Gluconeogenesis and Glucuronidation in Liver in Vivo and the Heterogeneity of Hepatocyte Function*

(Received for publication, May 8, 1995, and in revised form, July 10, 1995)

Karin Ekberg†, Visvanathan Chandramouliš, Kozikot Kumananš, William C. Schumannš, John Wahren‡, and Bernard R. Landau§

From the †Department of Clinical Physiology, Karolinska Hospital, S-176 71 Stockholm, Sweden and the §Departments of Medicine and Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106

Hepatocytes from the periporal and perivenous zones of livers of animals have been found by in vitro techniques to have differing metabolic capacities (1, 2). In gluconeogenesis, glucose-6-P formed from gluconeogenic substrates is hydrolyzed to glucose. In glucuronidation, glucuronic acid from UDP-glucuronic acid is conjugated. The UDP-glucuronic acid is formed from glucose-6-P with carbon skeletons unchanged. Thus, both glucose and glucuronic acid are formed from glucose-6-P with glucose-1-P and UDP-glucose as intermediates. The apparent explanation is the greater decrease in glycerol compared with lactate concentration as blood streams from the periporal to the perivenous zones of the liver lobule. Glucuronidation is then expressed in humans relatively more in the perivenous than periporal zones and gluconeogenesis from glycerol more in the periporal than perivenous zones.

In order to examine metabolic zonation in human liver, [2-14C]glycerol, which labels carbons 2 and 5 of glucose-6-P, and [1-14C]lactate, which labels carbons 3 and 4 of glucose-6-P, in the process of gluconeogenesis, were infused intravenously into healthy subjects who ingested acetaminophen and had fasted 36 h. Distributions of 14C were determined in glucose and in the glucuronic acid moiety of acetaminophen glucuronide excreted in urine. Ratios of 14C in carbons 2 and 5 to 14C in carbons 3 and 4 were significantly higher in blood glucose than in glucuronide. Since glucose and glucuronic acid are formed from glucose-6-P in liver without randomization of carbon, the differences in the ratios indicate that the pool of glucose-6-P in liver is not homogeneous. The glucuronide sampled glucose-6-P with more label from lactate than glycerol compared to the glucose-6-P sampled by the glucose. The apparent explanation is the greater decrease in glycerol compared with lactate concentration as blood streams from the periporal to the perivenous zones of the liver lobule. Glucuronidation is then expressed in humans relatively more in the perivenous than periporal zones and gluconeogenesis from glycerol more in the periporal than perivenous zones.

We infused into fasted normal subjects, given acetaminophen, [2-14C]glycerol, which labels carbons 2 and 5 of glucose-6-P, and [1-14C]lactate, which labels carbons 3 and 4 of glucose-6-P (Fig. 1). The ratios of the 14C in carbons 2 and 5 to the 14C in carbons 3 and 4 of blood glucose were compared with the ratios in the acetaminophen glucuronide excreted in urine.

EXPERIMENTAL PROCEDURES

Subjects—The subjects were four healthy women volunteers, ages 26–44 years, on weight-maintaining diets, and with a mean body mass index (kg/m²) of 19.8 (range 19.4–20.1). The experimental protocol was approved by the Human Investigation Committees at the Karolinska Hospital and University Hospitals of Cleveland. Informed consent was obtained from each subject.

Protocol—The subjects fasted for 36 h. A catheter was then inserted into a peripheral vein of one arm for blood sampling and into a peripheral vein of the other arm for infusion. In the first subject we infused 10 μCi of [2-14C]glycerol, and in each of the other three subjects 25 μCi, in trace quantity (specific activity 40 mCi/mmol; purchased from ICN Biomedical Inc., Irvine, CA). In the first subject we also infused 60 μCi of [1-14C]lactate, and in each of the other three subjects 55 μCi (10 μCi as a bolus), again in trace quantity (specific activity 61 mCi/mmol; also purchased from ICN Biomedical Inc.). The labeled glycerol and lactate were in solution in sterile isotonic saline, shown to be pyrogen-free, and the infusion rate was constant at between 10 and 15 ml/h. Duration of infusion in the first two subjects was 5 h and in the other two subjects 8 h. The first two subjects ingested 0.5 g of acetaminophen at the beginning and at 1 and 2 h into the infusion, the other two subjects at 3, 4, and 5 h into the infusion.

Blood samples, each 75 ml, were drawn between 3 and 5 h in the first two subjects and between 5 and 8 h in the other two subjects. Subjects were encouraged during the infusions to drink about 240 ml of water/h. Urine was collected from the first two subjects between 2–3.5 h and 3.5–5 h and in the other two between 5–6.5 h and 6.5–8.5 h. One ml blood samples were collected at the beginning and end of each infusion for determination of plasma glucose concentration.

Analyses—Glucose concentration in plasma was determined using glucose oxidase. Glucose was isolated from blood as described previously (6). Briefly, the blood at the time of collection was deproteinized by the addition of ZnSO₄ and Ba(OH)₂. The precipitate was removed by centrifugation and the supernatant frozen until it could be processed. It was denitized by passage through a column of ion exchange resins. Glucose in the effluent was isolated using preparative paper chromatography followed by high pressure liquid chromatography.

The acetaminophen glucuronide was isolated, reduced to its glucoside, and glucose isolated from the glucoside as described previously (17, 3). Briefly, urine at the time of collection was taken to pH 4.5 and frozen until processed further. The urine was concentrated, then brought to its original volume with methanol, and the precipitate that formed was discarded. The methanol was evaporated and the resulting concentrate made basic and applied to a column of AG1-X8 in the acetate form (Bio-Rad). The column was washed with water and then increasing concentrations of acetic acid. The fraction eluted containing the glucuronide, identified using carbazole (7), was evaporated to dryness. The
glucuronide was reduced with diboran to acetaminophen glucoside and the glucoside hydrolyzed with β-glucosidase. Glucose from the deionized hydrolysate was isolated as just described for glucose from blood.

A portion of each glucose from blood and urine was combusted to CO₂. The remainder of each glucose was degraded to yield each of its six carbons as CO₂. Each CO₂ was assayed for ¹⁴C specific activity (6, 7).

Calculations—The specific activity of each carbon of glucose divided by the sum of the specific activities of the six carbons of glucose time 100 is recorded as the percentage of ¹⁴C in that carbon. The sum of the six specific activities divided by the specific activity of the glucose carbons, determined by the combustion of the glucose to CO₂ × 100, is recorded as percentage of recovery. That provides a measure of the adequacy of the degradation.

Statistics—The significance of the differences in the ratios of incorporations of ¹⁴C in blood glucose to those in urinary glucuronide and other differences were assessed by a two-tailed paired t test (9).

RESULTS

Plasma glucose concentrations at the beginning of the infusions ranged from 3.9 to 5.1 mM. Over the duration of the infusions concentrations declined by 0.5–0.7 mM. Amounts of ¹⁴C in glucosides isolated from some blood samples were small, and therefore degradations of those glucoses were not done.

¹⁴C was in greatest percentages in carbons 2, 3, 4, and 5 (Table I) in accord with the pathways followed by the labeled carbons of the glycerol and lactate (Fig. 1). Recoveries were good. There was only about one-third as much ¹⁴C in carbons 2 and 5 as carbons 3 and 4 of glucose from the first subject, AD. This was the reason for giving 25 mCi of [2-¹⁴C]glycerol to the other three subjects, since the lower the amount of ¹⁴C in a carbon, the more likely an error in the determination of the amount.

¹⁴C in carbon 2 was more than in carbon 5 (p < 0.001), and ¹⁴C in carbon 4 was more than in carbon 3 (p < 0.001). This result is in accord with glycerol’s entrance into the triose-P pool via dihydroxyacetone-3-P and lactate’s via glyceraldehyde-3-P and incomplete isotopic equilibration of the triose phosphates. Distributions in the glucose from blood and from glucuronide in the periods of collection were remarkably similar. The ratios of ¹⁴C in carbons 2 and 5 to ¹⁴C in carbons 3 and 4 were significantly different in blood glucose and in glucose from glucuronide (p < 0.005), being higher by an average of 30% (range from 26 to 37%). Incorporations into carbons 1 and 6 occur in the formation of glucose-6-P from [2-¹⁴C]lactate formed from the [2-¹⁴C]glycerol. Subtraction of the incorporations of ¹⁴C from the [2-¹⁴C]lactate into carbons 2 through 5 (10, 11) will give a still greater difference in the carbons 2 and 5 to carbons 3 and 4 ratios.

DISCUSSION

The duration of infusion was increased from 5 to 8 h, with sampling during the last 3 h of infusion, to assure a steady state. However, just the very similar distributions at different times of blood and urine collections for each subject provide that assurance. Since glucose and glucuronic acid are both formed from glucose-6-P without randomization of carbon, the differences in the distributions, reflected in the differing C₂ + C₃/C₅ ratios, mean there was not a homogeneous pool of glucose-6-P in liver. The glucuronide sampled glucose-6-P in hepatocytes where there was overall more label from lactate than glycerol, than was the case for the glucose-6-P the glucose sampled.

The approach we introduce here for testing for metabolic heterogeneity in liver demonstrates differing metabolism in hepatocytes in humans in vivo. That conclusion rests on two assumptions. First, that the distribution of ¹⁴C in blood glucose reflects distribution in glucose-6-P in liver. Liver is the major source of blood glucose, but there is a report of a significant contribution of kidney to glucose production in dogs fasted overnight (12). Second, that the distribution of ¹⁴C in the glucuronide reflects distribution in glucose-6-P in liver. Liver is the major site of glucuronidation, although glucuronidation can occur in other tissues (13). The present results are in accord with our previous finding that when [U-¹³C]glycerol and acet...
aminophen are given to fasted subjects isotopomer patterns are different in blood glucose and glucuronic acid from urinary acetaminophen glucuronide (8).

Neese et al. recently reported (14) that rats infused with [2-13C]glycerol and acetaminophen, and fasted 24 h or infused with fructose, formed glucose and acetaminophen glucuronide from triose-P of the same enrichment. This seeming difference from our findings may be related to the difference in species, a quantity of glycerol rather than a trace amount being given to the rats, the conversion of glycerol to lactate, and the effect of the fructose load when that was also given.

A different gradient in the concentrations of glycerol and lactate along the liver lobule (3–5) is an apparent major reason for the differing distributions we observe. Other factors, such as differences in the activity of glycerokinase along the liver lobule, could play a role. Hence, hepatocytes in the hepatic vein zone were presumably exposed to more label from the lactate than glycerol compared to hepatocytes in the periporal zone. The glucuronide compared to the glucose then sampled glucose-6-P to a greater extent in the hepatic venous than periporal zone. That conclusion is in accord with a higher capacity for glucuronidation found in rat liver in vitro in hepatic venous than periporal zones.

Acknowledgment—We gratefully acknowledge the assistance of Dr. Paul K. Jones in the statistical analyses.

REFERENCES
1. Jungermann, K., and Katz, N. (1989) Physiol. Rev. 69, 708–764
2. Wals, P. A., Palacin, M., and Katz, J. (1988) J. Biol. Chem. 263, 4876–4881
3. Wahren, J., Efendic, S., Luft, R., Hagenfeldt, L., Bjorkman, O., and Felig, P. (1977) J. Clin. Invest. 59, 295–307
4. Bjorkman, O., Felig, P., and Wahren, J. (1980) Diabetes 29, 610–616
5. Bjorkman, O., and Felig, P. (1982) Diabetes 31, 516–520
6. Landau, B. R., Schumann, W. C., Chandramouli, V., Magnusson, I., Kumaran, K., and Wahren, J. (1993) Am. J. Physiol. 265, E636–E647
7. Magnusson, I., Chandramouli, V., Schumann, W. C., Kumaran, K., Wahren, J., and Landau, B. R. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 4682–4685
8. Landau, B. R., Fernandez, C. A., Previs, S. F., Ekberg, K., Chandramouli, V., Wahren, J., Kalhan, S. C., and Brunengraber, H. (1995) Am. J. Physiol., 269, E18–E26
9. Snedecor, G. W., and Cochran, W. G. (1980) Statistical Methods, 7th Ed., pp. 54–63, Iowa State University Press, Ames, IA
10. Magnusson, I., Schumann, W. C., Bartsch, G. E., Chandramouli, V., Kumaran, K., Wahren, J., and Landau, B. R. (1991) J. Biol. Chem. 266, 6975–6984
11. Hostetter, K. Y., Williams, H. R., Shreeve, W. W., and Landau, B. R. (1969) J. Biol. Chem. 244, 2075–2077
12. Cersosimo, E., Judd, R. L., and Miles, J. M. (1994) J. Clin. Invest. 93, 2584–2589
13. Dutton, G. J. (1980) Glucuronidation of Drugs and Other Compounds, pp. 249–158, CRC Press Inc., Boca Raton, FL
14. Neese, R. A., Schwarz, J.-M., Falik, D., Turner, S., Letscher, A., Vu, D., and Hellerstein, M. K. (1995) J. Biol. Chem. 270, 14452–14463