METABOLIC INTERMEDIATES IN LIVER OF RATS GIVEN LARGE AMOUNTS OF FRUCTOSE OR DIHYDROXYACETONE*

Helen B. Burch, Paul Max, Jr., Kyung Chyu

and Oliver H. Lowry

Department of Pharmacology
Washington University Medical School
St. Louis, Missouri 63110

Received January 22, 1969

Summary: The livers of rats given large injections of fructose or dihydroxyacetone were analyzed for glucose, glycogen, and 14 related metabolites. Dihydroxyacetone produced a large increase in glycogen and a modest increase in glucose and glucose 6-P. Fructose produced no increase in glycogen but a large increase in glucose and glucose-6-P. Changes in intermediates suggest in both cases diversion of part of the injected material into the Embden-Meyerhof pathway. Fructose caused a profound drop in ATP, UTP and UDPG. The fall in ATP is attributed to sequestration of phosphate into fructose-1-P, which increased to 18 mmole/kg. The decrease in ATP is believed to lead in turn to the fall in UTP and UDPG and the failure to deposit glycogen.

The very rapid conversion of fructose and dihydroxyacetone to glucose and glycogen in liver has been widely studied (1-12). Less information is available concerning changes in levels of metabolic intermediates after fructose (7,12,13), or dihydroxyacetone administration which is the subject of this report. The results show a marked difference in the utilization of these compounds for glycogenesis and help to indicate rate limiting steps in their metabolism.

METHODS

Sprague-Dawley male rats (Holtzman Rat Co., Madison, Wis.) weighing about 100 g were fasted for 24 hr, anesthetized with

* This work was supported by grants from the USPHS NB 1352, N. Sci. Found. GB 5397, and the American Cancer Society (P-78).

phenobarbital, and given 40 mmoles/kg of fructose or dihydroxyacetone intraperitonealy. Liver samples were removed while the circulation was still intact, and dropped within one sec into Freon-12 at -150°.

Samples were weighed at -20°, macerated in 2 M $HClO_4$ at -10°, and the extraction completed as described before (14).

Sixteen components of liver were measured, the majority of them by methods described earlier (14), UDPG (15), glycogen (16). Fructose, fructose-1-P, and UTP (H. B. Burch, P. Max, Jr. and O. H. Lowry, unpublished) were also measured by enzymatic fluorometric methods.

RESULTS

Glycogen and glucose synthesis (Fig. 1). Injection of fructose resulted in no significant increase in liver glycogen up to 1 hr, whereas dihydroxyacetone produced rapid glycogen formation (66 to 76 mmole/kg in 1 hr). In contrast, during the same interval glucose increased greatly after fructose injection (18 mmole/kg) but to a lesser degree after dihydroxyacetone (9 mmole/kg). The rapid formation of glucose from fructose agrees with the fructose ^{14}C experiments of Muntz and Vanko (13).

A minimal estimate of glucose formation can be made by taking into account the fact that hepatic glucose levels are very close to plasma levels and hence to extracellular fluid levels throughout the body. If the liver is 5 % of body weight and the remainder of the extracellular fluids is 20 % of body weight, the liver produced per kg in 1 hr at least 90 mmoles of glucose from fructose and 40 mmoles of glucose from dihydroxyacetone.

Thus both fructose and dihydroxyacetone were rapidly converted to glucosyl units, but subsequent glycogen synthesis was blocked in the case of fructose.

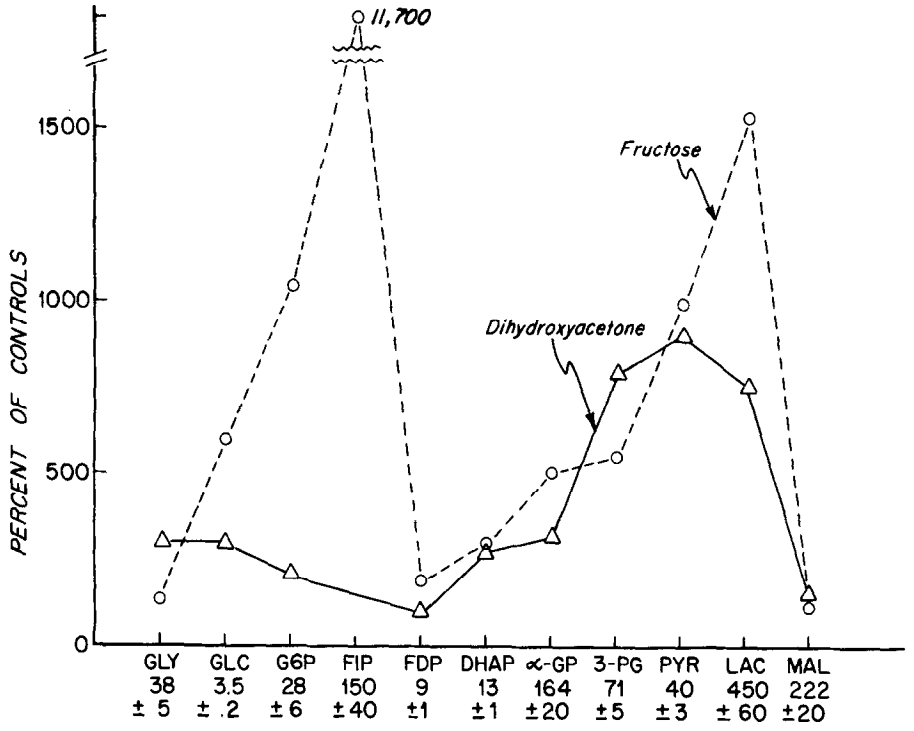

Fig. 1. Change in metabolites of rat liver 1 hr after fructose or dihydroxyacetone. Control levels are recorded as either mmole/kg (glycogen and glucose) or μmole/kg, and represent the averages and standard errors for 5 or 6 rats. The experimental points represent the mean for 5 rats in each case. The non-standard abbreviations are: glycogen, gly; glucose-6-P, G6P; fructose-1-P, F1P; fructose-1,6-P_2, FDP; dihydroxyacetone-P, DHAP; α-glycero-P, α-GP; 3-P-glycerate, 3-PG; pyruvate, pyr; lactate, lac; malate, mal.

Metabolic intermediates after fructose administration. At 60 min after fructose injection (Fig. 1) 9-fold to 14-fold increases in hepatic lactate, pyruvate, and glucose-6-P, 4- to 5-fold increases in α-glycero-P, 3-P-glycerate, and a 2-fold increase in dihydroxyacetone-P, were observed. Impressive accumulations occurred in the case of the fructose monophosphates, 100-fold for fructose-1-P, and not less than 5-fold for fructose-6-P. (The initial fructose-6-P level was too low to measure precisely). The formation of these phosphorylated intermediates was very rapid (Fig. 2) so that by 15 min the levels

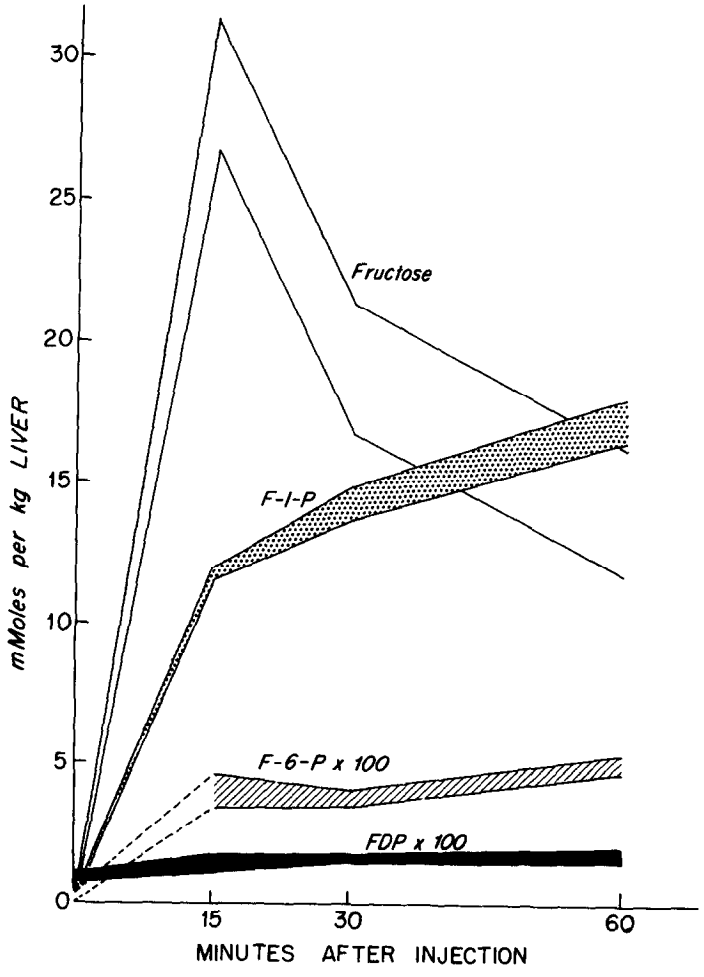

Fig. 2. Concentration of fructose and fructose phosphates in rat liver after a fructose load. Five animals are represented at each time and the width of each line equals 2 standard errors.

---

were 70-80 % of the 1 hr values. In contrast to the monophosphates, fructose-1,6-P_2 increased less than 100 %.

By 15 min ATP had undergone a dramatic decrease and it remained at about one-third of the control level for the rest of the hour (Table 1). UTP also fell to about the same degree but at a slower rate. UDPG fell in parallel with UTP.

The changes in pyruvate and fructose-1-6-P_2 are similar to

Table 1

Hepatic ATP, UTP and UDPG after injection of fructose or dihydroxyacetone (DHA). The averages ± the standard errors are shown in mmole/kg. The animals are those of Fig. 1.

Metab-olite	Control 0'	Substance injected	Time After Injection 15'	30'	60'
ATP	2.81 ±.12	Fructose	1.07 ±.03	1.01 ±.02	0.94 ±.04
		DHA	2.63 ±.11	2.47 ±.08	2.62 ±.20
UTP	0.23 ±.02	Fructose	0.19 ±.02	0.12 ±.01	0.09 ±.01
		DHA	0.29 ±.02	0.25 ±.01	0.25 ±.02
UDPG	0.24 ±0.01	Fructose	0.15 ±.01	0.11 ±.01	0.09 ±.01
		DHA	0.23 ±.01	0.19 ±.01	0.16 ±.01

those found by Lamprecht and Trautschold (7) in rats starved 5 days then fed fructose for 24 hours. Kjerulf-Jensen (2) first demonstrated fructose-1-P in rat liver after fructose injection. More recently, Heinz (21) found fructose-1-P levels of 1.9-3.9 mmole/kg after intraportal fructose injection.

Metabolic intermediates after dihydroxyacetone. At 60 min after the injection of dihydroxyacetone (Fig. 1) an increase of 7 to 8-fold had occurred in lactate, pyruvate, and 3-P-glycerate, but only 2-fold in dihydroxyacetone-P and α-glycero-P. Most of the other intermediates, including UTP and ATP, (Table 1) showed little or no change. The exception, UDPG, decreased about 30 % in 60 min. The most rapid change in UDPG occurred between 15 and 30 min.

DISCUSSION

Dihydroxyacetone. The first step in dihydroxyacetone

utilization is presumably conversion to dihydroxyacetone-P by glycerol kinase. If so, the results indicate exceedingly efficient subsequent conversion, (probably through fructose-1, 6-P_2 and fructose-6-P) to glucose-6-P and glucose. Dihydroxyacetone-P did not rise above 0.04 mmole/kg and fructose-1,6-P_2 did not rise at all, in spite of a rate of formation of glycosyl units of at least 110 mmole/kg/hr.

The major increases in lactate, pyruvate and 3-P-glycerate show that some of the triose-P formed was diverted into the Embden-Meyerhof pathway. It will be observed that proportionately 3-P-glycerate increased much more than did dihydroxyacetone-P, and lactate increased far more than did glycero-P. This suggests that something in addition to triose-P itself was accelerating the pathway below glyceraldehyde-P. This could be ADP formed by the rapid phosphorylation of dihydroxyacetone. The failure of malate to increase concomitantly suggests that the diversion of triose-P toward pyruvate did not extend as far as the citrate cycle. (Malate does increase markedly with large lactate loads (15)).

The rapid synthesis of glycogen was associated with a fall in its immediate precursor, UDPG. This suggests that glycogen synthetase had been stimulated. The modest increase in glucose-6-P does not seem an adequate explanation.

Fructose. Two pathways are recognized by which fructose can be converted to glucose. One begins with fructose-1-P, formed by the specific fructokinase and proceeds through the triose phosphates to fructose-1,6-P_2, fructose-6-P and glucose-6-P. The other begins with fructose-6-P formed directly by hexokinase. Although hexokinase has a high K_m for fructose (2 to 7 mM according to Walker (17) and Di Pietro (18)), the reac-

tion is favored in the present instance by the very high fructose levels (Fig. 2). The very large increase in fructose-1-P shows that fructose-1-P aldolase activity is inadequate to keep up with fructokinase and may represent a limitation to the first pathway. It is not unlikely that the hexokinase route predominates when the system is flooded with fructose, as in the present case, whereas fructokinase, with a more favorable K_m, (< 0.5 mM, (19,20)) may predominate when fructose levels are low.

Many of the intermediates were affected in a comparable way by fructose and dihydroxyacetone. However, there were several striking differences which may be the result of the large increase in the case of fructose in fructose-1-P, which reached the remarkable level of 18 mmole/kg. The amount of phosphate thereby tied up is equal to the total acid-soluble phosphate in a control liver. This sequestration of phosphate could explain the profound decrease in ATP and UTP. The UTP decrease could in turn account for the fall in UDPG and failure of glycogen synthesis.

The fall in ATP cannot be explained on simple imbalance between the rate of ATP synthesis and the rate of ATP consumption (to phosphorylate fructose), because calculation shows that much more ATP must have been used in the case of dihydroxyacetone.

REFERENCES

1. Cori, C. F., and Shine, W. M., J. Biol. Chem., 114, XXI (1936).
2. Kjerulf-Jensen, K., Acta Physiol. Scand., 4, 249 (1942).
3. Pany, J., Z. Physiol. Chem., 272, 273 (1942).
4. Cori, G. T., Ochoa, S., Slein, M. W., and Cori, C. F., Biochim. Biophys. Acta, 7, 304 (1951).
5. Hers, H. G., and Kusaka, T., Biochim. Biophys. Acta, 11, 427 (1953).
6. Spiro, R. G., and Hastings, A. B., J. Biol. Chem., 230, 751 (1958).

7. Lamprecht, W., and Trautschold, I., Z. Physiol. Chem., 311, 245 (1958).
8. Ross, B. D., Hems. R., and Krebs, H. A., Biochem. J., 102, 942 (1967).
9. Krebs, H. A., Nolton, B. M., and Hems, R., Biochem. J., 101, 607 (1966).
10. Exton, J. H., and Park, C. R., J. Biol. Chem., 243, 4189 (1968).
11. Muntz, J. A., J. Biol. Chem., 243, 2788 (1968).
12. Kupke, V. I., and Lamprecht, W., Z. Physiol. Chem., 348, 17 (1967).
13. Muntz, J. A., and Vanko, M., J. Biol. Chem., 237, 3582 (1962).
14. Lowry, O. H., Passonneau, J. V., Hasselberger, F. X., and Schulz, D. W., J. Biol. Chem., 239, 18 (1964).
15. Hornbrook, K. R., Burch, H. B., and Lowry, O. H., Mol. Pharm., 2, 106 (1966).
16. Passonneau, J. V., Gatfield, P. D., Schulz, D. W., and Lowry, O. H., Anal. Biochem., 19, 315 (1967).
17. Walker, D. G., Biochem. J., 87, 567 (1963).
18. Di Pietro, D. L., J. Biol. Chem., 239, 4051 (1964).
19. Hers, H. G., Le Métabolisme du Fructose, Editions Arscia, Bruxelles, p. 78 (1957).
20. Parks, R. E., Jr., Ben-Gershom, E. and Lardy, H. A., J. Biol. Chem., 227, 231 (1957).
21. Heinz, F., Z. Physiol. Chem., 349, 859 (1968).