

# Studies on Plasma Lactic Dehydrogenase in Mice with Myeloid Leukemia

## I. Relation of Enzyme Level to Course of Disease

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### SUMMARY

The plasma lactic dehydrogenase (LDH) activity was increased in RF mice with primary and transplanted radiation-induced myeloid leukemia. The degree of elevation in mice with transplanted leukemia varied irregularly with the time after transplantation and with the transplant generation, the mean of all values being about 14 times normal. The extent of LDH increase in any transplant generation was correlated with the severity of leukemic infiltration of the liver and other organs. This correlation indicates that LDH mirrors the severity of the leukemic process. Whole-body x-radiation in the absence of transplanted leukemia, also caused a transient elevation of plasma LDH.

Elevation of the plasma lactic dehydrogenase (LDH) activity has been noted in mice with a variety of neoplasms, including leukemia (7). The elevation has been ascribed in part to a filterable agent and in part to the effects of the tumor itself (4, 5). The present study was undertaken to determine whether the plasma LDH rises in RF mice with transplanted and radiation-induced myeloid leukemia and to explore the pathogenesis of any increase encountered.

### MATERIALS AND METHODS

*Induction and transplantation of leukemia.*—The primary myeloid leukemias had been induced in random-bred RF male mice by 150–300 r whole-body x-radiation at 8–12 weeks of age (6). Transplantation was carried out by intravenous injection of  $60 \times 10^6$  nucleated leukemic spleen cells suspended in 0.3–0.5 ml. of Tyrode solution. Recipients were 10-week-old random-bred RF male mice; in a few instances they were exposed to 300 r whole-body x-radiation within 24 hours before inoculation. The diagnosis of leukemia was made by the demonstration of primitive myeloid cells in

the peripheral blood and in (characteristic) infiltrations of bone marrow and spleen as described by Upton *et al.* (6). Mice were killed when splenomegaly was revealed by palpation.

*Measurements of LDH activity.*—Blood was obtained either from the orbital venous plexus or from the heart. The blood removed from the orbital plexus was collected in a sterilized capillary tube, and 0.005 or 0.01 ml. of plasma was used for LDH assay. Blood procured by heart puncture was collected in a heparin-treated syringe and centrifuged in the cold (0° C.) for 25 minutes at 2500 r.p.m. The plasma was then pipetted off, and its LDH activity was measured in 2.0 ml. of the following reaction mixture, modified from an earlier method (8): 0.1 ml. of undiluted or diluted plasma, 0.05 M sodium phosphate (pH 7.40),  $1.0 \times 10^{-4}$  M DPNH, and  $2.3 \times 10^{-3}$  M sodium pyruvate. The reaction was observed by measuring the decrease in optical density at 340 m $\mu$  at 27° C. in a thermoregulated Zeiss spectrophotometer. A 5- to 10-minute incubation prior to the addition of pyruvate was performed routinely to minimize minor turbidity changes and to deplete the reaction mixture of endogenous pyruvate. A linear relation between rate of DPNH oxidation and enzyme concentration was obtained under these conditions; one-third of this concentration of pyruvate did not give a linear response. LDH activity was calculated from linear decrease of DPNH absorption

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between 1 and 4 minutes after addition of the substrate. Activity was expressed in units per ml., one unit indicating a decrease of 0.001 in optical density per minute under the conditions described above.

infiltrated than bone marrow and spleen but was more likely to be infiltrated than the kidney, as illustrated in Table 1.

The extent of infiltration is compared with the level of plasma LDH in Table 2, showing that,

TABLE 1  
HISTOLOGIC CHANGES AMONG MICE WITH TRANSPLANTED MYELOID LEUKEMIA

ORGAN		SEVERITY OF INFILTRATION OR NECROSIS*							
		0	F±	F+	F++	F+++	D+	D++	D+++
		Percentage of mice affected (94 animals)							
Bone marrow	Infiltration	0	2	0	0	0	0	6	92
	Necrosis	9	1	0	1	0	2	47	40
Spleen	Infiltration	0	5	1	1	0	2	16	76
	Necrosis	91	1	5	3	0	0	0	0
Liver	Infiltration	4	5	4	8	0	7	32	40
	Necrosis	81	1	14	4	0	0	0	0
Kidney	Infiltration	93	0	4	1	0	1	1	1
	Necrosis	100	0	0	0	0	0	0	0

\* Infiltration and necrosis were termed focal (F) or diffuse (D), focal indicating only localized (e.g., perivascular) and diffuse more generalized involvement. The degree of infiltration, whether focal or diffuse, was scored as follows: none (0), very slight (±), slight (+), moderate (++), and advanced (+++), as indicated in Figs. 1-6.

## RESULTS

*Histologic changes in leukemic animals.*—The plasma LDH was correlated with histological observations in 94 mice. Leukemic infiltration was invariably noted in the bone marrow, and in order of decreasing involvement less often in the spleen (Fig. 1), liver (Figs. 2-5), and kidney (Table 1). The bone marrow was the only tissue consistently necrotic (Fig. 6). Occasionally focal necrosis was noted in the spleen and liver; none was observed in the kidney. Judged on the basis of organ involvement, the disease was not at the same stage of development at any given time after inoculation in all the mice with myeloid leukemia. Although the leukemic infiltrations became progressively less well differentiated with successive passages, this degree and distribution within the various organs did not change appreciably.

*Plasma LDH activity of leukemic mice.*—Plasma LDH levels were generally increased in mice with primary and transplanted leukemia. The mean of all values was 14,900 units/ml plasma, as opposed to the normal average value of 1100 units/ml; but the degree of elevation varied irregularly with the transplant generation (Chart 1). The irregularity could be a reflection of a role of the liver, since it, of the four organs examined histologically, showed the most variation; it was less likely to be diffusely

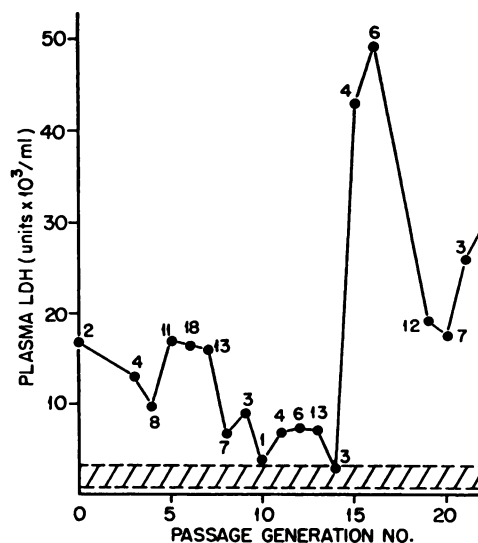


CHART 1.—Plasma LDH in relation to passage generation. The data for the zero passage were obtained from primary, nonpassaged leukemias. The control range, within 95 per cent confidence limits, is shown in the shaded area. The figure opposite each point indicates the number of mice examined.

when any of the three organs was found to be focally infiltrated, the plasma LDH was likely to be in the normal range, with liver showing some variation. When the liver was diffusely infiltrated,

the normal enzyme level in plasma was observed in only 6 per cent of cases. Among the leukemic mice examined, furthermore, a normal histologic picture was obtained in the liver in only four animals, and these were in the normal enzyme group. A summary of Table 2 reveals that the level of plasma LDH was high in animals showing diffuse infiltration and low in those with focal infiltration, the diffusely infiltrated liver showing the greatest tendency to be associated with a high level of plasma LDH. In a comparison of all the animals showing the lower plasma LDH level in Table 2, the liver was usually noninfiltrated or only focally infiltrated. In 92 animals a closer histological

grading was performed to determine whether the degree of liver infiltration would be reflected by the level of plasma LDH (Table 3). A correlation with a gradual transition did not appear, but instead an abruptly higher average LDH level seemed to occur in the diffusely infiltrated liver cases.

To study plasma LDH in relation to time after leukemia transplantation, twelve 10-week old animals were bled serially at intervals after being given injections intravenously of  $\sim 60 \times 10^6$  nucleated spleen cells (in 0.5 ml. of Tyrode solution) from donors in the fifteenth-passage generation. Another group, comprising eight mice, received 1.0 ml. of a cell-free filtrate prepared from the same suspension, and controls (eleven mice) were concomitantly given injections of 0.5 ml. of Tyrode solution. These three groups, designated the cell group, filtrate, and control groups, were

TABLE 2  
CORRELATION OF EXTENT OF INFILTRATION TO PLASMA LDH LEVEL IN LEUKEMIC MICE

HISTOLOGICAL APPEARANCE	PLASMA LDH (UNITS/ML)				Total
	0-2000	2000-5000	5000-15,000	Over 15,000	
<b>Focal infiltration:</b>					
Bone marrow	2	1	0	0	3
Spleen	9	1	0	0	10
Liver	19	4	2	0	25
<b>Diffuse infiltration:</b>					
Bone marrow	20	13	28	38	99
Spleen	20	18	37	57	132
Liver	7	16	36	55	114
<b>Normal:</b>					
Bone marrow	0	0	0	0	0
Spleen	0	0	0	0	0
Liver	4	0	0	0	4

The figures represent number of animals in each group. The bone marrow was necrotic in many cases, preventing any evaluation of extent of infiltration.

TABLE 3  
CORRELATION BETWEEN PLASMA LDH LEVEL AND SEVERITY OF LEUKEMIC INFILTRATION OF LIVER

INFILTRATION OF LIVER*	No. ANIMALS	PLASMA LDH	
		Average (units/ml)	Stand. dev.
None	4	1,500	460
F±	6	1,100	425
F+	3	4,800	6,170
F++	5	1,600	678
D+	6	16,000	9,800
D++	30	15,000	9,035
D+++	38	11,000	10,500

\*For explanation of scoring, see footnote to Table 1.

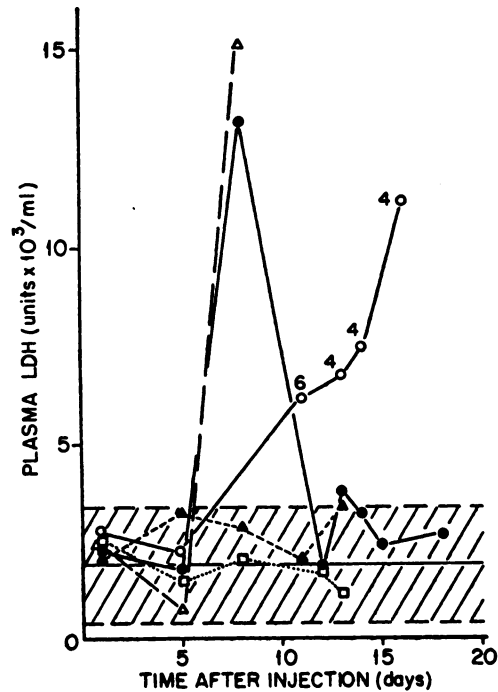


CHART 2.—Plasma LDH in relation to time after injection of leukemic cell or cell-free filtrate. Control mean and 95 per cent confidence limits indicated by shaded area.

● Group of three cell-injected mice surviving throughout observation period (see text).

○ Group of seven cell-injected mice dying with leukemia before end of observation period, each of which showed increase in LDH. Figures denote numbers per point.

△ One mouse dying with leukemia 9 days after injection of cells (marked terminal increase in LDH).

▲ One mouse (No. 10, see text) dying with leukemia 13 days after injection of cells (no LDH increase).

□ Group of eight filtrate-injected mice, none of which developed leukemia or showed increase in LDH within observation period.

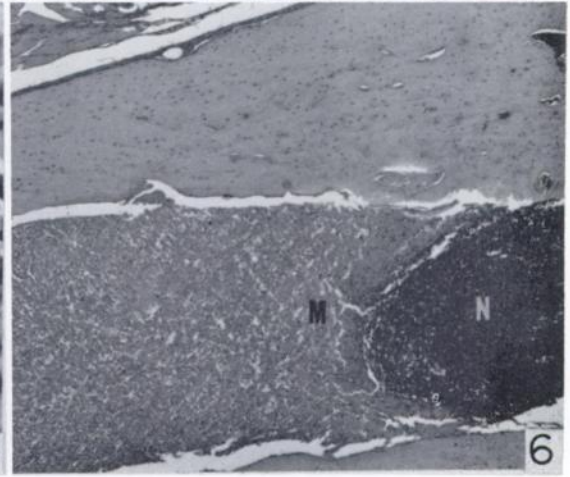
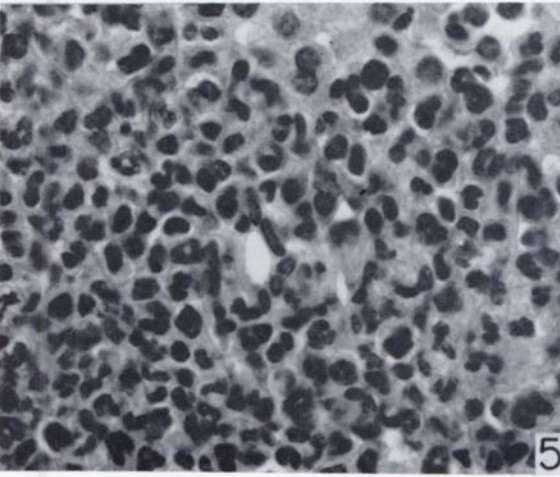
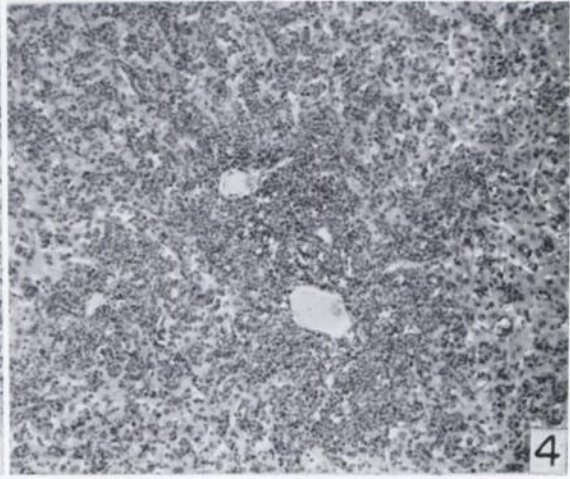
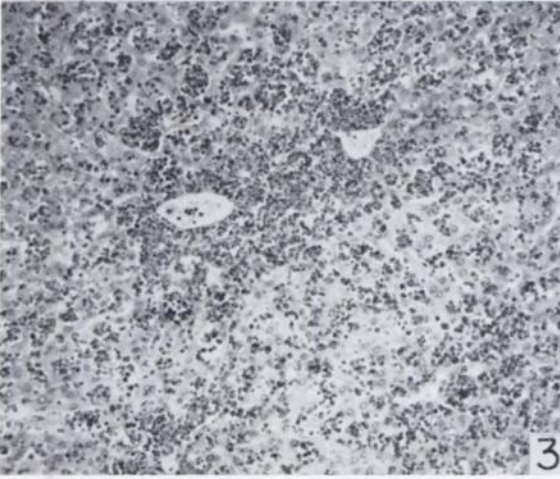


FIG. 1.—Spleen, showing diffuse infiltration of leukemic cells.  $\times 64$ , H. & E.

FIG. 2.—Liver, showing focal (grade F++) periportal infiltration of leukemic cells.  $\times 64$ , H. & E.

FIG. 3.—Liver, showing diffuse (grade D++) infiltration of leukemic cells.  $\times 64$ , H. & E.

FIG. 4.—Liver, showing diffuse (grade D+++ ) infiltration of leukemic cells.  $\times 64$ , H. & E.

FIG. 5.—Liver, with leukemic infiltration around central vein, showing myelogenous characteristics of leukemia cells. Note prominence of metamyelocytes with ring-shaped nuclei.  $\times 410$ , H. & E.

FIG. 6.—Bone marrow, showing sharply demarcated boundary between zone (N) of ischemic necrosis (grade D+) and viable leukemic marrow (M).  $\times 26$ , H. & E.

bled every 2–3 days from the orbit, and their plasma LDH levels were correlated with the presence or absence of splenomegaly as determined by palpation and other evidences of leukemia. Nine mice in the cell group died during the period from 9 to 16 days after inoculation, and elevation of plasma LDH activity was found associated with splenomegaly in all these except one (No. 10), which died accidentally from asphyxia on blood-sampling (Chart 2). Excluding this animal, the highest plasma LDH activity of the eight remaining mice was 5.8 times that of the control average, and the mean survival time after inoculation was 14 days. Examination of all three animals in the cell group still living 24 days after injection revealed splenomegaly, which had persisted from about the 15th day. In one of them a high plasma LDH activity was found 8 days after injection, but by 11 days the activity had returned to normal. The level in the other two animals remained normal or only transiently elevated throughout. No elevation of plasma LDH activity in the filtrate or control groups was detected.

*The effects of irradiation on plasma LDH.*—Since the leukemia had been induced originally by x-radiation, we examined the effect of irradiation on plasma LDH. The plasma LDH increased within 3 days after irradiation but appeared to be back to normal by the 14th day (Chart 3).

### DISCUSSION

More than 30 mouse tumors are associated with a transmissible virus-like agent(s) that induces an increase in plasma LDH activity (2, 3, 5). Although mouse tumors induced by radiation or chemical carcinogens have been found not to give regular evidence of such an agent, plasma LDH elevation has been noted in certain radiation-induced leukemias (2, 9). It is not altogether surprising, therefore, that the LDH level was increased in our mice with primary and transplanted radiation-induced myeloid leukemia.

The observed elevation of plasma LDH remains to be explained. Its correlation with the extent of leukemic infiltration or necrosis of the liver suggests that it may have been related to impairment of hepatic function. A more plausible explanation, however, is that the correlation was related to the number of leukemic cells in the body, since the enzyme appears to come preponderantly from leukemic leukocytes (1), which diffusely infiltrate the liver only late in the course of the disease. Our failure to induce an increase in LDH with a cell-free filtrate of leukemic spleen cannot be regarded as excluding a viral etiology, in view of the limited scope of the experiment and the possibility that the increase may depend on synergistic effects of

neoplasm and virus, as observed in other instances (4). The observed time course of the increase in plasma LDH is similar to that reported for tumors which do not contain the LDH agent (10).

### ACKNOWLEDGMENTS

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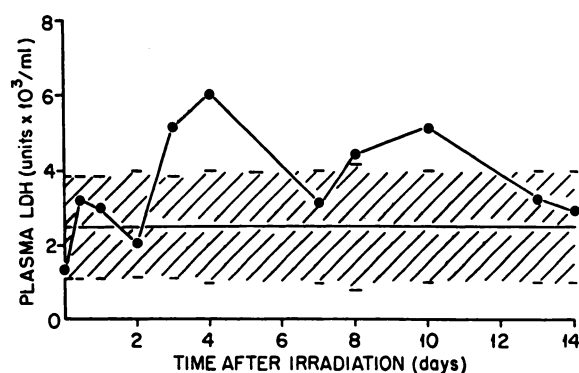


CHART 3.—Plasma LDH in relation to time after x-radiation. Experimental group consisted of five 10-week-old RF male mice exposed to 300 r 250 kvP whole-body x-rays, at a dose rate of 80 r/min.

- Mean of controls.
- — 95 per cent confidence limits.
- Observed mean for treated group.

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