MATERIALS AND METHODS

Tumors tested show increased activity of LDHk when compared to the purified enzyme is assayed in vitro (2). This association with forming virus encodes LDHk. That is, a mutant of Kirsten sarcoma shock (2).

Oxygen and by certain dinucleotides (4), and, as mentioned zymes. LDHk is a basic protein which is reversibly inhibited by other LDH isozymes, which we will call the standard LDH iso isozymes. Several characteristics distinguish LDHk from the related to the uncharacterized LDH VI originally noted by Markert and Moller (10) in their seminal paper on the separation of LDH (EC 1.1.1.27) activity and was therefore called LDHk. It may be found in the sera of many patients with malignant tumors, while the sera of healthy persons had little or no such activity. This isozyme was detectable only when assayed in a nitrogen atmosphere, and its activity showed little or no relationship to the total lactate dehydrogenase activity as measured by a standard clinical assay. The activity of serum LDHk appeared to be correlated with the presence of known metastases. Increased serum LDHk appeared in a wide variety of patients with cancer, although it appeared to be more common in certain types of cancer. Increased serum LDHk activity was also found in the sera of some patients with nonmalignant disease. The activity of serum LDHk may be useful to monitor recurrence or response to therapy in certain types of cancer.

INTRODUCTION

In 1979, Anderson et al. (3) described an antigen common to anaerobically shocked cells and cells transformed by Kirsten murine sarcoma virus. This protein was shown to have a LDH2 (EC 1.1.1.27) activity and was therefore called LDHk. It may be related to the uncharacterized LDH VI originally noted by Markert and Moller (10) in their seminal paper on the separation of LDH isozymes. Several characteristics distinguish LDHk from the other LDH isozymes, which we will call the standard LDH isozymes. LDHk is a basic protein which is reversibly inhibited by oxygen and by certain dinucleotides (4), and, as mentioned above, it can be induced in untransformed cells by anaerobic shock (2).

Genetic evidence suggests that an oncogene of Kirsten transforming virus encodes LDHk. That is, a mutant of Kirsten sarcoma virus which is temperature sensitive for transformation produces an LDHk activity which is similarly temperature sensitive when the purified enzyme is assayed in vitro (2). This association with malignant transformation was reinforced by the finding that most tumors tested show increased activity of LDHk when compared to adjoining normal tissue (1, 4). This finding prompted us to test sera of patients having malignant disease for the presence of LDHk.

A summary of some of the data in this study has been published previously (5).

MATERIALS AND METHODS

Serum Specimens. Sera were obtained from the Department of Laboratory Medicine, Roswell Park Memorial Institute, and the Clinical Laboratories of Buffalo General Hospital, Buffalo, NY. They were separated from the blood clots within 4 hr of collection and were stored at 4° until tested.

Serum samples from patients were assayed in order of receipt without knowledge of the diagnoses. Positive control samples were included with each set of serum assays.

LDH Isozyme Analysis. Isozymes were separated and measured by nondenaturing slab gel electrophoresis. The gel system for classical LDH isozymes was a modification of that of Dietz and Lubrano (8): a 5.5% polycrylamide slab cast in 0.4 M Tris-HCl, pH 8.3, with 0.05 M Tris-HCl and 0.38 M glycine for the reservoir buffers. The LDHk isozymes were separated using the system of Anderson and Kovacik (1), a 5.5% polycrylamide gel (with 0.15% bisacrylamide) photopolymerized with 5 μg of riboflavin and 0.27 μl of N,N',N'-tetramethylethylenediamine per ml. The gel buffer was 0.15 M potassium tetraborate, pH 8.3. The upper reservoir buffer contained 0.08 M imidazole and 0.02 M boric acid, pH 8.9; the lower, 0.075 M potassium tetraborate, pH 8.3. Electrophoresis was performed with reverse polarity, i.e., with the anode in the upper reservoir, for 16 hr at 200 V (20 V/cm) at 4°.

LDHk isozymes were visualized in these gels by the staining method of Dietz and Lubrano (8). When LDHk gels were stained, the staining mixture was supplemented with 2% glycerol to complex borate ions, and the staining was done under a nitrogen atmosphere for 4.5 hr at room temperature. Gels were scanned with an integrating densitometer (Quick Scan Jr.; Helena Laboratories, Beaumont, TX) to measure the intensity of staining. One cycle of the integrator pen was taken as 1 unit of LDH activity; this unit corresponds to about 10^-6 IU.

Statistical Analysis. For analysis of variance and correlation analysis, the dependent variable was log10(serum LDHk + 1). This transformation made the variance of the variable more constant from group to group. The analysis was done using the Statistical Package for the Social Sciences.

RESULTS

Characteristics of the Assay for LDHk. As described more fully below, an LDHk-like activity was detected in sera of many cancer patients. The activity closely resembled that described in transformed cultured cells (2) and in human tumors (4) with respect to its electrophoretic mobility and its reversible inhibition by oxygen. The enzymatic activity observed in the electrophoretic assay was proportional to the amount of serum added down to about 5 LDHk units. Below this point, the assay was nonlinear in a way which tended to diminish differences among weakly positive sera.

This LDHk activity was unchanged after storage for 3 weeks at 4° or a cycle of freezing and thawing.

LDHk in Sera of Patients with Cancer and in Healthy Individuals. Chart 1 presents the LDHk activities found in the sera of 206 patients with cancer and 30 healthy donors. The data are separated according to the site of the primary tumor or the type of malignant disease. Many patients had high LDHk activity compared to the control group. The value, 2 units/0.1 ml, being 2 S.D.s above the mean for the control group could be used as
a cutoff between normal and elevated levels. About one-half of
the patients had LDH\(_k\) activity above this level.

**Serum LDH\(_k\) in Sera of Patients with Nonmalignant Disease.**
The control sera in Chart 1 were obtained from healthy donors
(for plasmapheresis). To examine serum LDH\(_k\) in patients with
nonmalignant diseases, we obtained sera from patients admitted
at a general hospital, assayed them for LDH\(_k\) in a blind fashion,
and then consulted their hospital records for diagnostic infor-
mation. The patients whose diagnoses did not include cancer
are summarized in Table 1. Of 78 patients, 13 (17\%) had values
greater than 3 units, and 22 (28\%) had values greater than 2.
These groups could still have included patients with undetected
cancer; i.e., the diagnostic work-up could have been incomplete.

Two of these patients with the most increased serum LDH\(_k\)
were older than any of the cancer patients studied. We speculate
that advanced age may affect serum LDH\(_k\), but we do not
presently have enough data to test that speculation. As men-
tioned above, there was no apparent connection between age
and serum LDH\(_k\) within the population of cancer patients.

**LDH\(_k\) in Sera of Patients with Metastatic Malignant Disease.**
The data in Chart 1 seemed to show a correlation between the
serum LDH\(_k\) activity and the presence of metastases. To test
this possibility, we performed a one-way analysis of variance to
compare patients with metastatic disease to those with primary
tumors only. The difference between these groups was signifi-
cant (p = 0.018).

To allow a visual comparison of serum LDH\(_k\) activity among
healthy individuals, patients with nonmalignant disease, patients
with primary malignant disease, and patients with metastatic
malignant disease, we combined the data of Chart 1 and Table
1. The cases were grouped according to the activity of serum
LDH\(_k\) as shown in Chart 2. The distribution of cases among the
Table 1
LOH in patients without known cancer

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>LDH&lt;sub&gt;k&lt;/sub&gt; (units/0.1 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes, arteriosclerotic heart disease, anemia, small bowel obstruction (cancer not ruled out)</td>
<td>F</td>
<td>81</td>
<td>26</td>
</tr>
<tr>
<td>Cholangitis, perforation of gall bladder</td>
<td>F</td>
<td>70</td>
<td>17</td>
</tr>
<tr>
<td>Back pain, no cancer</td>
<td>F</td>
<td>73</td>
<td>12</td>
</tr>
<tr>
<td>Lumbar disc protrusion, no cancer</td>
<td>M</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>Leg ulcers, bedsores</td>
<td>F</td>
<td>90</td>
<td>9</td>
</tr>
<tr>
<td>Cataract</td>
<td>F</td>
<td>65</td>
<td>11</td>
</tr>
<tr>
<td>Phlebitis of leg</td>
<td>F</td>
<td>73</td>
<td>11</td>
</tr>
<tr>
<td>Diabetes, hypergastrinemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior septal myocardial infarction, left ventricular aneurysm, coronary artery disease</td>
<td>M</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Coronary spasm, mitral valve prolapse, cholecystitis</td>
<td>F</td>
<td>60</td>
<td>8</td>
</tr>
<tr>
<td>Right tympanoplasty</td>
<td>F</td>
<td>52</td>
<td>7</td>
</tr>
<tr>
<td>Common duct cyst</td>
<td>F</td>
<td>71</td>
<td>6</td>
</tr>
<tr>
<td>Inflammatory perineal cyst</td>
<td>F</td>
<td>56</td>
<td>6</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>M</td>
<td>63</td>
<td>6</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>F</td>
<td>71</td>
<td>5</td>
</tr>
<tr>
<td>Hematoma</td>
<td>F</td>
<td>74</td>
<td>5</td>
</tr>
<tr>
<td>Umbilical hernia</td>
<td>M</td>
<td>65</td>
<td>4</td>
</tr>
</tbody>
</table>

9 patients without cancer 3
57 patients without cancer <=2

3, there was little or no correlation between standard LDH activity and LDH<sub>k</sub> activity. The correlation coefficient between log(LDH<sub>k</sub> + 1) and log(LDH<sub>k</sub> + 1) was 0.44, confirming that the 2 parameters were not correlated. This was consistent with previous work which showed that LDH<sub>k</sub> differs from the standard isozymes both biochemically (2) and in its expression during the cell cycle (4).

Electrophoretic Variants of Serum LDH<sub>k</sub>. In a few cases, we detected electrophoretic variants of serum LDH<sub>k</sub>. Fig. 1 shows a section of one electrophoretogram which contained 3 variants, all of which migrated less rapidly than the usual form of serum LDH<sub>k</sub>. The patients with these apparent variants had different types of malignant tumors: colon; pharyngeal; and testicular. The serum enzyme in all except these 3 cases co-migrated with tumor LDH<sub>k</sub> but in no cases did we have tumor and serum enzyme from the same patient.

Inhibition by Diadenosine Tetraphosphate. One unusual characteristic of LDH<sub>k</sub> is that its activity is inhibited by diadenosine tetraphosphate and diguanosine tetraphosphate (4). The K<sub>i</sub> for these inhibitors is about 100 μM, and they are not competitive with NAD<sup>+</sup>. This property is shared by LDH<sub>k</sub> activities from cells.

Comparison with Standard LDH Isozymes. The activity of standard LDH isozymes was also sometimes increased in sera of patients with neoplastic disease. However, as shown in Chart 2, bottom. The ratio of metastatic cases to total malignant cases in each of the activity classes is shown in Chart 2, top.

Comparison with Standard LDH Isozymes. The activity of standard LDH isozymes was also sometimes increased in sera of patients with neoplastic disease. However, as shown in Chart 2, bottom. The ratio of metastatic cases to total malignant cases in each of the activity classes is shown in Chart 2, top.

Comparison with Standard LDH Isozymes. The activity of standard LDH isozymes was also sometimes increased in sera of patients with neoplastic disease. However, as shown in Chart 2, bottom. The ratio of metastatic cases to total malignant cases in each of the activity classes is shown in Chart 2, top.
transformed by Kirsten murine sarcoma virus, from human tumors, from human liver, and from rat retina (11). Human serum LDHₖ was, by contrast, not inhibited by diadenosine tetraphosphate (Chart 4). This property may reflect a unique source for the serum enzyme, or it may reflect modification of the enzyme by serum components.

**DISCUSSION**

In this study, we examined the presence of LDHₖ in the serum of a wide variety of patients. The variety of diseases in the patient population limits our conclusions. Nevertheless, serum LDHₖ seemed to be correlated with cancer and especially with the presence of metastases. The observation does not necessarily imply that metastatic disease leads to production of serum LDHₖ. Perhaps, for example, the types of tumor which were most likely to metastasize in our population also tended to be LDHₖ producing. However, we feel that there probably is a relationship between the extent of metastases and the level of serum LDHₖ for at least some types of cancer. We have recently observed such a correlation in some patients by following serum LDHₖ levels during disease progression or during treatment.

It also seems that different types of cancer may have different effects on the serum LDHₖ of the patient. Among the 23 groups of cancer presented in Chart 1, 8 were represented by samples from at least 10 patients. These groups were: colon; breast; ovarian; lung; oral and pharyngeal neoplasms; melanoma; Hodgkin's disease and malignant lymphoma; and acute myelocytic leukemia. Of these groups, patients with colon cancer had the highest mean serum LDHₖ, and patients with oral and pharyngeal tumors had the lowest. The difference between these 2 groups was significant at the 5% level when tested by the Wilcoxon 2-sample test (9).

Serum LDHₖ may also appear in patients with nonmalignant disease. The interpretation of our data on this point is somewhat limited by the fact that some patients may have had undocumented malignant disease. It will be important to define nonmalignant conditions which cause the appearance of serum LDHₖ and to establish the origin of serum LDHₖ in such patients.

Two previous reports also describe a cathodal LDH activity in the serum of some patients with nonmalignant disease (6, 7). This basic LDH could have been LDHₖ. Although LDH assays in these cases were presumably performed under aerobic conditions, LDHₖ does have some residual aerobic activity, and thus it might have been detected in some patients with a very high level of this enzyme in their sera. Since many of these patients had severe hypoxia, an extreme level of LDHₖ might not be surprising.

We do not know the source of serum LDHₖ in the patients studied. Since many tumors have high LDHₖ activity, it is reasonable to assume that they may secrete this enzyme or release it during necrosis. On the other hand, since anoxia can induce LDHₖ in rat cells and rat muscle (4), LDHₖ could be produced in nontumorous tissue as a result of circulatory disturbance caused by the tumor mass. The source of serum LDHₖ could be investigated by correlating the levels of LDHₖ in tumor and serum of individual patients or by correlating the presence of LDHₖ variants in the tumor and serum.

Further definition of the occurrence and origin of serum LDHₖ will show whether this is a valuable marker for any aspect of malignant disease. If serum LDHₖ were characteristic of metastatic disease, e.g., it might be useful to estimate the extent of metastatic disease and to monitor the response to treatment.

**ACKNOWLEDGMENTS**

The authors thank V. Onorato and W. Kovacik for technical assistance; B. Britten, A. Mittelman, N. Petrelli, and P. Reinagel for stimulating discussion; M. Held for preparation of the manuscript; and L. Emrich for advice on statistical tests and for performing the analysis of variance.

**REFERENCES**

An Unusual Oxygen-sensitive Lactate Dehydrogenase Isozyme Associated with Kirsten Murine Sarcoma Virus in Human Serum
