Lactic Dehydrogenase Activity in Plasma and Interstitial Fluid during Growth of Mouse Tumors*

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SUMMARY

Changes in the plasma lactic dehydrogenase (LDH) activity in mice bearing a solid mammary carcinoma or the Ehrlich-Landschütz hyperdiploid (ELD) ascites carcinoma have been studied. The Riley agent was associated with both tumors; therefore, an increase of 5—10 times the normal activity level of LDH occurred shortly after tumor inoculation. The sources of the larger increase of plasma LDH which appeared during later stages of tumor growth are discussed. The main emphasis was on the enzyme concentration gradients between the interstitial fluid compartments concerned. It was concluded that a large proportion of the increased LDH activity was released from the tumor cells. With reference to the anemia, it was shown that the contents of the destroyed red cells was only large enough to produce a small part of the observed LDH elevation.

The lactic dehydrogenase (LDH) activity in normal mouse plasma is low but increases significantly during tumor growth (10). Riley and Wróblewski (15) have described this rise as occurring in five stages. Stage 1 was a latent period lasting for about 72 hours after inoculation of the tumor. This was followed by a rapid increase in plasma LDH activity to about 5—10 times its normal level, prior to detectable growth of the tumor. The third stage was a plateau at this level for 4—5 days, followed by stage 4—a second increase during the logarithmic growth phase of the tumor, when levels of 50—100 times the normal could be reached. The 5th and final stage was a rapid fall in plasma LDH just before the death of the animal. Similar changes have been reported by Hsieh, Suntzeff, and Cowdry (9) and by Friend and Wróblewski (4) for a variety of transplanted and induced solid tumors and leukemias. Riley et al. (12, 14) then showed that a "virus-like" agent associated with many transplanted tumors was responsible for the five- to tenfold rise in plasma LDH activity during stages 2 and 3 mentioned above. He attributed higher increases to a "synergistic effect" of the agent with the growing tumor. In a later paper Riley (13) has established evidence indicating that the anemia of tumor cases was one operating factor, although not the only one, in the sense that red cell destruction contributed added amounts of LDH to tumor plasma.

It is clear that the plasma enzymes have different cellular origins, which observation, in the case of the LDH activity, is also reflected by the various subfractions called "isoenzymes" (3, 6). It is our aim to investigate further the various sources of the added plasma LDH activity and its relationship to tumor growth and to the LDH content of other extracellular fluid compartments. Recent data (1, 16) on the enzymic activity of extracellular tissue fluids now render possible an incomplete evaluation of the pertinent dynamic concentration and transport conditions between tumor and host compartments (17). Attention will be paid mainly to LDH rises during stages 4 and 5 of Riley, as observed in solid and ascites mouse tumor populations bearing the Riley agent.

MATERIALS AND METHODS

Mouse and tumor strains.—Only C3H and hybrids of C3H X DBA mice were used. The solid tumor was a nonhemorrhagic mammary carcinoma

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which originated as a spontaneous tumor in a CSH mouse. It was transplanted subcutaneously in such a way that the resulting tumors became unicentric. The ascites tumor was the hyperdiploid Ehrlich-Landschütz (ELD) carcinoma. The routine inoculum contained $25 \times 10^6$ cells.

Other tumor inocula were employed in certain experiments as indicated in the text. When suspensions of ascites cells containing $800 \times 10^6$ cells per ml. were required, the cells were obtained by centrifuging a 10- to 12-day ascites tumor and were resuspended in sterile physiological saline to give the required concentration.

All mice received food and water ad libitum. Ascites tumor and blood samples were obtained as described by Burgess and Sylvén (2).

Methods.—The growth rate of solid tumors was measured by removing and weighing them after the host was killed. No quantitative measure of the ratio of growing to necrotic regions could be obtained, but an estimate was always made by observation of the color and transparency of the vital tumor regions (5).

Growth of ascites tumors was assessed by serial measurements of total fluid volume and total ascites cell number as described previously (2).

Serial measurements of the hematocrit value of all tumor-bearing mice were made to establish the time of onset and degree of anemia. It is realized that changes in total blood volume may be different in mice bearing solid and ascites tumors, but this has not been taken into account, since the absolute value of the anemia was felt to be irrelevant to these results.

Lactic dehydrogenase was measured spectrophotometrically as described previously (1). The activities are expressed as Wróblewski units/µl (18). Wróblewski units were calculated per ml.; therefore, our values should be multiplied by $10^4$ to render them exactly equivalent to those of Wróblewski.

RESULTS

Normal LDH levels.—The normal baseline levels of LDH activity expressed on a per-volume basis in this strain of mice were on average: in blood plasma, 0.4–0.6 units/µl; in normal, cell-free, intraperitoneal fluid and in interstitial fluid sampled from subcutaneous fat tissue and also from skeletal muscle, 2–3 units/µl.

The extracellular tissue fluid, which later on is drained into the blood, thus contained LDH activity ca. 5 times higher than that in blood itself. This difference is still greater if the activity is expressed on a per-protein basis. Additional amounts of this enzyme are apparently added from local cellular sources.

It should be emphasized that the above values for LDH activity in intraperitoneal fluid were found in CSH mice. A strain of white Swiss stock mice available in our laboratory showed higher levels of about 7 units/µl in the intraperitoneal fluid, although the normal plasma level was the same as in CSH mice.

LDH in solid mammary carcinomas.—The pattern found by Riley and Wróblewski (15) for the increase in plasma LDH was confirmed during the growth of this solid mammary carcinoma (Chart 1). The rise in plasma LDH occurred in two phases with respect to other changes which were taking place simultaneously. During the first 11 days the tumors grew slowly, reaching about 1 gm. weight and presenting no or very little necrosis. There was a small rise in plasma LDH, a rapid loss of carcass weight, and a small fall in hematocrit. After the 11th day the tumors grew rapidly in size, reaching 7–8 gm. when the animals died at 22–25 days and developing a high degree of central necrosis. When the tumor weighed between 1 and 2.5 gm. the necrosis was of a dry type, and at this time the most rapid increase in plasma LDH occurred (while the rate of loss of carcass weight slowed down). No further change in hematocrit was seen after the 11th day of tumor growth.

A summary of the observations in other compartments of the same tumor-bearing strain of mice showed the following activity ranges (1): cell-free interstitial fluid at the tumor periphery, $\sim 140$ units/µl; in the necrotic tumor center, $\sim 220$ units/µl; intraperitoneal fluid in tumor cases not involving the peritoneal cavity, ranges, 15–20 units/µl.

As compared with the normal conditions the dynamic equilibria had now changed so that the
blood plasma had an activity concentration about twice that of the interstitial fluid of normal compartments not directly involved by tumor. The very high levels in the tumor compartment could be derived only from local cellular sources.

**LDH gradients in ascites tumors.**—A different plasma LDH activity pattern was obtained when the growth of the ELD ascites tumor was studied (Chart 2, A and B). The growth curves of the ascites tumor were similar to those described previously (2). A rapid growth, measured by the increase in total cell number, occurred soon after inoculation and continued for 8–10 days. This was accompanied by a larger rise in plasma LDH concentration than that found during the early phase of solid tumor growth (Chart 2, B). A still higher LDH concentration was observed in the ascites fluid at this time, but this subsequently fell somewhat, owing to the increased rate of dilution at later stages of growth. The animals lost no carcass weight but developed a high degree of anemia (Chart 2, B). The decreased growth rate of the ascites tumor at about 10 days, as well as the subsequent very marked loss in total cell number at 14–16 days, was accompanied by a further increase in the LDH activity of both ascites fluid and plasma. There was still no loss of carcass weight, and the proportion of red blood cells began to increase.

The LDH activity levels in the two compartments under study cannot be directly compared with those of solid tumors, since an enormous extra interstitial fluid pool was formed in the ascites case. Therefore, the total LDH content of plasma and ascites fluid (Chart 2, A) was calculated. The

![Chart 2](chart2.png)

**Chart 2.**—Changes in plasma and ascites fluid LDH, and hematocrit in mice during growth of the ELD ascites tumors.

A: ▲ ▲ Total ascites cell number;
● ●● LDH, total units in blood plasma and ascites fluid.
B: △ △ Ascites volume, ml.;
■ ■ Plasma LDH, units/μl.
○ ○ Ascites fluid LDH, units/μl.
□ □ Hematocrit.

The normal levels of plasma and intraperitoneal LDH are indicated by means of dotted lines across the graph. Each point represents the mean value of three mice.

results strongly suggest that the largest increase in total fluid LDH was associated with the terminal phase of growth characterized by extensive decay of ascites tumor cells.

The salient feature of the plasma/ascites activity ratio in this material is that the peritoneal compartment was mostly richer in LDH activity than the plasma. If calculated on a per-protein basis, the difference would become still greater. This condition is indicative of a local release of enzymes in the peritoneal cavity.

**Additional experiments and quantitative estimations.**—Further information as to the possible
sources of the amounts of enzyme added to the plasma in tumor cases may be obtained by the following experiments and considerations:

1. Riley et al. (14) have shown that injection of minute amounts of his agent will cause a permanent rise of 5–10 times in the plasma LDH activity after 2 or 3 days. This has been amply confirmed in this and other laboratories. No greater rise in the plasma LDH could be obtained by several repeated large injections of agent-containing ascites fluid. This rules out the possibility that additional increases are due to large amounts of agent, perhaps released from massive necrosis of tumor cells.

2. Inocula containing \(300 \times 10^6\) untreated ascites cells were injected every other day into mice. The mice had received cells equivalent to a 12-day ascites tumor after three such injections. After each injection some of the animals were killed, and measurements were made of plasma LDH, hematocrit, and ascites cell number. Chart 3 shows that after such a large inoculum many of the inoculated cells disappeared rapidly; and the more injections the mice received, the fewer cells survived. This massive destruction of cells in the animal was accompanied by a very large rise in the plasma LDH activity.

If, after three such large inoculations the mice were left for 4 days without further treatment, the ascites cell number began to increase again in the usual way, and the plasma LDH level fell somewhat from the maximum found immediately after the repeated injections. The animals did not become anemic until the inoculations had ceased and the total tumor cell number began to increase.

3. It should, furthermore, be considered that living ascites tumor cells continuously “secrete” or “leak out” fairly large LDH activities. Under in vitro conditions these amount to the order of 1,000 units/hr/\(10^6\) cells (7).

4. Since, among other factors involved, the specific rates of enzymic inactivation in tissue compartments and the rates of elimination in tumor-bearing mice are unknown and may be subject to variations in the course of tumor growth, a rough assessment of the pertinent quantitative aspects is premature. The following calculations, therefore, suggest only that in the case of ascites tumors there was sufficient LDH in the tumor cells which lysed to account for the added amounts of LDH observed in both fluid compartments. This does not preclude the possibility of other additional enzymic sources.

Ascites cells at the 12th day after inoculation, just before they start to deteriorate, contain ca. 12 units of LDH per \(20,000\) cells (2). In the ascites tumor shown in Chart 2 about \(650 \times 10^6\) ascites cells disappeared after the 13th day. These cells contained about 390,000 units of LDH, whereas the rise in total fluid LDH was about 200,000 units.

The inoculum of \(300 \times 10^6\) ascites cells represented about 180,000 units of LDH; thus, after three such inocula 540,000 units of LDH had been injected. Chart 3 shows that the maximum increase in plasma LDH after such a series of injections was about 30 units/\(\mu l\). A mouse has ca. 1 ml. of blood plasma; therefore, the total increase in LDH was only approximately 30,000 units.
observed degrees of anemia cannot alone account for the observed rises in LDH activity of blood plasma and ascites fluid.

DISCUSSION

The results show that the introduction of a growing tumor can inflict large changes in the normal pattern of LDH activity. Furthermore, the patterns vary both in different types of tumors and during the growth of a single tumor. In the case of solid mammary carcinomas the plasma LDH level reached a range 2 times higher than that of the intraperitoneal fluid of the same mouse. The time-activity curves gave no direct indication as to the sources of the added enzyme activity. Probably contributions have been added from both tumor cells and from host tissues, including muscle and lyed red cells. The relative size of these contributions from different sources cannot yet be estimated. In the case of ascites tumor growth, the ascites fluid LDH concentration was higher from the 5th to 14th days after inoculation than that of the plasma. During this time a marked anemia developed, but there was no decrease in carcass weight. The degree of protein retardation in the peritoneal cavity was probably increasing, but at the same time there was also an increasing degree of dilution. The total LDH activity of ascites fluid and blood plasma was at this time roughly proportional to the total tumor cell number (Chart 2, A). During later stages of ascites tumor growth the total LDH activity showed a remarkably high increase, while the free tumor cell number rapidly decreased. This may suggest that large additional quantities of LDH have originated from the lysed tumor cells. The assumed LDH contributions from decaying tumor cells was further evidenced by independent experiments (Chart 3), in which the plasma LDH activity showed an early twofold rise over the maximum plasma level during ascites tumor growth without any association with host anemia or decrease in carcass weight.

Available data do not justify a strict quantitative evaluation of the dynamic transport conditions involved (17). This is partly because of our lack of independent marker experiments aimed to supply the necessary rate coefficients of enzymic elimination and transport between the different body compartments. At this preliminary stage it does seem appropriate, however, to call attention to the possible sources of LDH activity, among which the tumor cells should not be neglected. Our evaluations actually show that the tumor cells constitute a much larger pool of LDH activity than that which could possibly be released from red cells in the course of anemia. We agree with Riley (13) that in some mouse tumors anemia alone may explain a five- or tenfold rise in plasma LDH activity but cannot give a sufficient explanation for larger contributions as generally observed in both solid and ascites tumors. This point is no matter of controversy; the specific LDH sources in different tumor types may possibly become delineated in the future by data obtained from starch gel electrophoresis experiments.

Several other questions remain to be more closely studied in connection with enzymatic plasma changes in tumor diseases. From the in vitro experiments by Holmberg (7) and others it may be inferred that vital tumor cells release rather marked quantities of soluble cytoplasmic enzymes. Now the question arises whether, and to what extent, necrotic tumor cells constitute a source. We have a feeling, only circumstantially supported by the present data, that large rises in plasma LDH activity are often associated with massive tumor necrosis. However, since the parameters of tumor necrosis cannot be easily measured by independent means, no clear-cut correlations have been possible between plasma enzyme levels and vital tumor mass, or the extent or rate of necrotic changes. In discussions on the complex and still obscure operativi mechanisms of systemic changes evoked by a rapidly growing tumor, protein degradation products may play an important role. We have witnessed the discovery of the toxohormone (11), but other more specific diffusible agents with a cytotoxic activity directed against certain host cells (8) may be released from tumors.

REFERENCES


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