The current study was conducted as part of a program to characterize the influence of drugs on advanced leukemia in mice. Treatment of leukemia in advanced stages of the disease is considerably more difficult than treatment in early stages because of the increased number of leukemic cells (4, 5), the systemic infiltration, and the accompanying debility of the host. Despite these difficulties, it was demonstrated in previous studies (1, 3, 5, 8) that with drugs such as A-methopterin, 6-mercaptopurine, or reserpine it was possible to increase significantly the survival time of mice bearing advanced leukemia. The extent of the antileukemic effect was influenced by the schedule of treatment employed as well as by the dosage of drug. For example, in studies employing continuous treatment, it was demonstrated that daily treatment with A-methopterin elicited a greater extension in the survival time of mice with advanced leukemia than various other schedules ranging from treatment twice daily to treatment every 4 days (5). With all the schedules of treatment employed, the toxicity of the drug for the host limited its therapeutic effectiveness. Typically, the antileukemic effect increased with the dosage until the maximum extension in survival time was attained. Further increases in dosage resulted in a decreased extension in survival time because of increased toxicity for the host (5, 8).

The latter observation suggested that some advantage might be derived from the discontinuance of therapy prior to the time when the toxic effects on the host outweighed the antileukemic effect. The present study was conducted to determine the influence of the duration of treatment with A-methopterin on mice bearing advanced leukemia (L1210).

MATERIALS AND METHODS

General.—The general procedures employed for continuous daily treatment of mice bearing advanced leukemia (L1210) have been previously described (5, 8). In the present experiment, 8–12-week-old CDBA hybrid1 male mice were given inoculations into the right thigh of 0.1 ml of a uniform saline suspension (33.4 X 10^6 cells/ml) of leukemic cells (L1210). The inoculum was 100 times the inoculum which produced 100 per cent deaths from leukemia in untreated mice.

A-methopterin2 was dissolved in 2 per cent aqueous sodium bicarbonate and was administered subcutaneously in the constant volume of 0.01 ml/gram of body weight. Treatment was initiated on the 8th day following tumor inoculation, at which time all the mice displayed palpable tumor growths at the site of inoculation. As a further test of the stage of the disease, ten untreated leukemic mice were selected at random on the initial day of treatment, and their blood was inoculated into normal mice. All the recipient mice grew large local tumors at the site of inoculation and succumbed to the disease.

Experimental.—In addition to various control and tumor titration groups, the present experiment included 61 different groups of tumor-bearing mice receiving A-methopterin treatment. Six groups of ten mice each received daily injections of A-methopterin beginning on the 8th day following tumor inoculation and continuing until death. The daily doses employed in these groups were 0.41, 0.62, 0.93, 1.4, 2.1, and 3.1 mg/kg. In addition, there were 55 groups (eleven groups at each of the five higher dose levels) of eight mice, each of which was scheduled to receive a limited number

1 (BALB/cAn X DBA/2J)F1.
2 A-methopterin was provided by Dr. J. M. Ruegsegger of the Research Division, American Cyanamid Co., Pearl River, N.Y.
of consecutive daily treatments. The scheduled number of daily treatments ranged from five to 25 for doses of 0.62, 0.93, and 1.4 mg/kg, and from three to 23 for doses of 2.1 and 3.1 mg/kg. On alternate days, treatment was discontinued for one group of mice at each dose level. There were no limited treatment groups at the 0.41 mg/kg/day dose level. In previous experiments (5, 8), dose levels approximating this had been consistently below the optimal level of daily treatment.

Statistical.—For each of the various treatment groups, two summary statistics are of interest. These are the average survival time subsequent to tumor inoculation and the average remaining lifetime of survivors subsequent to discontinuance of treatment. These statistics permit the simultaneous comparison of average survival time for the various durations of therapy. Further, the average remaining lifetime of survivors when treatment was discontinued can be compared with the average remaining lifetime on that day for mice continuing to receive treatment.

Estimates of average survival times and of average remaining lifetimes were obtained for this experiment by employing a procedure which pooled the results for the various treatment groups. This procedure is described briefly below. The pooling procedure takes advantage of the fact that, prior to the discontinuance of treatment for a group, it is essentially on the same regimen as groups receiving further treatment. The relative comparisons of two treatment groups then depend only on differences occurring after their treatments begin to differ and do not reflect differences occurring while the treatments were identical. When analyzed in this way, there is no difference in the relative comparison of continued and discontinued treatment yielded by average survival time and by average remaining lifetime. Comparisons based on observed average survival time and observed average remaining lifetime for individual groups could, however, have been contradictory.

The procedure employed for effectively combining results of experiments of this type has been used frequently in medical statistics. It is the actuarial or modified life table method (7). In principle, the procedure applies very simply in the present instance. To evaluate the effectiveness of a particular therapy (dosage and duration of daily treatment), it is necessary to determine, for each day, the proportion of survivors among those mice which had received all prior treatments in conformity with the scheduled therapy and were alive on the preceding day. The number of mice on which survival proportions are based may be reduced each day by deaths and by the elimination of mice from treatment in conformity with the planned schedule of therapy.

The proportion of survivors for each day provides an estimate of the probability of surviving from one day to the next. Serial multiplication of these proportions from the initial day provides estimates of the probabilities of surviving from the initial day through each particular day. Differencing of the latter estimated probabilities in turn provides estimates of the probabilities of animal death on any particular day or, effectively, an estimate of the distribution of days of death of animals on a therapy. Any other characteristic of interest can be obtained routinely from this estimated distribution—in particular, one can estimate average survival time from the initial day and the average remaining lifetime of survivors from any subsequent day.

In practice, the computations required by the modified life table method are simple and routine.

RESULTS

The principal results of the current experiment are summarized in Charts 1 and 2.

Chart 1 compares the average survival time at each dose level and each scheduled duration of therapy employed.4 The right terminal of each curve represents the average survival time when treatment was scheduled to be continued indefinitely. For each dose level, the average survival time curve characteristically rises with the scheduled number of treatments and then plateaus at a level corresponding to the survival time attained with the uninterrupted schedule. None of the curves of Chart 1 indicates any definite tendency for an interrupted therapy schedule to be optimal, and there is no clear indication that it would ever have been worth while to discontinue therapy.

Of the mice treated continuously until death, those receiving 0.62 and 0.93 mg/kg daily were al-

4 In some instances the average survival time for a schedule could not properly be computed because of the failure of any mice on the schedule to survive long enough to have their treatment discontinued, even though actuarial computations indicated a definite probability for such survival. In these cases conservative estimates of the average survival time were made by assuming that any survivors would have lived only three additional days beyond discontinuance. Since in most of these cases the expected survivorship was only about 5 per cent, it is unlikely that the conservative estimates made could be much in error. This difficulty could have been avoided by employing an experimental plan under which randomization procedures were used from day to day to determine which mice were to have their therapies discontinued. In laboratory application, such an experimental plan on the scale of the present study would be extremely awkward.
forded the maximum extension in survival time. It may be noted that, although these two dose levels on continuous treatment elicited similar average survival times, there were observable differences in response. At 0.93 mg/kg daily, most of the mice died with reduced tumors and considerable weight loss, indicating that they eventually succumbed primarily to drug toxicity. In contrast, at 0.62 mg/kg daily many mice died with large tumors and little weight loss.

At 0.41 mg/kg A-methopterin daily, although no interrupted schedules of treatment were planned, all mice died prior to the twentieth scheduled treatment. For this group of mice an identical survival time would have been observed if nineteen or any greater number of daily treatments had been scheduled. Accordingly, Chart 1 shows a stable survival time for nineteen or more treatments.

Chart 2 compares the average remaining lifetime of survivors following discontinuance of treatment on any particular day with the average remaining lifetime from that same day for groups in which treatment was continued without inter-

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**CHART 1.**—Average survival time of leukemic mice as a function of the scheduled number of treatments with A-methopterin. For each schedule of treatment the average survival time was estimated according to the actuarial procedure as described in the text. The abscissa corresponding to the vertical bar on each curve indicates the longest individual survival time observed during the course of treatment—the average survival time is not changed by scheduling treatments beyond the maximum individual survival time. Estimated average survival times are not shown for schedules extending beyond the maximum individual survival time observed during treatment. In some instances it was necessary to estimate the average survival time employing a conservative procedure as indicated in footnote 8.
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The results are shown for dose levels of 0.62 and 1.4 mg/kg daily only, but essentially similar results were obtained with the other dose levels employed. Typically, whenever treatment was discontinued, the remaining mice survived, on the average, 2–3 days following discontinuance. There were a few isolated instances of individual mice surviving 5 or 6 days following discontinuance. It should be noted that the average survival time of control mice was 10.2 days or 2.2 days beyond the initiation of A-methopterin treatment (Chart 1). Thus, the average remaining lifetime in any group where treatment was discontinued was approximately that of untreated mice from the day of treatment initiation (Chart 2).

In contrast, the average remaining lifetime for mice on continued therapy from any day is initially high, but gradually decreases (Chart 2). Just as long as it remains higher than the average remaining lifetime when therapy is interrupted, it is worth while to continue treatment.

DISCUSSION

The toxicity of the known antineoplastic drugs for the host is a prime limiting factor in their employment in therapy. To obtain the greatest increases in survival time of tumorous animals it is necessary to employ dosages and schedules of treatment which afford the most extensive inhibition of tumor without permitting host toxicity to become limiting.

Against leukemia, it is necessary to choose an

![Chart 2](chart2.png)

**Chart 2.**—Average remaining lifetime of leukemic mice as a function of the number of prior treatments with two selected levels of A-methopterin. Figures in parentheses represent the number of mice on which the average remaining lifetime is based. For continued treatment groups these figures represent the remaining cohort, and the average was obtained by the actuarial procedure. For discontinued treatment groups the simple average of the individual remaining lifetimes is shown.
optimal dosage for that interval. Against early leukemia in mice, treatments spaced 4 days apart permitted the employment of high doses of antifolic without undue increase in undesirable host toxicity, thereby resulting in marked increases in survival time (2, 5). Against terminal leukemia daily treatment was optimal, and it was advantageous to employ lower doses of the drugs (3).

If the treatment is carcinostatic, merely holding the tumor in check, and the toxicity of the drug for the host is not exceeded, it should be theoretically possible to treat and maintain the animal indefinitely. Or, if the treatment is resulting in progressive diminution of the leukemic process, within the safe limits of host toxicity, treatment may be continued until a cure is achieved. Such situations have been observed in the limited case in which treatment is initiated relatively early following leukemic inoculation, even with leukemic inocula well above that required for 100 per cent takes in untreated controls (9). In the latter situation, if it could be determined that a cure had been achieved, it would be desirable to discontinue treatment and so curtail any further risk of drug toxicity.

Where the treatment is resulting in progressive decrease in leukemia but the toxicity for the host is becoming limiting, temporary discontinuance of treatment may provide a means of permitting the host to recover sufficiently so that treatment may be resumed (9). The success of such a procedure is contingent upon the host's recovering sufficiently rapidly relative to the recovery of the leukemia.

Against advanced leukemia such procedures have not, as yet, been practical. As the number of leukemic cells is increased, antifolics such as A-methopterin and aminopterin are less effective (4, 5). Compensation for an increased number of leukemic cells can be made by increasing the dosage of A-methopterin, but the tolerated dose range is soon exceeded. This may be aggravated by the general debility of the host and accompanying reduced drug tolerance. The relative reduction in the effectiveness of treatment does not permit wide spacing of treatment schedules or temporary discontinuance of treatment. Treatment of advanced leukemia every 4 days did permit the employment of higher doses of drug, but apparently the leukemic cell population recovered too rapidly between treatments to make this procedure practical.

At optimal doses on daily treatment, the tumor is apparently held in check, but discontinuance of treatment results in increased tumor growth and death of the host in 2–3 days. At higher dose levels, where the reduction in toxicity for the host due to discontinuance of treatment might have resulted in some therapeutic advantage, it was counterbalanced by a corresponding reduction in antitumor effect.

Although the data indicate that, with advanced leukemia, it was not advantageous to discontinue therapy entirely (or even to interrupt therapy for a moderate period, since mice die relatively quickly following discontinuance), the possibility remains that some other modification in treatment schedule might be useful. For example, there is the possibility that some advantage might be gained by initiating daily treatment of advanced leukemia at a relatively high dose level and subsequently lowering the dose, thereby minimizing the risk of cumulative toxicity for the host (6).

The employment of a second drug in combination with the A-methopterin might be useful. For example, it has been shown (8) that the combination of A-methopterin given every 3 days with 6-mercaptopurine daily was more effective against advanced leukemia than A-methopterin alone given every 3 days. It has been suggested that 6-mercaptopurine, which by itself was only moderately effective, served to shorten the interval between antileukemic treatments, thus providing a more nearly optimal schedule of treatment (8).

The data of the current experiment further emphasize the importance of detailed studies designed to elucidate factors affecting the therapeutic usefulness of antitumor drugs.

SUMMARY

In the treatment of advanced mouse leukemia L1210 with daily injections of A-methopterin, it was found advantageous to continue the treatment indefinitely. Whenever daily treatment was discontinued, the leukemic mice succumbed, on the average, in 2–3 days. The data suggest that the failure to derive any benefit from the discontinuance of treatment resulted from the inability to obtain sufficiently extensive regression of advanced tumors with regimens of treatment which are not lethal to the host.

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