Further Studies on the Dietary Control of Pyruvate Kinase Activity[1] (34243)

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The activity of pyruvate kinase has been found to be inversely related to the state of gluconeogenesis in dietary and hormonal experiments (1). However, the activity of the enzyme increased on the first day after a high-carbohydrate to high-protein dietary shift (2) and also after the reverse dietary shift (3). These transitory elevations in pyruvate kinase activity have been attributed to the relief by the high-protein diet of a translational limitation of synthesis in the rats prefed the 90% glucose diet and to the relief of this limitation in the rats prefed the 90% casein diet (2, 3). Subsequent experiments using actinomycin D and 8-azaguanine, both which inhibit transcription, or cycloheximide, which inhibits translation, have yielded results consistent with the previous hypothesis (4). Since rats treated with either actinomycin D or cycloheximide do not eat, the experiments were performed by force-feeding 4 g of casein hydrolysate in 2 doses or 6 g of carbohydrate in 3 doses. Force-feeding, however, had two disadvantages; (i) the amount of food taken in was less than the rats would normally have eaten, and (ii) force feeding caused a considerable amount of stress. It was desirable, therefore, to repeat earlier experiments with *ad libitum*-fed rats using a transcriptional blocking agent which would have minimal effect on food intake allowing the experiments to be performed under conditions free of the stress due to force-feeding.

The antibiotic 8-azaguanine, which has inhibited protein synthesis in animals (5, 6) apparently at the level of transcription, appeared suitable for this purpose. In preliminary work, 8-azaguanine was relatively nontoxic, it caused only a small reduction in food intake and it did not cause diarrhea even at dose levels of 30 mg/rat per day.

Procedure. Male Sprague-Dawley rats weighing 150–170 g were prefed for 4 days a diet consisting of 90% glucose, 5% corn oil, 4% P.H salt (7) and 1% vitamins (8). The rats were then fed *ad libitum* a diet in which casein was substituted for carbohydrate, but otherwise identical to the previous diet. In the reverse dietary change the high-protein diet was prefed for 5 days after which the animals were fed the high-carbohydrate diet. 8-Azaguanine was administered intraperitoneally in two doses, 15 mg/dose. The first dose was administered at least 2 hr before the diets were changed and the second dose approximately 12 hr after the dietary change. The animals were killed by a sharp blow to the head and decapitation, 24 hr after they were given the second diet. Carcasses were exsanguinated for approximately 30 sec, the liver was removed, rinsed, blotted, weighed, and chilled over ice. Liver glycogen was determined by a nephelometric procedure (9). A 10% liver homogenate was prepared with 0.14 *M* KCl, pH 7.4, using a Potter–Elvehjem homogenizer. The crude homogenate was centrifuged at 0–5° for 30 min at 20,000*g* and the clear supernatant solution was used for the determination of soluble protein (10) and pyruvate kinase (11).[2]

Results and Discussion. Rats prefed the 90% glucose diet consumed an average daily amount of this diet equaling 7.8% of their body weight. After the dietary change the

[1] Supported in part by Public Health Service Research Grant No. AM-04732 from the National Institute of Arthritis and Metabolic Diseases.

[2] The procedure of Bucker and Pfleiderer was modified by increasing the amount of phosphoenolpyruvate added to 3 μmoles/1 ml of assay mixture.

TABLE I. Response of Pyruvate Kinase to Diets and 8-Azaguanine.

Dietary treatment	8-Azaguanine	RLS[a]	(mg/100 g of body wt) Liver glycogen	(mg/100 g of body wt) Soluble liver protein	Pyruvate kinase (units[b]/100 g of body wt)
4 days 90% glucose	—	4.68 ± 0.45[c]	85.9 ± 23	369 ± 53	23.2 ± 4.0
+ 1 day 90% casein	—	3.81 ± 0.16	31.9 ± 7.7	352 ± 17	55.1 ± 4.1
	+	3.93 ± 0.18	19.6 ± 2.1	350 ± 57	67.9 ±13
5 days 90% casein	—	5.71 ± 0.16	59.6 ± 11	443 ± 20	26.2 ± 2.5
+ 1 day 90% glucose	—	4.87 ± 0.13	206 ± 11	443 ± 29	47.9 ± 3.7
	+	4.66 ± 0.08	229 ± 22	391 ± 16	27.5 ± 1.6

[a] RLS = relative liver size = (liver wt × 100)/(body wt).

[b] One unit of enzyme is defined as that amount of enzyme which can produce 1 μmole of measured product under the conditions of the assay.

[c] SE of mean; values represent the average of four animals.

daily consumption of the 90% casein diet averaged 7.1% of the body weight in the nontreated rats and 6.1% in the azaguanine-treated rats. Concurrent experiments indicated that this difference was without significance with respect to the magnitude of the metabolic response. The rats which were prefed the 90% casein diet consumed on the average an amount of food equal to 7.7% of their body weight. The consumption of the 90% glucose diet after the dietary change was similar in magnitude and was only slightly reduced by the antibiotic. The significance of these figures becomes clear if we consider that a daily food intake amounting to 7.0% of the body weight equals 10.5 g in a 150 g rat; considerably more than we were able to force feed previously (4).

The effects of diets and 8-azaguanine on relative liver size, liver glycogen, soluble liver protein and pyruvate kinase activity are summarized in Table I. It should be noted that the value of relative liver size was reduced by feeding the second diet, a tendency we have noted previously (4). The level of liver glycogen was reduced after feeding the 90% protein diet and increased after feeding the 90% gluose diet. In contrast to the results in force-fed rats, however, liver glycogen was not completely depleted by the high-protein diet, even in azaguanine-treated rats. Similarly, the antibiotic did not reduce the level of liver glycogen in the rats prefed the 90% casein diet and then the 90% glucose diet. The results, therefore, indicate that any possible stress resulting from the administration of the antibiotic was minimal.

Values of soluble liver protein were similar in groups of animals prefed the same diet irrespective of subsequent treatment. Pyruvate kinase activity could, therefore, be expressed as specific activity (units per mg of soluble protein) and this would not substantially change the relative changes in activity.

Previously we have proposed that in rats fed the 90% glucose diet the rate of pyruvate kinase synthesis is limited at the level of translation by a limited supply of amino acids, whereas in the rats prefed with the 90% casein diet the limitation is at the level of transcription due to reduced availability of mRNA arising as a consequence of an elevation in the concentration of amino acids in the liver (2, 3). Therefore, in the high-glucose to high-protein dietary shift, the second diet will cause the removal of the translational limitation without the necessity of increased transcription. The subsequent increase in pyruvate kinase activity should not then be inhibited by 8-azaguanine. In the reverse dietary shift (high-protein to high-glucose) as a requirement for increased transcription has been postulated, the antibiotic is expected to inhibit the increase in pyruvate kinase activity. Table I shows that these expectations were indeed realized; the antibiotic inhibited the increase in pyruvate kinase activity after feeding the 90% glucose

diet, but not the increase in the reverse dietary shift.

Summary. Rats prefed a 90% glucose diet for 4 days were fed *ad libitum* a 90% casein diet for a period of 24 hr. The transitory elevation in pyruvate kinase activity in rats fed the 90% casein diet was not inhibited by 30 mg of 8-azaguanine administered in 2 equal doses 12 hr apart. In rats prefed for 5 days a 90% casein diet, *ad libitum* feeding of the 90% glucose diet increased pyruvate kinase activity and in this case the increase in activity was inhibited by 8-azaguanine. The results confirm earlier data and are consistent with the hypothesis that in rats prefed the high-carbohydrate, protein-free diet the synthesis of pyruvate kinase is limited at the level of translation, whereas in rats prefed the high-protein, carbohydrate-free diet the synthesis of the enzymes is limited at the level of transcription.

The authors wish to thank Mrs. Margaret Hill for her technical assistance.

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Received June 9, 1969. P.S.E.B.M., 1969, Vol. 132.