.05-0.08 µmol/min per g from 50 mM glucose. When the sodium was completely replaced with potassium (140 mM), glycogen synthesis from glucose was increased fivefold. Even so, the maximal rate with 50 mM glucose (0.4 μ mol/min per g) was much less than rates obtained in vivo. The mechanism for the stimulation by extreme extracellular concentrations of potassium is obscure.

With rat hepatocytes, Hems et al. (26) and Seglen (15) found that efficient glycogen synthesis required the presence of glucose plus gluconeogenic precursors (fructose, glycerol, lactate, etc.). Katz et al. (18, 27) showed that efficient glycogen synthesis also required the presence of glutamine or alanine. These observations have been amply confirmed, as illustrated by the data from Boyd et al. (2) shown in Table I. In such a system glucose and glycogen are synthesized concurrently at the expense of the gluconeogenic precursor. The rate of glycogen deposition is similar to that attained in vivo. Exogenous glucose is essential for glycogen synthesis but it serves as an activator rather than a substrate. The effect of the amino acids is to divert the gluconeogenic flux of glucose-6-phosphate (glucose-6-P)⁴ from glucose production into the formation of UDP-glucose and glycogen. The mechanism of the amino acid effect is still unclear.

^{3.} In many studies the incorporation of ¹⁴C from glucose into glycogen has been taken as a measure of glycogen synthesis. Such incorporation frequently represents an exchange of ¹⁴C with unlabeled carbon and may occur in the absence of net glycogen deposition as a result of futile cycling between glucose, glucose-6P, and glycogen (19, 20).

^{4.} Abbreviations used in this paper: F-2,6-P₂, fructose-2,6-bisphosphate; F-2,6-P₂ase, fructose-2,6-bisphosphatase; glucose-6P, glucose-6-phosphate; glucose-6Pase, glucose-6-phosphatase.

Table I. Glycogen Synthesis in Hepatocytes from Fasted Rats

Substrate	Net glycogen deposition
	mg/g cells after 2 h
Glucose (20 mM)	2.1
Fructose (5 mM)	6.7
Lactate-Pyruvate (10-1 mM)	0
Glutamine (10 mM)	0.4
Glucose; Fructose	10.8
Glucose; Fructose; Glutamine	15.6
Glucose; Lactate-Pyruvate	7.5
Glucose; Lactate-Pyruvate; Glutamine	13.5

Hepatocytes from 20-h fasted rats were incubated with the indicated substrates (after Boyd et al. [2]).

In vivo also, gluconeogenic compounds are better precursors of glycogen than is glucose. It has been repeatedly observed that rats fed fructose (a gluconeogenic precursor²) deposit more glycogen in liver than when fed glucose. This was also seen in humans in a rare study where liver and muscle biopsies were taken from volunteers (28). As shown in Table II, after glucose infusion, plasma glucose rose to 310 mg/100 ml, and 13 mg/g of glycogen was deposited in liver. The infusion of the same amount of fructose increased blood glucose only from 90 to 110 mg/ml, but hepatic glycogen deposition was now 45 mg/g.

More direct evidence that under most conditions in vivo the major sources of liver glycogen are three carbon compounds, even in the presence of a glucose load, comes from studies with isotopic substrates. Moriwaki and Landau (29) found that 2 h after the intragastric administration of 360 mg/100 g body weight of $[U-^{14}C]$ glucose to fasted rats, the specific activity of liver glycogen-glucose was about half that of the circulating glucose. When glycogen deposition was stimulated with cortisol, the specific activity of glycogen was still only 60% that of

Table II. Glycogen Synthesis After Infusionof Glucose or Fructose into Humans

		Maximum blood glucose	Liver glycogen			Muscle glycogen	
Infusion	n		Before	After	Δ	Before	After
		mg/100 ml	mg/g			mg/g	
Glucose	6	310	46	59	13	15	18
Fructose	5	110	43	88	45	15	19

Volunteers were fasted overnight and infused intravenously for 4 h with ~ 1 g/kg per h of either glucose or fructose. Liver and muscle (quadriceps) biopsies were taken at the start and the end of the experiment. Initial blood glucose was 90 mg/100 ml (after Nilsson and Hultman [28]).

circulating glucose. When 360 mg of glucose and 180 mg of glycerol were administered together, these authors estimated that <20% of the carbon of glycogen was derived from glucose.

That the gluconeogenic pathway itself is important for glycogen synthesis in vivo was clearly shown by the studies of Shikama and Ui (30). NaH¹⁴CO₃ was injected intraperitoneally into fasted rats and an unlabeled glucose load was given intravenously. CO₂ is introduced into the carboxyl carbon of phosphoenolpyruvate (through carboxylation of pyruvate and subsequent reversible interconversion of oxaloacetate and fumarate) so that ¹⁴C-incorporation into glycogen becomes a measure of de novo synthesis from three carbon precursors. As shown in Table III, the glucose load increased greatly the incorporation of ¹⁴CO₂ into glycogen, lowered the specific activity of circulating glucose, but increased markedly that of glycogen, indicating that the latter was derived mainly from small precursors. Similar observations were made by Newgard et al. (31).

To obtain a quantitative assessment of the directness versus indirectness of hepatic glycogen synthesis during glucose loading of rats, Newgard et al. (31) administered [3-³H, U-¹⁴C]glucose to fasted animals at a rate sufficient to suppress endogenous glucose production. In the direct conversion of glucose into glycogen, ³H and ¹⁴C would appear in glycogen glucose without loss of specific activity relative to that in circulating glucose. But to the extent that glucose first traversed the glycolytic sequence (at whatever site) prior to glycogen synthesis, ³H would be lost at the triose phosphate level, whereas ¹⁴C could still enter glycogen from labeled three carbon precursors. As shown in Table IV, when the doubly labeled glucose was given intragastrically, the specific activity of ³H and ¹⁴C in newly synthesized glycogen was only 12 and 33%, respectively, of that in the blood glucose. Thus, only a small fraction of glycogen carbon could have been derived directly from glucose. Similar results were obtained when glucose was infused intravenously or consumed by the rats in their food.

Radziuk (32), using a similar experimental approach in man, obtained much the same results. After feeding 96 g of labeled glucose to fasting volunteers, he calculated that at most

Table III. Specific Activity of Glucose and Glycogen after Injection of NaH¹⁴CO₃

Injection	Blood glucose		Liver glyco	ogen
	Level	Sp act	Level	Sp act
	mg/100 ml	cpm/mg	mg/g	cpm/mg
Saline	69	9,200	1.9	4,400
Glucose	155	3,000	4.2	7,100

Rats were injected intraperitoneally with NaH¹⁴CO₃ and intravenously with either saline or 100 mg/100 g body weight of glucose. The experiment was terminated 30 min after intravenous injection (after Shikama and Ui [30]).

Table IV. Glycogen Synthesis after Intragastric Administration of Glucose

Portal plasma glucose	7.8 mM
Liver glycogen	16.5 mg/g
Relative specific activity	
Arterial glucose- ³ H	100
Arterial glucose-14C	100
Liver glycogen- ³ H	12
Liver glycogen- ¹⁴ C	33

20-h starved rats were infused intragastrically with 167 mg/100 g body weight per h of $[3^{-3}H, U^{-14}C]$ glucose for 2 h. Relative specific activity is expressed as a percent of that in the infused glucose. Initially, portal glucose was 5 mM and liver glycogen 0.7 mg/g (after Newgard et al. [31]).

one third of the liver glycogen deposited could have come directly from circulating glucose and that at least two thirds had a gluconeogenic origin.

The lack of randomization of ¹⁴C in glycogen after the injection of specifically labeled [14C]glucose has been previously taken as evidence that glucose is directly converted into glycogen without prior cleavage. Hers (33) injected rats with [1-14C]glucose and [1-14C]fructose and isolated liver glycogen. Glycogen formed from fructose had 50% of the ¹⁴C in C-1 and 30% in C-6. With glucose, 85% of the ¹⁴C in glycogen was in C-1 and very little in C-6. These observations have been repeatedly confirmed. However, the conclusion that glucose is predominantly converted to glycogen without cleavage is not warranted. If the glycogen was formed in part directly from glucose and in part after glucose cleavage to pyruvate, the ¹⁴Cyield in glycogen would depend on the relative specific activities of these precursors. After a single injection, the specific activity of glucose is initially high but decays rapidly, and that of lactate attains only a low value. Thus, a short period after injection, the incorporation of ¹⁴C by direct conversion of glucose will much exceed that from other labeled precursors, and most of the ¹⁴C will be incorporated in the early period (minutes) after injection. Also, the administration of large boluses of glucose is attended by severe hyperglycemia which will serve to force glucose through the sluggish glucokinase reaction in liver, and thus, favor the direct pathway of glycogen synthesis (see below). A valid analysis requires conditions of physiological concentrations and constant specific activities of glucose and lactate, as are approximated with a continuous infusion of [14C]glucose. Indeed, we found that when a glucose load labeled either in C-1 or C-6 was infused for 2 or 3 h, the isolated glycogen had 27% of its activity in the "opposite" end of the glucose molecule compared with that of the infused material (Table V). Randomization in circulating glucose at that time was only \sim 10%. A minimal value for the contribution of the nonglucose precursors to glycogen carbon may be obtained from the specific activity of glycogen which was 40-

Table V. Analyses of Liver Glycogen, Plasma Glucose, and Plasma Lactate after Infusion of [1-¹⁴C]- or [6-¹⁴C]Glucose into Fasted Rats

¹⁴ C on glucose	C-1	C-6
Liver glycogen (mg/g)	16.3±1.7	23.8±1.9
Relative specific activity*:		
Plasma glucose	0.75±0.03	0.83±0.04
Plasma lactate	0.31±0.02	0.30±0.02
Liver glycogen	0.43±0.02	0.42±0.03
Randomization [‡] in:		
Plasma glucose	0.095±0.01	0.11±0.02
Liver glycogen	0.27±0.01	0.27±0.02

20-h fasted rats were infused with glucose (labeled as indicated) at a rate of 167 mg/100 g body weight per h for 2 h. Values are means±SEM for three to six animals. (Data from unpublished studies by Newgard, Golden, Foster, McGarry, and Katz).

* Expressed as counts per minute per micromoles with the specific activity of infused glucose set at 1.00.

 \ddagger Expressed as C-4, C-5, C-6/(C-1–C-6), or C-1, C-2, C-3/(C-1–C-6) in experiments with [1-1⁴C] and [6-1⁴C]glucose, respectively.

50% of that of circulating glucose. However, since the precursors are also labeled, this is an underestimate. For a reliable calculation, determination of the specific activity of phosphoenolpyruvate is required. Assuming that the specific activity of phosphoenolpyruvate is one half that of plasma lactate, and taking into account the randomization in glucose, calculations to be presented elsewhere indicate that at least 70% of the glycogen arises from three carbon precursors, in spite of the infusion of a large glucose load. It should be stressed that the ¹⁴C-data apply to the relative incorporation of carbon into glycogen rather than net conversion of glucose into glycogen. In the experiments of Table V, there was still hepatic output of glucose at 20-25% of the basal rate. ¹⁴C-incorporation in the absence of net uptake of glucose or in the presence of substantial glucose breakdown has been frequently observed in vivo and in vitro. Irrespective of the ¹⁴C-balance, this means that glucose, as far as hepatic balance is concerned, does not contribute net carbon to liver constituents. The ¹⁴C-incorporation may be formally interpreted as an exchange of labeled for unlabeled carbon.³ Alternatively, glucose uptake and production proceed in different cell populations. We are concerned here with the physiological function of liver as a whole in glucose balance.

The studies described above establish that, contrary to widespread belief, continued carbon flow through the gluconeogenic reactions of liver plays an important role in hepatic glycogen synthesis during the fasted-to-fed transition. The fact that this process is essential for efficient glycogen synthesis postprandially has recently been convincingly demonstrated by independent studies from two laboratories (Sugden et al. [34] and Newgard et al. [35]). In both cases, fasted rats were treated with 3-mercaptopicolinic acid (an inhibitor of phosphoenolpyruvate carboxykinase) prior to the administration of glucose. This resulted in a reduction in liver glycogen deposition of 80–90% despite similar elevation of the blood glucose concentration in control and experimental groups. Moreover, when the infused glucose was labeled with ¹⁴C in the 1 position, the liver glycogen formed in the absence of 3-MPA showed extensive label randomization, whereas little randomization was seen in the small amount of glycogen formed in the presence of the inhibitor (indicating that when gluconeogenesis was blocked only the relatively inefficient, direct pathway for hepatic glycogen synthesis was operative [35]).

In summary, there is overwhelming evidence that in rats raised on "mixed" or sucrose diets, the capacity for hepatic glucose utilization is limited and glycogen deposition in liver is predominantly a gluconeogenic process. The administration of glucose stimulates glycogen deposition, but the effect is mainly indirect with the products of glucose cleavage in the periphery acting as the major proximate precursors of glycogen. In rats and dogs conditioned on high glucose or starch diets where the capacity for hepatic glucose utilization appears to be increased, glucose might become an important source of liver glycogen. Available evidence in humans, at least those on a mixed Western diet, shows a limited capacity for hepatic glucose utilization and that the major sources of liver glycogen after a glucose meal are its cleavage products. There are no data from human populations consuming predominantly starch diets.

Hepatic lipogenesis

Using rat hepatocytes, Clark et al. (36) noted that lactate was greatly superior to glucose as a precursor for fatty acids. As illustrated in Table VI, 2 mM lactate, alone or in the presence of glucose, was a much better precursor than was 10 mM glucose. A preferential use of lactate over glucose for lipogenesis has also been shown in perfused rat liver by Brunengraber et al. (37) and Salmon et al. (38). The findings in hepatocytes have been amply confirmed (Boyd et al. [2]).

Hems et al. (26) compared in rats the incorporation of

Table VI. Incorporation of Glucose andLactate into Lipid by Rat Hepatocytes

		¹⁴ C recovered in					³ HOH in	
Sub- strate		Glucose	CO₂	Fatty acids	Lipid glycerol	Fatty acids	Lipid glycerol	
	тM			µatom C	or H/g per h	!		
Lactate	2	116	60	9.4	3.8	34	20	
Glucose	10		40	1.4	4.6	24	15	
Lactate and	2	42	⁸²] ₁₂₄	⁶⁰ 61	9.6	64	20	
glucose	10	-	42	1∫.	4.2	04	20	

Rats were meal fed a high carbohydrate diet. Lactate and glucose were labeled uniformly with 14 C (after Clark et al. [36]).

[¹⁴C]glucose and ³HOH into liver and depot fat over a 24-h period. (In adipose tissue, glucose is the preferred substrate for lipogenesis.) They found that even after a meal most of the liver fat was derived from nonglucose precursors. Baker et al. (39) came to the same conclusion. These authors fed mice [¹⁴C]glucose and injected them with ³HOH. Feeding promptly stimulated hepatic lipogenesis but, as shown in Table VII, under most conditions the fatty acids were derived from nonglucose was administered to fed mice were most of the fatty acids derived directly from this substrate.

Rats refed a high carbohydrate diet after a fast exhibit a very high liver glycogen content and very efficient hepatic lipogenesis. Clark et al. (36) have shown that in hepatocytes from these rats, glycogen is the major source of lipid. It is likely that refeeding after a fast leads first to replenishment of the glycogen stores which then serve as a source of pyruvate and acetyl-coenzyme A for lipogenesis. The results of Boyd et al. (2) support this sequence in hepatocytes.

Thus, the evidence in rodents is clearcut; glucose administration acts as a trigger for lipid synthesis, but the hexose serves only indirectly (via lactate or glycogen) as a precursor for this process in liver. There are no relevant experiments with humans.

There is no "glucose paradox" with Japanese quail

The studies reviewed above were with rodents, dogs, and humans; very little relevant information is available for other animal species. The exception is our study with a bird, the Japanese quail, which is strikingly different in that this species uses glucose as a major precursor for hepatic glycogen and lipid synthesis. The Japanese quail (*Coutournix coutournix japonica*) is a small (200 g) domestic bird. Plasma glucose is 15–20 mM and the in vivo rate of hepatic lipogenesis is very high, especially during egglaying (Riesenfeld et al. [1] and references therein). Isolated quail hepatocytes, when incubated with glucose as sole substrate, utilize the sugar at a moderate rate, but compared with lactate its incorporation into fatty

Table VII. Contribution of Glucose and Nonglucose Carbon to Fatty Acid Synthesis in Mouse Liver In Vivo

Dietary state	Glucose intubated	Plasma glucose	Micromoles of fatty acid per minute per mouse from		
			Glucose carbon	Nonglucose carbon	Total
-		mg/ml			
Fasted		1.1	0.3	2.5	2.8
Fed		2.2	10.0	73.0	83.0
Fasted	250 mg*	5.5	18.0	53.0	71.0
Fed	250 mg*	3.2	220.0	110.0	330.0

* Given by stomach tube as 50% glucose, equivalent to 12 g/kg body weight (after Baker et al. [39]).

Labeled substrate	Alanine	Δ		¹⁴ C in			
		Glucose	Lactate	CO2	Glucose	Lipid	³ H from ³ HOH in lipid
	тM	µmol/g/h		µatom C or	H/g/h		
[U-14C]Glucose	none	-8	_	34	_	8	54
	2.5	-54	_	104	_	64	188
	10.0	-80		120	_	186	242
[U-14C]Lactate	none	+84	-282	330	324	94	94
	10.0	+16	-232	320	44	198	272

Table VIII. Effect of Alanine on Glucose and Lactate Metabolism in Hepatocytes from Japanese Quail

-, uptake; +, production. Cells were from mature egglaying birds. The concentration of glucose was 10 mM and of lactate 20 mM. Some 80-90% of the added alanine was taken up by the cells (after Katz et al. [40]).

acids is limited (1, 40). However, when supplemented with several amino acids, glucose uptake, its incorporation into glycogen, and most noticeably, its incorporation into fatty acids increases many fold. In the presence of alanine, glucose is equal or superior to lactate as a precursor of glycogen and fatty acids, as illustrated in Table VIII. In the preparation of these hepatocytes, there is an almost complete loss of glutamine and alanine from the cells. The latter is taken up very rapidly from the medium, with replenishment of near normal amino acid content of the tissue. Thus, to date the quail hepatocyte is the only liver cell system found in which under physiological conditions glucose uptake is high and where this substrate is an efficient source of glycogen and fatty acids.

Enzymes of glucose phosphorylation and uptake

The liver content of hexokinase is very low, and the major enzyme for glucose phosphorylation is glucokinase (for review, see reference 41). In the rat, the enzyme is unaffected by fasting periods up to 20 h (31), but is depressed by prolonged starvation (48-72 h) and in diabetes (41); it is elevated only to a limited extent by a high carbohydrate diet. The K_m for glucose was reported to be $\sim 10 \text{ mM}$ (41), but a more recent value, obtained with a highly purified rat liver enzyme, was \sim 5 mM (42). Maximal activity with cell extracts measured at 100 mM glucose is 2-3 µmol/min per g, but at physiological concentrations of substrate it is much less, 0.35 and 0.6 μ mol/ min per g for fed rats at 5 and 10 mM glucose, respectively. and also, considerably less than the rate of glycogen deposition seen in vivo (31). Moreover, rates of glucose phosphorylation as measured by enzyme assays in liver homogenates do not correspond to rates of glucose uptake in isolated hepatocytes. In the latter, much of the glucose-6P is hydrolyzed back to glucose, due to the action of glucose-6-phosphatase (glucose-6Pase). The phosphorylation of glucose in intact cells can be measured by the loss of tritium from position 2 of glucose (20). As illustrated in Table IX, the uptake of glucose at concentrations below 20 mM was only a small fraction of the rate of phosphorylation. Futile cycling can also be extensive in vivo (19).

Glucose uptake by hepatocytes and perfused liver becomes substantial at unphysiologically high substrate levels (2 μ mol/ min per g at 50 mM) and it shows no saturation even at 80 mM (43). The K_m (or $K_{0.5}$, half maximal rate) appears to be ~30-40 mM (44) rather than the 5 mM calculated for the purified enzyme. The results support the existence of another form of glucose phosphorylating enzyme in liver with a very high $K_{0.5}$. Nordlie (45) suggested that this function might be subserved by the microsomal glucose-6Pase with pyrophosphate or carbamyl phosphate acting as donors of the high energy phosphate. So far, definite proof that such a mechanism operates physiologically is lacking.

To conclude, the limited capacity for hepatic glucose utilization in the rat, and probably also in man,⁵ is probably due to the low level of glucokinase combined with variable rates of futile cycling caused by the activity of glucose-6Pase.

Regulation of hepatic glucose-6P metabolism

If during the fasted-to-fed transition the major fraction of liver glucose-6P is gluconeogenic in origin, how is this centrally located metabolite diverted away from free glucose formation and into the pathway of glycogen synthesis? A widely accepted theory is that proposed by Hers (4). Its essential features are that glucose loading causes activation of glycogen synthesis secondary to inhibition of glycogen phosphorylase. This is expected to "pull" UDP-glucose and glucose-6P into glycogen. The predicted fall in glucose-6P concentration is considered sufficient to attenuate glucose-6P flux through the glucose-6Pase step since hepatic glucose-6P levels (generally in the region of 0.1 mM) are far below the K_m of glucose-6Pase for this substrate (2–3 mM). An opposing view, based primarily on theoretical grounds, is that with glucose loading glucose-6P is "pushed" into glycogen as a result of inhibition of glucose-

^{5.} In a survey of different mammalian species for hepatic glucokinase activity, Lauris and Cahill (46) stated that the activity of this enzyme "was from extremely low to absent in the toadfish, guinea pig, cat and man." To our knowledge this interesting observation, made almost twenty years ago, has never been followed up.

Table IX. Glucose Phosphorylation and Glucose-6-Pase in Rat Hepatocytes

Diet	Lactate	Change in glucose*	Glucose phos- phorylation	Glucose 6-Pase
•••••		µmol/h/g wet w	veight	
	_	-24	106	82
Meal fed	+	+22	102	124
Forder 4	-	+6	32	38
Fasted	+	+34	26	60
Fed	-	+4	7.8	12
diabetic	+	+46	4.0	50

-, uptake; +, production.

* Glucose uptake was measured by analysis of the medium; initially [glucose] was 15 mM, [lactate] was 209 mM. Glucose phosphorylation was determined by the ³HOH yield from [2-³H]glucose corrected for incomplete detrition. Glucose-6-Pase was obtained by difference (after Katz et al. [20]).

6Pase (47). More recent studies suggest that both "pull" and "push" mechanisms might in fact be operative. Niewoehner et al. (48) administered glucose by gavage to fasted rats in the amount of 4 g/kg body weight. This huge load increased portal glucose concentration to 15 mM; hepatic glycogen synthesis was rapid and the tissue UDP-glucose level was halved. However, the concentration of glucose-6P increased from 0.095 to 0.15 μ mol/g liver at 20 min. Newgard et al. (49) infused glucose intragastrically to fasted rats for 3 h at a rate of 167 mg/100 g body weight per h. Both UDP-glucose and glucose-6P levels fell at 1 h. Although the former remained low, the latter rebounded to basal levels by 2 h, at which time glycogen synthesis was brisk and carbon flow through glucose-6Pase was markedly suppressed.

These observations suggest that, in addition to causing activation of glycogen synthase, glucose loading results in inhibition (or deactivation) of glucose-6P hydrolysis. A similar phenomenon might have been at work in the hepatocyte studies of Katz et al. (20) and Okajima et al. (50) where amino acids and mercaptopicolinate were shown to divert gluconeogenically derived glucose-6P away from glucose and into glycogen formation concomitant with rising levels of the hexose phosphate. From the work of Arion and co-workers (51), it appears that the phosphohydrolase itself is a nonspecific enzyme that resides within the lumen of the endoplasmic reticulum. Specificity for glucose-6P is conferred by the presence in the membrane of a translocase that transports glucose-6P from the extra- to the intramicrosomal compartment. Our bias is that the translocase rather than the phosphohydrolase itself is subject to metabolic regulation. A search for potential regulators seems worthwhile.

Role of fructose-2,6-bisphosphate $(F-2,6-P_2)$ in the fastingto-fed transition of liver metabolism

The newly discovered regulatory molecule, F-2,6-P₂, has added a new and important dimension to our understanding of the control of the glycolytic-gluconeogenic sequences in liver (for review, see reference 52). Its concentration is low in the fasted state and increases dramatically with refeeding, consistent with its established role as an activator of phosphofructokinase-1 and inhibitor of fructose-1,6-bisphosphatase (F-1,6-P₂ase). Moreover, its concentration in hepatocytes from fasted rats rises many fold within minutes after exposure of the cells to elevated levels of glucose and insulin (53-57). In the context of the present discussion, the latter observations presented another curious paradox. If hepatic F-2,6-P₂ levels increase acutely in vivo with refeeding of fasted animals, how could glucose-6P continue to be generated so efficiently from three carbon precursors (since F-1,6-P₂ase should now be shut down)? Studies by Kuwajima et al. (58) promise to resolve this metabolic dilemma. Fasted rats were given a continuous intragastric infusion of glucose or were allowed to refeed on a regular chow diet ad lib. In both cases, despite acute elevation of the blood glucose (and presumably insulin) concentration, hepatic F-2.6-P₂ levels remained low for the first 3-4 h. They began to rise towards fed values only after liver glycogen stores had been largely repleted (58). Similar observations were made by Claus et al. (59). Thus, a theoretical obstacle to active gluconeogenic carbon flow into glycogen for several hours into the postprandial period was removed. The interesting possibility is raised that the late rise in hepatic F-2,6-P₂ levels serves to prevent excessive glycogen deposition from gluconeogenic precursors so that the latter, together with glycolytically derived pyruvate, are shunted into the pathway of lipogenesis (58). The precise biochemical mechanisms underlying this temporal sequence of events with refeeding and the basis for the in vitro/in vivo discrepancy remain to be elucidated.

Role of insulin

As noted earlier, when isolated hepatocytes from fasted animals were provided with appropriate substrate mixtures, insulin was neither necessary for nor stimulatory to the process of glycogen (or fat) synthesis. Yet, ever since the classic studies of Madison and co-workers, (60) it has been recognized that in the intact animal insulin is essential for these anabolic events to occur. How can these apparently conflicting observations be reconciled? Although a simple answer has yet to emerge, it seems likely that the essentiality of insulin for the catabolic-toanabolic transition of liver metabolism in vivo stems, at least in part, from its ability to suppress the secretion of glucagon and to antagonize the catabolic actions of the α -cell hormone (and other counterregulatory hormones). Such a role for insulin in liver is to be contrasted with its well-known ability to promote glucose uptake and metabolism in muscle and fat tissue when present as the sole hormone. It is also fully in accord with the early studies of Exton and Park (61) on the opposing roles of insulin and glucagon in the control of hepatic cyclic AMP levels and with the bihormonal hypothesis for the regulation of glucose homeostasis (62) and ketone body production (63).

Conclusion

Caution is required in extrapolating from observations made in vitro to metabolic and regulatory events operative in vivo. In the former situation, normal metabolic effectors might be lost or subtle damage to the cell membrane might alter cellular behavior and response to hormones. However, most of the in vitro studies cited above are consistent with the experimental observations made with intact animals.

When taken together, the data presented support the concept that in most conditions only with excessive glucose loads, which are rarely encountered by rat or man, would glucose be taken up efficiently in direct manner by liver. Rather, it appears that in the immediate postprandial phase, dietary carbohydrate is converted into liver glycogen and fat largely via an indirect mechanism involving the sequence: glucose $\rightarrow C_3$ unit \rightarrow glycogen and lipids. Although the nature of the three carbon intermediate has not been firmly identified, lactate (and possibly alanine) would seem to be a likely candidate since it is produced during glucose absorption from the gut (Boyd et al. [2]; Nicholls et al. [64]) and by muscle and erythrocytes.⁶ Implicit in this formulation is that carbon flow from the C₃ level to glucose-6P must remain active for at least several hours after carbohydrate ingestion, but that the glucose-6P formed is now diverted away from the glucose-6Pase reaction and into the pathway of glycogen synthesis. The mechanism of this crucial metabolic switch remains to be delineated. One factor is probably a glucose-induced activation of glycogen synthase. An additional possibility, and one that we find intuitively attractive, is that glucose loading somehow leads to inhibition (or deactivation) of the glucose-6Pase system, which would direct glucose-6P carbon away from glucose and into glycogen formation. In any event, clarification of the nature of this permissive glucose effect, predictable from the early work of Soskin and colleagues, will fill a longstanding void in our understanding of glucose homeostasis.

The observations we have cited were made predominantly with rodents, dogs, and man on standard diets. Care is required in generalizing to other animal species and to varied environmental and dietary conditions. In dogs and rats, prolonged conditioning to a high carbohydrate diet alters the metabolic patterns. Studies with human populations with a dietary regimen differing from that of Western man would be of interest.

Finally, it should be emphasized that we do not challenge the fact that in the intact organism a major fraction of dietary glucose is ultimately converted into liver glycogen and fat. What we question is whether, in quantitative terms, the ingested glucose is the immediate precursor of these storage materials and whether liver is the primary site of glucose disposal. As to these issues, which undoubtedly will be interpreted by many as a recent departure from accepted dogma, we might do well to recall that the notion of an indirect pathway from glucose to liver glycogen was expressed as long ago as 1944 by Boxer and Stetton (68) but subsequently fell into disfavor. Even more thought provoking are the words of Claude Bernard written over a century ago, some 20 years after his discovery of liver glycogen. The question under discussion was: What is the source of liver glycogen when fasted animals are refed? His response was as follows: "I will carefully refrain from making a definite decision on such a fundamental question. 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." (Claude Bernard, "Lecons sur le Diabète," Paris, 1877).

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^{6.} While studies in the rat would be consistent with this interpretation (Table V), the situation in other species is less clear. For example, Cherrington and co-workers (65) have recently concluded that dogs consuming one mixed meal per day exhibit no net uptake of glucose by the liver, yet display a marked postprandial hyperlactatemia that is hepatic in origin. They postulated that in this model postprandial hepatic glycogen synthesis must derive from sources other than circulating lactate and glucose. Also to be noted are the studies by Jackson et al. (66) and Radziuk and Inculet (67) in which substrate balance across the human forearm showed a net uptake rather than a net output of lactate in response to oral glucose loading. Clearly, further work is needed to elucidate the nature and sources of liver glycogen precursors in the postprandial period.

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