Comparison of Phosphohexose Isomerase and Lactic Dehydrogenase Activities in Plasma, Liver, and Tumor Tissue of Tumor-bearing Rats

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The mechanisms underlying increases in the activity of various enzymes in the serum or plasma are, in general, incompletely understood. It has been stated that the plasma enzymes are derived from cellular elements either in the tissue or in the blood and that the level of circulating enzymes is therefore an index of the rate of disintegration of such cells (8). On the other hand, in some instances an elevation in activity of a particular plasma or serum enzyme is specifically related to a tissue process involving that enzyme, as, for example, the high serum alkaline phosphatase associated with increased osteoblastic activity in bone or with obstruction of the biliary system.

In 1943, Warburg and Christian (19) postulated that the excessive glycolytic activity of tumors might be manifested by increased activity of various glycolytic enzymes in the serum. In pursuance of this concept, several investigations have explored the serum levels of aldolase (1, 15-17, 19), phosphohexose isomerase (4-6), and lactic dehydrogenase (2, 10) in animals with transplanted tumors or in patients with neoplastic disease. Several concepts concerning the factors controlling the level of serum glycolytic enzyme activity have been suggested as a result of those studies (5, 6, 16).

It was believed that further information concerning these mechanisms might be obtained by comparing the changes occurring in the activities of two glycolytic enzymes in the serum with the changes in the neoplastic tissue, liver, and erythrocytes during the growth of a transplanted tumor. The enzymes, phosphohexose isomerase and lactic dehydrogenase, were chosen for this purpose.

MATERIALS AND METHODS

A total of 88 CFN-Wistar rats, a specially bred strain free of respiratory disease (7), were used in the present study. In each of three experiments, about sixteen to twenty rats were each given injections subcutaneously in the flank of 0.1 cc. of a 15 per cent suspension of an 8- to 9-day-old Walker carcinosarcoma 256, and eight to eleven animals were kept as controls under similar conditions. The rats weighed about 80-125 gm. at the beginning of the experiment; because of the limited supply, it was not possible to match the weights completely in the control and experimental subgroups. Food (Purina Laboratory Chow) and water were available ad libitum. At 3 or 4 days, 7 days, 10, 13, and 18 or 20 days after tumor implantation, four or five of the tumor-bearing rats and two or three of the control animals were sacrificed following ether anesthesia and withdrawal of blood from the abdominal aorta into heparinized syringes. In general, both the control and tumor-bearing animals doubled their weight during the 18-20-day experiment. The three experiments were so designed as to afford a duplicate study of the alterations in the phosphohexose isomerase and lactic dehydrogenase activities and contents of the liver and tumor after implantation of the tumor. The plasma enzyme activities were studied in all three experiments and the erythrocyte enzymes in one experiment.

Determination of phosphohexose isomerase activity.—As described in an earlier paper (4), this involved the extent of conversion at pH 7.4 and 37°C of 0.002 M glucose-6-phosphate to fructose-6-phosphate by a suitable volume of plasma or homogenate of tissue. Isomerase activity is defined as the reciprocal of that concentration, expressed as cubic centimeters of plasma or as grams of tissue per cubic centimeter of reaction mixture, that would cause the formation of 25 mg. of fructose as fructose-6-phosphate in 30 minutes/cc of reaction mixture.
under the stated conditions. The total phosphohexose isomerase content of tissue, blood, or serum was calculated on the basis of activity in a proportionately larger reaction mixture. For example, a rat liver weighing 6 gm. and having an isomerase activity of 28,000 units would have a total isomerase content of 28,000 x 25 x 6 or 8.75 x 10^4 µg-equivalents.

**Determination of lactic dehydrogenase activity.**—This was based on the interaction of reduced DPN with Na pyruvate and followed essentially the method of Meister (13) and of others (18) for the determination of this enzyme activity in tissues. Five-tenths cc. of an appropriately diluted serum or plasma (1:10-1:40 with saline), of liver or tumor homogenate (1:1000 or 1:5000), or of an hemolysate (1:1000) was heated to 37°C. and added to the following mixture at 37°C.: 2.3 cc. of 0.065 m phosphate buffer of pH 7.4; 0.1 cc. of 0.01 m sodium pyruvate, adjusted to pH 7.4; and 0.1 cc. of a 0.2 per cent saline solution of the disodium salt of reduced DPN (Sigma, approximately 90 per cent pure). The reaction mixture, 8 cc. in volume, was then rapidly transferred to a quartz cuvette in the cell compartment of the Beckman, maintained at 37° C. by circulation of water through a thermostar inserted into the Beckman. Changes in the optical density at 340 mµ were observed during the initial, zero-order portion of the reaction. The enzyme activity was expressed in units as the change in optical density X 10^3 effected per minute under these conditions by a concentration of 0.006 cc. of serum or plasma or 0.006 gm. tissue/cc of reaction mixture. It can be shown mathematically that a change of 0.001 in the optical density is equal to 1/63 µM. Since ε_m, the millimolar extinction coefficient of DPNH, is equal to 6.22 (11), one unit of activity represents an oxidation of 0.161 µM DPNH/minute. The total lactic dehydrogenase content of the liver or tumor was expressed in units and obtained by the following expression:

$$\text{Total weight of tumor or liver (gm.)} \times \text{activity.}$$

$$\frac{0.006 \text{ gm.}}{}$$

It should be noted that the measures of reaction velocity employed above are such that the activity of the enzyme is directly proportional to its concentration in plasma or tissue (8).

**RESULTS**

**Liver and tumor weights.**—Chart 1 shows the increase in the weight of the liver during the growth of tumor. Each point usually represents the average of two to three control animals or four to five tumor-bearing animals. The increase in weight of the liver was more manifest when the calculations were based on the carcass, or body minus tumor, weight. This relative increase in liver weight during tumor growth has, of course, been observed previously (18).

**Enzyme activities and contents of liver, tumor, and erythrocytes.**—The phosphohexose isomerase activity of the liver remained constant, within biological variation, during the course of tumor growth and was essentially the same as that of the control animals (Chart 2, A). In contrast, the lactic dehydrogenase activity of liver in the tumor-bearing animals increased (Chart 2, B), but this change was not distinctive, since, within biological variation, the control animals showed a rise of the same extent. The increase of liver lactic dehydrogenase in normal growing rats has been reported previously (14). Chart 2, C shows that the tumor lactic dehydrogenase activity was about 6 × 10^4 units at the earliest stages, 3 days, and rose only slightly to about 8 × 10^4 units at 20 days. The isomerase activity rose to a somewhat greater extent, namely, from about 8 × 10^4 units at 3 days to about 18 × 10^4 units at 20 days. Since there were no differences between the enzyme activities of the livers in the control and tumor-bearing animals and since the weight of the liver in the latter...
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to $6 \times 10^4$ for the first 13 days, and again at 20 days the lactic dehydrogenase activity of the erythrocytes was somewhat higher in the tumor-bearing than in the control rats. This experiment indicated that there was at least no marked passage of enzymes from erythrocytes to serum. However, further experiments are necessary to establish whether there are any significant differences in erythrocyte enzymes between control and tumor-bearing animals.

Relation between total enzyme contents of tumor and enzyme levels in plasma.—Chart 4 shows the relationship between the total contents of lactic dehydrogenase and phosphohexose isomerase in the tumor and the levels of these enzymes in the plasma. The level of plasma phosphohexose isomerase remained normal, about 30 units, until a tumor content of log 5.4, or $2.5 \times 10^6 \mu\text{g-equivalents}$, was reached. Then the plasma level began to rise so that it reached an average level of about 270 units or 9 times the normal mean value at a tumor content of about $1.5 \times 10^7 \mu\text{g-equivalents}$. In contrast, the average plasma lactic dehydrogenase level remained normal until a tumor dehydrogenase content of about $1 \times 10^7$ units, then rose slightly until it attained at 20 days an average value of 88 units, or about twice the normal mean value at a tumor content of $5.2 \times 10^7$ units.

The disparity between the alterations in the plasma level of phosphohexose isomerase and that of lactic dehydrogenase is brought out more clearly in Chart 5. The individual values for the plasma isomerases were plotted against the plasma dehydrogenase values for each of the 29 control and 54 tumor-bearing rats used in these experiments. It may be seen that at plasma isomerase activities up to about 8 times the upper limit of the normal range, there were no significant rises in plasma lactic dehydrogenase. Moderate increases in dehydrogenase occurred occasionally at the higher plasma isomerase activities, namely, between about 500 and 750 units.

DISCUSSION

The plasma phosphohexose isomerase activity was in the normal range until sometime between
the 7th and 10th day of tumor implantation, then rose to parallel the increasing content of isomerase in the tumor. This parallelism was consistent with the theory that the tumor was the source of the enzyme. The average plasma lactic dehydrogenase activity rose only slightly between the 7th and 10th day, and definitely elevated activities were obtained only in association with very high plasma isomerase activities. Sibley, Fleisher, and Higgins (15) have recently reported that, in rats similarly implanted with Walker carcinosarcoma 256, the serum aldolase activity remained within normal limits for 1 week and rose at some time between then and 14 days. Sibley and associates have offered substantial proof that the tumor is the source of the elevated serum aldolase, and their work, as well as that of others (20), suggests that foci of necrosis in the tumor and/or anoxia of certain areas of the tumor may be the factors involved in the liberation of aldolase and possibly other glycolytic enzymes into the circulation.

The varying extents to which these serum glycolytic enzyme activities are elevated by the end of the 3d week of tumor growth are of interest. As has been noted, the average serum isomerase activity is about ninefold the normal mean value, that of serum lactic dehydrogenase is about two-
fold, and that of serum aldolase is, as may be seen from the work of Sibley et al. (15), about three- to fourfold the normal mean value. The plasma volume has been estimated as about 4.0 per cent of the weight of the normal or tumor-bearing adult rat (9). Accordingly, the isomerase content of the plasma compartment of the normal adult rat may be calculated as about 30 × 25 × 4 or 3 × 10^4 μg-equivalents/100 gm of body weight, and the lactic dehydrogenase content as 20/0.006 × 4 × 10^4 or 13.3 × 10^3 units/100 gm of body weight. The contents of isomerase and dehydrogenase of the plasma compartment of the tumor-bearing rats would be about 27 × 10^4 μg-equivalents and 25 × 10^3 units, respectively. The ratio of these is about 1.1. On the other hand, the ratio of the contents of these enzymes in the tumor at 20 days is 1.5 × 10^7 μg-equivalents: 5.2 × 10^7 units, or about 0.29. These results would indicate either that phosphohexose isomerase is liberated relatively more rapidly than lactic dehydrogenase from the tumor, or that it leaves the circulation relatively more slowly. Experiments to test these alternatives are in progress.

SUMMARY

The phosphohexose isomerase and lactic dehydrogenase activities of liver, plasma, and tumor were determined at intervals after the implantation of Walker carcinosarcoma 256 in CFN-Wistar rats. These enzyme activities in the livers of the tumor-bearing animals did not differ significantly

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CHART 5.—Relationship between plasma phosphohexose isomerase and lactic dehydrogenase activities in individual control and tumor-bearing rats. (Lower limit of dehydrogenase activity was less than zero and is not shown on chart.)
at any time from those of the control animals. The contents of the enzymes in the liver rose after about the 10th day of tumor implantation, but this rise was attributable to the relative increase in liver weight of the tumor-bearing animals. The plasma enzyme levels in the tumor-bearing animals remained within normal limits until the 7th day after implantation of the tumor. The plasma phosphohexose isomerase activity then rose until it attained a level at 20 days that was about 9 times the mean normal value, whereas the serum lactic dehydrogenase activity reached a maximum that was only twice its mean normal value. Consideration of individual values showed that at plasma isomerase activities up to about 8 times the upper limit of the normal range, there were no significant rises in plasma lactic dehydrogenase.

The relationship of these plasma enzyme levels to the enzyme content of the tumor and to other factors is considered.

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