Plasma Enzymes in Tumor-bearing Rats

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Summary

The plasma enzymes, lactic dehydrogenase, glutamic oxaloacetic transaminase, and malic dehydrogenase, increased progressively in tumor-bearing rats. The amount of increase varied markedly with the type of tumor and the site of implantation. A lactic dehydrogenase agent could not be demonstrated in any of these animals. Isozyme patterns indicated the tumor was a significant contributor to the increased lactic dehydrogenase in the plasma of these rats. The plasma lactic dehydrogenase was increased less in animals that bore a tumor contaminated with bacteria. The clearance rate of lactic dehydrogenase was slower than normal in tumor-bearing rats, and it decreased progressively with tumor growth.

Introduction

Plasma enzymes are altered during the course of a number of diseases. The usual alteration is an increase in enzyme activity that can be attributed to one or more of the following: (a) increased production of enzyme by some tissue; (b) destruction of tissue, which causes the release of enzymes; (c) increased cellular permeability to the enzymes; (d) decreased plasma clearance of the enzymes.

Most studies on plasma enzyme alterations in tumor-bearing animals have been done on mice. Riley et al. (16) have shown that in most transplantable mouse tumors the elevated plasma LDH was due to a transmissible factor. This transmissible factor, or LDH agent, caused a number of other plasma enzymes to be elevated (13, 15). Later evidence suggests that increases in plasma enzymes in the presence of the LDH agent are due to impaired clearance (1, 9, 14).

The LDH agent could not be demonstrated in either tumor-bearing rats (12) or cancer patients (3), indicating that the mechanism of plasma enzyme elevation in these species is different and that it requires further investigation. We describe experiments to investigate the possibility of an LDH agent in tumor-bearing rats; to correlate elevations in plasma enzyme activity to tumor growth; to reveal the effects of different tumors, different transplant sites, and presence of bacteria on plasma LDH; and to provide information on the mechanism of the elevation of plasma LDH in the host rat.

Materials and Methods

Female Holtzman rats weighing approximately 200 gm, female Swiss mice from A. R. Schmidt Company, Madison, Wisconsin, and female C3H mice bred in our laboratory were used. The tumors were Walker carcinoma 256, Jensen sarcoma, Novikoff hepatoma, MDAB hepatoma (10), Sarcoma 180, and a primary hepatoma induced with dietary 3'-methyl-4-dimethylaminoozo-benzene. Tumor transplantation was performed by the following technique: Tumors were obtained from rats bearing 6- to 8-day-old i.m. tumors. Viable portions of the tumor were sliced into small pieces, separated from connective tissue and necrotic portions, and forced through a 30-mesh stainless steel wire cloth in the barrel of a syringe. The cells were suspended in an equal volume of 0.9% sterile saline containing 200 μg/ml each of sodium penicillin G and streptomycin sulfate. Transplants were made by inoculating 0.2 ml of this suspension into the desired site. When comparisons were made between different sites of inoculation, portions of the same suspensions were used for all sites. Lipo polysaccharide from Escherichia coli 055:B5 (Lot No. 460630) was obtained from Difco Laboratories, Detroit, Michigan.

LDH was assayed by the method of Wróblewski and LaDue (19), MDH as described by Mehler et al. (11), and GOT by the spectrophotometric method of Karmen et al. (7). One unit of activity for each of these enzymes is defined as the amount of enzyme in 1 ml of plasma that produced a decrease of 0.001/min in the optical density at 340 μm when the appropriate substrate was added in the presence of reduced nicotinamide adenine dinucleotide.

The LDH isozymes were separated by vertical starch bed electrophoresis (17) and stained by the procedure described by Fine and Costello (4). The clearance rate of plasma LDH was determined by giving an i.v. injection of 20,000 units of LDH and measuring its rate of disappearance. Plasma from rats bearing large i.m. Jensen tumors was used as the source of LDH, and usually no more than 1 ml of this plasma was required per animal. The disappearance rate was determined by periodically sampling the blood and assaying the LDH activity; at least 3 time intervals were used for the determination of the half time.

Results

The 1st experiment was designed to show the effect of growth of Walker carcinoma, transplanted i.m., upon LDH, MDH, and GOT. A progressive increase in plasma enzymes with tumor growth is depicted in Chart 1. All 3 enzyme activities were significantly increased by the 7th day of tumor growth, when the tumor weight averaged approximately 5% of the total b.w. The least elevation was observed in GOT, but even this activity was increased 10-fold when the tumor accounted for 30% of the total b.w. Plasma LDH was affected most by tumor growth, and this enzyme was therefore selected for studies on the mechanism of plasma enzyme increases in tumor-bearing rats.

The effects of tumor implantation site on plasma LDH are presented in Table 1. The same tumor suspension was used to inoculate i.m. in the rectus femoris, i.p., or s.c. along the back. Determinations of LDH were made when the tumors at all sites averaged approximately 20 gm (range, 10–32 gm). To at-
tain this weight usually required 6-8 days for the i.p. tumors, 8-10 days for the i.m. tumors, and 13-15 days for the s.c. tumors. All tumors caused an elevation in the plasma LDH when they were relatively small. The differences in LDH elevation attributed to the different implantation sites are striking. Although the smallest increases generally occurred with s.c. transplants and the largest with the i.m., there were notable exceptions, i.e., the Walker carcinoma and the i.p. Novikoff hepatoma.

We attempted to demonstrate an LDH agent in these rats. Injections of plasma from tumor-bearing rats or of saline extracts of the tumor failed to elevate the plasma LDH in either rats or mice. The LDH agent in the plasma of mice bearing Sarcoma 180, which increased the LDH in normal mice, had no effect upon the rat. When normal rats were given repeated injections of plasma from a rat bearing a large i.m. Jensen tumor and then inoculated with Novikoff or MDAB tumors, plasma LDH was not elevated above the expected value.

LDH zymograms were used to indicate the source of the plasma LDH in tumor-bearing rats. Table 2 summarizes the distribution of lactic dehydrogenase (LDH) isozymes in tumors and plasma from tumor-bearing rats and various normal tissues.

The LDH isozymes are numbered with respect to their rate of movement toward the anode. No. 1 is the slowest and No. 5, the fastest. The number of plus signs gives an approximation of the quantity of each isozyme. The LDH isozymes were separated by vertical starch bed electrophoresis (17) and stained by the method of Fine and Costello (4).

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>LDH IS MEYS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Muscle</td>
<td>+++</td>
</tr>
<tr>
<td>Kidney</td>
<td>++</td>
</tr>
<tr>
<td>Spleen</td>
<td>+++</td>
</tr>
<tr>
<td>Plasma</td>
<td>+++</td>
</tr>
<tr>
<td>Liver</td>
<td>++</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>++</td>
</tr>
<tr>
<td>Walker carcinoma</td>
<td>++</td>
</tr>
<tr>
<td>Plasma</td>
<td>+++</td>
</tr>
<tr>
<td>Primary hepatoma</td>
<td>+++</td>
</tr>
<tr>
<td>Plasma</td>
<td>+++</td>
</tr>
<tr>
<td>Jensen sarcoma</td>
<td>++</td>
</tr>
<tr>
<td>Plasma</td>
<td>++</td>
</tr>
</tbody>
</table>
* The 3rd band is occasionally found in normal liver.

TABLE 3

Effect of Bacterial Contamination of the Tumor upon Plasma Lactic Dehydrogenase

<table>
<thead>
<tr>
<th>Tumor</th>
<th>% Body weight</th>
<th>LDH units*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncontaminated Walker</td>
<td>13.6</td>
<td>2520 ± 310</td>
</tr>
<tr>
<td>Walker with S. typhimurium#</td>
<td>13.5</td>
<td>1760 ± 270</td>
</tr>
<tr>
<td>Uncontaminated Jensen</td>
<td>13.2</td>
<td>16020 ± 1680</td>
</tr>
<tr>
<td>Jensen with S. typhimurium#</td>
<td>14.4</td>
<td>1666 ± 247</td>
</tr>
</tbody>
</table>
* Average value for 12 animals ± S.E.
# 10⁶ bacteria were mixed with the tumor inoculum and injected i.m.
The increased plasma LDH found in tumor-bearing rats could come from a number of tissues, but the predominant source in our experiments was the tumor tissue. The similarity between the LDH zymograms of the plasma of tumor-bearing rats and the tumor suggests that a substantial portion of the LDH was from the tumor. Similar conclusions have been reached by Burgess and Sylvén (2) in tumor-bearing mice. We could observe no correlation between erythrocyte loss and plasma LDH. For example, the i.p. Jensen shown in Table 1 was the most anemic; yet the plasma LDH was only 13% as high as the same tumor implanted i.m. The Walker tumor implanted i.m. produced less anemia in the rat than either the Novikoff or MDAB; yet it had the highest plasma LDH. Rats bearing Novikoff or MDAB tumors have about the same hemoglobin concentration when their tumors are i.m. and s.c.; yet the i.m. tumors always produced more increase in plasma LDH. It also appears unlikely that the amount of necrosis of the tumor was directly related to the plasma LDH. All of the tumors (Table 1) were as necrotic when implanted s.c. as i.m., but the s.c. implants produced less increase in plasma LDH. We have observed that contaminating the tumor with S. typhimurium reduces the amount of necrotic tumor tissue in rats bearing either the Walker or the Jensen tumor (5, 6). The contamination also reduced the plasma LDH in these rats, and it is possible that the decreased necrosis was partially responsible. A more likely explanation would be the effect of bacterial contamination upon the clearance rate of LDH.

Wakim and Fleisher (18) reported that the RES was involved in the removal of plasma enzymes and that various enzymes were cleared at different rates. Perhaps LDH from different tissues or various isozymes are cleared at different rates. Clearance rates in normal, endotoxin-treated, and tumor-bearing rats were determined with the use of plasma containing isozymes 1 and 2 from a rat bearing the Jensen tumor.

Since the RES is involved in the clearance of plasma enzymes, it is not surprising that endotoxins and bacteria alter the concentrations of these enzymes in plasma. Effects of endotoxin are not restricted to LDH but have also been shown for GOT and 2-hydroxybutyric dehydrogenase (8). Konttinen et al. (8) found that plasma LDH reached a maximum 6 hr after injection of endotoxin and returned to normal at 24 hr. Two hr after endotoxin, we observed a slow clearance of plasma LDH, which could have been responsible for the maximum activity at 6 hr; however, enhanced clearance must start much earlier than our observation for the activity to be normal at 24 hr. We observed an increased clearance of plasma LDH and a decreased activity at 48 hr after endotoxin.

Discussion

The effect of the tumor upon the LDH clearance rate was examined, and these data are presented in Table 4. We selected a tumor that did not produce a large increase in LDH so that there was a high ratio between the injected LDH and the endogenous amount. Tumor-bearing rats consistently had a depressed clearance rate. The depression in clearance rate and elevation in plasma LDH activity appeared to progress with increasing tumor size.
Our results agree with those of Notkins et al. (12), which indicate that an LDH agent is not involved in the increased plasma LDH in transplantable rat tumors. Increased plasma LDH in rats bearing transplantable tumors appears to be due to a decreased clearance of the enzyme and an additional source of enzyme, the tumor. It is impossible with our present information to determine the relative importance of these factors.

References

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