EVIDENCE FOR THE PRESENCE OF TWO TYPES OF PYRUVATE KINASE IN RAT LIVER

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In the gluconeogenetic state, it has been proposed that the newly synthesized pyruvate which is formed from lactate, alanine and serine is converted to phosphoenolpyruvic acid via oxaloacetic acid, the reaction being catalyzed by pyruvate carboxylase and phosphoenolpyruvate carboxykinase (Utter, Keech and Scrutton, 1964). However, this phosphoenolpyruvic acid which is formed at the expense of two high energy phosphate bonds would simply be reconverted to pyruvic acid again if the pyruvate kinase level was maintained. Thus, to diminish such wasteful cycling, there should be some mechanism controlling the pyruvate kinase level in gluconeogenetic organs, such as the liver and kidney.

In the present paper it is reported that the pyruvate kinase level in the liver is under hormonal and dietary regulation, like the levels of other key glycolytic enzymes. In contrast, no change in the enzyme level in skeletal muscle was found under various conditions. This difference between the response of liver and muscle to hormonal and dietary conditions was demonstrated by zone electrophoresis and an immunological method. There are at least two types of pyruvate kinase in mammals, tentatively named type M and type L. Pyruvate kinase M is an usual muscle type enzyme, and its characteristics have already been described in detail (Boyer, 1962). In crude extracts of liver both types of enzyme are present. Pyruvate kinase L is not neutralized with anti-pyruvate kinase M serum and it is the type L that fluctuates in activity under various physiological conditions. Recently, Weber et al. (1965) reported a similar phenomenon, showing that the overall activity of liver pyruvate kinase was reduced in diabetic animals and returned to normal on insulin treatment.

MATERIAL AND METHOD

Male Sprague Dawley albino rats, weighing 150 gm to 200 gm, were used throughout. Animals were fed on laboratory chow and water ad libitum, unless otherwise mentioned. Animals were sacrificed by a blow on the head and were exsanguinated. The livers were rapidly isolated and chilled, and 3 gm of liver tissue were homogenized with 12 ml of cold KCl solution (0.05 M) containing EDTA (0.005 M) using a Potter type glass homogenizer. The homogenates were centrifuged at 105,000xg for 60 minutes. The supernatants were used for assay of pyruvate kinase activity and for protein estimations by the Biuret method (Layne, 1957). A 1.5 gm sample of tissue from left femoral muscle was also homogenized with 13.5 ml of the same solution used for the liver. The homogenates were centrifuged and the supernatants were employed for experiments as with liver material. Pyruvate kinase was assayed by measurement of the pyruvate formed from phosphoenolpyruvate in the presence of ADP, according to a slight modification of the method of Kimberg and Yielding (1962). Incubation was carried out at 37°C. One unit of pyruvate kinase activity was expressed as the amount of enzyme which catalyzed the formation of one micromole of pyruvate in one minute under the conditions described above. Zone electrophoresis of crude extracts of organs was carried out on potato starch blocks (5 x 20 x 350 mm), with 0.05 M potassium phosphate buffer (pH 8.0). After 13 hours electrophoresis in a cold room the starch block was cut into 10 mm lengths, and each piece was mixed thoroughly with 1 ml of 0.1 M KCl solution to extract the enzyme. The pyruvate kinase in the supernatant was assayed as described above. Rat muscle pyruvate kinase (type M) was purified by ammonium sulfate fractionation (50 60 % saturation), heat treatment (5 min. at 55°C), dialysis, linear elution (0.0 to 0.05 M KCl) from a DEAE cellulose column and ammonium sulfate fractionation. After these purification procedures, the enzyme preparation showed only one peak on ultracentrifugal and electrophoretic analyses, and its specific activity was 650. This purified preparation was injected into rabbits with complete adjuvant to obtain anti-pyruvate kinase M serum (Leskowitz and Waksman, 1959). One ml of this serum neutralized 44 units of the enzyme immediately after mixing.

RESULTS AND DISCUSSION

As shown in Table 1, the level of pyruvate kinase in the liver was markedly reduced in fasting animals and those treated with alloxan, or fed on a high protein diet. Under these three conditions, gluconeogenesis in the liver is known to be stimulated. In contrast the enzyme level in muscle did not change under the above conditions, as demonstrated by the absence

of gluconeogenesis in muscle. With regard to the response to dietary conditions, the enzyme level was shown to be highest in animals fed on a 10% casein diet and lowest in those fed on a 90% casein diet. Since this

Table 1. Pyruvate Kinase Levels of Liver and Muscle of Rats in the Gluconeogenetic State

Treatment	No. of Animals	Pyruvate Kinase Activity[5] Liver	Muscle
None (normal)[1]	4	0.633±0.10	25.7± 1.4
Fasted for 24 hours	5	0.530±0.02	26.5± 1.6
Fasted for 48 hours	6	0.360± 0.04	29.6± 0.7
Diabetic[1),2)]	6	0.130±0.01	27.9±2.5
Insulin treated Diabetic[1),2),3)]	5	0.635±0.14	
Fed on 90% Casein Diet[4]	4	0.340±0.05	28.2±1.7
Fed on 10% Casein Diet[4]	4	0.965±0.06	24.5±2.2

1) These rats were fed on laboratory chow (Oriental Yeast Co. Ltd.)
2) Seven mgm per 100 gm body weight of alloxan monohydrate were injected intravenously and animals were used 5 days after the injection. Rats, with blood sugar levels of more than 300 mgm per dl, were used for experiments.
3) Fifteen units of insulin per 100 gm body weight were injected subcutaneously 48, 24 and 15 hours before sacrifice.
4) Synthetic diets contained 4 gm of inorganic salt mixture, 1 gm of vitamin mixture, 2 gm of cellulose powder, 0.01 gm of choline chloride, and the indicated amounts of casein. The diets were made up to 100 gm by addition of appropriated amounts of dextrin. The animals were fed on the synthetic diets for 3 days before sacrifice.
5) The activity was expressed as units of enzyme per mgm of protein of the crude extract.

response to the composition of the diet was no longer observed in alloxan diabetic animals, the enzyme level in the liver seems to be regulated mainly by insulin. As shown in the table, injection of insulin (Novo Lente) restored the enzyme level to normal in alloxan diabetic animals.

The fact that the level of pyruvate kinase in the liver, but not the muscle, is greatly influenced by the diet and by hormones, suggests that the enzymes in these two organs are different. As seen in Fig. 1, on starch block zone electrophoresis, crude liver extracts showed only a single peak. Peak M of the liver enzyme had the same mobility as that of the muscle enzyme. Three other peaks were tentatively named L_1, L_2 and L_3, respectively. It should be noted that brain and heart had the same type of electrophoretic pattern as muscle, while kidney showed the same pattern as liver. The differences in the natures of the L group enzymes have not yet been elucidated.

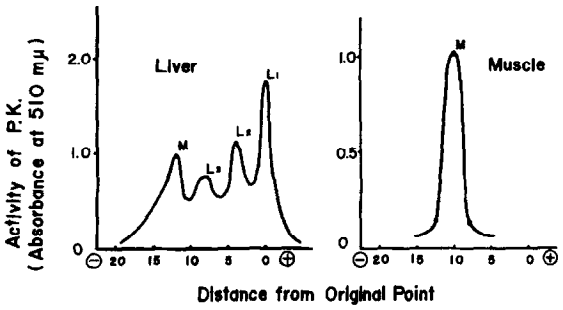

Fig. 1. The Patterns of Zone Electrophoresis of Pyruvate Kinase of Liver and Muscle

Table 2. Summary of the Differences between Pyruvate Kinase M and Pyruvate Kinase L

	Type M	Type L
Distribution	Muscle, Brain, Heart Liver and Kidney	Liver and Kidney
Anti-Muscle Enzyme Serum	Neutralized	Not Neutralized[1)]
Amm. Sulfate Precipitation	50 60 % Saturation	25 45 % Saturation
DEAE Cellulose Column Elution	0.02 M KCl	0.13 M KCl [2)]
Insulin Administration to Diabetic Rats	Not Significantly Increased	Significantly[1)] Increased

1) Details of the experiments are given in Table 3.
2) The enzymes were eluted from a DEAE cellulose column with a linear gradient increase of KCl concentration. The concentrations of KCl at which the enzymes were eluted were calculated from the volumes of the eluates.

However, type L and type M are quite distinct, as summarized in Table 2. Type M of liver is identical to, or at least, very similar to that of muscle. Anti-rat muscle pyruvate kinase serum neutralized not only the enzyme activity of the muscle enzyme, but also that of type M from other sources. However in even 60 times excess, this antibody had no effect on type L activity. Because of this the rapid assay of type M and type L enzyme in tissue homogenates was possible. Table 3 shows the ratios of Type L to M in liver extracts under various physiological and pathological conditions. The type L level increased very markedly after administration of insulin or a high carbohydrate diet, whereas that of type M showed only a slight change. In regenerating liver and hepatoma cells (AH 130, ascites form), most or all of the pyruvate kinase

was of the type M. These facts strongly suggest that type L enzymes is the more directly related to the metabolic control of gluconeogenesis in the liver,

Table 3. Changes of Ratio of Type L to Type M in the Liver under Various Physiological and Pathological Conditions

	No. of Animals	Pyruvate Kinase Activity[1] Total	Type L	Type M	L/M
Fed on 10% Casein Diet	3	0.965 ± 0.055	0.677 ± 0.017	0.288	2.35
Fed on 90% Casein Diet	3	0.340 ± 0.050	0.159 ± 0.025	0.181	0.88
Diabetic[2]	3	0.277 ± 0.040	0.107 ± 0.044	0.170	0.63
Insulin Treated Diabetic[2]	4	0.700 ± 0.120	0.534 ± 0.134	0.166	3.22
Hepatoma Cell AH 130[3]		6.4	0.000	6.4	0.00
Regenerating Liver[4]	5	0.496 ± 0.109	0.109 ± 0.051	0.387	0.28

1) Total pyruvate kinase activity was assayed in the absence of the anti-muscle enzyme serum. Type L activity was measured in the presence of the antibody 20 times in excess. Type M activity was expressed as the difference of total and type L activity.
2) The animals were fed on a 10% casein diet. Other experimental conditions were the same in Table 1.
3) The cancer cells were obtained one week after inoculation into the peritoneal cavity.
4) The enzymes was assayed 10 days after resection of two thirds of the liver.

while type M is a less differentiated form of pyruvate kinase, because its level is not affected by external conditions. Physiological aspects and more detailed information of these heteroenzymes will be reported in the near future.

SUMMARY

Two types of pyruvate kinase were identified by electrophoresis and an immunological procedure and tentatively named type L and M. Type M is distributed in muscle, brain, heart, liver and kidney, having the same nature in all these organs. Type L, which was not neutralized with anti-type M serum, was only found in liver and kidney. Anti-serum for type M was used in estimating the levels of the two types of enzyme in tissue extracts.

The level of type L varied greatly under various physiological conditions, whereas that of type M only changed slightly. In alloxan diabetic animals and those fed on a high protein diet or fasted for 48 hours, the total level of pyruvate kinase as well as the ratio of type L to type M were greatly decreased, and on subsequent insulin administration or administration of a normal diet the levels returned to normal. On the basis of comparative

studies on the two types of pyruvate kinase in regenerating liver and tumor cells, the possibility that the control mechanism might depend on the stage of differentiation of the enzyme was discussed.

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