# Catalase Activity in Mouse Leukemia L1210[1]

FREDRIK I. LOTTSFELDT, CAROLE PEHOUSHEK, AND WILLIAM KRIVIT

*(Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota)*

SUMMARY

Serial determinations of liver, kidney, spleen, and blood catalase were made on BDF1 mice with leukemia L1210. Total liver catalase was significantly decreased in the terminal stage of the disease. Spleen catalase specific activity was significantly increased in the terminal stage. Kidney and blood catalase were unchanged. In leukemic mice treated with amethopterin, the same changes in catalase occurred, but at a delayed rate. After prolonged therapy, kidney catalase was also depressed.

The decrease in liver catalase occurring in malignancy has received much attention. Decreased liver catalase in patients with malignancy was first reported in 1910 and was one of the first demonstrations of a systemic biochemical alteration produced by malignancy (3–5, 17). Not until more than 30 years later did Greenstein *et al.* (10, 11) "rediscover" this phenomenon in rats with transplanted hepatoma and sarcoma. Extensive investigation has revealed depression of liver catalase to be almost universal in humans with malignancy and in animals with spontaneous or transplanted tumors. The demonstration, first by Nakahara and Fukuoka (15) and then by others (1, 7), that this effect could be obtained by injecting a tumor extract into normal mice, and also by *in vitro* incubation of tumor with normal mouse liver (13), produced further documentation of the systemic biochemical alterations induced by malignancy. The work on liver catalase alterations has been summarized by Greenstein (8) and by Nakahara and Fukuoka (16).

Liver catalase studies in leukemia have been scanty. Greenstein and Andervont (9) found that in A strain mice with transplanted lymphoma (no. 7292) of 9 days' duration liver catalase was reduced to 30% of normal activity. Spector *et al.* (19) and Lutz *et al.* (14) demonstrated decreased liver catalase activity in rat leukemia LK2 and IRC741 respectively.

Mouse leukemia L1210 has been widely used as an animal tumor for testing of chemotherapeutic agents in recent years, but there is little knowledge regarding systemic biochemical alterations induced by this tumor. As the initial phase of a study into alterations of catalase activity in leukemia, we have made serial determinations of liver, spleen, kidney, and blood catalase activities during the course of mouse leukemia L1210. We have also studied the effect of amethopterin therapy on these modalities.

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## METHODS

### INDUCTION OF LEUKEMIA

Leukemia L1210 was induced in BDF1 male mice[2] (22–25 gm) by injection of 0.1 ml of 1% spleen suspension S.C. into the right groin area. The spleens were obtained from DBA/2 male mice, weighing 22–25 gm, which were in a terminal stage from L1210 leukemia. Those mice receiving therapy were given 1.0 mg/kg of amethopterin in 0.1 ml saline, S.C. in the left groin area, starting at the 5th day after leukemia transplant and continuing daily until the animals were sacrificed.

### OBTAINING OF SPECIMENS FOR CATALASE DETERMINATION

Untreated leukemic mice were chosen randomly for sacrifice each day after leukemia transplant. Controls were also sacrificed each day. Amethopterin-treated mice were sacrificed after 3 and 17 days of therapy (8 and 22 days respectively after leukemia transplant). Blood for hemoglobin, WBC, differential, and catalase determinations was obtained from the tail vein. Mice were sacrificed by cervical dislocation; the animals were immediately perfused with cold isotonic saline through the left ventricle, after the hepatic vein had been cut. Liver, spleen, and kidneys were removed. Analyses for each animal were run individually. All homogenization was done at high speed for 2 min. in a Virtis "45" homogenizer at 4°C.

A 4% homogenate of liver (w/v) was prepared in distilled water. Of this, 0.5 ml was diluted to 50 ml in phosphate buffer (0.01 M, pH 7.0, temperature 4°C.).

A 0.5% homogenate of spleen was prepared in phosphate buffer.

A 1% homogenate of kidney was prepared in distilled water and 5 ml of this diluted to 50 ml in phosphate buffer. Whole blood (0.02 ml) was diluted to 10 ml in phosphate buffer. The different concentrations of tissues were chosen for convenience in measuring rate of hydrogen peroxide degradation. Catalase determinations were not affected by the type of solution used for homogenization.

[2] Graciously supplied by the National Cancer Institute, National Institutes of Health.

### CATALASE DETERMINATION

Catalase activity was determined by a modification of the method described by Beers and Sizer (2). Of the material to be analyzed, 0.5 ml was added to 2.5 ml of hydrogen peroxide (20 mM) at room temperature (0.1 ml 30% Superoxol, Merck, diluted to 50 ml in 0.01 M phosphate buffer, pH 7). The disappearance of hydrogen peroxide was measured at 240 mμ in a recording spectrophotometer (Beckman Model DK1) at room temperature. The reaction remained linear only for the first 20–30 sec. under these conditions. The hydrogen peroxide utilization per minute was extrapolated from the linear portion of the reaction. One unit of catalase is defined as the amount of enzyme required to decompose 1 μmole of $H_2O_2$ in 1 min. Specific activity is defined as units catalase activity per milligram of wet weight tissue. The blood determination is expressed as units catalase activity per milligram of hemoglobin. Each sample was run in duplicate.

## RESULTS

Survival under the above conditions in the untreated animals is 8–9 days. Animals used on the 8th and 9th days are in a terminal state. Selection of animals by the 9th day is no longer random because most mice have already expired. From previous experiments under these conditions, survival of amethopterin-treated mice would be anticipated to be 24–28 days from the time of leukemia transplant.

### HEMOGLOBIN AND WBC

Changes in the peripheral blood picture are shown in Table 1. A moderate anemia and marked leukocytosis appeared late in the disease. Up to 9% lymphoblasts and immature lymphocytes also appeared late in the disease. A mild leukopenia was consistently seen in the early stages of leukemia (3d and 4th day). In addition to leukopenia, the mice treated over a prolonged period with amethopterin exhibited a marked anemia.

TABLE 1

HEMOGLOBIN AND WBC IN MICE WITH LEUKEMIA L1210

| No. of days following leukemia transplant | No. of mice | Hemoglobin (gm %)[a] | WBC per cu mm × $10^{3}$[a] | Lymphoblasts and immature lymphocytes (%)[a] |
|---|---|---|---|---|
| Control | 21 | 16.7 (15.0–18.2) | 14.2 (7.0–18.9) | 0 |
| 3 | 3 | 16.3 (15.4–17.0) | 7.0 (6.7–7.7) | 0.3 (0–1) |
| 4 | 5 | 16.5 (14.2–18.2) | 7.3 (5.9–8.8) | 2.6 (1–4) |
| 5 | 3 | 16.5 (16.3–17.0) | — | — |
| 6 | 3 | 16.3 (15.2–18.2) | 14.9 (11.7–20.3) | 4 (4) |
| 7 | 4 | 12.9 (10.5–15.4) | 20.5 (10.7–43.5) | 5.0 (2–10) |
| 8 | 6 | 10.8 (7.8–14.6) | 41.7 (9.9–61.4) | 6.5 (2–15) |
| 9 | 3 | 11.9 (10.8–13.1) | 47.2 (34.7–54.8) | 9.3 (9–10) |
| 8[b] | 4 | 14.5 (13.8–15.2) | 13.0 (11.4–15.5) | 3.3 (1–5) |
| 22[b] | 4 | 4.8 (3.0–7.2) | 6.0 (4.3–8.5) | 7.8 (0–17) |

[a] Mean (range).

[b] Mice received 25 μg amethopterin daily, starting 5 days after leukemia transplant.

TABLE 2

LIVER CATALASE IN MICE WITH LEUKEMIA L1210

| No. of days following leukemia transplant | No. of mice | Liver weight (gm)[a] | Specific activity (units[b]/mg liver)[a] | Total activity (units[b]/liver × $10^{3}$)[a] |
|---|---|---|---|---|
| Control | 30 | 1.24 ± 0.16 | 132 ± 11 | 162 ± 25 |
| 1 | 6 | 1.35 ± 0.28 | 139 ± 8 | 189 ± 20 |
| 2 | 6 | 1.20 ± 0.13 | 135 ± 8 | 162 ± 18 |
| 3 | 3 | 1.29 ± 0.06 | 144 ± 14 | 186 ± 18 |
| 4 | 6 | 1.12 ± 0.15 | 144 ± 9 | 162 ± 25 |
| 5 | 3 | 1.29 ± 0.12 | 131 ± 6 | 170 ± 18 |
| 6 | 4 | 1.22 ± 0.20 | 133 ± 11 | 163 ± 35 |
| 7 | 6 | 1.37 ± 0.20 | 109 ± 31[c] | 146 ± 42 |
| 8 | 8 | 1.91 ± 0.31[c] | 63 ± 27[c] | 117 ± 34[c] |
| 9 | 3 | 2.10 ± 0.37[c] | 59 ± 21[c] | 121 ± 26[c] |
| 8[d] | 4 | 1.64 ± 0.13[c] | 87 ± 6[c] | 142 ± 7 |
| 22[d] | 4 | 1.33 ± 0.52 | 70 ± 27[c] | 91 ± 24[c] |

[a] Mean ± standard deviation.

[b] One unit is the amount of catalase required to degrade 1 μmole $H_2O_2$ in 1 min.

[c] Probability of difference of this magnitude occurring by chance alone has a $P$ value of $< 0.015$.

[d] Mice received 25 μg amethopterin daily, starting 5 days after leukemia transplant.

TABLE 3

KIDNEY CATALASE IN MICE WITH LEUKEMIA L1210

| No. of days following leukemia transplant | No. of mice | Kidney pair weight (gm)[a] | Specific activity (units[b]/mg kidney)[a] | Total activity (units[b]/kidney pair × $10^{3}$)[a] |
|---|---|---|---|---|
| Control | 24 | 0.32 ± 0.04 | 65 ± 5.9 | 21 ± 3.0 |
| 3 | 3 | 0.35 ± 0.05 | 65 ± 5.6 | 23 ± 2.3 |
| 4 | 6 | 0.28 ± 0.03 | 66 ± 5.8 | 18 ± 2.5 |
| 5 | 3 | 0.32 ± 0.03 | 70 ± 0.5 | 24 ± 2.1 |
| 6 | 4 | 0.30 ± 0.04 | 69 ± 2.7 | 21 ± 3.0 |
| 7 | 6 | 0.29 ± 0.05 | 62 ± 6.8 | 18 ± 3.8 |
| 8 | 8 | 0.28 ± 0.03 | 64 ± 6.7 | 18 ± 2.8 |
| 9 | 3 | 0.28 ± 0.03 | 65 ± 0.9 | 18 ± 1.5 |
| 8[c] | 4 | 0.30 ± 0.03 | 64 ± 7.4 | 19 ± 4.0 |
| 22[c] | 4 | 0.25 ± 0.03[d] | 49 ± 2.3[d] | 12 ± 1.6[d] |

[a] Mean ± standard deviation.

[b] One unit is the amount of catalase required to degrade 1 μmole of $H_2O_2$ in 1 min.

[c] Mice received 25 μg amethopterin daily, starting 5 days after leukemia transplant.

[d] Probability of difference of this magnitude occurring by chance alone has a $P$ value of $<0.015$.

### LIVER CATALASE

Liver catalase data are presented in Table 2. No significant changes were seen during the 1st 6 days of the leukemia. On the 7th day a slight increase in liver weight and concomitant decrease in specific activity of catalase occurred. However, total catalase content in the liver was still normal. On the 8th and 9th days marked increases in liver weight and decreases in catalase specific activity were observed. In addition, total liver catalase activity was significantly depressed to under 75% of control levels on these days ($P < 0.015$). In the animals

treated with amethopterin, the drop in both catalase specific activity and total catalase activity per liver was not prevented. In fact the lowest values obtained for total liver catalase occurred in leukemic mice under amethopterin treatment for 17 days, at which time the activity was 56% of normal.

### Kidney Catalase Activity

Kidney catalase data are presented in Table 3. No significant alterations were seen in the untreated leukemic mice with regard to kidney weight, specific catalase activity, or total catalase activity per kidney pair. In mice which received amethopterin for 17 days, there was a significant reduction of catalase specific activity (to 76% of control) and total kidney catalase activity (57% of control).

TABLE 4

Spleen Catalase in Mice with Leukemia L1210

| No. of days following leukemia transplant | No. of mice | Spleen weight (gm)[a] | Specific activity (units[b]/mg spleen)[a] | Total activity (units[b]/spleen $\times 10^3$)[a] |
|---|---|---|---|---|
| Control | 24 | 0.064 ± 0.011 | 4.9 ± 2.0 | 0.32 ± 0.11 |
| 3 | 3 | 0.094 ± 0.012[c] | 6.8 ± 0.9 | 0.65 ± 0.17[c] |
| 4 | 6 | 0.080 ± 0.006[c] | 6.0 ± 4.5 | 0.49 ± 0.36 |
| 5 | 3 | 0.107 ± 0.010[c] | 4.5 ± 0.5 | 0.48 ± 0.4 |
| 6 | 4 | 0.100 ± 0.016[c] | 4.7 ± 0.7 | 0.47 ± 0.6 |
| 7 | 6 | 0.177 ± 0.080[c] | 4.3 ± 1.1 | 0.78 ± 0.58[c] |
| 8 | 8 | 0.354 ± 0.078[c] | 7.3 ± 1.8[c] | 2.67 ± 0.68[c] |
| 9 | 3 | 0.411 ± 0.005[c] | 7.6 ± 0.5[c] | 3.12 ± 0.2[c] |
| 8[d] | 4 | 0.252 ± 0.092[c] | 6.2 ± 1.1 | 1.58 ± 0.85[c] |
| 22[d] | 4 | 0.704 ± 0.297[c] | 9.2 ± 1.6[c] | 6.54 ± 3.3[c] |

[a] Mean ± standard deviation.

[b] One unit is the amount of catalase required to degrade 1 μmole of $H_2O_2$ in 1 min.

[c] Probability of difference of this magnitude occurring by chance alone has a $P$ value of <0.015.

[d] Mice received 25 μg amethopterin daily, starting 5 days after leukemia transplant.

TABLE 5

Blood Catalase in Mice with Leukemia L1210

| No. of days following leukemia transplant | No. of mice | Blood specific activity (units[a]/mg hemoglobin)[b] |
|---|---|---|
| Control | 17 | 78 ± 9 |
| 3 | 3 | 90 ± 2 |
| 4 | 5 | 84 ± 6 |
| 5 | 2 | 72 ± 11 |
| 6 | 3 | 80 ± 11 |
| 7 | 3 | 73 ± 5 |
| 8 | 6 | 93 ± 14[c] |
| 9 | 3 | 87 ± 11 |
| 8[d] | 3 | 78 ± 5 |
| 22[d] | 3 | 76 ± 14 |

[a] One unit is the amount of catalase required to degrade 1 μmole of $H_2O_2$ in 1 min.

[b] Mean ± standard deviation.

[c] Probability of difference of this magnitude occurring by chance alone has a $P$ value of <0.015.

[d] Mice received 25 μg amethopterin daily, starting 5 days after leukemia transplant.

### Spleen Catalase Activity

Table 4 contains data on spleen catalase activity. As anticipated, increasing duration of leukemia was accompanied by a great increase in the size of the spleen. An unexpected observation, however, was the increase of 49% in specific catalase activity of the spleen which also occurred late in the course of the leukemia. This increase was also seen in amethopterin-treated mice. Total spleen catalase activity was greatly increased, largely because of the increase in spleen size.

### Blood Catalase Activity

Levels of blood catalase are given in Table 5. Changes in blood catalase were somewhat erratic and probably not meaningful, even though a slightly higher blood catalase was observed on the 8th day of the leukemia.

## DISCUSSION AND CONCLUSIONS

In L1210 mouse leukemia, the total liver catalase becomes significantly depressed as the disease progresses. In order to determine "true" liver catalase it is necessary to correct the obtained values for leukemic cell infiltration into the liver. Therefore, the catalase activity of the leukemic cells as well as the amount of leukemic tissue present are needed in order to adjust the liver catalase data presented in Table 2. Separation of liver parenchymal cells from leukemic cells for quantitative biochemical studies is not possible. Likewise, direct determination of leukemic cell catalase activity is difficult because of the necessity to completely exclude red blood cells which have an extremely high catalase content.

However, a maximal figure for catalase activity of leukemic cells can be estimated from the spleen data. On the 9th day the perfused spleen microscopically appears to consist almost completely of leukemic cells. If it is assumed that the entire increase in spleen weight from day 0 (control) to the 9th day (347 mg) and increase in total spleen catalase activity (2,800 units) is due to leukemic cells, then the specific activity of leukemic tissue is approximately 8.1 units per mg. Any red blood cells or results of hemolysis which remain in the spleen following whole animal perfusion would contribute substantially to the measured activity. Therefore 8.1 units per mg represents a maximal figure. If the total weight increase of the liver (860 mg) were due to leukemic cells, then the total liver catalase would have been increased only by $7.0 \times 10^3$ units. In other words, less than 6% of total liver catalase measured at the 9th day is accounted for by leukemic cell infiltration. Therefore, the decrease in activity of total liver catalase reflects changes in hepatic tissue *per se*, whereas the decrease in specific activity is largely a dilutional effect from leukemic infiltration.

It also must be taken into consideration that the increase in liver weight most likely is not completely due to leukemic infiltration. Frei *et al.* (6) and Wolf and Klemperer (20) have demonstrated increased liver size in leukemic patients in the absence of leukemic infiltration. In this

study the liver had extensive leukemic infiltration on the 8th and 9th days, but it could not be determined if this accounted for the entire weight increase.

In conclusion, it appears that the decrease in liver catalase in mice with leukemia L1210 is smaller in magnitude than seen in most animal tumors. This is especially true in view of the extreme state of disease present on the 8th and 9th days.

Studies of kidney catalase activity in tumor-bearing rodents have shown less marked depression than that seen in liver catalase activity (8). It usually is observed only in those animals which have a very pronounced decrease in liver catalase activity. Therefore, it was not surprising to see no decrease in kidney catalase in the untreated leukemic mice in this study.

The determinations of catalase activity in leukemic mice receiving amethopterin were made only at 2 time intervals (the 8th and 22d days after leukemia transplant), but the alterations followed a consistent trend. In both liver and spleen, the values obtained on the 8th day in amethopterin-treated mice were intermediate between those obtained on the 7th and 8th days in untreated leukemic mice (Tables 2 and 4). On the 22d day liver catalase was even further depressed than on the 8th and 9th days in untreated mice, while spleen specific activity showed a further increase. In addition, kidney catalase became significantly depressed for the first time (Table 3). The possibility that prolonged amethopterin therapy by itself could produce these changes in normal mice was not ruled out. Amethopterin therapy for 4 days in normal mice produced no changes in liver, spleen, kidney, or blood catalase activity.

These observations were consistent with data presented by Skipper *et al.* (18) on the cell kinetics of L1210. With this particular dosage schedule of amethopterin, Skipper's data showed a progressive increase in the number of leukemic cells, but at a slower rate than in untreated mice, until the lethal number of leukemic cells was reached. Likewise from our data, the catalase changes also occurred at a slower rate in amethopterin-treated mice. Liver catalase activity may be a useful parameter in evaluating the effectiveness of various forms of chemotherapy in eradicating leukemia cell population, but more extensive studies have to be performed using other chemotherapeutic agents in several different dosage schedules.

Hano *et al.* (12) found a partial correlation between the effectiveness of several chemotherapeutic agents in mice with Ehrlich ascites tumor in prolonging survival and in preventing liver catalase depression. Amethopterin was not an effective chemotherapeutic agent in this particular test system, nor was the decrease in liver catalase prevented in tumor-bearing mice receiving the drug.

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