Influence of Dietary Composition on the Capacity of Glucose Formation in Liver of Rats

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The influence of the levels of dietary carbohydrate, protein, and fat on the capacity of glucose formation in liver slices and on the activities of gluconeogenic and glycolytic enzymes in the liver of rats were investigated. Incorporation of radioactivity from pyruvate- ^{14}C into glucose by liver slices not only increased in carbohydrate deficient groups, but also was affected by fat content of diets. PEPCK, FDPase, and G6Pase of rat liver were affected by the levels of dietary carbohydrate. Moreover, PEPCK and FDPase were increased by increasing the protein content of diets and G6Pase was increased by increasing the fat content of diets. The ratios PEPCK/PK and G6Pase/GK were increased significantly by decreasing the carbohydrate content of diets, but the ratio FDPase/PFK did not show any significant change among dietary groups. From these experiments, it was concluded that the capacity of glucose formation in the liver of rats was changed by carbohydrate and fat content of diets and changes in gluconeogenic capacity with diets were correlated with changes in G6Pase activity.

Unless carbohydrate is continually ingested with food, the needs for glucose in the animal must be met by the degradation of the reserve polysaccharide of the body (glycogenolysis) and/or by the conversion of non-carbohydrate precursors to glucose (gluconeogenesis).

It has been shown that the sum of percent incorporation of ^{14}C from amino acid- ^{14}C or pyruvate- ^{14}C into blood glucose and liver glycogen was increased in rats fed a low carbohydrate diet high in protein^{1,2)} or high in fat²⁾ than in rats fed a high carbohydrate diet. It was also increased in fasted rats than in normally fed rats.^{3,4)} Increased incorporation can be the consequence of either increased

glucose synthesis, or decreased utilization of glucose, or both. Evidences considered to support all these possibilities have accumulated by studies utilizing tissue slices⁵⁾ and measuring activities of gluconeogenic and glycolytic enzymes of the liver and kidney cortex⁶⁾ which are the main site of gluconeogenesis. Further, in recent studies on diabetic animals and cortisone treated normal animals, increased formation of glucose has been shown *in vivo*⁷⁾ and *in vitro*.^{8~10)}

From the facts, it is assumed that the capacity of glucose formation in rat liver depends

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3) G. B. Nadkarni, B. Friedmann and S. Weinhouse, *J. Biol. Chem.*, **235**, 420 (1960).

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upon the levels of carbohydrate in diets. Therefore, the purpose of this study is to investigate the situation in more detail by tissue slices. Moreover, since it is possible to deduce the capacity of glucose formation in tissues from the activity of gluconeogenic enzyme or the ratio of gluconeogenic enzyme activity to glycolytic enzyme activity, the influence of the levels of dietary carbohydrate on the activities of gluconeogenic and glycolytic enzymes was investigated.

METHODS AND MATERIALS

Animals and Diets. Donryu strain rats were purchased from Nippon Rat Co., Urawa. Unless otherwise stated, all animals were females, about 100–170

g in body weight. The animals were housed in individual cages placed in a temperature regulated room at about 25°C and 12-hr per day of darkness and light and were weighed daily. Diets and water were available *ad libitum* unless stated otherwise. Diets were prepared by substituting; (a) fat for carbohydrate at constant protein, (b) protein for fat at constant carbohydrate. The composition of diets are shown the Tables I and II. All the test diets contain the same amounts of salts per 100 calculated calories. The amounts of the mixture of vitamins used were proportional to the levels of protein in diets, namely, 8.5 g for 250 g of casein and 25.5 g for 750 g of casein. The stock diet was pellets delivered from Central Laboratories for Experimental Animals, Tokyo.

Materials. Glucose 6-phosphate dehydrogenase (from yeast), aldolase (from rabbit muscle), ATP-Na₂H₂-3H₂O, ADP-Na₃, ITP-Ba₂, FDP-Na₃H, F6P-Ba, G6P-Na₂, PEP-Na-H₂O, OAA and NADP-NaH₂ were purchased from C. F. Boehringer & Soehne GmbH, Mannheim. Sodium pyruvate-3-¹⁴C was purchased from Radiochemical Centre, Amersham, Buckinghamshire, England. Chemicals were used without further purification.

Preparation of liver slices and assay of glucose formation from pyruvate-3-¹⁴C. Slice were cut from livers by sliding a razor blade along the surface of a glass slide with a 0.3 mm deep groove which was pressed on to the liver samples. Liver slices (100 mg) were transferred to vessels (25 ml vials fitted with rubber

TABLE I. COMPOSITION OF DIET^a—1

	25 P-S High carbohydrate diet (g)	25 P-F High protein diet (g)	75P-S High fat diet (g)
Casein	250	750	250
α -Starch	640	123	—
Corn oil (or Lard)	50	50	306
Mineral mixture B ^b	50	50	50
Vitamin mixture ^b	8.5	25.5	8.5
Choline chloride	1.5	1.5	1.5

TABLE II. COMPOSITION OF DIETS^a—2

	75 P-F (g)	50 P-F (g)	25 P-F (g)	12 S-P (g)	35 S-P (g)	63 S-P (g)
Casein	750	500	250	250	250	250
α -Starch	—	—	—	123	358	640
Lard ^c	99	203	306	257	163	50
Mineral mixture B	50	50	50	50	50	50
Vitamin mixture	25.5	17	8.5	8.5	8.5	8.5
Choline chloride	1.5	1.5	1.5	1.5	1.5	1.5

^a Diets were supplemented further with 6000 IU of vitamin A, 600 IU of vitamin D and 100 mg of vitamin E per kg of diet. In order to prepare diets isocaloric per g of diet, cellulose powder was used.

^b A. E. Harper, *J. Nutrition*, **68**, 405 (1959). See reference 32).

^c Butter was used as dietary fat instead of lard in Expt. 2.

stoppers and hanging glass center wells) containing 2.5 ml of a high potassium medium¹¹⁾ (40 mM KHCO_3 , 10 mM KCl, 20 mM MgCl_2 , 10 mM CaCl_2 , and 60 mM potassium pyruvate containing 1 μCi of sodium pyruvate-3- ^{14}C , pH 7.4) and incubated for 60 min at 37°C in an atmosphere of O_2 - CO_2 (95:5) with shaking. At the end of the incubation, 0.2 ml of the mixture of ethanol amine and ethylene-glycol monomethylether (1:2 v/v) was introduced through the rubber stopper with a syringe and hypodermic needle on to the filter paper in the center well. To ensure complete liberation of CO_2 , 0.5 ml of 0.2 N H_2SO_4 was injected into the medium and shaking was continued for 30 min. The filter paper containing the CO_2 was allowed to dry, flattened to the bottom of a vial, and 10 ml of dioxane scintillation solution (4 g of PPO, 0.2 g of POPOP, 60 g of naphthalene, 20 ml of ethylenglycol, 100 ml of methanol and dioxane to one liter) was added. Reaction medium was transferred to a centrifuge tube and centrifuged at 2500 rpm for 10 min. One ml of supernatant was passed through an Amberlite MB-2 column (0.8×15 cm). The column was eluted with water and the first 15 ml of eluates was collected. An aliquot of purified eluate was used for determination of glucose- ^{14}C . Tissue slices were digested with 2 ml of 30% KOH in boiling water for 40 min, and glycogen was precipitated by the addition of 5 ml of ethanol and purified according to Hassid *et al.*¹²⁾ All samples were counted in a Packard Tri-Carb 314 EX type liquid scintillation spectrometer. Medium glucose and liver glycogen were determined by glucose-oxidase method and phenol-sulfuric acid method, respectively.

The results were expressed as μmoles of substrate converted into glucose per 100 g of body weight. In all experiments, the incorporation rate of ^{14}C into glucose, liver glycogen, and CO_2 was expressed as counts per minute per 100 g of body weight. The incorporation of ^{14}C continued at a roughly linear rate throughout the incubation period.

Preparation of enzyme extracts and assay of enzyme activities. Rats were killed by blows on the head and allowed to bleed freely by decapitation before excision of tissue. The liver was quickly removed, cooled on ice, weighed, cut into small pieces and homogenized in a Potter-Elvehjem type glass homo-

genizer with a Teflon pestle.

PEPCK was assayed by a modification of the method of Nordlie *et al.*¹³⁾ The assay was based on the measurement of PEP formed during incubation of the enzyme with saturating levels of OAA and ITP. PEP was cleaved with mercuric ion and the liberated P_i was estimated by the method of P. S. Chen *et al.*¹⁴⁾ One g of rat liver was homogenized in 4 volumes of ice cold 0.25 M sucrose and the homogenate was centrifuged at $20,000 \times g$ for 90 min at 0°C. The supernatant fraction was used for the enzyme assay. PEPCK activity in the supernatant was stable for at least one week when stored in the frozen state at about -15°C. Inorganic phosphate derived from the enzyme solution (endogenous) and from reagents were determined under the condition of the enzyme assay without treatment of HgCl_2 .

PK was assayed by the method of Tanaka *et al.*¹⁵⁾ which was based on the measurement of pyruvic acid formed during incubation of the enzyme with saturating levels of PEP and ADP. The red color of 2,4-dinitrophenylhydrazone of pyruvic acid in the alcoholic alkaline solution was estimated at 530 m μ . The liver was homogenized with 3 volumes of 0.04 M KCl solution and the homogenate was centrifuged at $20,000 \times g$ for 90 min at 0°C. The supernatant was diluted with 10 volumes of water and used for the enzyme assay. PK activity was stable for several weeks in the frozen state. Blank assay was performed using water instead of the substrate.

G6Pase assay was performed by a slight modification of the method of Fitch *et al.*¹⁶⁾ which was based on the measurement of P_i formed during incubation of the enzyme with saturating levels of G6P. P_i was determined colorimetrically by a modification of the method of Furchgette *et al.*¹⁷⁾ The homogenates were prepared by the same procedure as the PEPCK assay and filtered through two layers of gauze and stored in a deep freezer at about -15°C. Under these conditions, the enzyme activity was stable for several weeks. Frozen homogenates were thawed and aliquots of these homogenates were supplemented with neutral

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16) W. M. Fitch, R. Hill and I. L. Chaikoff, *J. Biol. Chem.*, **234**, 1045 (1959).

17) R. E. Furchgette and T. deGubareff, *ibid.*, **223**, 377 (1956).

TABLE III. ENZYME ASSAYS

Enzyme	Assay medium	Volume of reaction mixture (ml)	Products determined	References
PEPCK	OAA, 3.35 μ moles; GSH, 0.80 μ mole; ITP, 4.50 μ moles; MgSO ₄ , 11.2 μ moles; NaF, 10 μ moles; Tris-HCl, 73.0 μ moles; pH 8.0	0.65	HgCl ₂ -labile P _i	13)
PK	MgSO ₄ , 5 μ moles; KCl, 100 μ moles; ADP, 2 μ moles; PEP, 2 μ moles; 1.0 Tris-HCl, 50 μ moles; pH 7.5	1.0	Pyruvic acid	15)
G6Pase	G6P, 20 μ moles; maleate-NaOH, 20 μ moles; pH 6.8	1.0	P _i	16)
HK (GK)	ATP, 9 μ moles; NADP, 2.25 μ moles; MgCl ₂ , 22.5 μ moles; glucose, 300 μ moles; G6P dehydrogenase, 0.84 unit (International Unit); Tris-HCl, 132 μ moles; pH 7.5	3.0	NADPH	18)
FDPase	FDP, 0.5 μ moles; cysteine, 100 μ moles; MgSO ₄ , 50 μ moles; Tris-HCl, 250 μ moles; pH 7.5	5.0	P _i	19)
PFK	ATP, 2 μ moles, hydrazine, 2 μ moles; F6P, 55 μ moles; aldolase, 1 μ g; Tris-HCl, 50 μ moles; pH 7.4	1.0	Alkaline-labile P _i	20)

sodium deoxycholate solution (to a final concentration of 0.2%, w/v) prior to use for the assay of G6Pase.

Glucokinase assay was a modification of the method of Sharma *et al.*¹⁸⁾ The method was based on the measurement of the rate of production of NADPH at 340 m μ in the presence of saturating levels of glucose, ATP, NADP, and an excess of G6P dehydrogenase. One g of rat liver was homogenized with 2 volumes of 0.15 M KCl solution containing 5 mM MgCl₂, 5 mM EDTA, and 10 mM 2-mercaptoethanol (pH 7.3), and the homogenate was centrifuged at 20,000 $\times g$ for 90 min at 0°C. The supernatant fraction was dialyzed against 500 volumes of the buffer solution used for homogenization at 4°C for 150 min. Dialysate was diluted to 2-folds with water and used immediately for the enzyme assay because the enzyme activity was unstable. As the molar coefficient of absorbancy of NADPH at 340 m μ , the value 6.2 $\times 10$ cm²mole⁻¹ was used for calculation of the enzyme unit.

FDPase was assayed by the method of Taketa and Pogell¹⁹⁾ with a slight modification. One g of rat liver was homogenized in 4 volumes of ice cold 0.154 M KCl solution and the homogenate was centrifuged at 20,000 $\times g$ for 90 min at 0°C. The supernatant was diluted to 50-folds with ice cold water prior to use for the enzyme assay. The determination of liberated inorganic phosphate was carried out by the

method of Chen *et al.*¹⁴⁾ Enzyme activity was stable for at least two weeks in a deep freezer at about -15°C.

PFK assay was performed by a modification of the method of Weber *et al.*²⁰⁾ Triose phosphate formed from FDP by the action of aldolase was measured by the procedure of Sibley and Lehninger.²¹⁾ Namely, triose phosphate trapped with hydrazine was hydrolyzed by sodium hydroxide and alkali-labile phosphate was determined by the method of Chen *et al.*¹⁴⁾ Blank assay was performed using water instead of the substrate. One g of rat liver was homogenized with 3 volumes of 20 mM Tris-HCl buffer solution containing 5 mM MgSO₄ and 0.1 mM EDTA (pH 8.0) and the homogenate was centrifuged at 20,000 $\times g$ for 90 min at 0°C. The supernatant was used for the enzyme assay.

All enzyme assays were performed under conditions in which the amount of product formed was proportional to enzyme concentration and to incubation time, and conditions of enzyme assay were summarized in Table III.

One unit of enzyme activity was defined as the amount required to form one μ mole of product per minute under the assay condition, and the activities of enzymes were expressed as units of enzyme per 100 g of body weight.

18) C. Sharma, R. Manjeshwar and S. Weinhouse, *J. Biol. Chem.*, **238**, 3840 (1963).

19) C. A. Taketa and R. M. Pogell, *ibid.*, **240**, 651 (1965).

20) G. Weber and R. L. Singhal, *Life Science*, **4**, 1993 (1965).

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Plan of experiments.

Experiment 1. In order to determine the influence of a high carbohydrate, a high fat, and a high protein diet on the capacity of glucose formation in liver slices and on the activities of gluconeogenic and glycolytic enzymes in the liver, rats were maintained on the stock diet for at least two days since arrival, divided into three groups of five animals and fed 25 P-S (high carbohydrate diet), 25 P-F (high fat diet), or 75 P-S (high protein diet), respectively, for two weeks.

Experiment 2. Rat were maintained on the stock diet for two days, divided into four groups of six animals, and fed 75 P-F, 25 P-F, 35 S-P, or 63 S-P, respectively, or divided into six groups of five animals and fed 75 P-F, 50 P-F, 25 P-F, 12 S-P, 35 S-P, or 63 S-P, respectively, for two weeks. In order to change the level of carbohydrate in diets, 75 P-F, 50 P-F and 25 P-F diets were prepared by isocaloric substitution of calories from protein for calories from fat at constant very low carbohydrate and 25 P-F, 12 S-P, 35 S-P and 63 S-P diets were prepared by isocaloric substitution

pyruvate-3- ^{14}C into medium glucose increased in rats fed the high fat diet, but a slight increase was observed in the high protein group. Respiratory $^{14}\text{CO}_2$ showed a significant increase in rats fed the high fat diet, but the incorporation of radioactivity was less than 1/50 of that into glucose, and, moreover, the incorporation of ^{14}C into liver glycogen was negligible. Amounts of glucose formed did not change among test groups (Table IV). Although increased incorporation of ^{14}C from pyruvate-3- ^{14}C into glucose was observed in rats fed low carbohydrate diets, the rates of incorporation were constant when the carbohydrate content was above 35% of diet (Table V). Thus, the higher response to low content of carbohydrate and the lower response to high content of protein in diets were indicated in experiments 1 and 2.

Recently, Friedmann *et al.*²²⁾ showed that the recovery per cent of ^{14}C from pyruvate-3- ^{14}C into blood glucose was increased in rats

TABLE IV. INFLUENCE OF A HIGH CARBOHYDRATE DIET, A HIGH PROTEIN DIET, AND A HIGH FAT DIET ON INCORPORATION OF ^{14}C FROM PYRUVATE-3- ^{14}C INTO GLUCOSE, GLYCOGEN, AND CO_2 BY LIVER SLICES OF RATS

Diet	CO_2 cpm $\times 10^{-4}$	Glycogen		Glucose	
		Amounts mg/100 g of body weight	Total counts	Amounts mg/100 g of body weight	Total counts cpm $\times 10^{-6}$ /100 g of body weight
High carbohydrate diet	6.11 ^{a)} (5.88, 6.33)	77.3 (58.1, 96.5)	— ^{b)}	97.2 (83.3, 111)	4.84 (4.24, 5.40)
High protein diet	8.98 (7.35, 10.6)	30.0 (24.9, 35.4)	—	104 (94.3, 114)	5.31 (5.18, 5.34)
High fat diet	12.0 (11.9, 12.3)	29.0 (19.1, 38.8)	—	91.4 (70.8, 112)	6.94 (6.18, 7.69)

^{a)} Mean for two animals. Individual value is indicated in parentheses.

^{b)} Counts of glycogen- ^{14}C was negligible in this study.

of calories from fat for calories from carbohydrate at constant protein. Caloric intake of each animal was restricted to about 40 cal per 100 g of body weight per day.

fed a high fat and a high protein diet, and the higher rate of incorporation was observed in the fat-fed group than that of the protein-fed group. Our experimental results by liver slices agree closely with those of *in vivo* study

RESULTS AND DISCUSSION

As shown in Table IV, incorporation of

22) B. Friedmann, E. H. Goodman and S. Weinschouse, *J. Biol. Chem.*, **242**, 3620 (1967).

TABLE V. INFLUENCE OF DIETARY CARBOHYDRATE, PROTEIN, AND FAT ON INCORPORATION OF ^{14}C FROM PYRUVATE-3- ^{14}C INTO GLUCOSE BY LIVER SLICES OF RATS

	Diet			
	75 P-F	25 P-F	35 S-P	63 S-P
Number of animals	4	6	5	6
Amounts of glucose mg/100 g of body weight	118 $\pm$ 30 ^{a)}	123 $\pm$ 33	125 $\pm$ 32	117 $\pm$ 23
Total counts of glucose- ^{14}C cpm $\times$ 10 ⁻⁶ /100 g of body weight	11.1 $\pm$ 1.0	12.0 $\pm$ 2.3	8.3 $\pm$ 1.7	8.9 $\pm$ 2.0
Carbohydrate cal %	—	—	35.0	62.5
Fat cal %	24.0	74.6	39.7	12.5
Protein cal %	73.2	24.4	24.4	24.4

^{a)} Mean $\pm$ standard deviation

TABLE VI. INFLUENCE OF A HIGH CARBOHYDRATE DIET, A HIGH PROTEIN DIET, AND A HIGH FAT DIET ON THE ACTIVITIES OF GLUCONEOGENIC AND GLYCOLYTIC ENZYMES IN THE LIVER OF RATS

Enzyme	High carbohydrate diet	High protein diet	High fat diet
G6Pase (A)	89.2 $\pm$ 17.8 ^{a)}	91.0 $\pm$ 6.08	123 $\pm$ 9.80 ^{b)}
GK (B)	5.30 $\pm$ 0.35	2.83 $\pm$ 0.67 ^{b)}	1.26 $\pm$ 0.31 ^{b)}
(A)/(B)	16.9 $\pm$ 3.31	33.3 $\pm$ 8.15 ^{b)}	100 $\pm$ 23.6 ^{b)}
FDPase (C)	72.8 $\pm$ 5.05	84.3 $\pm$ 1.29 ^{b)}	77.8 $\pm$ 3.66
PFK (D)	9.57 $\pm$ 0.39	9.53 $\pm$ 0.45	8.33 $\pm$ 0.34 ^{b)}
(C)/(D)	7.50 $\pm$ 0.30	8.88 $\pm$ 0.57 ^{b)}	8.84 $\pm$ 0.80
PEPCK (E)	30.2 $\pm$ 7.50	61.4 $\pm$ 16.5 ^{b)}	47.7 $\pm$ 3.54 ^{b)}
PK (F)	376 $\pm$ 56.0	305 $\pm$ 30.7	202 $\pm$ 13.3 ^{b)}
(E)/(F) $\times$ 100	7.97 $\pm$ 0.69	20.8 $\pm$ 7.03 ^{b)}	21.4 $\pm$ 1.82 ^{b)}

^{a)} Mean $\pm$ standard deviation^{b)} Differs significantly from corresponding values in animals fed the high carbohydrate diet at the 5% levels by *t* test.

TABLE VII. INFLUENCE OF DIETARY CARBOHYDRATE, PROTEIN, AND FAT ON THE ACTIVITIES OF GLUCONEOGENIC AND GLYCOLYTIC ENZYMES IN THE LIVER OF RATS

Enzyme	Diet					
	75 P-F	50 P-F	25 P-F	12 S-P	35 S-P	63 S-P
G6Pase (A)	90.7 $\pm$ 15 ^{a)}	105 $\pm$ 8.7	118 $\pm$ 16	92.5 $\pm$ 13	82.2 $\pm$ 13	78.3 $\pm$ 6.7
GK (B)	2.54 $\pm$ 0.2	2.02 $\pm$ 0.4	1.70 $\pm$ 0.2	2.34 $\pm$ 0.3	3.64 $\pm$ 0.7	5.35 $\pm$ 0.5
(A)/(B)	36.0 $\pm$ 7.2	54.6 $\pm$ 15	74.4 $\pm$ 9.2	40.6 $\pm$ 8.6	23.0 $\pm$ 4.8	14.8 $\pm$ 2.2
FDPase (C)	92.1 $\pm$ 4.4	92.4 $\pm$ 6.3	83.6 $\pm$ 4.3	80.3 $\pm$ 4.3	75.1 $\pm$ 3.5	73.3 $\pm$ 3.3
PFK (D)	10.7 $\pm$ 1.9	11.1 $\pm$ 1.9	10.5 $\pm$ 0.6	9.25 $\pm$ 1.0	9.43 $\pm$ 1.3	8.45 $\pm$ 0.7
(C)/(D)	8.84 $\pm$ 1.4	8.33 $\pm$ 0.9	7.97 $\pm$ 0.7	8.74 $\pm$ 0.7	8.05 $\pm$ 0.8	8.71 $\pm$ 0.7
PEPCK (E)	60.7 $\pm$ 4.0	53.2 $\pm$ 2.9	42.3 $\pm$ 3.5	37.1 $\pm$ 5.4	28.1 $\pm$ 5.2	22.1 $\pm$ 3.8
PK (F)	273 $\pm$ 22	251 $\pm$ 8.7	254 $\pm$ 17	285 $\pm$ 5.6	291 $\pm$ 20	398 $\pm$ 75
(E)/(F) $\times$ 100	22.2 $\pm$ 2.2	21.8 $\pm$ 1.7	15.9 $\pm$ 0.8	13.0 $\pm$ 2.4	8.38 $\pm$ 1.7	6.18 $\pm$ 0.3
Carbohydrate cal %	—	—	—	12.0	35.0	62.5
Protein cal %	73.2	48.8	24.4	24.4	24.4	24.4
Fat cal %	24.0	49.4	74.6	62.6	39.7	12.5

^{a)} Mean $\pm$ standard deviation for five animals

of Friedmann *et al.* Thus, it is suggested that the hepatic gluconeogenesis play an important role on the homeostatic control of blood glucose at least under the condition of feeding of low carbohydrate diets high in fat.

23) G. Weber, S.K. Srivastava and R.L. Singhal, *J. Biol. Chem.*, **240**, 750 (1965).

24) A. E. Harper and F. G. Young, *Biochem. J.*, **71**, 696 (1959).

25) S. R. Wagle and J. Ashmore, *Biochim. Biophys.*

It is known that rats fed low carbohydrate diets have the higher activities of hepatic gluconeogenic enzymes than those of rats fed high carbohydrate, ²³⁻²⁷⁾ and the activities of some hepatic glycolytic enzymes of rats fed

Acta, **97**, 142 (1965).

26) S. R. Wagle and J. Ashmore, *J. Biol. Chem.*, **239**, 1289 (1964).

27) J. W. Young and H. A. Lardy, *Biochemistry*, **3**, 1687 (1964).

low carbohydrate diets are lower than those of rats fed high carbohydrate diets.²⁸⁻³⁰ Thus, influences of dietary composition on the activities of gluconeogenic and glycolytic enzymes in the liver of rats were investigated and the results are shown in Tables VI and VII.

The activities of PEPCK of two groups fed low carbohydrate diets were higher than those of the high carbohydrate group, moreover, the activity of the high protein group was higher than that of the high fat group. On the other hand, the activities of PK of rats fed the high protein diet tended to be lower than that of the high carbohydrate group, though there was no statistically significant difference. The high fat group showed about 50% decrease in the activity as compared with that of the high carbohydrate group. PEPCK activity increased progressively in response to the progressive decrease in calories from carbohydrate at constant protein content and also increased in response to the increase in calories from protein at constant carbohydrate content. PK activity appeared to be sensitive to dietary carbohydrate, because the activity changed only when diets contained this nutrient and depended on the carbohydrate content in diets. The ratio of PEPCK to PK activity was influenced by both carbohydrate and protein. Whereas there were limits to the extent to which changes in the enzymatic activity can occur in response to the increase in the protein content in diets. Thus, there was an increase in PEPCK activity and a decrease in PK activity in response to the absence or deficiency of carbohydrate in diets. This result was consistent with the concept that mammalian bodies maintain blood glucose by means of changing activities of gluconeogenic and glycolytic enzymes and shifting the direction of the overall metabolite flow through

the gluconeogenic or glycolytic system towards glucose formation when supply of exogenous glucose is meager.

The high fat group showed a significant increase in G6Pase activity in comparison with those of the high carbohydrate and the high protein group. The higher response to low content of carbohydrate (below 35% carbohydrate) in diets at constant protein content and the lower response to the protein content in diets at constant carbohydrate level was observed. In another words, G6Pase activity appeared to respond to the level of fat in addition to a response to the level of carbohydrate in diets and differed from PEPCK activity in respect of the higher response to fat content of diets. The high fat group also caused a marked decrease in GK activity. GK activity showed a progressive increase with increasing the level of carbohydrate in diets at constant protein and when the protein content increased at constant carbohydrate in diets, the higher activity was obtained. Therefore, GK activity also appeared to respond to the level of fat in diets, but the response occurred in opposite direction against that of G6Pase activity, namely, GK activity decreased progressively with increasing the level of fat in diets. The response to the level of fat in diets can be shown more clearly from the ratio of G6Pase to GK activity.

FDPase activity showed an increase with decreasing carbohydrate level in diets, and with increasing protein level in diets. Thus, changes of FDPase activity in response to the level of dietary components were similar to that of PEPCK activity. But a markedly different situation was observed in the case of PFK activity as compared with those of GK and PK. PFK activity of rats fed the diets containing moderate and high levels of carbohydrate tended to decrease as compared with other low carbohydrate groups. Consequently, changes in PFK activity in response to levels of dietary components occurred in the same direction of those of FDPase acti-

28) H. A. Krebs and L. V. Eggleston, *Biochem. J.*, **94**, 3C (1965).

29) H. Abraham, B. Borrebaek and I. L. Chaikoff, *J. Nutrition*, **83**, 273 (1964).

30) N. Perez, L. Clark-Turri, E. Rabajille and H. Niemeyer, *J. Biol. Chem.*, **239**, 2420 (1964).

vity. As a matter of course, the ratio of FD-Pase to PFK activity was similar among such dietary groups.

As described above, increased activities of G6Pase were observed in rats fed low carbohydrate diets high in fat under conditions of constant protein or constant carbohydrate content. Moreover, in our unpublished data G6Pase activity did not show any changes under the condition of constant fat in diets. No experiment have yet been undertaken which showed influence of the content of dietary fat on gluconeogenic enzymes. Therefore, it should be emphasized to consider that G6Pase activity in rat liver is affected significantly by fat feeding. In addition to the influence of fat content in diets on G6Pase activity, the carbohydrate content in diets had a small but definite influence on the activity because activities of carbohydrate deficient groups were higher than those of groups fed high carbohydrate diets.

These observations were in good agreement with the increased formation of glucose obtained *in vitro* studies with liver slices as described above. Consequently, the strongest correlation between the capacities of glucose formation by liver slices and gluconeogenic enzyme activities was observed between the rate of incorporation of ^{14}C into glucose by liver slices and G6Pase activity.

The increased formation of glucose from saturating levels of various precursors in slices of rat kidney cortex was also observed by Krebs *et al.*³¹⁾ and Fuwa *et al.*³²⁾ in rats fed high protein diets. In kidney cortex slices,

the capacity of glucose formation was more significantly influenced by protein content in diets than fat or carbohydrate content in diets. In our previous report,³³⁾ it was described that changes in G6Pase activity in rat kidney cortex were reflected in the capacity of glucose formation by rat kidney cortex slices, namely, G6Pase activity in rat kidney cortex also increased significantly in rats fed the high protein diet.

It is clear from the above results and discussion that the capacity of glucose formation in liver of rats was changed both by carbohydrate content and by fat content in diets and changes in gluconeogenic capacity with diets were correlated with changes in G6Pase activity. Furthermore, as stated in the introduction, the needs of glucose in mammalian bodies must be met by the conversion of non-carbohydrate precursor to glucose in the body, unless carbohydrate is continually ingested with food, whereby the bodies fed the low carbohydrate diet high in fat may be supplied with glucose by increased capacities of gluconeogenesis in the liver, while the bodies fed the low carbohydrate diet high in protein may be supplied with glucose by those in the kidney.

33) H. Fuwa, H. Suzuki and J. Nagata, *Agr. Biol. Chem.*, **33**, 1470 (1969).

The following abbreviations were used in this report: ATP=adenosine triphosphate; ITP=inosine triphosphate; ADP=adenosine diphosphate; NADP or NADPH=nicotinamide adenine dinucleotide phosphate oxidized or reduced form; OAA=oxaloacetic acid; PEP=phosphoenolpyruvate; G6P=glucose 6-phosphate; F6P=fructose 6-phosphate; FDPase=fructose diphosphatase; G6Pase=glucose 6-phosphatase; PEPCK=phosphoenolpyruvate carboxykinase; PK=pyruvate kinase; GK=glucokinase; PFK=phosphofructokinase; P_i =inorganic phosphate; GSH=glutathione, reduced form.

31) H. A. Krebs, D. A. H. Bennet, D. deGasqust, T. Gascoyne and Y. Yoshida, *Biochem. J.*, **86**, 22 (1963).

32) H. Fuwa, Y. Aoyama and T. Yamada, *Agr. Biol. Chem.*, **33**, 1464 (1969).