Change of the Gluconeogenic Capacity of Rat Kidney Cortex Slices with Dietary Components

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Substituting protein for carbohydrate in diets significantly enhanced the rates of glucose formation from pyruvate, glutamate, or glycerol in rat kidney cortex slices. The tissue, however, increased slightly its gluconeogenic capacity in response to low carbohydrate, high fat diet. The rates of glucose taken up per unit weight of kidney cortex of rats fed a high carbohydrate diet was higher than those of rats fed diets high in protein or fat. Kidney weight in g per 100 g body weight (relative kidney size) of rats fed diets high in protein was significantly higher than that of rats fed diets high in carbohydrate or fat.

Kidney cortex slices are suitable for comparison of the rate of glucose formation under different physiological conditions, since glucose formation from a variety of noncarbohydrate precursors (gluconeogenesis) is readily measured in the tissue preparation without concomitant decomposition of glycogen and without extensive glycolysis.¹⁾

Krebs *et al.*²⁾ showed that keeping rats for 3 to 5 days on a low carbohydrate diet consisting of 3 parts of casein and 1 part of margarine plus vitamin and mineral supplements increased the gluconeogenic capacity of the tissue. They concluded that the increased gluconeogenic capacity on the low carbohydrate diet was an adaptive reaction and the body responded by synthesizing glucose when the dietary supply of glucose did not satisfy the metabolic demands of the animal.

This paper deals with measurements of the rates of renal gluconeogenesis from pyruvate, glutamate, or glycerol of rats fed low carbo-

hydrate diets high in protein or fat as compared with those of rats fed a high carbohydrate diet containing adequate protein in order to test a hypothesis that the increased gluconeogenic capacity of rat kidney cortex slices depends on the content of carbohydrate in the diet. The results presented show that substituting protein for carbohydrate in diets significantly enhances the renal gluconeogenic capacity of the rat, although, rat kidney cortex increases slightly its gluconeogenic capacity in response to diets low in carbohydrate and high in fat.

MATERIALS AND METHODS

Animals and diets. Male or female Donryu strain rats were purchased from Nippon Rat Co., Urawa. The animals were housed in individual cages in a temperature regulated room at about 25°C and 12 hr per day of darkness and light. Rats were fed 30, 40, and 45 kcal per 100 g body weight per day for Expts. 1, 3, and 2, respectively, and fed *ad libitum* for Expt. 4. Calorie intakes per 100 g body weight were predetermined by separate preliminary experiments of three groups of five rats fed 25CS *ad libitum* under conditions comparable with Expts. 1, 2, and 3, respectively. Water was available *ad libitum* for

1) H. A. Krebs, *Proc. Roy. Soc.*, **159B**, 545 (1964).

2) H. A. Krebs, D. A. H. Bennet, P. deGasquet, T. Gascoyne and T. Yoshida, *Biochem. J.*, **86**, 22 (1963).

TABLE I. COMPOSITION OF DIET (1)

	25CS (%)	40CS (%)	55CS (%)	65CS (%)	75CS (%)
Casein	25	40	55	65	75
Corn oil	5	5	5	5	5
Salts mixture ¹⁾	5	5	5	5	5
Vitamin mixture ^{1,2)}	0.85	1.36	1.87	2.21	2.55

 α -Starch

Made to 100%

1) A. E. Harper, *J. Nutrition*, **68**, 405 (1959).

2) Thiamine HCl 0.059%

Riboflavin 0.059

Niacin 0.294

Ca pantothenate 0.235

Pyridoxine HCl 0.029

Menadione 0.006

Biotine 0.001

Folic acid 0.002

Vitamin B₁₂ 0.0002

Inositol 1.176

Ascorbic acid 0.588

Lactose Made to 100%

Diet also contained per 100 g: 0.15 g of choline chloride, 600 IU of vitamin A, 60 IU of vitamin D₂, and 10 mg of vitamin E acetate.

TABLE II. COMPOSITION OF DIET (2)

	75CB (g)	25CB (g)	25CL (g)	25C- 12SB (g)	25C- 65SB (g)
Casein	75	25	25	25	25
Butter	11	34	0	28.5	5.55
Lard	0	0	31.6	0	0
Salts mixture ¹⁾	5	5	5	5	5
Vitamine mixture ^{1,2)}	2.55	0.85	0.85	0.85	0.85
α -Starch	0	0	0	12.3	64

1) See footnote of Table I.

2) Each diet also contained 0.15 g of choline chloride, 600 IU of vitamin A, 60 IU of vitamin D₂, and 10 mg of vitamin E acetate.

animals throughout the experiments. The composition of test diets was shown in Tables I and II. Diets were prepared by substituting (a) protein for carbohydrate at constant fat (shown in Table I); (b) fat for carbohydrate at constant protein (shown in Table II); (c) protein for fat at constant and very low carbohydrate (shown in Table II). All the test diets contained the same amount of salts per 100 calculated calories. The amounts of the mixture of vitamins (mainly of water-soluble vitamins) used were proportional to the levels of protein in diets, namely, for example 8.5 g for 250 g

of casein and 25.5 g for 750 g of casein. Commercial pellets were delivered from Central Laboratories for Experimental Animals, Tokyo and contained about 20% of protein.

Plan of experiments. The experiments were carried out in four phases, namely, Expts. 1~4.

In Expt. 1, male rats maintained on a high carbohydrate diet (25% casein-starch diet) for 3 weeks, weighing from 180 to 190 g, were divided into three groups of six rats. Each group was fed the following diets: group 1, the high carbohydrate diet (25CS); group 2, a high protein diet (75% casein-starch diet, 75CS); group 3, a high fat diet (25% casein-butter diet, 25CB). After 1 week on the test diet, glucose formation from pyruvate, glutamate, or glycerol was estimated in kidney cortex slices.

In Expt. 2, female rats maintained on the pellets, weighing from 50 to 60 g, were divided into four groups of five rats. Each group was fed 75CB, 25CB, 25C12BS, and 25C62SB, respectively. The former two diets were prepared by isocaloric substitution of calories from protein for those from fat at constant and very low carbohydrate. The latter three diets were prepared by isocaloric substitution of calories from fat for those from carbohydrate at constant protein. After 2 weeks on the test diet, glucose formation from pyruvate was estimated in kidney cortex slices.

In Expt. 3, female rats maintained on 25CS for 1 week, weighing from 100 to 120 g, were divided into four groups of five rats. Each group was fed 25CS, 40CS, 55CS, and 65CS, respectively, in order to test the effect of substituting protein for carbohydrate at constant fat. After 1 week on the test diet, glucose formation from pyruvate was estimated in kidney cortex slices.

In Expt. 4, female rats maintained on the pellets, weighing from 180 to 190 g, were divided into 3 groups of five rats. Each group was fed 25CS, 75CS, and 25CL, respectively. After 1 week on the test diet, glucose uptake of kidney cortex slices was estimated.

Incubation of kidney cortex slices. (a) *Glucose formation.* The animals were killed between 10:00 a.m. and noon by a sharp blow on the head followed by decapitation. The kidneys were removed rapidly and completely, weighed, and chilled. The capsule was stripped and kidney halved. The preparation of the slices and incubation procedures were similar to

those of Krebs *et al.*²⁾ Kidney cortex slices were prepared with a Stadie-Riggs micrometer (maximum tissue thickness 0.5 mm) and dropped into Krebs-Henseleit buffer pH 7.4. The slices were shaken for 1 min at room temperatures to remove residual glucose, blotted on a filter paper, and weighed. About 50 mg of slices (8~12 mg dry weight) were transferred to a 50 ml Erlenmeyer flask containing 5.0 ml of Krebs-Henseleit bicarbonate buffer pH 7.3~7.4 including each of following substrates (0.01 M): sodium pyruvate, sodium L-glutamate, and glycerol. Flasks were stoppered with a serum stopper, gassed for 1 min with 95% O₂-5% CO₂ via two needles, and shaken for 1 hr about 100 oscillation per min at 37°C. The control slices obtained from kidneys of the same rat were incubated exactly in the same manner except for the omission of substrate. After the incubation the flasks were cooled in ice and slices were removed, dried in an oven for 2 hr at 105~110°C and weighed. The medium was deproteinized with HClO₄ (final concentration 0.27 N) and the protein precipitates removed by centrifugation. Glucose in the supernatant was determined colorimetrically by the glucose-oxidase method of Huggett and Nixon³⁾ as modified by Krebs *et al.*²⁾ except for the following modifications. Glucose-oxidase reagent was prepared daily from 24 mg of glucose oxidase (Deoxin from Nagase Sangyo Co., Osaka), 100 ml of 0.5 M sodium phosphate buffer pH 7.0, 0.4 ml of 0.1% peroxidase solution (Degree of purity II from C. F. Boehringer und Soehne, GmbH, Mannheim), and 0.4 ml of *o*-dianisidine solution (1% in 95% ethanol). To 1.0 ml of glucose solution (5~25 μ g in 0.27 N HClO₄) 3.0 ml of the glucose-oxidase reagent was added.

The results are reported as μ moles of extra glucose formed as a result of adding the substrate per hr per g dry weight of tissue.

(b) *Glucose uptake.* Incubation procedures were similar to those of Underwood and Newsholme,⁴⁾ About 50 mg of slices prepared as described above were shaken for 15 min at 37°C in Krebs-Henseleit bicarbonate buffer pH 7.4 without substrate with 95% O₂-5% CO₂ as the gas phase until glucose release of slices ceased. The slices were transferred to another flask with medium containing 2 mM D-glucose with 95% O₂-5% CO₂ as the gas phase and incubated for 2 hr at 37°C with shaking. After the incubation the weight of slices and glucose in the medium were determined as described in (a).

The results are reported as μ moles of glucose taken up per hr per g dry weight of tissue.

RESULTS

Table III shows the results of Expt. 1. There was a significant increase of 41%, 33%, and 41% in the rates of renal gluconeogenesis per unit weight with pyruvate, glutamate, and glycerol, respectively, as substrate in rats fed the high protein, low carbohydrate diet as compared with rats fed the high carbohydrate diet. Because the weight of kidneys increased in response to the high protein diet on average about 30%, the effect of the diet expressed as the rates of gluconeogenesis per kidney per 100 g body weight might be highly

TABLE III. CHANGES OF THE RATES OF GLUCOSE FORMATION FROM PYRUVATE, GLUTAMATE, OR GLYCEROL IN KIDNEY CORTEX SLICES AND RELATIVE KIDNEY SIZE OF RATS FED HIGH CARBOHYDRATE, HIGH PROTEIN, OR HIGH FAT DIETS

	25% Casein-starch diet	75% Casein-starch diet	25% Casein-butter diet
	μ moles of extra glucose formed/g dry weight/hr		
Pyruvate	135 $\pm$ 24.2 ¹⁾ (5)	191 $\pm$ 22.3* (6)	149 $\pm$ 15.6 (6)
Glutamate	62.7 $\pm$ 11.7 (5)	89.6 $\pm$ 20.3** (6)	63.8 $\pm$ 6.2 (6)
Glycerol	58.3 $\pm$ 10.9 (5)	82.4 $\pm$ 5.9* (4)	74.0 $\pm$ 6.9** (5)
	Kidney weight in g/100 g body weight		
	0.822 $\pm$ 0.042 (6)	1.08 $\pm$ 0.052* (6)	0.818 $\pm$ 0.041 (6)

1) Mean $\pm$ standard deviation for number of rats indicated in parentheses.

*,** Differs significantly from corresponding values in animals fed 25CS at the 1% and 5% levels, respectively, by *t* test.

3) A. St G. Huggett and D. A. Nixon, *Biochem. J.*, **66**, 12P (1957).

4) A. H. Underwood and E. A. Newsholme, *Biochem. J.*, **104**, 300 (1967).

TABLE IV. EFFECT OF SUBSTITUTING PROTEIN OR FAT FOR DIETARY CARBOHYDRATE ON THE RATE OF GLUCOSE FORMATION FROM PYRUVATE IN KIDNEY CORTEX SLICES AND RELATIVE KIDNEY SIZE OF RATS

25% Casein-62% starch butter	25% Casein-12% starch butter	25% Casein-butter	75% Casein-butter
μ moles of extra glucose formed from pyruvate/g dry weight/hr			
227 $\pm$ 11.7 ¹⁾	253 $\pm$ 18.3	285 $\pm$ 25.7	303 $\pm$ 21.5*
Kidney weight in g/100 g body weight			
0.984 $\pm$ 0.064	0.961 $\pm$ 0.084	0.988 $\pm$ 0.145	1.33 $\pm$ 0.065*

1) Mean $\pm$ standard deviation for five rats.* Differs significantly from corresponding values of animals fed 25C62SB at the level of 0.1%, by *t* test.

TABLE V. EFFECT OF SUBSTITUTING PROTEIN FOR DEITARY CARBOHYDRATE ON THE RATE OF GLUCOSE FORMATION FROM PYRUVATE IN KIDNEY CORTEX SLICES AND RELATIVE KIDNEY SIZE OF RATS

25% Casein-starch	40% Casein-starch	55% Casein-starch	65% Casein-starch
μ moles of extra glucose formed from pyruvate/g dry weight/hr			
178 $\pm$ 29.6 ¹⁾⁽⁵⁾	190 $\pm$ 17.0(5)	232 $\pm$ 27.2(5)	249 $\pm$ 23.9*(4)
Kidney weight in g/100 g body weight			
0.884 $\pm$ 0.033(5)	0.996 $\pm$ 0.072*(5)	1.04 $\pm$ 0.096*(5)	1.18 $\pm$ 0.173*(4)

1) Mean $\pm$ standard deviation for number of rats indicated in parentheses.* Differs significantly from corresponding values in animals fed 25CS at the level of 5%, by *t* test.

TABLE VI. CHANGES OF THE RATES OF GLUCOSE UPTAKE IN KIDNEY CORTEX SLICES AND RELATIVE KIDNEY SIZE OF RATS FED HIGH CARBOHYDRATE, HIGH PROTEIN, OR HIGH FAT DIETS

25% Casein-starch	75% Casein-starch	25% Casein-lard
μ moles of glucose taken up/g dry weight/hr		
79.2 $\pm$ 10.7 ¹⁾	60.2 $\pm$ 6.9*	57.1 $\pm$ 5.9*
Kidney weight in g/100 g body weight		
0.692 $\pm$ 0.028	0.965 $\pm$ 0.081*	0.830 $\pm$ 0.101*

1) Mean $\pm$ standard deviation for five rats.* Differs significantly from corresponding values in animals fed 25CS at the level of 5%, by *t* test.

significant. Tissues from rats fed the high fat, low carbohydrate diet showed about the same gluconeogenic capacity as that of rats fed the high carbohydrate diet except for glycerol as substrate. The animals showed almost the same gain in body weight per day among each group from the 3rd to 7th day of the experimental duration, although rats fed the high protein diet lost or maintained their body weight for one or two days at the beginning of the experiment.

Results of Expts. 2 and 3 are shown in

Tables IV and V. The capacity of glucose formation from pyruvate in rat kidney cortex slices increased when more carbohydrate in diet was replaced with protein at constant fat content. Substituting fat for carbohydrate in diet at constant protein content, however, showed slight and insignificant increase of the gluconeogenic capacity of the tissue.

Table VI shows the results of Expt. 4. The rates of glucose taken up per unit weight of kidney cortex of rats fed the high carbohydrate diet was significantly higher than those

of rats fed diets high in protein or fat. However, owing to the increased weight of the kidneys in response to the high protein diet, the rates per kidney per 100g body weight might be similar between the high carbohydrate and the high protein groups.

DISCUSSION

The experimental data described in Tables III, IV and V indicate the capacity of glucose formation in rat kidney cortex slices increases when the content of protein in diets increases. Although an increased capacity of glucose formation in the tissue of rats fed a low carbohydrate diet has been observed by Krebs *et al.*,²¹ the increased capacity depends on the high protein content of diets and not on the low carbohydrate content.

The data described in Table VI indicate the capacity of glucose uptake of kidney cortex slices of rats fed the high carbohydrate diet is higher than those of rats fed diets high in protein or fat. This is not enough to account for the increased capacity of glucose formation in the tissue of rats fed the high protein diet, however, especially when the capacity is expressed as the rate per kidney per 100g body weight, since relative kidney size of rats increases in response to the high protein diet.

Thus, it is possible to conclude that administration of diets high in protein to rats produces a marked increase in renal glucose synthesis from noncarbohydrate precursors. The regulation of gluconeogenesis in liver and kidney by means of dietary components has received little attention. It has been suggested that two types of mechanisms can be distinguished in case of control of lipogenesis.⁵¹ Namely, one is short term and the other is long term. The long term effects have been found to correlate with "adaptive" changes

in the activity of the enzymes involved in the system. The reversibility of glycolysis has been considered by Krebs⁶¹ and Weber⁷¹ to depend on the activity of glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase. In addition, transamination and deamination of glucogenic amino acids has been considered. It is possible that an increase in renal gluconeogenesis of rats fed diets high in protein may be due to an increase in the activity of these enzymes in the kidney.⁸¹

It has been shown that the rates of incorporation of radioactivity in blood glucose and liver glycogen from ¹⁴C-pyruvate or ¹⁴C-amino acids increased more in rats fed diets high in protein^{9,101} or fat¹⁰¹ than in rats fed diets high in carbohydrate. The increased incorporation of ¹⁴C can be in consequence of either increased glucose synthesis, or decreased utilization of glucose, or both. It is probable, however, that the capacity of glucose formation increased in rats fed diets low in carbohydrate and high in protein or fat.

Liver and kidney cortex are unique in higher animals in that they are capable of both degradation and synthesis of glucose. The quantitative roles of the liver and kidneys in gluconeogenesis has been discussed by Krebs.¹¹ Although Krebs *et al.*¹¹¹ have shown that a low carbohydrate diet high in fat did not increase the gluconeogenic capacity of mouse liver slices, we have observed an increased capacity of glucose formation in rat liver slices¹²¹ and homogenates⁸¹ when rats

6) H. A. Krebs, *Johns Hopkins Hosp. Bull.*, **95**, 19 (1954).

7) G. Weber, *Advances in Enzyme Regulation*, **1**, 1 (1963).

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

9) T. Kimura and K. Ashida, *Agr. Biol. Chem.*, **32**, 1440 (1968); Unpublished observation.

10) B. Friedman, E. H. Goodman, Jr. and S. Weinhouse, *J. Biol. Chem.*, **242**, 3620 (1967).

11) H. A. Krebs, B. M. Notton and R. Hims, *Biochem. J.*, **101**, 607 (1966).

12) H. Suzuki and H. Fuwa, Unpublished observation.

5) J. M. Lowenstein in "The Metabolic Roles of Citrate", ed. by T. W. Goodwin, Academic Press Inc., New York, N.Y., 1968, p. 82.

were fed with diets high in protein or fat. This may account for the increased capacity of *in vivo* glucose formation of rats fed diets high in fat and low in carbohydrate. An alternative explanation is stimulatory effects

of free fatty acids on gluconeogenesis in liver¹³⁾ and kidney cortex.¹⁴⁾ The control mechanisms of gluconeogenesis in liver and kidney by means of dietary components are under investigation.

13) R. J. Haynes, *Advances in Enzyme Regulation*, **3**, 118 (1965); E. Struck, J. Ashmore and O. Wieland, *Biochem. Z.*, **343**, 107 (1965); J. R. Williamson, *Advances in Enzyme Regulation*, **5**, 229 (1967).

14) H. A. Krebs, R. N. Speake and R. Hems, *Biochem. J.*, **94**, 712 (1965); A. H. Underwood and E. A. Newsholme, *Biochem. J.*, **104**, 300 (1967); H. Fuwa and Y. Asano, Unpublished observation.