

The Concentration of Glucose in Mammalian Liver

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ABSTRACT Data on barium-zinc filtrates of liver homogenates and of boiled liver indicate that the free glucose content of liver exceeds the blood glucose level. For instance, for boiled liver, the glucose level is 10.1, compared with a blood level of 5.4 mm/kg. Method of preparation of the tissue is important for the interpretation of the final results, as has been shown in appropriate control experiments. Various methods including paper chromatography were used to show that the reducing substance in liver is glucose. The relation of the high glucose content of liver to hexokinase activity, phosphate activity, and to glucose transport between liver cell and blood is discussed.

INTRODUCTION

In previous experiments, an increase of osmotic activity was observed in homogenates of liver standing at 0°C., or at room temperature (1). Glucose was suspected as a solute which could contribute to the increase, and in fact, data (2) indicated that reducing substances could account for as much as one-third of the increase of total solute in liver homogenate, provided that reducing activity was due to glucose, or to a compound of similar molecular weight.

During such studies, an important problem arose. Data suggested that the *in vivo* concentration of *glucose* in liver cells could be over twice that of perfusing blood. This was because the concentration of *reducing substance* in frozen (3) or in boiled liver, processed rapidly after excision, was approximately twice that of blood.

The purposes of the present report were: (1) to determine how much of the reducing activity in filtrates of liver was due to glucose; and (2) to estimate, if possible, the magnitude and direction of a gradient of concentration

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of free glucose between parenchymal liver cell and concurrently sampled blood.

If the "free" or "true" glucose concentration of liver were really greater than that of blood, one would be forced to draw unusual inferences concerning transport of glucose into the liver cell, and concerning reactions of glucose, once it is within the liver cell. Such considerations led to an examination of data of other investigators who had attempted to determine the glucose content of the liver.

While levels of liver glucose greater than those of blood were reported by some (4-6), levels equal to or less than those of blood were reported by others (7, 8). Such apparent discrepancies could have been due to differences in methods of preparation of tissue prior to actual analyses for reducing activity.

Conditions under which high values of liver glucose were found were: (a) boiling plus repeated extractions in boiling water of freshly excised, tared pieces of liver from dogs, rabbits, and guinea pigs (4, 5) and (b) 24 hour extraction in "uranium solution" of liver slices from rabbits and guinea pigs (6). On the other hand, conditions under which low values were found were: (a) immersion of small pieces of liver (80 to 200 mg.) in liquid air followed by extraction of tissues in ice cold alcohol or in ice cold $ZnSO_4-NaOH$ for an unstated period of time (7); and (b) immersion of rat liver in liquid oxygen, followed by homogenization at $0^\circ C.$ in $Ba(OH)_2$. After 20 minutes at room temperature, the Ba-deproteinized tissues were centrifuged, and the supernatant fluids were removed for analyses (8).

The aforementioned procedures raised questions concerning: (a) the degree of attainment of diffusion equilibrium between glucose in tissue and in ambient fluid at a given temperature and within a given interval of time; and (b) the extent of catabolic formation of glucose in the intact center of a piece of liver, the surface of which is coagulated.

The plan of the experiments reported herein was as follows: (1) methods of preparation of tissue; (2) effect of temperature and time of standing on the concentration of reducing substance in barium-zinc filtrates of liver; (3) identification of the reducing material; and (4) estimate of the glucose concentration of liver prepared under various specified conditions.

Methods of Preparation of Tissue

A. Large pieces of liver (20 to 50 gm.), quickly removed from nembutalized dogs, were immersed immediately into liquid nitrogen. Frozen tissue was crushed in a Carver press, and 0.4 to 0.6 gm. of undiluted homogenate was added (within 1 minute) to tared tubes containing 2 cc. of 0.3 N $Ba(OH)_2$. Then 2 cc. of 5 per cent $ZnSO_4$ was added, and the total volume was increased to 15 cc. with water. Such depro-

teinizing mixtures remained at room temperature for 2 to 4 hours after which they were centrifuged. Supernatant fluids were removed for determination of reducing activity.¹

B. Large pieces of liver, freshly excised, were pulverized in a mortar filled with liquid nitrogen. Aliquots of frozen tissue powder (0.4 to 0.6 gm.) were added to tared tubes containing 2 cc. of 0.3 N Ba(OH)₂. Deproteinization was performed in the manner described under section A.

C. Small pieces of liver (0.1 to 0.2 gm. each) were excised without hemostasis from nembutalized dogs, and transferred within 2 seconds, one by one, directly from the dog to hot water (98°C.) in a manner described previously (9). Beakers, each containing 5 to 10 pieces of liver (total tissue weight 0.5 to 1.0 gm.) and 10 ml. of water, were kept at 98°C. for 30 to 60 minutes. Next, beakers were cooled, and their contents transferred quantitatively to glass homogenizers. Homogenized fluids were weighed and divided into two aliquots: one aliquot of known weight was deproteinized with Ba-Zn solution (as in A and B), and filtrates obtained were analyzed for reducing activity; the second aliquot of known weight was placed in an oven at 105°C. for 24 hours to obtain dry weight of tissue in the sample. Original wet weight of tissue added to boiling water was calculated from dry weight of the boiled, homogenized material, together with dry weights of four separate aliquots of the same tissue removed at the same time (as the boiled aliquots) from the same dog. These data plus those on reducing activity were used to estimate the concentration of reducing substance per unit weight of tissue or of tissue water. Each observation on reducing activity was the average value of six replicate determinations.

Analytical procedures performed on filtrates of tissues were: determination of reducing substance, or "glucose" by the Nelson method (10); determination of fructose by the resorcinol method (11); determination of fermentable reducing activity by obtaining the difference of reducing activity in filtrates before and after addition of washed bakers' yeast.

Additional procedures for identification of the reducing material were: ascending paper chromatography with ethyl acetate-pyridine-water (2:1:2 by volume) as solvent (12), and with ammoniacal silver nitrate as spray. Paper chromatography was used for both qualitative and quantitative purposes. A supplementary qualitative test was performed on eluates of chromatographic washings with glucose oxidase paper (clinistix).

RESULTS

Effect of Time of Extraction and of Temperature

In order to test the effect of time of extraction on the content of reducing substance in filtrates, homogenates of liver were treated as follows: weighed aliquots of homogenate (0.5 to 1.0 gm), immediately after recovery from the

¹ Henceforth, such deproteinizing mixtures will be referred to as Ba-Zn solutions.

Carver press, were added to deproteinizing solutions in the fashion described under Methods. At specified intervals of time (20 to 250 minutes), after exposure to deproteinizing solutions, aliquots of supernatants were removed for analysis.

Fig. 1 presents a plot of values of reducing substances in a set of liver filtrates from the same homogenate *versus* time. Zero time was defined as that of removal of homogenate from the Carver press; *i.e.*, the time just prior to immersion of tissue in deproteinizing solution. Reducing activity was 13.6 mM/kg. of whole liver after 22 minutes in the Ba-Zn solution, and and this rose to a level of 17.1 mM/kg. after 90 minutes. Apparent diffusion equilibrium between reducing substances in tissue and in ambient solution

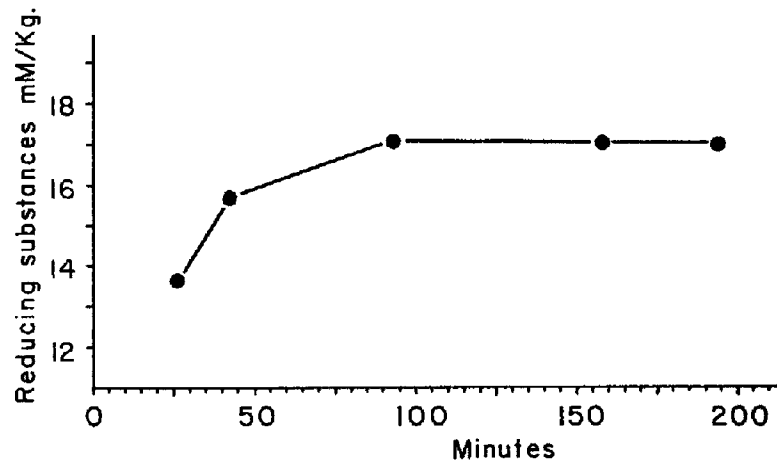


FIGURE 1. Reducing substance in liver homogenate *versus* time of extraction. Zero time defined as time of removal of homogenate from Carver press.

had been attained after 90 minutes, as judged by the constant level of reducing activity maintained between 90 and 200 minutes. Thus, the amount of reducing substance detected in filtrates of liver depends upon the time interval of exposure of tissue to a Ba-Zn solution. Diffusion of glucose from tissue to ambient solution, and/or glycogenolytic processes within the liver could have caused a pattern of concentration *versus* time, such as is depicted in Fig. 1.

Homogenates of liver, quickly removed from the Carver press, were immersed in hot (98°C.) Ba-Zn solutions. High temperatures were maintained for 4 minutes by immersing the tube containing hot Ba-Zn solution plus tissue into a boiling water bath. The assumption implicit in this experiment was that enzymatically catalyzed reactions of glycogen and of other cellular metabolites would cease abruptly at 98°C. After gradually cooling to room temperature, the deproteinization mixtures were allowed to stand for 20 to 200 minutes. Tubes were then centrifuged, and supernatant fluids withdrawn

for analysis of reducing activity. The plot of values of reducing substances *versus* time was practically identical to that of Fig. 1, and consequently it is not presented herein. This means that increases of reducing activity in both heated and non-heated filtrates of liver must have been due to diffusion of glucose into ambient solution, and not to degradation of glycogen.

The average glycogen content of three dog livers prepared under our conditions was 3 per cent or 164 mm/kg. (expressed as glucose), while fermentable reducing activity was 10 mm/kg. Extent of glycogen breakdown and of appearance of fermentable reducing substance was observed after 4 hours and after 24 hours at room temperature. After 4 hours, glycogen content had decreased to 48 mm/kg., while fermentable reducing activity had increased to 51 mm/kg. Hence, additional glucose accounted for 36 per cent of the glycogen breakdown. After 24 hours, glycogen content was 10, while glucose content was 116 mm/kg., whence the 24 hour increase of glucose was 70 per cent of observed disappearance of glycogen.

This suggests that increases of liver glucose of 3 to 5 mm/kg. (as seen in Fig. 1) would not be associated with a detectable decrease of glycogen content. However, if glycogenolysis continued for 20 to 200 minutes in the center of the tissue mass in Ba-Zn solution, one would observe a continuing increase of reducing activity rather than a levelling after 90 minutes (Fig. 1).

However, boiling in Ba-Zn solution or in water would stop glycogen breakdown in a few seconds. It follows that fermentable reducing substance, measured in suddenly heated tissues, could not have originated from glycogen breakdown.

A technique employing extraction of tissues in alcohol or in Ba-Zn at 0°C. (7) would provide conditions for slow diffusion of glucose, as well as for continued catabolism of glycogen with formation of reducing substances—mainly glucose. Hence, one would expect to find a low value of liver glucose under such conditions—even though some glycogen breakdown had occurred. The procedure of deproteinization, after only 20 minutes of immersion of liver in Ba(OH)₂ at room temperature (8), would not permit sufficient time for diffusion equilibrium to be reached (see Fig. 1).

Aforementioned considerations account for discrepancies in reported values of the “glucose” content of the liver. Our data are in accord with those of Palmer (4), Cori *et al.* (5), and others (6), all of which suggest that the concentration of glucose in liver is greater than that in blood.

Effect of Time Interval between Excision and Immersion in Hot Water

The time interval between excision of each bit of liver and its immersion into the boiling water was *ca.* 2 seconds. The mass of liver (0.1 gm.) was such as to induce achievement of thermal equilibrium between tissue and water within a few seconds or less. Glycogen breakdown in an intact piece of liver between time of excision and time of achievement of thermal equilibrium (at 98°C.)

was assumed to be negligible. This was checked in three experiments. Freshly excised bits of liver were held in the forceps for intervals of 2 to 10 seconds prior to immersion in the boiling water. After boiling as described herein, glucose determinations were performed on deproteinized homogenates of the boiled tissue. Results in a representative experiment were as follows: Values of glucose in millimoles per kilo of whole liver were 6.92 for the 2 second interval; 7.13 for the 5 second interval and 8.81 for the 8 second interval. The plot of such concentration *versus* time intervals yielded a curve with concavity facing upward. Consequently, we assumed that the concentration value extrapolated to zero time would not be measurably different in millimoles per kilo from 6.92, the 2 second value.

Identification of Reducing Substance in Liver

Methods for identification of the reducing substance in liver filtrates were: (a) fermentation; (b) reducing activity of various hexose phosphates before and after treatment with Ba-Zn solution; (c) ascending paper chromatography for qualitative identification of hexoses or pentoses; (d) elution of chromatographic paper for quantitative recovery of reducing activity in the R_f zone of glucose; (e) glucose oxidase test.

A. FERMENTATION Washed bakers' yeast (Fleischmann's) in suspension of 20 per cent volume was added to equal volumes of liver filtrates, and to standard solutions of glucose, fructose, galactose, mannose, arabinose, lactose, and maltose. Reducing activity of filtrate and of standards was determined before, and 1 hour after incubation with yeast at room temperature.

In freshly frozen liver, from the Carver press or from the mortar, 80 to 90 per cent of reducing activity disappeared after incubation with yeast. In boiled liver, 100 per cent of reducing activity disappeared after incubation with yeast. This means that most of, or all of the sugar in our liver filtrates was a fermentable reducing material. Similar tests showed that *all* of the reducing substance of blood filtrates, obtained simultaneously with liver, was fermentable.

B. EXCLUSION TESTS FOR HEXOSE PHOSPHATE ESTERS Glucose-1- PO_4 (potassium salt) had no reducing activity under our testing conditions. Glucose-6- PO_4 (barium salt) and fructose-1-6- PO_4 (barium salt) did show evidence of reducing activity. Such reducing activity was eliminated in the Ba-Zn filtrates of the two-hexose phosphates, suggesting that they were quantitatively precipitated in the deproteinization mixture. Hence, glucose-1- PO_4 , glucose-6- PO_4 , and fructose-1-6- PO_4 could not have contributed quantitatively to the observed reducing activity of Ba-Zn filtrates of tissues.

C. ASCENDING PAPER CHROMATOGRAPHY Aliquots (0.02 to 0.04 ml.) of Ba-Zn filtrates of boiled liver (prepared by method C) were placed on paper strips permeated with pyridine-ethyl acetate and water. Control

aliquots of standard solutions of glucose, fructose, mannose, arabinose, lactose, maltose, and galactose were placed in parallel positions. After 24 hours, ammoniacal AgNO_3 was sprayed on the paper to detect reducing activity of material from liver tissue and from standards. The distance between point of application of original material and appearance of a stained spot after 24 hours of diffusion was used as an estimate of the R_f value of a given substance.

Ba-Zn filtrates of liver, glucose, and galactose diffused over the same distance in 24 hours; i.e., they had equal R_f values in the solvent system used. Since galactose was not fermented by the yeast, while glucose and liver filtrates were, the chromatographic data indicated, at least qualitatively, that the reducing substance found in liver was glucose.

D. QUANTITATIVE RECOVERY OF GLUCOSE FROM CHROMATOGRAPHIC PAPER

Aliquots of 0.1 to 0.2 ml. of boiled liver filtrate and of standard glucose solution were applied to chromatographic paper strips. After 24 hours, the site of diffusion was located by spraying the paper along the diffusion path of the glucose standard, but not along that of the liver filtrate. The zone of unstained paper parallel to that of the "glucose spots" was cut free. Water was dripped slowly over the paper; 1 to 2 ml. of the dripping eluate was collected, and analyzed for reducing activity.

In one experiment on boiled liver, an aliquot of Ba-Zn filtrate, containing 88 micrograms of reducing substance, was applied to the paper. After 24 hours of diffusion, the unstained zone of paper, adjacent to the zone into which glucose had diffused, was eluted. The eluate, so obtained, contained 80 micrograms of reducing substance. This means that at least 90 per cent of the reducing material in filtrates of liver moved in a manner identical to that of glucose. Two other experiments yielded recoveries of 55 and 75 per cent.

E. GLUCOSE OXIDASE REACTION The material eluted from chromatographic strips reacted with glucose oxidase paper (clintix) imparting to it a deep blue color.

In summary, we had identified in the filtrate a fermentable reducing substance with a chromatographically measured coefficient of diffusion equal to that of glucose. The reducing substance could be recovered quantitatively after diffusion on chromatographic paper, and reacted positively in the qualitative test with glucose oxidase.

Glucose Content of Liver

Aforementioned tests suggested that the reducing material in Ba-Zn filtrates of liver was really glucose, and that the concentration measured was a close approximation to that present in the liver of an intact dog.

Table I presents concentration values for reducing substances in blood and in frozen livers of four dogs. All of the reducing substance found in blood was fermentable, while 80 per cent of that in freshly frozen liver was fermentable.

TABLE I
VALUES OF REDUCING ACTIVITY OF BLOOD
AND LIVER HOMOGENATE IN MILLIMOLES PER KILO
OF WHOLE TISSUE

Homogenate was prepared in the mortar and pestle under liquid nitrogen.
Fructose content was determined on the same filtrate.

Experiment	Blood		Liver homogenate		
	Total	Fermentable	Total	Fermentable	Fructose
1	4.9 (3)	4.9	7.2 (3)	6.2	0.34
2	5.1 (3)	5.1	6.7 (3)	5.5	0.28
3	6.8 (2)	6.8	10.6 (2)	8.7	
4	5.9 (3)	5.9	13.0 (3)	11.8	

Numbers in parentheses designate the number of determinations on each tissue.

Resorcinol tests indicated that "fructose" content of two livers (Experiments 1 and 2) was 0.28 and 0.34 mm/kg. of whole tissue, or *ca.* 5 per cent of the fermentable reducing substance. This is a maximal value for fructose content since glucose contributes a small amount of color in the resorcinol reaction.

The concentration of glucose in liver was always greater than that in the simultaneously obtained blood. The minimal excess found was in Experiment 2, in which blood glucose was 5.1 mm/kg., while liver glucose was 5.5 mm/kg. The maximal difference was that of Experiment 4, in which blood glucose was 5.9, while liver glucose content was 11.8 mm/kg. of whole tissue. Such differences of glucose concentration must have been minimal ones, because no corrections were made for content of extracellular and intracellular water in the liver.

Boiling tissue, used to eliminate autolysis in freshly excised tissue, was expected to eliminate glycogen breakdown in freshly excised liver. The mass of liver at 38°C., relative to that of water in a heat reservoir maintained at 98°, favored rapid attainment of thermal equilibrium (at 98°C) between tissue and ambient fluid.

Table II presents data similar in character to those of Table I, except that

TABLE II
VALUES OF REDUCING ACTIVITY OF BLOOD AND BOILED LIVER
IN MILLIMOLES PER KILO OF WHOLE TISSUE

Experiment	Blood		Boiled liver	
	Total	Fermentable	Total	Fermentable
1	4.5 (4)		10.3 (6)	
2	6.8 (5)	6.8	7.9 (6)	7.9
3	5.4 (1)	5.4	10.1 (6)	10.1
4	4.3 (1)	4.3	8.5 (6)	8.5

Numbers in parentheses designate the number of determinations on each tissue.

the tissues had been boiled, and not frozen, in preparation for analytical procedures. Fermentable reducing activity in such tissue was over twice that in simultaneously sampled blood. In Experiment 1, the total reducing substance in blood was 4.5 while in liver it was 10.3 mm/kg. of whole tissue. Blood samples were taken from the anesthetized animal just prior to excision of pieces of liver—a procedure lasting for from 4 to 8 minutes. We assumed that the blood glucose level remained constant during the excision procedure. The pH of liver extracts immediately after boiling was *ca.* 8.0—a condition which is not favorable for hydrolysis of glucose esters, or of glycogen. Consequently, we inferred that the reducing content under conditions described, reflected the *in vivo* concentration of free glucose in the liver.

DISCUSSION

If the free glucose concentration of hepatic cells were maintained at levels greater than those of perfusing blood, conditions would be conducive to diffusion of glucose *out of*, but not into, liver cells. This implies that the minimal concentration of extracellular glucose required for net uptake of glucose by the liver must be equal to or greater than that present in liver cells. If our values of 10.1 mm/kg. for whole liver, and 5.4 mm/kg. for blood are correct (see Experiment 3, Table II), then net uptake of glucose by this liver would require a minimal concentration in perfusing blood of 16 mm/kg. or three times the usual value of blood.² Such a prediction has been confirmed by data of others who studied net uptake of glucose in perfused liver and in liver slices.³

Perfusion experiments have demonstrated net uptake of glucose and net synthesis of glycogen only when the concentration of glucose in perfusing fluid was raised to levels of 330 to 870 mg./100 ml. (15, 16). Interestingly, Corey and Britton (17) could find no glycogen synthesis in perfused cat and rat livers with perfusion concentrations as high as 5 per cent or 150 mm/liter. Cortical extract added to the perfusion caused increase of liver glycogen while insulin did not. On the other hand, Sokal *et al.* (16) showed evidence of net synthesis of glycogen in isolated rat livers perfused for 5 to 6 hours with concentrations of 100 to 200 mg./100 ml. of perfusion fluid. Such livers obtained from fasting rats were practically devoid of glycogen (*ca.* 0.05 per cent). If the free glucose content of glycogen-free livers falls to values approaching those of systemic blood, then small increases in glucose concentration (within "physiological" limits) would account for Sokal's observations. However, no

² The latter figure was derived by making appropriate corrections for liver water content (80 per cent) and extracellular water (25 per cent of total tissue weight).

³ The observed flux between C¹⁴-labelled glucose, given intraportally, and various liver metabolites of rats treated with amytal is not considered a clean cut experimental demonstration of net glucose uptake (13, 14).

data on liver glucose were presented, and consequently, one cannot interpret such results in a comprehensive manner.

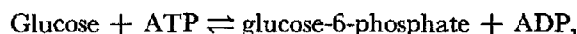
Conditions conducive to glucose diffusion *out of* liver cells have been demonstrated in experiments with data on concentrations of glucose in hepatic and in portal veins. Thus hepatic vein concentration exceeded that of portal vein in fasting and in non-fasting dogs (18–20) as well as in fasting man (21, 22).

On the other hand, net entry of glucose into the liver required infusions of exogenous glucose. Thus, glucose concentration of the portal vein exceeded that of the hepatic vein when the infusion had raised portal vein glucose to levels of 167 to 444 mg./100 ml. in non-fasting dogs (19), and to 100 to 180 mg./100 ml. in fasting dogs (23). Data on hepatic glycogen or on hepatic glucose content were not given in any of the aforementioned reports.

In isolated liver slices from rats and rabbits, net synthesis of glycogen could be induced only when the concentration of glucose in the medium reached values of 30 to 55 mM/liter (24–26).

This implies that non-glucose substrates of blood of the intact animal constitute the major source of liver glycogen, and consequently of liver glucose. Such metabolic pathways for 3-carbon compounds, as well as for fructose, are well known, having been described in detail by Cori (27) and by others (18).

Given a glucose content of 10 to 15 mM/liter of cellular water in the liver, one can calculate roughly the free energy decrease of the hexokinase reaction with constituents at physiological levels. Thus,



and

$$\Delta F = \Delta F^\circ + RT \ln \frac{[\text{glucose-6-phosphate}]}{[\text{glucose}]} \frac{[\text{ADP}]}{[\text{ATP}]},$$

in which $\Delta F^\circ = -4000$ kcal./mole (28, 29); $[\text{glucose-6-phosph}] = 0.55$ mM/Kg. (30); $\text{ADP} = 1.96$ mM/kg. (31); $\text{ATP} = 1.09$ mM/kg. (31); and $\text{glucose} = 10$ mM/kg. (present data). With such assumed values, the decrease of free energy for the hexokinase reaction would be -5430 kcal./mole at physiological concentrations of reactants and products. This means that the reaction has a strong tendency to reach an equilibrium state wherein the reactants would be reduced to low levels. If tissue glucose is *maintained* at levels of 0.01 molar, one can explain the pattern of “physiological” concentrations on the basis of the following: (1) the chemical potential of ATP near equilibrium is not 1×10^{-3} molar, but 10^{-8} molar—a vanishingly low value;⁴ or (2) the glucose-6-phosphate content near equilibrium is greater

⁴Chance has postulated that the chemical potential of ATP may be much less than its chemical concentration in ascites tumor cells (32).

than that reported above—in fact, it would be maintained at levels of *ca.* 1.0 molar, a concentration in excess of the osmotic activity of liver tissue; or (3) the reaction is maintained at a state far removed from equilibrium and proceeds toward equilibrium at a slow rate. The first two possibilities clash with reality. The third implies the existence of little or no hexokinase activity in glycogen-containing cells of the liver. Such a bizarre prediction has been confirmed, at least partially, by the work of others. Vestling (33) noted that slices and homogenates of rat liver consumed little or no glucose from substrates at initial concentrations of 1 per cent or >50 mM/liter. The failure of glucose consumption was duplicated under several controlled conditions of pH, ionic strength, ionic composition, and of osmotic activity. On the other hand, Vestling noted that addition of small amounts of crude hexokinase from brain tissue resulted in effective catalysis with consequent consumption and phosphorylation of glucose in apparently inert slices of liver. However, Vestling postulated that there was a finite, but small amount of hexokinase in liver. Long (34) found that the hexokinase activity of rat liver was lower than that of every other rat tissue assayed. Moreover, he calculated that the phosphorylating capacity of such livers would be too small to account for the observed rate of glycogenesis. It is possible that such activity of hexokinase, as was detected by Vestling and Long, might have been that in non-glycogen-storing cells of the liver.

There remains the possibility that our estimate of liver glucose was erroneously high, and that there is plenty of hexokinase in the liver. Consequently, the concentration of free glucose in the liver cell would be lower than that in the blood. This means that an energy-rich machinery would be needed to move the liver glucose from the cell to the blood. Apart from thermodynamic considerations, such a gradient of glucose would not fit the concept which holds that the liver is a reservoir of quickly available glucose for the body. For example, an injection of epinephrine would have to result in sudden activation of a “glucose pump” in order to account for the sudden increase of blood glucose after such injections.

An alternative possibility is that glucose is metabolized in the liver by way of a reaction with DPN to form gluconate (35). However, the standard free energy decrease for this reaction is -6920 kcal./mole of glucose oxidized, which leads to the same difficulties encountered in assuming that glucose is metabolized by the hexokinase reaction.

Teleological considerations lead us to accept the validity of a high value for hepatic glucose. When peripheral tissues require extra glucose (epinephrine, exercise, etc.), either the free glucose concentration or the permeability of the cell membrane increases. Then a large quantity of glucose would move from a high intracellular concentration to a low extracellular concentration. Even though the teleological approach is not a rigorous one, it does provide an escape from the dilemma of a glucose pump in the liver, and it does fit ob-

servations herein and elsewhere (3) on high concentrations of glucose in the liver.

If there is little or no hexokinase activity within the liver cell, one may infer that glucose therein remains near equilibrium with glucose-6-phosphate *via* catalysis with phosphatase. The reaction is



in which the standard free energy decrease (ΔF°) of the hydrolysis is -3000 kcal./mole (28, 29, 36)

$$\Delta F = -3000 + RT \ln \frac{[\text{glucose}] [\text{phosphate}]}{[\text{glucose-6-phosphate}] [\text{H}_2\text{O}]}$$

in which values derived from the literature are $[\text{glucose-6-PO}_4] = 0.55$ mM/kg. (30); and inorganic phosphate = 3 mM/kg. (30). Substitution of such values for conditions at 38°C. leads to a ΔF of -5260 kcal./mole. Thus at physiological levels of reactants and products, the free energy decrease of the reaction favors formation of products—particularly of glucose. Moreover, if the reaction approaches equilibrium, where $\Delta F = 0$, the standard free energy decrease, ΔF° , is such as to favor formation of additional glucose. This accounts for the observed increase of glucose concentration in liver homogenates kept at room temperature for periods of from 5 minutes to 24 hours. Under such conditions, the reaction could be expected to approach an equilibrium state where the ratio of free glucose to glucose-6-phosphate would be high. On the other hand, equilibrium conditions in the hexokinase reaction demand that the ratio of glucose to glucose ester be low—a requirement in apparent contradiction to actual findings.

In summary, the implications of a high level of glucose in liver cells relative to that in plasma are: (1) that conditions are conducive for outward, but not inward, diffusion of glucose in liver cells. Such conditions fit the concept of the liver as a reservoir of readily available glucose; (2) that the main extracellular source of liver glycogen is non-glucose metabolites; *e.g.*, lactate, fructose, etc., and that glucose *per se* cannot move across the membrane in the direction ISF \rightarrow cell unless the concentration of perfusing glucose is raised to levels greater than those in liver cells; and (3) that there is little or no hexokinase activity, but abundant phosphatase activity in liver cells.

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