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Effect of Fructose, Dihydroxyacetone, Glycerol, and Glucose on Metabolites and Related Compounds in Liver and Kidney*

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SUMMARY

The effects of large intraperitoneal doses of fructose and dihydroxyacetone on the levels of many metabolites of carbohydrate metabolism are reported for liver, kidney cortex, and medulla of the rat. Levels were also measured in liver after glycerol and glucose administration, and in kidney cortex and medulla, after glycerol. In both liver and kidney there were large accumulations of fructose-1-P from fructose and of glycero-P from glycerol. The accumulations were associated with marked depletion of ATP, UTP, and total adenylate, and, in liver at least, of P_i.

The increases in glycero-P were not associated with comparable changes in dihydroxyacetone-P, and the ratio between the two rose from 18:1 to as high as 1000:1, with relatively minor changes in lactate to pyruvate ratios. Riboflavin deficiency, which drastically lowers glycero-P oxidase activity, caused an increased accumulation of glycero-P. These results suggest that the newly formed glycero-P is chiefly oxidized by glycero-P oxidase rather than by glycero-P dehydrogenase. The disproportionate increase in glycero-P seen after glycerol was also observed after fructose administration in kidney cortex but not in liver.

In the case of each of the four nutrients, fructose, dihydroxyacetone, glycerol, and glucose, UDP-glucose decreased in liver. Concomitantly, metabolites of the Embden-Meyerhof pathway increased. The increases tended to be smaller for fructose 1,6-diphosphate and dihydroxyacetone-P than for members above and below them on the pathway. The increases were generally greatest after fructose and least after glucose. Fructose caused rapid large increases in glucose but there was little glycogen formation during the first hour.

A useful strategy in the analysis of control sites and mechanisms is to impose a sudden change in the metabolic situation and to observe the consequent alterations in appropriate metabolite levels. In this study fasted animals were given large doses of glucose precursors or glucose, and the resulting changes were determined in liver, kidney cortex, and kidney medulla. The glucose precursors were fructose, glycerol, and dihydroxyacetone, all of which should enter the Embden-Meyerhof pathway at the triose-P level. Although the pathway may be entered at the same point in each case, there are nevertheless certain major differences in response which arise from differences in the speed at which the organs can act on the initial enzyme product. Dihydroxyacetone enters directly into the pathway. Glycerol metabolism is delayed at the glycero-P stage; fructose metabolism is delayed at the fructose-1-P stage. The accumulation of glycero-P or fructose-1-P is sufficient to trap large amounts of phosphate and to cause severe decreases in ATP leading to other secondary changes in metabolite levels.

In the case of glycerol, additional experiments were conducted in riboflavin-deficient animals, in order to observe the consequence of the decreased activity of glycero-P oxidase which this deficiency causes.

The effects on metabolites after giving glucose, or these glucose precursors, have been compared with changes previously observed when the glucose precursor was lactate.

A preliminary report has been published of some of the findings in liver after giving fructose and dihydroxyacetone (1). The effects of fructose on metabolites in liver have also been the subject of a number of other recent investigations (2–6).

EXPERIMENTAL PROCEDURE

Animals and Materials

Animals—Male Sprague-Dawley rats (Holtzman Rat Company, Madison, Wisconsin) were used. For most of the experiments they were grown on Purina chow to weights of 100 to 185 g.

For the riboflavin deficiency study rats received synthetic diets (7) for 6 or 7 weeks beginning at 22 days of age. The basal diet was riboflavin-free; the control diet differed from it only by the addition 15 mg of riboflavin per kg. Litter mates were distributed among three experimental groups. The first received the riboflavin-free diet; the second received the high riboflavin diet *ad libitum*; and the third (weight controls) received the high riboflavin diet in a daily quantity adjusted to maintain their weight equal to the deficient group.

Preparation of Material—After fasting for 24 hours, the rats

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were anesthetized by intraperitoneal injection of sodium phenobarbital, 150 mg per kg. Thirty minutes later they were treated by intraperitoneal injection with 20 ml per kg of solution containing the particular nutrient. Controls received the same volume of 0.9% NaCl. After the desired time interval (an hour in the case of controls) the peritoneum was opened and the kidneys were removed and immersed in Freon 12 at −150° within 1 sec after clamping the renal vessels. A piece of liver was similarly frozen. Cortex and medulla were dissected from the frozen kidney at −20°. The outer portion of the cortex and the straight tubular section of the medulla were taken. The corticomedullary junction was avoided.

Frozen tissues were stored at −60° until used. Samples were weighed at −20°, extracted with perchloric acid at −10°, and subsequently treated as described earlier (8). Samples for glycogen were homogenized in 36 volumes of 0.2 N HCl at 0°, diluted 5-fold with 1 mM EDTA, heated 10 min at 100°, and analyzed without removing insoluble material (9).

Analytical Methods

Enzymes and Reagents—Most enzymes were obtained either from Boehringer Mannheim or from Sigma. Crystalline UDP-glucose pyrophosphorylase was kindly supplied by Dr. R. G. Hansen, Utah State University. Fructose-1-P aldolase from rabbit liver was a gift of Sigma.

General Procedures for Metabolites—All but the protein (10) and acid-soluble phosphate (11) procedures are based on specific enzyme reactions which result in oxidation or reduction of pyridine nucleotides. The increase or decrease in reduced nucleotides is measured fluorometrically to achieve high sensitivity. Measurements were made in a Farrand fluorometer on 1-ml volumes in 3-ml fluorometer tubes (Pyrex test tubes, 10 × 75 mm, selected for uniform size and freedom from scratches). When the anticipated level of a metabolite was low (fructose-1,6-di-P, dihydroxyacetone-P, pyruvate) the neutralized liver extracts were treated with 60 mg per ml of Florisil (Floridin Company, Tallahassee, Florida), in order to remove a large part of the interfering fluorescent material (12). Before use, the Florisil was heated for 1 hour at 100° in 1 N HCl, washed free of acid, and dried at 100°.

Except as noted, analyses were made as originally described for glycogen (9), glucose, glucose-6-P, fructose-1,6-di-P, dihydroxyacetone-P, glycero-P, 3-P-glycerate, P-pyruvate, pyruvate, ATP, ADP, AMP (8), lactate (13), and P_i (14).

New or significantly revised procedures are summarized below and in Table I.

Fructose—Glucose and fructose were measured in the same sample with the aid of yeast hexokinase, glucose-6-P dehydrogenase, and P-glucoisomerase. The previous glucose method (8) was unchanged except for the addition of 0.5 mM dithiothreitol to the reagent. Extract equivalent to 0.15 mg of tissue was used. After the glucose reaction was complete (3 to 5 min), 4 μg of P-glucoisomerase were added in a small volume. The final reading was made after another 5 min. Because of the danger of contamination of the other enzymes with isomerase, the reaction time at the glucose step was carefully controlled, and as a check, fructose standards were incubated with all components present except isomerase.

UDP-glucose and UTP—The method previously described (12, 15) for UDP-glucose was modified slightly to permit subsequent UTP measurement on the same sample. UDP-glucose dehydrogenase was allowed to react first with UDP-glucose. When the conversion to UDP-glucuronate was complete, crystalline UDP-glucose pyrophosphorylase was added to convert UTP and glucose-1-P to UDP-glucose and pyrophosphate.

Fructose-1-P—This was measured by means of a system

TABLE I

Analytical conditions

Analyses were made in 1 ml of reagent in fluorometer tubes (10 × 75 mm) plus neutralized $HClO_4$ extract equivalent to the amount of liver or kidney indicated (wet weight). Enzymes were added in volumes of 5 μl or less.

Substance	Buffer	Enzymes per ml	Other additions	Tissue	Incubation time
				mg	*min*
UDP-glucose	Tris, 50 mM, pH 8.4	20 units (0.16 μmole/ml/min) UDP-glucose dehydrogenase	0.1 mM DPN^+, 1 mM glucose-1-P, 0.25 mM magnesium acetate	2–3	30
UTP	Tris, 50 mM, pH 8.4	20 units (0.16 μmole/ml/min) UDP-glucose dehydrogenase plus 0.15 μg crystalline UDP-glucose pyrophosphorylase	0.1 mM DPN^+, 1 mM glucose-1-P, 0.25 mM magnesium acetate	2–3	30
Fructose-1-P	Imidazole, 50 mM, pH 7.6	2 μg glycero-P dehydrogenase[a]; after 3 min, 500 μg muscle aldolase[a, b]	0.005 mM DPNH	0.12–1	120[c]
Glycerol	Hydrazine, 400 mM, pH 9	7 μg glycero-P dehydrogenase[a]; after 15 min, 1 μg glycerokinase[a]	1 mM $MgCl_2$ 0.2 mM DPN^+, 1 mM ATP	0.8–2	20
Dihydroxyacetone	Imidazole, 50 mM, pH 7.6	4 μg glycero-P dehydrogenase,[a] 25 μg glycerokinase	1 mM EDTA, 1 mM mercaptoethanol, 2 mM $MgCl_2$, 5 mM sodium Amytal, 0.02% bovine serum albumin, 1 mM ATP, 0.007 mM DPNH	0.12	60

[a] These enzymes were centrifuged (see text) and redissolved in Tris-HCl buffer (pH 8) before use.

[b] Or sufficient rabbit liver aldolase to give maximum activity toward fructose-1-P of 7.5 nmoles per ml per min.

[c] Time shortened to 45 min when liver aldolase was used.

coupling aldolase and glycero-P dehydrogenase. Heinz (16) has described a similar procedure for the spectrophotometer. Two different aldolase preparations were used, one from muscle, the other from liver. Since large amounts of the muscle enzyme were needed, most of the $(NH_4)_2SO_4$ was removed from the preparation by centrifugation to avoid inhibition of glycero-P dehydrogenase. Because of the low activity toward fructose-1-P, incubation had to be extended to 120 min. With the more active enzyme from liver, incubation could be reduced to 45 min. Both aldolases gave nearly the same results for fructose-1-P. Further confirmation of the validity of the assay was obtained by measuring the increase in fructose upon heating in 1 N HCl at 100° for 15 min.

α-Glycero-P and Glycerol—After measuring glycero-P in the tissue sample, as previously described (8), glycerokinase from *Candida mycoderma* was added to convert glycerol to an additional quantity of glycero-P (a fluorometric adaptation of a published spectrophotometric method (17)). Since glassware and glycero-P dehydrogenase were frequently found to be contaminated with glycerol, and glycerokinase as purchased actually had added glycerol to stabilize it, care was taken on the one hand to avoid touching glassware unnecessarily and on the other to sediment both enzymes by centrifugation and wash them with 5 volumes of 2.5 M $(NH_4)_2SO_4$.

Dihydroxyacetone—This fluorometric, one-step assay uses the reaction of dihydroxyacetone with glycerokinase described by Bublitz and Kennedy (18), and Wieland (19), and uses glycero-P dehydrogenase as an auxiliary enzyme. The small amount of dihydroxyacetone-P in the extract is removed by the dehydrogenase before addition of glycerokinase. A much larger amount of glycerokinase is necessary than in the case of glycerol. Most of the sulfate was removed from the enzymes by centrifugation, for the reason given above.

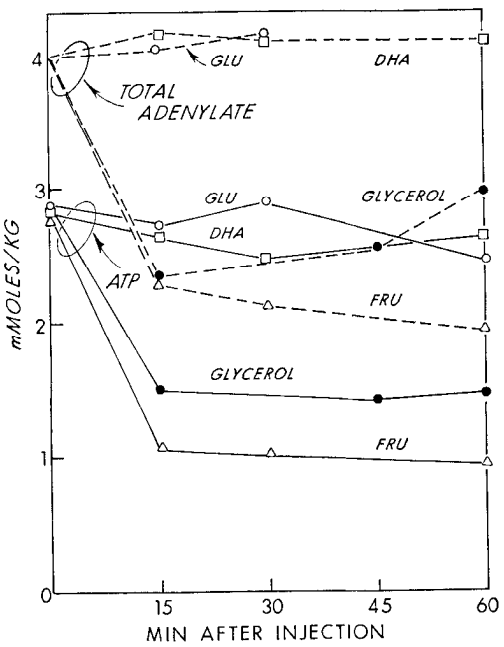

FIG. 1. Changes in hepatic ATP and total adenylate compounds after injection of large amounts of four different nutrients (40 mmoles per kg). Each *point* represents the average of four to six animals for dihydroxyacetone (*DHA*), fructose (*FRU*), and glycerol and of two animals for glucose (*GLU*).

Enzymes—α-Glycero-P oxidase was measured in a two-step fluorometric procedure with methylene blue as the electron acceptor, as described by McDougal *et al.* (20); α-glycero-P dehydrogenase was determined as previously reported (21).

Glycerokinase was assayed by a fluorometric adaptation of the methods of Bublitz and Kennedy (18) and Wieland (22). Homogenates (1:50) were prepared in ground glass homogenizers in 0.025 M imidazole-HCl buffer (pH 7) containing 1 mM EDTA, 1 mM dithiothreitol, and 1 mM glycerol. Assays were started within 15 min after homogenizing. The activity of the homogenates was stable for at least 1 hour at 0°. The enzyme in tissue stored at −60° was stable for at least a year but large losses occurred in homogenates stored overnight either at 0° or at −60°.

Activity was measured at 25° with reagent containing 400 mM hydrazine, 20 mM HCl (giving a pH of 9), 1 mM EDTA, 1 mM dithiothreitol, 2 mM $MgCl_2$, 5 mM sodium Amytal, 10 mM glycerol, and 0.2 mM DPN^+ (added just before use). To 1 ml of reagent in a fluorometer tube were added homogenate equivalent to 50 μg of liver and 6 μg of α-glycero-P dehydrogenase. After 2 min an initial fluorescence reading was made and the reaction was started with 10 μl of 100 mM ATP. A lag period of 6 to 8 min occurred, after which the rate was linear with time for at least 20 min. For tissue and reagent blanks ATP was omitted. Glycero-P, standardized enzymatically in the spectrophotometer, was used to test the efficiency of the auxiliary enzyme.

RESULTS

Effects of Adenine Nucleotides, UTP, and P_i—Before presenting the metabolite data it seems useful to describe certain changes in adenine nucleotides, UTP, and P_i, which took place when two of the glucose precursors were given. This may make it easier to understand some of the metabolite changes.

Large amounts of dihydroxyacetone and glucose (40 mmole per kg) had little effect on ATP levels, but fructose, as shown by Mäenpää *et al.* (23), and glycerol as well, produced marked decreases in ATP in liver and kidney (Figs. 1 and 2, Table II). The difference may reasonably be attributed to the fact that

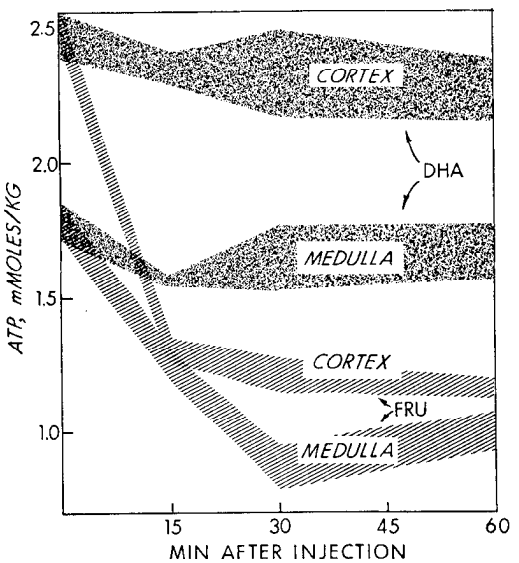

FIG. 2. Changes in ATP of renal cortex and medulla after injection of fructose (*FRU*) or dihydroxyacetone (*DHA*). The width of each band represents two standard errors.

TABLE II

Effects of glycerol on ATP and phosphorylated intermediates of kidney cortex and medulla

Rats were anesthetized with phenobarbital and treated by injection with 40 mmoles of glycerol per kg.

Metabolite	Time after injection			
	0 min	15 min	45 min	60 min
	μmoles/kg			
Kidney cortex				
ATP	2,470 ± 80	1,550 ± 66	1,630 ± 70	1,400 ± 170
Glycero-P	215 ± 48	9,240 ± 750	9,960 ± 280	11,700 ± 540
Dihydroxyacetone-P	12 ± 1	19 ± 2	9 ± 2	26 ± 2
Fructose-1,6-di-P	24 ± 4	17 ± 6	41 ± 4	49 ± 10
Ratio of glycero-P to dihydroxyacetone-P	18	495	1,070	457
Kidney medulla				
ATP	1,780 ± 70	1,290 ± 26	1,590 ± 37	1,550 ± 80
Glycero-P	109 ± 17	1,260 ± 90	1,650 ± 230	1,770 ± 170
Dihydroxyacetone-P	<10	38 ± 5	29 ± 4	29 ± 4
Fructose-1,6-di-P	83 ± 7	73 ± 16	72 ± 11	64 ± 5
Ratio of glycero-P to dihydroxyacetone-P		33	57	61

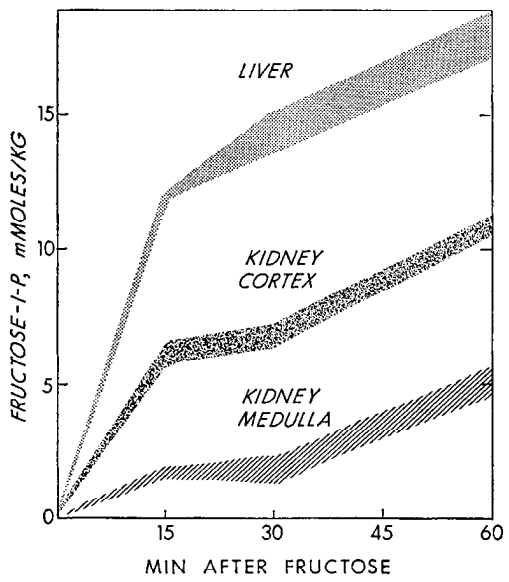

FIG. 3. Increase in hepatic and renal fructose-1-P after fructose injection. Fructose-1-P was less than 0.1 mmole per kg in 0.9% NaCl controls (represented as zero time).

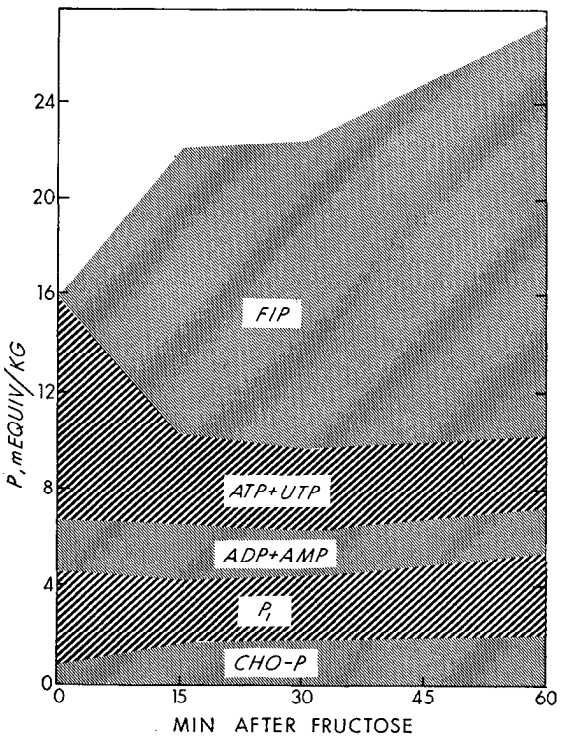

FIG. 4. The phosphate balance in liver after administration of fructose. Milliequivalents of phosphate per kg of liver are indicated for fructose-1-P (*F1P*), various nucleotides, P_i, and the sum of other measured carbohydrate phosphates (*CHO-P*).

with fructose and glycerol there occur large accumulations of the first phosphorylated product, in one case fructose-1-P (Fig. 3), in the other case glycero-P (Table II, Fig. 10). This apparently sequesters so much phosphate that there is insufficient P_i to permit maintenance of normal ATP levels.

This interpretation is supported by the fact that, in liver at least, P_i levels decreased after fructose (Fig. 4). The P_i levels were partially restored by 60 min. Fig. 4 also records the phosphate balance for all phosphate compounds measured. There is a net increase at 60 min of nearly 12 mmoles per kg, with a net decrease of 7 mmoles per kg in the sum of P_i plus mononucleotide phosphate. The net increase in phosphate was confirmed by measurement of total acid-soluble phosphate (Table III).

The possibility, however, that the fall in ATP after fructose may not be entirely due to P_i sequestration is raised by the results in kidney medulla. Here ATP levels fell almost as much as in liver or kidney cortex (Fig. 2), and yet fructose-1-P levels during the first 30 min were below 2 mmoles per kg.

Four hours after giving fructose, hepatic ATP, total adenylate,

TABLE III

Changes in hepatic acid-soluble phosphate after injecting fructose, dihydroxyacetone, or glycerol

Average values are shown for the number of animals in parentheses. The time after nutrient injection is given.

Nutrient given	Total acid-soluble phosphate	Phosphate of measured compounds	Undetermined acid-soluble phosphate
	mmoles/kg wet wt		
None (3)	30.1	16.5	13.6
Fructose, 30 min (3)	38.5	24.7	13.8
Difference	+8.4	+8.2	+0.2
Dihydroxyacetone, 30 min (3)	34.9	18.0	16.9
Difference	+4.8	+2.5	+2.3
Glycerol, 45 min (2)	36.8	24.3	12.5
Difference	+6.7	+7.8	−1.1

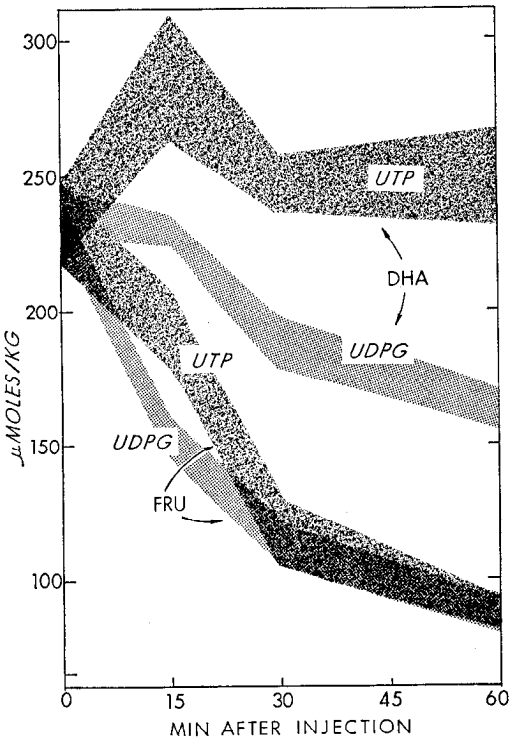

FIG. 5. Changes in hepatic UTP and UDP-glucose (*UDPG*) after injection of fructose (*FRU*) or dihydroxyacetone (*DHA*). The width of each band represents two standard errors.

and UTP had returned to control values, and fructose-1-P had fallen to 4% of the concentration at 60 min (not shown).

Fructose given at half the dosage of the above experiment (20 mmoles per kg) produced a fall in ATP at 30 min to 50% of normal in both liver and kidney cortex (not shown). Fructose-1-P levels in this case were about equal in liver, kidney cortex, and kidney medulla (3.5 to 4 mmoles per kg). Fructose at 5 mmoles per kg produced only a slight rise in fructose-1-P and no change in ATP.

Glycerol also caused a large increase in acid-soluble phosphate (Table III) which was accompanied by a 40% decrease in P_i at 15 min, and return to normal by 45 min (not shown). Some increase in acid-soluble phosphate occurs even with dihydroxyacetone (Table III), but this is not at the expense of high energy phosphate compounds, and P_i actually increased 20% (not shown).

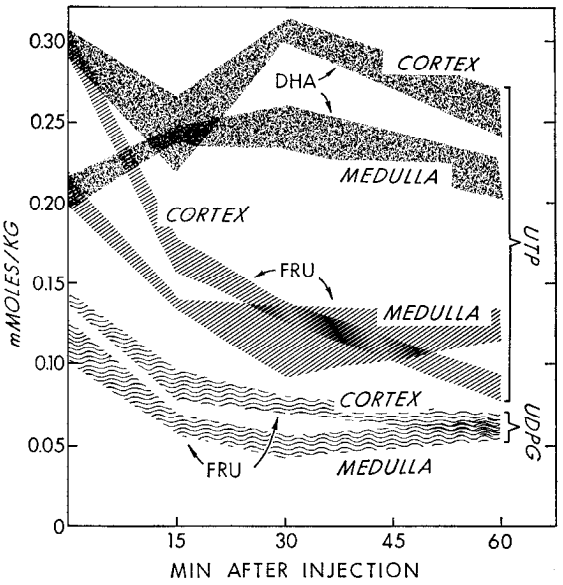

FIG. 6. Changes in UTP and UDP-glucose of renal cortex and medulla after injection of fructose (*FRU*) or dihydroxyacetone (*DHA*). Representation is the same as in Fig. 5.

It will be noted that the decreases in ATP after glycerol and fructose were associated with large drops in the sum of ATP, ADP, and AMP (Fig. 1). This is presumably the result of the well known phenomenon of deamination of AMP when its level is increased (see also "Discussion").

UTP in liver and kidney is sharply decreased by fructose (Figs. 5 and 6). The fall is somewhat slower than in the case of ATP. The percentage change is less in kidney medulla than in cortex, as was also true for ATP. UTP levels were not significantly affected by dihydroxyacetone or glucose. Curiously, after glycerol administration, in spite of the severe ATP depletion, a significant fall in UTP was not shown. With a dose of 40 mmoles of glycerol per kg no changes in UTP were observed at 15 min or 45 min, and at 60 min there may have been a decrease but it was not statistically significant (not shown). Similarly, in another experiment, with half the glycerol dose, ATP fell 45% without change in UTP (Table IV).

Riboflavin Deficiency—It was found that glycerol causes a greater rise of hepatic glycero-P in riboflavin-deficient rats than in controls (see below) and a more profound fall in ATP (Table IV). (It is to be noted that these marked changes occurred in spite of the fact that only half as much glycerol was given as in the experiment of Figs. 1 and 3.) This enhanced sensitivity to glycerol is attributed to the fact that in riboflavin deficiency there occurs a large decrease in glycero-P oxidase (see below).

A significant fall in P_i was evident in the deficient animals at 60 min, but not in the controls (Table IV). Again there is a discrepancy between the large (65%) fall in ATP and the modest (25%), and not statistically significant, fall in UTP.

Comparison of Control Levels of 10 Metabolites in Liver and Kidney—It order to appreciate the differences in response of kidney and liver to the several nutrients, it is desirable to compare the control metabolite levels in the two organs (Table V).

TABLE IV

Effects of glycerol on nucleotide phosphates in liver during riboflavin deficiency

Anesthetized weight controls or riboflavin-deficient rats were treated by injection with glycerol (20 mmoles per kg) or 0.9% NaCl 60 min before sampling the liver. The averages and standard errors are shown for the number of rats in parentheses.

Conditions	ATP	ADP	AMP	Total adenylate	UTP	P_i
			μmoles/kg			
Control (4)	3040 ± 100	820 ± 160	230 ± 30	4090 ± 100	788 ± 80	4120 ± 390
Glycerol (6)	1730 ± 100	940 ± 24	279 ± 35	2950 ± 100	782 ± 133	5400 ± 290
Deficient (3)	2480 ± 360	1330 ± 180	382 ± 42	4160 ± 590	237 ± 40	4750 ± 50
Deficient, glycerol (6)	810 ± 50	940 ± 20	567 ± 48	2320 ± 90	175 ± 11	3402 ± 300

TABLE V

Hepatic and renal metabolites of control animals given injections of 0.9% NaCl

Levels of metabolites are expressed in micromoles per kg, wet weight, except that of glycogen which is in millimoles per kg. Liver and kidney data in Columns 2 and 3 were obtained from the same six animals. Averages for four weight controls and three deficient animals appear in Column 4. Standard errors are shown.

1. Metabolite	2. Liver	3. Kidney		4. Liver[a]	
		Cortex	Medulla	Weight controls	Riboflavin-deficient
Glycogen	38 ± 5	0.77 ± 0.05	2.07 ± 0.20	32 ± 14	7.0 ± 2.5
Glucose	3500 ± 170	2970 ± 70	1550 ± 150	4340 ± 140	4290 ± 210
Glucose-6-P	28 ± 6	68 ± 6	73 ± 6	85 ± 8	53 ± 7
UDP-glucose	236 ± 9	141 ± 4	113 ± 13	438 ± 20	374 ± 30
Fructose-1,6-di-P	9 ± 1	24 ± 4	83 ± 7	11 ± 2	11 ± 2
Dihydroxyacetone-P	13 ± 1	12 ± 1	<10	12 ± 3	21 ± 3
Glycero-P	164 ± 22	215 ± 19	109 ± 17	178 ± 37	687 ± 155
3-P-glycerate	71 ± 5	251 ± 48	43 ± 7	60 ± 20	338 ± 48
Pyruvate	40 ± 3	46 ± 3	153 ± 17	37 ± 7	23 ± 10
Lactate	449 ± 6	473 ± 91	795 ± 230	538 ± 15	766 ± 21

[a] From riboflavin deficiency study. The weight controls were kept to the weight of the deficient animals by calorie restriction (see "Animals").

Glycogen of course is much lower in kidney than in liver. The medulla contains more glycogen and less glucose than cortex, as observed by Needleman, Passonneau, and Lowry (24). The other metabolite concentrations in liver tend to be closer to those of the renal cortex than to those of the medulla. The relatively high levels of medullary fructose diphosphate and the low levels of dihydroxyacetone-P, glycero-P, and 3-P-glycerate may be noted. The differences are in keeping with the greater glycolytic capacity of medulla than of either renal cortex or liver. Thus, high pyruvate kinase activity of medulla would tend to maintain a low 3-P-glycerate to pyruvate ratio as seen.

Kidney cortex is especially rich in 3-P-glycerate. Presumably in these fasting animals both liver and kidney cortex are converting pyruvate to glucose. Dihydroxyacetone-P levels are the same. Apparently under the circumstances the conversion of 3-P-glycerate to glyceraldehyde-P is more effective in liver than in kidney cortex.

Effects of Dihydroxyacetone on Metabolites in Liver and Kidney—After the intraperitoneal administration of dihydroxyacetone (40 mmoles per kg) the tissue levels of the nutrient itself rose promptly. In liver at 15, 30, and 60 min the respective concentrations were 27, 22, and 14 mmoles per kg. At 60 min the levels in kidney cortex and medulla were 14 and 19 mmoles per kg, respectively.

An hour after dihydroxyacetone administration (Fig. 7), there was a 200% rise of glycogen in liver with trivial changes in kidney. The glucose increase, but not the absolute level, was greater in medulla and probably indicates beginning spillage of glucose into the urine (24).

There was a striking increase in glucose-6-P in medulla, where it reached a level at the time shown (434 μmoles per kg), which is 6 times its initial value, and which on an absolute basis is 5 times the level attained in liver. The medulla is low in glucose 6-phosphatase activity (25), but it is even lower in fructose diphosphatase activity;[1] it is also relatively rich in hexokinase (25). Therefore it might be supposed that the glucose-6-P is formed from the increased glucose coming to it rather than from triose phosphate generated by the dihydroxyacetone. The only difficulty is that when glucose itself was administered to test this point, although even higher glucose levels were produced in the medulla, average glucose-6-P levels rose only a fifth as much as after dihydroxyacetone (not shown).

The UDP-glucose level fell in liver but not in kidney. This fall suggests that there was an enhancement of glycogen synthetase activity. This may have been due to one or more of the following: (*a*) stimulation by the rise in glucose-6-P; (*b*) the effect of insulin (26, 27) which would be released by the glucose rise; or (*c*) a direct effect of glucose (28).

In view of the fact that dihydroxyacetone-P must be the first product formed from dihydroxyacetone, there was a sur-

[1] R. G. Narins, unpublished observations.

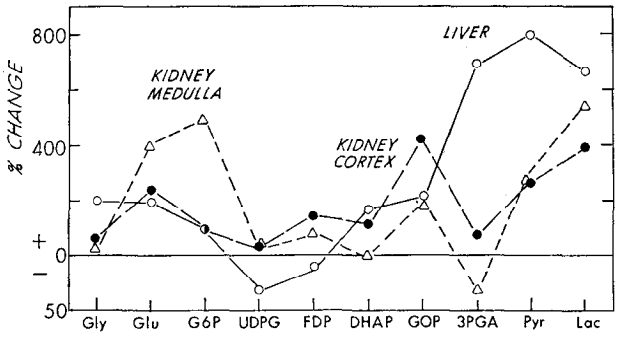

FIG. 7. Effects of dihydroxyacetone on hepatic and renal metabolites. Changes in tissue concentrations 60 min after injection of dihydroxyacetone are shown as the increments relative to 0.9% NaCl-injected controls. Control levels are given in Table IV. *Gly*, glycogen; *Glu*, glucose; *G6P*, glucose-6-P; *UDPG*, UDP-glucose; *FDP*, fructose-1,6-di-P; *DHAP*, dihydroxyacetone phosphate; *GOP*, glycero-P; *3PGA*, 3-phosphoglycerate; *Pyr*, pyruvate; *Lac*, lactate. O——O, liver; ●- - -●, kidney cortex; △- - -△, kidney medulla.

prisingly small effect in liver or kidney on dihydroxyacetone-P levels. There were likewise relatively modest changes in fructose diphosphate, which should be readily formed from dihydroxyacetone-P. At 15 and 30 min when, as noted, dihydroxyacetone levels were higher than at 60 min, dihydroxyacetone-P levels in liver were even lower than in Fig. 7 (22 and 28 μmoles per kg, respectively). Clearly phosphorylation of dihydroxyacetone is the limiting step in its consumption.

Glycero-P levels increased in all three tissues. The renal cortex which had the highest initial value was most affected, reaching a level 3 times that in medulla.

The metabolite which showed the biggest difference in response among the tissues was 3-P-glycerate. In liver it increased 7-fold, but in renal medulla, where the level was low to begin with, an actual decrease occurred. P-pyruvate levels are not shown, since they were not measured routinely. However, in both liver and kidney, the levels have been found to parallel those of 3-P-glycerate, at approximately the equilibrium ratio (1:2 for P-pyruvate to 3-P-glycerate). In the present case, for example, P-pyruvate rose 230% in renal cortex and fell 20% in medulla.

Lactate rose markedly in all three tissues. Pyruvate gains in kidney cortex and medulla were intermediate between the changes for lactate and those for 3-P-glycerate.

The pattern of change below the triose phosphates suggests that pyruvate kinase activity keeps ahead of glyceraldehyde-P dehydrogenase activity in kidney cortex and medulla, but not in liver.

All of the metabolites were also measured in liver at 15 and 30 min after dihydroxyacetone administration. The changes were similar to those at 60 min except that they were progressive, *i.e.* they were about half as great at 15 min as at 60 min (not shown). Some of the metabolites were also measured in kidney at 15 and 30 min and the results concur in general with those of Fig. 7.

Effects of Fructose on Metabolite Levels (Figs. 8 and 9)—The effects of fructose are not strictly comparable to those of dihydroxyacetone, since each was given at the same molar level (40 mmoles per kg), *i.e.* twice as much fructose was given by weight.

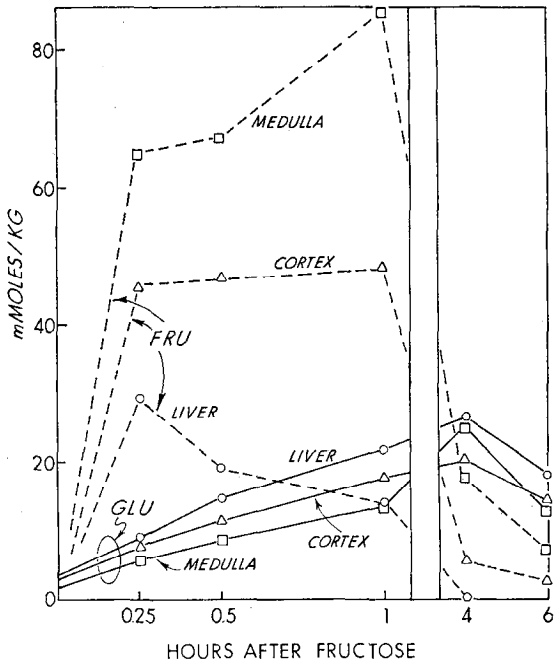

FIG. 8. Effects of fructose on hepatic and renal fructose (*FRU*) and glucose (*GLU*). Fructose of 0.9% NaCl controls was less than 0.2 mmole per kg.

Nevertheless, differential effects on the various metabolites may properly be compared.

In general, the metabolite changes in liver after giving fructose are quite similar to those observed by Exton and Park in perfused livers (5). The levels of glucose rose most rapidly in liver and most slowly in medulla, whereas fructose levels were just the reverse (Fig. 8). Presumably the very high fructose levels in medulla reflect the presence of unreabsorbed sugar concentrated in the tubules. At 4 and 6 hours, when fructose had disappeared from liver, there were still substantial levels in cortex and high levels in medulla. At 4 hours, when glucose levels were very high, the glucose level in medulla exceeded that in cortex, just as was seen in the case of dihydroxyacetone (Fig. 7).

An hour after giving fructose, glucose levels were much higher than after dihydroxyacetone. A few measurements with lower fructose dosage indicate that this may be due in part to the larger weight of fructose administered. The fact that very little glycogen was deposited (see below) is probably also a factor.

Heinz and Junghänel (6), after giving a single intravenous fructose injection (4.2 mmoles per kg), observed an increase in glucose levels in liver but not in whole blood, even after 20 min. This does not agree with the present findings. In a separate experiment from that of Fig. 8, following intraperitoneal injection of fructose (20 mmoles per kg), there occurred a prompt rise in plasma glucose (55% at 5 min, 115% at 10 min). By 30 min the increase was 175% and at that time nearly the same levels were found in plasma (12.1 mmoles per liter) and in liver (11.8 mmoles per liter). Conceivably the fact that whole blood was analyzed by Heinz and Junghänel and plasma was analyzed here may help to explain the discrepancy. Most experience shows little barrier for glucose between plasma and liver.

A major difference between the results with dihydroxyacetone and those with fructose is that after fructose injection, in spite

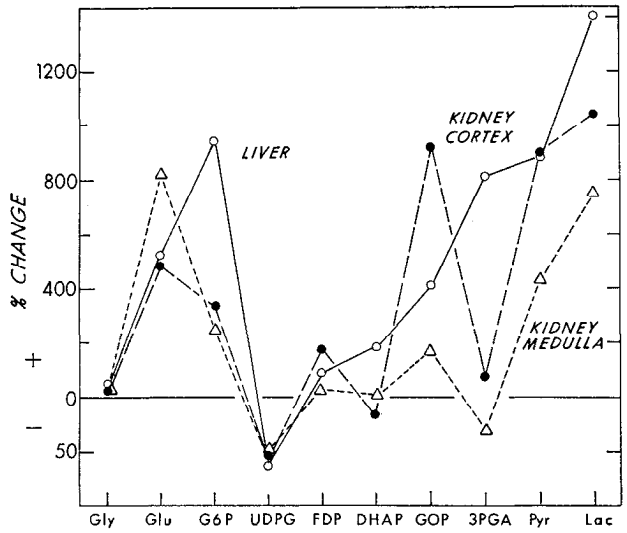

FIG. 9. Effects of fructose on hepatic and renal metabolites. Changes in tissue concentrations 60 min after injection of fructose are shown as the increments relative to 0.9% NaCl-injected controls. Control levels are given in Table IV. For abbreviations see legend to Fig. 7.

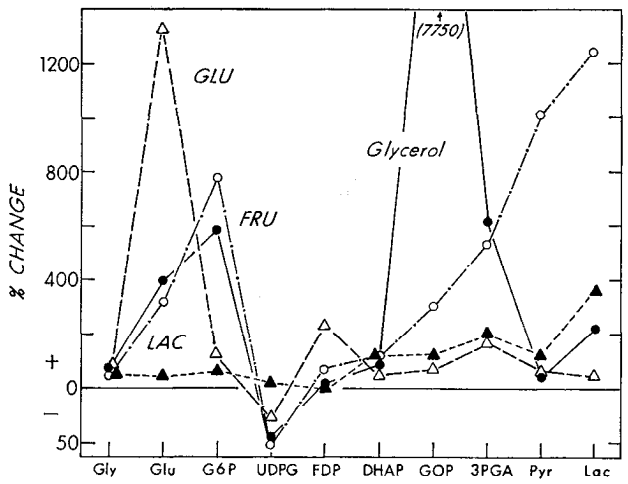

FIG. 10. Comparative effects on hepatic metabolites of injection of large amounts of glucose (*GLU*), fructose (*FRU*), glycerol and lactate (*LAC*). Since control values varied somewhat in the different experiments, the results have been put on the same basis by expressing the respective increments as the percentage of the levels for the controls of the fructose experiment (Table IV). All results are for 30 min after injection except with glycerol, in which case the time was 45 min. Results are from the same groups of animals as in Fig. 1 except for the lactate data which have been calculated from Hornbrook *et al.* (12). For abbreviations see legend to Fig. 7.

of the very high levels of glucose, there was almost no increase in liver glycogen (Fig. 9). Possibly this is due to the large fall in UDP-glucose which seems to be related in turn to the fall in UTP (Fig. 2), and to the P_i shortage. Arguing against this, however, is the very large increase in glucose-6-P, reported also by others (5, 6), which should have compensated for the low UDP-glucose level by stimulating glycogen synthetase. Conceivably the severe fall in ATP may have triggered the activation of phosphorylase, although the high glucose-6-P level would be expected to inhibit phosphorylase action. Fructose-1-P was tested on hepatic UDP-glucose pyrophosphorylase, and it has also been tested on glycogen synthetase (29) and in both cases was found not to inhibit. These observations do not imply that fructose does not ordinarily form glycogen. Over a longer time interval, fructose has been shown to be one of the best glycogen precursors (30).

Glucose-6-P increased more in liver and kidney cortex and less in medulla than was true after dihydroxyacetone administration. In liver the increase was dramatic. Possibly this is the result of very rapid glucose-6-P formation with very little diversion to glycogen. Glucose 6-phosphatase may simply have been overloaded. The possibility that fructose-1-P may inhibit glucose 6-phosphatase has not, however, been tested. Within analytical limits, fructose-6-P levels (not shown) kept pace with those of glucose-6-P, suggesting near equilibrium at the isomerase step.

As with dihydroxyacetone, the changes in dihydroxyacetone-P and fructose diphosphate after fructose addition are remarkably small, if it is true that most of the influx is via fructose-1-P and triose phosphates. Heinz and Junghänel (6) observed a large percentage increase in fructose diphosphate immediately after an intravenous injection of fructose, but this had subsided by the end of 15 min and the peak increase in absolute terms was only 50 μmoles per kg. Exton and Park (5) found an increase of only 20 μmoles per kg in liver perfused with 20 mM fructose. It would appear, therefore, that fructose-1-P cleavage is the limiting step in fructose utilization.

The changes in glycero-P, 3-P-glycerate, pyruvate, and lactate are similar to those seen after adding dihydroxyacetone except that they are generally greater, especially in renal cortex and liver. Increases in some or all of these after fructose in intact or perfused liver have been previously reported by a number of investigators (2–6, 31–35). Presumably the great influx of triose-P forces metabolites toward lactate and toward glycero-P (see "Discussion"). A special feature in kidney cortex is the 9-fold increase in glycero-P without increase in dihydroxyacetone-P, or any substantial change in the ratio of lactate to pyruvate. The changes in metabolites of liver at 30 min (Fig. 10) concur with those at 60 min. At 15 min the changes were similar but not quite so extensive (not shown).

Effect of Glycerol on Metabolites—Glycerol (Fig. 10) was given in the same molar quantity as in the case of fructose and dihydroxyacetone, *i.e.* only half as much by weight as for fructose. The effects were similar to those of fructose on hepatic glucose, glucose-6-P, UDP-glucose, fructose diphosphate, dihydroxyacetone-P, and 3-P-glycerate. There was much less effect on pyruvate and lactate than with either fructose or dihydroxyacetone.

As already noted, there occurs after glycerol administration an enormous increase in glycero-P. (Large increases after glycerol have previously been reported in intact (31, 36) and perfused (35) liver.) In view of the magnitude of the glycero-P increase, the failure of dihydroxyacetone-P to rise more than a small amount is quite surprising. The ratio of glycero-P to dihydroxyacetone-P increased from 18:1 in the controls to 670:1 at 45 min. There was no comparable rise in the lactate to pyruvate ratio which only increased from 21:1 to 40:1. In kidney cortex (Table II), 45 min after giving glycerol, the ratio of glycero-P to dihydroxyacetone-P had risen from 18:1 to 1070:1. In medulla, the ratio did not reach such impressive values (the highest ratio was 61:1).

The limiting step in glycerol use in liver and kidney is clearly glycerol-P oxidation. It is a question why this is so. Glycero-P dehydrogenase is an exceedingly active enzyme in both organs—far more active than glycerokinase. With glycero-P at such high levels the dehydrogenase would be expected to produce a corresponding increase in dihydroxyacetone-P or in the DPNH:DPN$^+$ ratio. The first did not occur, the second should have driven up the lactate to pyruvate ratio, which likewise did not occur. It is therefore possible that the new glycero-P is not freely presented to the dehydrogenase, but is instead oxidized by glycero-P oxidase, a much less active enzyme than the dehydrogenase. The oxidase is located in mitochondria, whereas the dehydrogenase is extramitochondrial. This presents a paradox, since according to Robinson and Newsholme (37), and as we have confirmed, glycerol kinase appears from measurements after differential centrifugation to be located outside of mitochondria and microsomes, *i.e.* glycero-P ought to be formed in the vicinity of the dehydrogenase rather than the oxidase.

Riboflavin Deficiency and Glycerol—That glycero-P oxidase (a flavoprotein), rather than glycero-P dehydrogenase, is the limiting factor in gluconeogenesis from glycerol seems confirmed by the experiment with riboflavin-deficient rats (Fig. 11). In these animals there was a 73% reduction in hepatic glycero-P oxidase but no difference (compared to weight controls) in glycero-P dehydrogenase or glycerokinase (Table VI). For the experiment, glycerol administration was reduced to half of that used above. In control animals, glycero-P increased 12-fold. In deficient animals with much higher initial values (Table V) the level increased 26-fold, reaching a level 9 times that attained by the control group. The ratio of glycero-P to dihydroxyacetone-P rose from 33:1 to 920:1 in the deficient liver. There was little or no sign of gluconeogenesis, but the increases in metabolites of the Embden-Meyerhof pathway below the triose phosphates suggest a stimulation of flux in that direction, perhaps secondary to the profound fall in ATP.

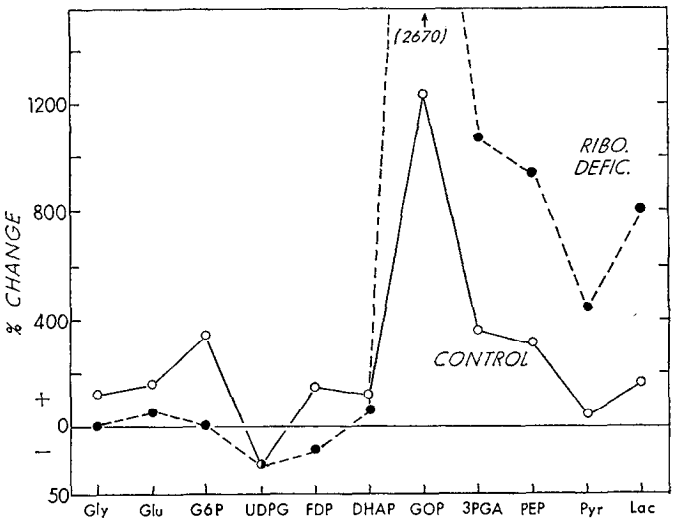

FIG. 11. Effect of glycerol on hepatic metabolites in riboflavin deficiency. Glycerol (20 mmoles per kg) was given to riboflavin-deficient rats and to weight controls (see "Animals"). Tissues were frozen 60 min after injection. There were six or seven animals in each group. The changes due to glycerol in control and deficient groups have been put on the same basis by calculating the respective increments as the percentage of the levels in control animals that did not receive glycerol. Values for control and deficient animals that did not receive glycerol are shown in Table IV. For abbreviations see legend to Fig. 7.

The over-all rate of glycerol utilization was reduced in the deficient animals, as shown by the fact that hepatic glycerol levels were twice as high in the deficient as in the control livers (not shown).

Effect of Glucose on Metabolites (Fig. 10)—Only a few animals have been studied after having been given glucose, but these are probably sufficient to show that in liver, aside from the expected very great increase in glucose, and the rise in glycogen, metabolite changes are generally smaller than in the case of the other three nutrients. An exception may be fructose diphosphate which was elevated 200%, both at 30 min, as shown, and also at 15 min. Of special interest is the relatively small increase in glucose-6-P, less than with any other nutrient tested except lactate. This would seem to show that glucose-6-P can be formed much more rapidly from triose-P than from glucose, and illustrates the well known impoverishment of the liver in regard to glucose-phosphorylating enzymes, particularly after fasting. Similarly, in renal cortex and medulla glucose administration resulted in much smaller increases in glucose-6-P (not shown) than in the case of either fructose or dihydroxyacetone.

Comparison with Changes after Lactate—An earlier study was made with similar methods and procedures on the effect of lactate on hepatic metabolites (38). It may be of interest to compare the data obtained with the present results (Fig. 10), since with lactate the influx is from the terminal end of the Embden-Meyerhof pathway rather than from the middle or at the beginning. The dosage was somewhat smaller than in the present studies (18 mmoles per kg).

Considering the smaller dosage used, it would appear that with lactate there is a larger effect than with glucose on metabolites below fructose diphosphate, but a smaller effect above. On the other hand, lactate seems to have a smaller effect on metabolites below glyceraldehyde-P than either fructose or

TABLE VI

Effect of riboflavin deficiency on three hepatic enzymes

Glycerokinase was measured on tissue from rats described in Table IV which had been 7 weeks on the experimental diets. Some of these animals had received glycerol 60 min prior to sampling the liver; this had no discernible effect on the enzyme activities and the results are pooled. The other two enzymes were measured on animals fed the same diets as in Table IV, but for only 6 weeks, and they were not fasted. Standard errors are shown for the number of animals given in parentheses.

Treatment	Weight	Glycerokinase	Glycero-P dehydrogenase	Glycero-P oxidase
	g	*moles/kg protein/hr*		
Riboflavin-deficient	75 ± 6 (15)	1.10 ± 0.02 (8)	35 ± 2 (6)	0.048 ± 0.008 (6)
Weight controls	78 ± 1 (12)	1.03 ± 0.04 (9)	29 ± 1 (6)	0.178 ± 0.014 (6)
Normal controls	333 ± 7 (10)	1.26 ± 0.05 (4)	53 ± 2 (6)	0.185 ± 0.007 (6)

dihydroxyacetone. Exton and Park (5) made the same observation in comparing lactate with fructose in perfused liver. Presumably a flood of carbohydrate can travel downward faster from triose phosphates than gluconeogenesis can create a flow upward from lactate. It is remarkable that all five nutrients produce similar modest increases in dihydroxyacetone-P. Both liver and kidney are clearly well equipped to transfer metabolites from the center of the Embden-Meyerhof system to the extremes.

DISCUSSION

Accumulation of Fructose-1-P and Glycero-P and the Consesequences—Kjerulf-Jensen first observed accumulation of fructose-1-P in intact liver after fructose administration (36). Since then this has been confirmed by several groups (3, 6) although levels in the liver have not been reported as high as those in the present experiments. Trapping, in the form of fructose-1-P, of very large amounts of phosphate is the apparent cause of the lowering of levels of ATP, P_i, and total adenylate. The present findings completely confirm the recent report of Mäenpää *et al.* (23). These investigators, who gave fructose intravenously, found that hepatic P_i fell to half within 1 min and to a third within 5 min. ATP and total adenylate fell almost as much but more slowly, suggesting that the P_i effect was primary. The decrease in total adenylate was associated with a large rise in plasma allantoin and uric acid, indicating that adenylate degradation did not stop at the IMP stage.

In contrast to these results in the intact animal, Exton and Park (5) found that in livers perfused with fructose, in spite of a high rate of glucose formation, no fall in ATP or total adenylate occurred. Presumably, the P_i supply from the perfusion fluid was adequate to balance any diversion to fructose-1-P, although the latter was not in fact measured.

The fall in ATP after fructose administration is presumably the cause of the fall in UTP. The surprising thing is that ATP and UTP changes are not better correlated. In liver there was a 15-min lag between the fall in ATP and that of UTP, and after glycerol administration ATP fell sharply in liver with at most a questionable fall in UTP. Conversely, in renal cortex after fructose the percentage fall in UTP at 60 min exceeded that of ATP. Examination of data for individual animals, which provide a broader range of values, confirms the lack of correlation between the two triphosphates. Although the fall in ATP results in a fall in total adenylate, total uridylate may not be similarly affected. If not, this would explain the more rapid and greater decreases in ATP than in UTP.

Pathway of Fructose Utilization—A number of investigators have discussed the question whether all fructose utilization proceeds via fructose-1-P, to dihydroxyacetone-P and glyceraldehyde (39–41). The present results add only a little to the conclusion of the majority that most but perhaps not all of the traffic follows this route. The very high levels of fructose-1-P which accumulate, and the fact that these levels fall rapidly when fructose has largely gone, certainly show that fructose-1-P is on a major pathway. Compatible with this is the fact that the metabolite profile after fructose is much like that after dihydroxyacetone administration (especially when the failure of fructose to form glycogen during the first hour is taken into account).

The data presented may be more decisive in regard to the fate of the glyceraldehyde moiety. As Kattermann *et al.* (33) point out, liver contains enzymes which could permit glyceraldehyde to be used by three different routes: (*a*) by direct conversion to glyceraldehyde-P (triose kinase), (*b*) by reduction to glycerol followed by phosphorylation, and (*c*) by oxidation to glycerate followed by phosphorylation to 2-P-glycerate. The metabolite changes reported here indicate that the first route is probably favored. Since fructose is utilized faster than glycerol, if glycerol were the first product, glycerol-P levels should have been very high, whereas they are little higher than those observed with dihydroxyacetone. Similarly if glycerate were the first product, higher 3-P-glycerate levels than those produced with dihydroxyacetone might have been expected. In kidney the data suggest the possibility that more of the glyceraldehyde is metabolized via glycerol (see below).

Accumulation of Metabolites Below Triose Phosphates—In the case of dihydroxyacetone, fructose, and glycerol, there is an accumulation in liver and renal cortex of metabolites below the triose phosphates on the glycolytic pathway. All three nutrients enter the metabolic pool, in part at least, at the triose-P level. The changes could therefore be due either to forcing metabolites downstream or to inhibition of gluconeogenesis from lactate entering the liver and kidney from the periphery. Both processes may contribute, but favoring the first alternative is the fact that Exton and Park (5) observed equally marked increases in these metabolites with fructose addition in the case of perfused liver, where no outside lactate would be available. Another argument on this side is the fact that fructose induced larger increases in 3-P-glycerate and pyruvate than did administration of lactate itself (Fig. 10).

In the case of fructose and glycerol a third possibility exists for the rise in 3-P-glycerate, pyruvate, and lactate, *i.e.* stimulation of glycolysis by the decreases in ATP. Since increases of the same magnitude occurred after dihydroxyacetone administration with no change in ATP, it is believed that this is not a major factor, except possibly in the case of the riboflavin-deficient animals. A point of interest, in confirmation of earlier studies (35), is the fact that fructose causes a greater increase than glycerol in hepatic pyruvate levels. In the present case the pyruvate increase was 20-fold greater after fructose, whereas the 3-P-glycerate response was about the same after fructose as after glycerol (Fig. 10). Each nutrient should have facilitated the pyruvate kinase step because of the lowered ATP. Glycero-P at levels as high as those observed were only very slightly inhibitory to pyruvate kinase *in vitro*.[2] Nevertheless, something seems to have produced a block at this point. (P-pyruvate measurements in another experiment (Fig. 11) show that there is no limitation after glycerol between 3-P-glycerate and P-pyruvate.)

The failure of glucose to produce much change in metabolite levels below fructose diphosphate seems attributable to its entering the metabolic pool above the triose phosphates, and shows the degree of protection possessed by the liver against unnecessary glycolysis.

Fructose Diphosphate to Triose Phosphate Ratios—It has been repeatedly shown that in various tissues the ratio between fructose diphosphate and triose phosphates is much greater than the equilibrium ratio. Ordinarily under conditions of glycolysis, with flow from fructose diphosphate to triose-P, this might be the result of aldolase inactivity. In the present case,

[2] M. H. Gay, personal communication.

liver and renal cortex, except after glucose administration, the flow should be in the opposite direction. Nevertheless, fructose diphosphate was still in excess in every case. Thus, in renal cortex the calculated equilibrium levels for dihydroxyacetone-P before and after dihydroxyacetone administration are 245 and 380 μmoles per kg, respectively, whereas observed values were only 12 and 25 μmoles per kg. (At 38° the equilibrium constant for (dihydroxyacetone-P) (glyceraldehyde-P):(fructose diphosphate), is approximately 100 μmoles per liter.) Similar discrepancies were seen after treatment with fructose and glycerol. In liver, after fructose injection, the dihydroxyacetone-P level rose from 13 to 37 μmoles per kg, but for equilibrium with fructose diphosphate the expected change would have been from an initial level of 150 μmoles per kg to a final level of 206 μmoles per kg. In no instance in kidney or liver were dihydroxyacetone-P levels as high as the equilibrium constant would predict. (These calculations are based on the assumption that glyceraldehyde-P is in equilibrium with dihydroxyacetone-P (1:25 ratio). Glyceraldehyde-P levels were below the level of sensitivity of the methods, but free glyceraldehyde-P levels could scarcely exceed the assumed equilibrium levels if the source of glyceraldehyde-P is dihydroxyacetone-P.) The conclusion would seem to be that part of the fructose diphosphate measured is either bound or otherwise sequestered from the free pathway between triose-P and fructose-6-P.

Ratios between Glycero-P and Dihydroxyacetone-P—Bücher and Kingenberg (42) and Hohorst *et al.* (43, 44) have found that under a variety of experimental conditions the ratios of lactate to pyruvate and those of glycero-P to dihydroxyacetone-P are related to each other in the manner expected if both systems are in equilibrium with a common pool of DPN^+ and DPNH. This does not appear to be true for some of the present experiments. It was seen that after glycerol administration the ratio of glycero-P to dihydroxyacetone-P increased 20 times more than the ratio of lactate to pyruvate. This may be the result of a special situation, since glycero-P is the first product. More difficult to explain on the basis of the Bücher-Hohorst concept is the observation that in renal cortex, after giving fructose, the ratio of glycero-P to dihydroxyacetone-P increased 13-fold with scarcely any change in the lactate to pyruvate ratio. Possibly in the kidney a large fraction of the glyceraldehyde produced by fructose-1-P cleavage is metabolized via glycerol and therefore causes the same disproportionate increase in glycero-P as seen after giving glycerol itself. Heinz (45) has reported that kidney contains a very high level of a TPN-linked alcohol dehydrogenase (46) which might be responsible for glyceraldehyde reduction. Heinz found that this enzyme increased with fructose feeding.

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