Effect of Dietary Composition on Rat Liver Phosphorylase Activity. (24464)

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In continuation of our studies on changes in enzymatic activity due to dietary adaptation we investigated the effect of feeding fat and carbohydrate diets on activity of liver phosphorylase. One of us recently reported that during absorption of fat in the rat the activity of liver alkaline phosphatase increased as compared to that of carbohydrate fed animals(1). This activity returned to normal after 12 hour fast, even though the animals had been adapted to the fat or carbohydrate diets over an extended period. Since these results failed to indicate the presence of a specific adaptative mechanism, we could only attribute the increase in enzyme activity to a transitory effect of fat absorption. It has also been shown that substitution of protein, fat, galactose or fructose in the diet for a direct glucose source, produces an increase in activity of liver glucose-6-phosphatase in the rat(2). Adaptation to a high fat diet increases rate of utilization of fat and decreases that of carbohydrate in normal and hypophysectomized rats during subsequent fasting period(3). Fat adapted rats are also able to maintain more efficiently their blood sugar levels during periods of glycogenolysis (4). A correlation between level of phosphorylase activity and rate of glycogen breakdown has been found by several investigators. When liver slices are incubated in a medium containing high levels of Na there is an increase in phosphorylase activity parallel with increase in rate of glycogenolysis(5). Increases in rate of glycogenolysis and in phosphorylase activity were also shown to take place in liver of obese hyperglycemic mice (6). Pancreas HGF, epinephrine and other sympathomimetic drugs induce similar effects (7). This apparent relationship between activity of liver phosphorylase and rate of glycogenolysis suggested the possibility of associating this enzyme with metabolic and biochemical changes that occur as a consequence of adaptation of rats to fat or carbohydrate diets.

Materials and methods. Animals used were albino rats of CNEA strain. They were housed in individual cages. High fat and high carbohydrate diets were similar to those of previous experiments† (1). In long term feeding experiments, diets were offered *ad lib.* for 45 days. Growth was satisfactory for both groups. For determination of phosphorylase activity, rats were sacrificed by a blow on the neck. The liver was removed, homogenized in a Potter-Elvehjem homogenizer at high speed, and diluted with 0.1 M NaF to obtain a 33% homogenate. During homogenization the tube was immersed in ice bath. The phosphorylase activity was determined not later than 1 hour after removal of liver. For determining enzymatic activity an aliquot of the homogenate (0.1-0.2 ml) was incubated with a substrate containing 0.022 M glucose-1-phosphate,‡ 0.073 M citrate buffer (pH 6.2) and 0.083 M NaF, in total volume of 0.65 ml, for 30 minutes at 38°C. Fluoride was used for its ability to inhibit phosphoglucomutase(8) and acid phosphatase (9). At end of incubation, enzyme activity was stopped by addition of 1 ml of 10% TCA. Inorganic phosphate was determined in supernatant by the method of Fiske and Subba-Row(10). A phosphorylase activity unit or micromolar unit is the amount of enzyme that will liberate 1 μM of P from Gl-1-P in the presence of fluoride, during incubation of 30 minutes at 38°C at pH 6.2.

Results. Table I shows effects of feeding 3 g of either fat or carbohydrate diet to rats previously fasted for 24 hours. These results are compared to those obtained with unfed fasted rats that had been previously maintained on

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† Vitamin supplement was VINUTRIN generously supplied by Lepetit, S. A.

‡ Gl-1-P was a generous gift of Sigma Chem. Co., St. Louis, Mo.

TABLE I. Effect of a Single Meal of a High Fat or a High Carbohydrate Diet, and of Fasting on Liver Phosphorylase Activity during Absorption.

Group	No. rats	Diet	Units of phosphorylase/g liver
I	8	High CHO	167 (±46)
II	11	" fat	16.3 (± 7.7)
III	20		21.6 (± 7.8)

regular stock diet. The animals fed the test meal were sacrificed 3 hours after feeding. In all instances food was still present in stomach and intestines at this time. A significant increase in phosphorylase activity was established in the carbohydrate fed group as compared to that of fat fed and non-fed animals.

Table II summarizes effects of dietary adaptation and a subsequent 24-hour fast on activity of liver phosphorylase. These results show that fat adapted animals maintain a higher activity than carbohydrate fed rats. In this case adaptation has seemed to change radically the pattern of enzyme activity, as seen by comparing these results with those obtained with the non-adapted animals in Table I.

Discussion. Carbohydrate feeding greatly stimulated activity of liver phosphorylase during the absorptive period, while feeding of fat seemed to slightly depress such activity. This increase of phosphorylase activity which follows ingestion of carbohydrate is compatible with augmentation of glucose metabolism that presumably occurs in liver as a consequence of increased supply of glucose.

When animals were adapted to fat or carbohydrate diets the level of enzyme activity during fasting was significantly higher in animals fed high fat diet. This increase in phosphorylase activity would seem to result from adaptive adjustment which takes place in the animal to conform its metabolic pattern to its specific dietary intake, and could conceivably explain the mechanism by which the fat adapted rat is able to maintain its glycogen stores and blood sugar at higher levels during fasting than the carbohydrate adapted animal.

TABLE II. Effect of Prolonged Feeding of High Fat and High Carbohydrate Diets on Liver Phosphorylase Activity after 24 Hours Fasting.

Group	No. rats	Diet	Adaptation period, days	Units of phosphorylase per g liver
I	7	High fat	45	133 (±55)
II	5	" CHO	"	24.6 (± 6.7)

Summary. During absorption of carbohydrate by the rat there is a significant increase of liver phosphorylase activity. When animals were adapted to high fat and high carbohydrate diets over 45 days and were then fasted 24 hours, the fat adapted rats maintained a higher level of liver phosphorylase activity. These changes in enzymatic activity would seem to be consistent with metabolic and biochemical changes which have been shown to occur as a consequence of the specific dietary composition.

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Received September 30, 1958. P.S.E.B.M., 1958, v99.