

The Effects of Triethylene Melamine and Related Compounds on the Leukocytes of Mouse Leukemia

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IN THE SCREENING of compounds for their possible chemotherapeutic effect against transplanted mouse leukemia triethylene melamine (TEM) (2,4,6-triethylenimino-*s*-triazine) has been found to be effective in prolonging the survival time of mice with the Ak4 strain of transplantable mouse leukemia.¹⁻² Since mice with this leukemia show a marked increase in white blood count between the fifth and eighth day of the disease³ it was felt worthwhile to investigate the effect of triethylene melamine (TEM) and related compounds on the blood picture.

Various investigators using several different strains of transplanted mouse leukemia have made similar studies on urethane⁴⁻⁶, a crude antagonist of pteroyl-glutamic acid (PGA),⁷ 4-amino-PGA⁸ and 4-amino-N¹⁰-methyl-PGA.³ In these studies suppression of the leukemic process was demonstrated by a delay in the appearance of immature cells in the peripheral blood and in the infiltration of the organs by the white cells and by the prevention of a marked increase in the total leukocyte count. In the work herewith reported the effect of TEM on the total and differential leukocyte count and visceral infiltration of animals with both developing and advanced leukemia was studied. In view of the chemotherapeutic effect of certain other ethylene imines closely related to TEM, hexamethylene-diethylenurea, 2,4-diethylenimino-6-amino-*s*-triazine, and 2-ethylenimino,4,6-dimethoxy-*s*-triazine were tested for their effect on advanced Ak4 leukemia.

METHOD

As has been described previously,³ young mice of the Akm stock weighing approximately 20 Gm. were used for this study. The leukemia was transmitted by intraperitoneal inoculation of 0.1 cc. of a saline suspension of leukemic spleen so diluted as to contain 1,000,000 cells.

Three types of experiments were used to study the effect of TEM. In experiments I and II treatment was started forty-eight hours after the inoculation of the leukemic spleen suspension. All injections of the compound were given intraperitoneally in 0.2 cc. of saline three times a week for ten doses. The treated mice received the maximum tolerated dose,

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0.75 mg./Kg. of TEM in saline, while the controls were given only saline. The mice were weighed weekly and the dose adjusted on a weight basis to conform to the average weight of the animals in the cage. As a rule, the mice remained at a constant weight or lost slightly on this therapy. All mice were kept on a standard diet of Purina laboratory chow.

In experiment I the effect of this standard course of therapy was evaluated by comparing total leukocyte and differential counts of the 17 treated leukemic mice with those of the 15 leukemic controls. These counts were done before either the inoculation of the leukemic spleen suspension or the start of treatment with TEM and were then repeated on the eighth day and every four days thereafter until the death of the animals. In order that the counts

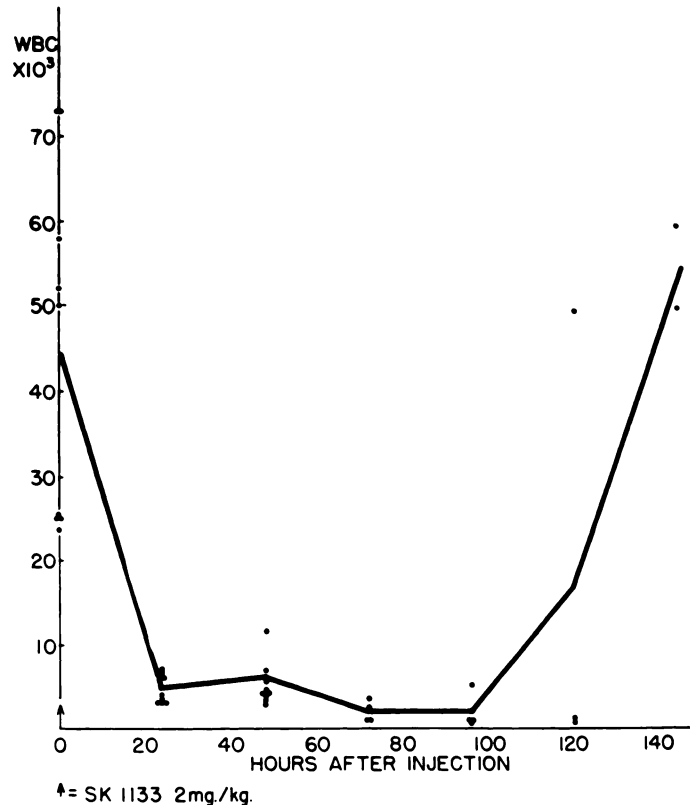


FIG. 1.—The effect of 2,4,6-triethlenimino-s-triazine (SK 1133) on advanced Ak4 leukemia

be made on free flowing blood, the mice were warmed briefly under an electric lamp. A small cut was then made in a tail vein with a razor blade and the studies were made on the blood obtained in this manner. Blood smears were stained with Wright's stain.

All the animals that died during the course of the experiment were autopsied and the spleen, liver and lymph nodes were examined for gross evidence of leukemia. Microscopic sections were taken if the diagnosis was in doubt.

Experiment II was devised to compare the amount of visceral infiltration in the treated mice to that in the leukemic controls. Sixty mice were inoculated with 0.1 cc. of leukemic spleen suspension containing 1,000,000 cells. Forty mice were then started forty-eight hours later on treatment three times weekly with 0.75 mg./Kg. of TEM while the other 20 mice served as controls. Approximately every second day total leukocyte counts were done on 2 control mice and 2 treated mice which were then sacrificed in order to obtain sections of liver, spleen, kidney, sternum and femur.

TABLE 1.—*The Effect of 2,4,6-Triethylenimino-s-Triazine on the Total Leukocyte Count of Leukemic Mice*

| Cont. or Treat. | Dose in mg./Kg.* | No. of Mice | Day of Experiment | | | | | | | | | | | |
|-----------------------|------------------|-------------|-------------------|-----------|------|------------|------|---------|------|---------|------|---------|--|--|
| | | | 0 | | 8 | | 12 | | 16 | | 20 | | | |
| | | | WBC | | WBC | | WBC | | WBC | | WBC | | | |
| | | | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range | | |
| Control exp. I a..... | | 9 | 10.9 | 6.0-15.0 | 97.7 | 54.0-156.0 | | | | | | | | |
| Treated..... | 0.75 | 10 | 12.0 | 6.0-19.5 | 2.7 | 1.0- 5.5 | 2.6 | 0.5-9.4 | 1.4 | 0.2-3.3 | 2.5 | 0.7-4.0 | | |
| Control exp. I b..... | | 6 | 16.2 | 12.6-19.3 | 75.4 | 21.2-159.0 | | | | | | | | |
| Treated..... | 0.75 | 7 | 12.1 | 9.9-14.7 | 1.7 | 0.9- 2.9 | 3.4 | 1.8-6.2 | 4.4 | 1.5-9.3 | | | | |

* The drug was dissolved in saline just prior to each injection. The mice received the drug three times weekly throughout the experiment.

TABLE 2.—The Effect of 2,4,6-Triethylenimino-s-Triazine on the Differential Leukocyte Counts of Mice with Ak4 Leukemia

| Day of Experiment | Number of Mice | Control or Treated | Dose in mg/Kg * | Average Total Leukocyte Count | % Polys. | % Lymphs. | % Leukemic Cells |
|-----------------------|----------------|--------------------|-----------------|-------------------------------|----------|-----------|------------------|
| <i>Experiment I a</i> | | | | | | | |
| 0 | 9 | Control | | 10,900 | 22.1 | 76.3 | 1.6 |
| 0 | 10 | Treated | 0.75 | 12,000 | 29.9 | 68.8 | 1.3 |
| 8 | 9 | Control | | 97,700 | 31.6 | 24.9 | 43.5 |
| 8 | 9 | Treated | 0.75 | 2,700 | 27.0 | 63.3 | 9.7 |
| 12 | | Control† | | | | | |
| 12 | 9 | Treated | 0.75 | 2,600 | 20.6 | 70.6 | 8.8 |
| 16 | 8 | Treated | 0.75 | 1,400 | 20.0 | 75.0 | 3.0 |
| 20 | 4 | Treated | 0.75 | 2,500 | 69.5 | 25.5 | 5.0 |
| <i>Experiment I b</i> | | | | | | | |
| 0 | 6 | Control | | 16,200 | 23.2 | 76.1 | 0.7 |
| 0 | 10 | Treated | 0.75 | 12,100 | 20.9 | 78.1 | 1.0 |
| 8 | 6 | Control | | 75,400 | 31.5 | 31.3 | 37.2 |
| 8 | 10 | Treated | 0.75 | 1,700 | 13.3 | 72.4 | 14.3 |
| 12 | | Control† | | | | | |
| 12 | 6 | Treated | 0.75 | 3,400 | 47.8 | 40.0 | 12.2 |
| 16 | 6 | Treated | 0.75 | 4,400 | 57.8 | 36.0 | 6.2 |

* Drug dissolved in saline just prior to injection. Mice injected three times weekly throughout the experiment. † Mice all dead.

TABLE 3.—Effect of 2,4,6-Triethylenimino-s-Triazine on Development of Leukemic Infiltration

| Number of Days After Transmission of Ak4 Leukemia | WBC of Untreated Leukemic Controls | Leukemic Controls | | | | WBC of Treated Leukemic Mice | Treated Leukemic Mice | | | |
|---|------------------------------------|-------------------|--------|--------|-------------|------------------------------|-----------------------|--------|--------|-------------|
| | | Liver | Kidney | Spleen | Bone Marrow | | Liver | Kidney | Spleen | Bone Marrow |
| 6 | 17,200 | 2 | 1 | 2 | 0 | 7,400 | 0 | 0 | 0 | 0 |
| 6 | 13,400 | 1 | 0 | 2 | 0 | 1,000 | 0 | 0 | 0 | 0 |
| 8 | 149,000 | 3 | 3 | 4 | 3 | 1,500 | 0 | 0 | 0 | 0 |
| 8 | 128,900 | 4 | 3 | 4 | 4 | 0,900 | 0 | 0 | 0 | 0 |
| 8 | 42,800 | 3 | 3 | 4 | 1 | 2,900 | | | | |
| 8 | 83,000 | 4 | 3 | 4 | 2 | 3,600 | 0 | 0 | 0 | 0 |
| 10 | 118,000 | 4 | 4 | 4 | 3 | 2,000 | 1 | 0 | 3 | 2 |
| 10 | 5,600 | 2 | 2 | 4 | 3 | 3,300 | 1 | 0 | 3 | 3 |
| 10 | 219,000 | 4 | 4 | 4 | 3 | 0,700 | 0 | 0 | 0 | 0 |
| 10 | | | | | | 5,300 | 2 | 1 | 3 | 0 |
| 12 | | | | | | 1,100 | 1 | 1 | 4 | 4 |
| 12 | | | | | | 4,200 | 0 | 0 | 3 | 1 |
| 12 | | 4 | | 4 | 0 | 4,600 | 1 | 1 | 3 | 1 |
| 12 | | 4 | 2 | 4 | 3 | 3,100 | 1 | 0 | 1 | 0 |
| 14 | | | | | | 1,400 | 1 | 0 | 3 | 0 |
| 14 | | | | | | 1,000 | 0 | 0 | 3 | 0 |
| 14 | | | | | | 2,500 | 2 | 1 | 3 | 2 |
| 14 | | | | | | 0,100 | 1 | 0 | 3 | 1 |
| 16 | | | | | | 35,200 | 2 | 2 | 3 | 3 |
| 16 | | | | | | 10,200 | 3 | 3 | 4 | 3 |
| 16 | | | | | | 1,900 | 1 | 1 | 3 | 1 |
| 16 | | | | | | 1,200 | 3 | 0 | 3 | 1 |
| 18 | | | | | | 0,700 | 1 | 0 | 4 | 1 |
| 18 | | | | | | 4,400 | 2 | 2 | 4 | 3 |
| 20 | | | | | | 1,100 | 1 | 1 | 4 | 3 |

In experiment III, 18 mice were inoculated in the usual way with the leukemic spleen suspension and remained untreated until the seventh or eighth day of the disease. At this time when the total leukocyte count was high, the mice were injected with 2 mg./Kg. of

TABLE 4.—*The Effect of 2,4,6-Triethylenimino-s-Triazine on the Total and Differential Counts of Mice with Advanced Ak4 Leukemia*

| Hours After Injection of Compound | No. of Mice | Total WBC $\times 10^3$ | | % Neutrophils | % Lymphocytes | % Leukemic Cells |
|-----------------------------------|-------------|-------------------------|-----------|---------------|---------------|------------------|
| | | Mean | Range | | | |
| <i>Experiment III a</i> | | | | | | |
| 0† | 10 | 41.4 | 8.2-73.0 | 30.3 | 34.1 | 36.8 |
| 24 | 10 | 4.9 | 3.2- 7.1 | 50.0 | 36.0 | 14.0 |
| 48 | 10 | 5.6 | 3.3-11.8 | 66.8 | 25.6 | 7.7 |
| 72 | 7 | 2.3 | 1.1- 3.5 | 60.0 | 32.0 | 8.4 |
| 96 | 5 | 2.1 | 0.8-11.4 | 13.3 | 59.5 | 27.2 |
| 120 | 3 | 17.2 | 0.6-49.6 | 0.0* | 13.0* | 87.0* |
| 144 | 2 | 54.8 | 50.0-59.5 | 2.0 | 28.0 | 70.0 |
| <i>Experiment III b</i> | | | | | | |
| 0† | 8 | 47.8 | 17.6-82.0 | 34.2 | 40.8 | 25.0 |
| 24 | 8 | 8.9 | 6.2-11.3 | 71.0 | 23.0 | 6.0 |
| 48 | 8 | 4.5 | 1.8- 8.1 | 70.0 | 24.5 | 5.5 |
| 72 | 7 | 3.0 | 0.5- 8.0 | 46.8 | 45.1 | 8.1 |
| 96 | 4 | 6.9 | 1.2-15.0 | 39.3 | 55.0 | 4.7 |
| 120 | 4 | 3.2 | 1.2- 6.0 | 4.0* | 76.0* | 20.0* |
| 144 | 4 | 7.1 | 2.5-18.4 | 8.2 | 59.5 | 32.3 |

* Only one smear used in this average.

† Mice received a single injection of 2 mg./Kg. of the compound on the seventh day of leukemia. 0 hour count taken just prior to injection of the compound.

TABLE 5.—*The Effect on Advanced Ak4 Leukemia of Compounds Related to 2,4,6-Triethylenimino-s-Triazine*

| Compound | Dose in mg./Kg. | Pre-Injection WBC $\times 10^3$ | | 24 Hour WBC $\times 10^3$ | | 48 Hour WBC $\times 10^3$ | | 72 Hour WBC $\times 10^3$ | |
|---|-----------------|---------------------------------|------------|---------------------------|------------|---------------------------|-------------|---------------------------|----------|
| | | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| Control | | 50.2 | 10.0-101.0 | 194.6 | 70.0-560.0 | 300.2 | 113.0-481.0 | | |
| 2,4-Diethylenimino-6-amino-s-triazine | 1.5 | 44.7 | 10.0-151.0 | 13.2 | 5.0- 23.0 | 9.8 | 8.0-16.0 | 3.7 | 1.0- 8.0 |
| | 0.4 | 67.7 | 20.0-173.0 | 17.8 | 6.0- 39.0 | 20.7 | 4.0-47.0 | | |
| Hexamethylene diethylenurea | 4.0 | 49.2 | 11.0- 99.0 | 25.6 | 5.0- 89.0 | 7.5 | 2.0-13.0 | 7.1 | 4.0-13.0 |
| | 1.0 | 55.1 | 20.0-110.0 | 29.0 | 10.0- 62.0 | 27.8 | 19.0-47.0 | | |
| 2-Ethylenimino-4,6-dimethoxy-s-triazine | 150 | 26.2 | 14.0- 39.0 | 37.5 | 10.0- 54.0 | 33.8 | 11.0-73.0 | | |
| | 250 | 37.2 | 30.0- 49.0 | 15.0 | 8.0- 20.0 | 14.3 | 6.0-24.0 | | |

TEM. This dose was at approximately the LD50 level for a single injection as shown by Thiersch and Philips.⁹ Total and differential counts were obtained before the injection of TEM and approximately every twenty-four hours thereafter until the death of the animal.

Hexamethylene diethylenurea (SK 1903), 2,4-diethylenimino-6-amino-s-triazine (SK

2390), and 2-ethylenimino-4,6-dimethoxy-s-triazine (SK 2683) were all tested for their effect upon the high total leukocyte count of advanced Ak4 leukemia in the manner described in experiment III. In each case up to the maximum tolerated dose for a single injection of the compound was used as determined by the Department of Pharmacology of The Sloan-Kettering Institute. The compounds were injected on the seventh day of the disease and total leukocyte counts were done before the injection and twenty-four and forty-eight hours later.

A detailed description of the leukemic cells and the natural history of this particular form of transmitted leukemia has been given previously.³ A high total leukocyte count

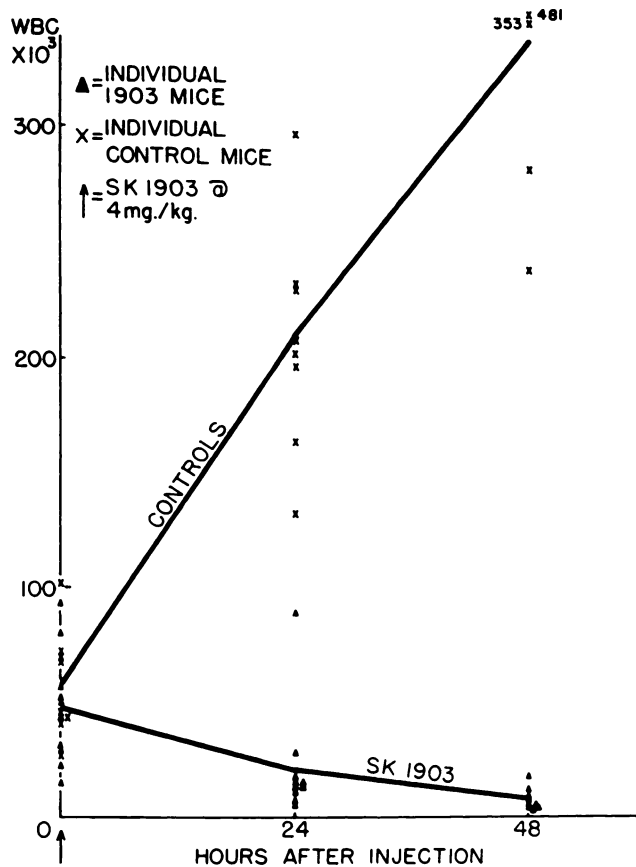


FIG. 2.—The effect of hexamethylene diethylenurea (SK 1903) on the total leukocyte count of advanced Ak4 leukemia.

ranging from 40,000 to 100,000 is usually seen six to seven days after inoculation with leukemia and at this time there is a definite increase in the percentage of polymorphocytes in the peripheral blood.

RESULTS AND DISCUSSION

The average leukocyte count of leukemic mice receiving therapy with TEM remained low while those of the controls rose steadily until death (table 1). Those mice which received TEM at 0.75 mg./Kg. three times weekly lived approximately twice as long as the controls and the differential counts showed a

marked suppression of the prolymphocytes allowing a proportional increase of the mature lymphocytes and neutrophils (table 2).

Histologic sections from the group of 40 mice treated with TEM 0.75 mg./Kg. on the standard dose schedule demonstrated a definite effect of the compound on the development of leukemic visceral infiltration (table 3). At eight days control animals showed extensive infiltration of the liver, spleen, kidney and bone marrow, whereas sections taken at the same time from mice under therapy showed no infiltration of the organs and were essentially normal. In the treated

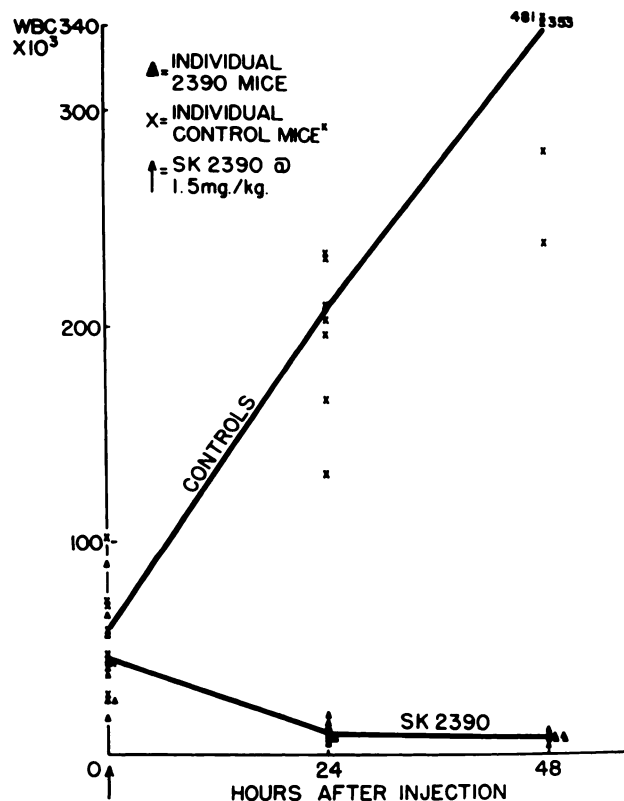


FIG. 3.—The effect of 2,4-diethylenimino-6-amino-s-triazine (SK 2390) on the leukocyte count of advanced Ak4 leukemia.

mice, infiltration of these organs was first noted on the tenth day of disease and reached a degree at sixteen days comparable to that of the eight day untreated mice.

In experiment III the leukemic mice which received a single dose of TEM 2.0 mg./Kg. on the eighth day of the disease showed a definite fall in the total leukocyte count. As seen in figure 1, the first post-treatment counts were taken at twenty-four hours, at which time a marked drop in the total leukocyte count was noted. A state of leukopenia was maintained in the surviving mice until approximately one hundred twenty hours after the injection of the compound at which time the total leukocyte count began to rise.

In addition to the leukopenia TEM caused a depression in the number of

immature lymphocytes seen in the peripheral blood with a corresponding increase in the number of mature lymphocytes and neutrophils (table 4).

Essentially the same results were obtained with single LD50 injections of hexamethylene diethylenurea, 2,4-diethylenimino-6-amino-*s*-triazine, and 2-ethylenimino-4,6-dimethoxy-*s*-triazine on advanced Ak4 leukemia (table 5, figs. 2, 3, 4). 2-Ethylenimino-4,6-dimethoxy-*s*-triazine, however, demonstrated somewhat less leukotoxic effect presumably because it contains only a single ethylen-

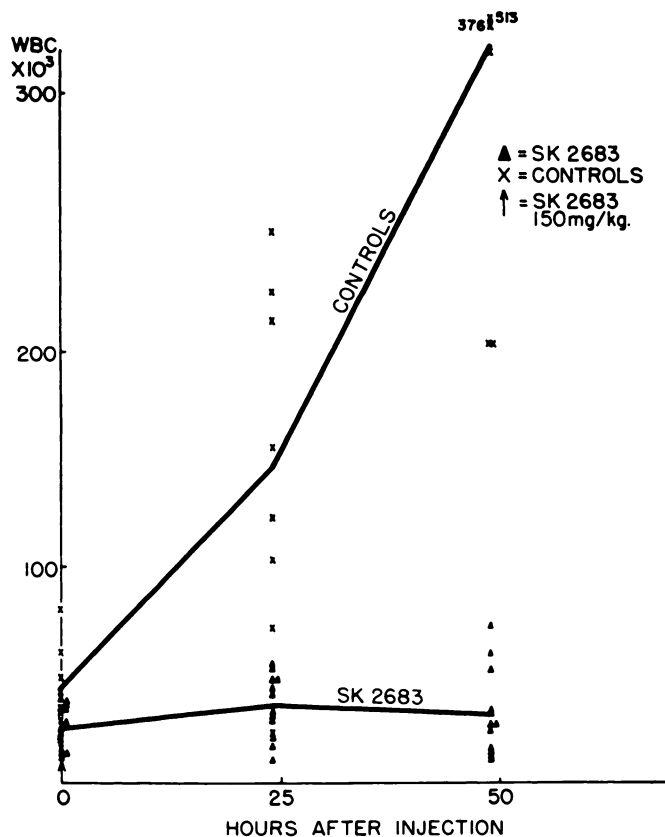


FIG. 4.—The effect of 2-ethylenimino-4,6-dimethoxy-*s*-triazine (SK 2683) on advanced Ak4 leukemia.

imine ring. The results of these experiments generally agree with those noted with the nitrogen mustard methyl-bis(2-chloroethyl)amine.¹⁰ The severe leukopenia shown in these experiments after chronic or single acute administration of the drug, plus the fact that these compounds have a definite inactivating effect on leukemia Ak4^{11, 12} lends support to the hypothesis that these agents exert their anti-leukemic action, as would be expected, by mechanisms more like nitrogen mustard derivatives than like the antagonists of folic acid.²

SUMMARY

1. In the usual therapeutic dosage in mice with the Ak4 strain of leukemia, TEM caused a prolongation of survival time, held the total leukocyte count

down at severely leukopenic levels, delayed the appearance of immature lymphocytes in the peripheral blood, and caused a marked delay in the appearance of leukemic infiltrations of the liver, spleen, bone marrow and kidney.

2. A single massive dose of TEM, administered on the seventh day of the disease when the total leukocyte count was high, caused a rapid fall in the count and depressed the number of prolymphocytes in the peripheral blood.

3. Single massive doses of such structurally related compounds as hexamethylene diethylenurea, 2,4-diethylenimino-6-amino-*s*-triazine, and to a lesser degree, 2-ethylenimino-4,6-dimethoxy-*s*-triazine caused a similar fall in the total leukocyte count when administered on the seventh day of the disease.

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