Serum Lactic Dehydrogenase Activity in Mice with Transplantable Leukemia*

BORROUGHS R. HILL AND RUSSELL T. JORDAN†

(Departments of Biochemistry and Microbiology, Division of Research, City of Hope Medical Research Institute, Duarte, Calif.)

Previous studies in man have shown that an elevated level of serum lactic dehydrogenase (SLDH) occurs in many individuals with neoplastic disease (1, 3). This elevation has been noted most consistently in patients with untreated leukemia (1). In an extension of these studies, experiments were undertaken to determine the level of SLDH in the blood of AKR mice before, and at various intervals after, the transplantation of lymphatic leukemia by various routes. A preliminary report has been made of this work (2).

MATERIALS AND METHODS

The 6- to 7-week-old mice used in this study were obtained from the Jackson Memorial Laboratory (AKR/Jax), and from Carworth Farms (AKR/C). Animals from different sources were chosen because of the difference in susceptibility to the tumor used. AKR/Jax mice are uniformly susceptible to the transplantable leukemia with a 100 per cent mortality rate. In AKR/C mice, however, the mortality rate is considerably less, with tumors growing in some animals for 4-8 days, and then regressing and disappearing. The L4946, line B, "ascites form" of transplantable leukemia was supplied by Dr. Lloyd Law, Bethesda, Maryland.

Groups of ten mice were given inoculations intracerebrally, intraperitoneally, or subcutaneously with approximately 1 × 10⁶ ascites tumor cells counted in a hemocytometer. Control animals received comparable numbers of trypsinized spleen cells from healthy AKR mice. Trypsin-dispersed spleen cells were obtained by the method described by Melnick (6) for kidney tissue. Splenic tissue was used, because it was the most satisfactory lymphoid tissue obtainable in sufficient quantity for the experiment. A second control group of untreated AKR mice was bled at the same time as the other groups to determine any changes which might have occurred in SLDH levels following repeated sampling for blood.

The diagnosis of leukemia in mice was based on the presence of typically enlarged pea-sized peripheral lymph nodes, enlarged spleen and liver, bone marrow examination, and white blood cell counts. In all instances the diagnosis of leukemia was confirmed by microscopic examination of liver or spleen and the transplantation of suspensions of these organs into normal mice.

Tail blood was examined at intervals for SLDH activity. Blood was drawn into a certified white blood cell diluting pipette, with physiological saline used as diluent to a final dilution of 1:20. The diluted blood was centrifuged for 4 minutes at 1,500 r.p.m. Blood clots were loosened with a small wooden applicator, and the suspension was recenterfuged. Since uncentrifuged diluted blood allowed to stand longer than 10 minutes often showed a slow, steady increase in SLDH activity, diluted blood samples were always centrifuged within 10 minutes after withdrawal. During the 10-minute period no significant increase in enzyme activity occurred. The supernatant fluid was then transferred to another tube, and all samples showing obvious hemolysis were discarded. A light straw color was ignored, since it was found that this amount of hemolysis did not significantly affect the results. Samples of 0.05 ml. of each of the supernatant fluids were diluted to 0.1 ml. with water and assayed for SLDH activity by a method described in a previous communication (3). Enzyme activity is expressed as mg. of dihydrodiphosphopyridine nucleotide oxidized/min/ml of original blood.

RESULTS

In Charts 1, 2, and 3 SLDH levels were plotted for only that period of time when all animals in the inoculated groups were still alive. The plotted values represent averages for ten mice, and the vertical lines show the standard errors (SE) of the means.
In mice given intracerebral inoculations of tumor cells (Chart 1) there was an increase in SLDH soon after transplantation, and the increase continued until a plateau was reached on the 4th day; this continued until the 12th day. The terminal stage of the disease was characterized by a rise in SLDH (Chart 4), the levels in some animals becoming 6–8 times higher than those of the controls. Although SLDH levels were increased within 2 days after the intracerebral transplantation of tumor cells, it was not until 8 days after the transplant that leukemia could be detected by blood count or bone marrow aspiration. The first death in the intracerebral group occurred on the 14th day (Table 1). SLDH levels of mice receiving spleen suspension intracerebrally were similar to those found in mice which were bled as controls but received no tissue transplant.

Within 2 days after inoculation, mice given ascites cells intraperitoneally (Chart 2) showed a rise in SLDH which continued progressively until death. Serum LDH in moribund mice showed a marked increase in activity just before death (Chart 4). Although the initial rise occurred within 2 days after intraperitoneal transplantation, it was not until the 7th day that leukemia could be detected by other methods. The first deaths occurred in this group on the 11th day (Table 1). Mice receiving spleen suspensions intraperitoneally showed
a slight rise in SLDH on the 5th day as compared with mice bled as controls.

Mice given subcutaneous inoculations of tumor cells (Chart 3) showed a rise in SLDH as early as 2 days after transplantation. Serum LDH activity in the subcutaneous group reached a higher level than in the group receiving cells by the intracerebral route. Although a rise in SLDH occurred in mice 2 days after subcutaneous transplantation of ascites cells, it was not until the 18th day that bone marrow and blood showed the presence of leukotic cells. The first death in this group occurred on the 21st day (Table 1). The inoculation of the period when SLDH activity was elevated, tumor growth was confirmed by other methods. In 93 days the serum enzyme activity had returned to near-normal levels, and at this time no tumors could be detected by other methods.

DISCUSSION

The results obtained in the present investigation are in agreement with those reported by Hsieh, Suntzeff, and Cowdry for several transplanted and induced tumors in mice (4, 5). They found an early rise in SLDH activity followed by a sharp rise in the terminal stage of the disease.

### TABLE 1

<table>
<thead>
<tr>
<th>Route of inoculation*</th>
<th>Rise in blood LDH</th>
<th>First sign of tumor take†</th>
<th>First death (Days after inoculation)</th>
<th>50 per cent mortality</th>
<th>50 per cent mortality mortality (No. mice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracerebral</td>
<td>2</td>
<td>8</td>
<td>14</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Subcutaneous</td>
<td>2</td>
<td>11</td>
<td>21</td>
<td>54</td>
<td>5</td>
</tr>
</tbody>
</table>

* Groups of ten mice.
† Evidenced by palpation or hematological and cytological examination.

They also observed a decline in activity to normal levels in cases of tumor regressions. It is of interest to note that the early rise in SLDH occurred well in advance of any clinical or hematological evidence of tumor growth.

SUMMARY

1. Two different sublines of AKR mice varying in susceptibility to the lethal effects of the L4946 transplantable leukemia of mice were studied to determine the level of serum lactic acid dehydrogenase (SLDH) following transplantation of tumor cells by various routes.
2. All mice given injections intracerebrally, intraperitoneally, and subcutaneously showed a rise in serum lactic dehydrogenase as early as 48 hours following inoculation, at a time when tumor growth was not palatable or detectable by hematological or cytological examination.
3. In mice uniformly susceptible to the lethal effects of the tumor cells, serum lactic dehydrogenase activity increased until the animals died and showed a marked rise just before death.
4. In mice bearing tumors which grew and then regressed following transplantation, an initial rise in serum lactic dehydrogenase occurred which gradually returned to within normal limits when the tumor disappeared.
5. The results indicate that serum lactic dehydrogenase activity is a sensitive indicator for.
determining the growth, development, and regression of the L4946 transplantable lymphatic leukemia in AKR mice.

REFERENCES


Serum Lactic Dehydrogenase Activity in Mice with Transplantable Leukemia

Borroughs R. Hill and Russell T. Jordan

Cancer Res 1957;17:144-147.

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