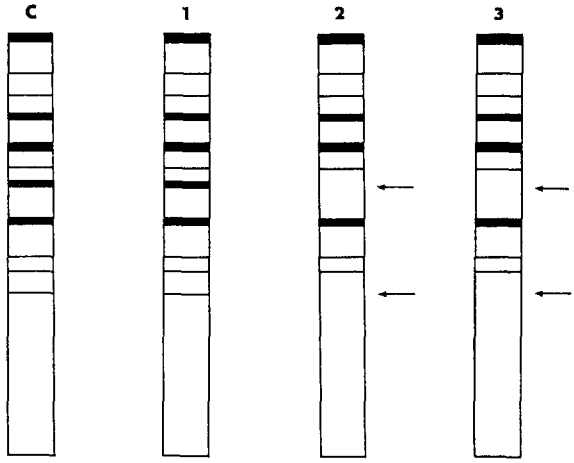

FIG. 2. Diagrams of polyacrylamide gel electrophoretic patterns of rat liver microsomal structural proteins obtained with pooled samples containing the material from two animals of each group. Cathode at the bottom; area of application at the top. C: Control. 1–3: Days after 1000 R X-irradiation of the heads.

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Enhanced antitumor effect of cytosine arabinoside given in a schedule dictated by kinetic studies *in vivo*

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THE SCHEDULING of cancer chemotherapy has been largely empirical because of the lack of precise information about the site, mode and duration of action of many of the presently useful antitumor

agents. Furthermore, the response of the normal host tissues *vis a vis* the tumor tissue has been definable only in a general way and has usually been limited to qualitative clinical toxicity studies. Nevertheless, there is growing evidence in animals and in man that dose scheduling which takes into consideration the kinetics of both the recovering normal and tumorous tissue can result in enhanced antitumor effect. For example, several studies,[1-3] have demonstrated striking dose schedule dependency of cytosine arabinoside (ARA-C) by comparing a series of empiric dose schedules. Furthermore, in man, twice weekly administration of methotrexate in acute lymphocytic leukemia has resulted in enhanced duration of remission over a daily dose schedule.[4]

For some antitumor agents, the site and mode of action are sufficiently well understood that this information can be used to study the effect of the drugs *in vivo*. ARA-C blocks reduction of cytidylic to deoxycytidylic acid,[5] inhibits DNA polymerase,[6] and inhibits DNA synthesis *in vivo*, blocking the incorporation of tritiated thymidine. The time to recovery of DNA synthesis, as measured by tritiated thymidine uptake, can then be measured in both normal and tumor tissues, and scheduling of additional chemotherapy can be designed which takes advantage of any differences therein. We have been able to demonstrate enhanced antitumor effect of a schedule designed in this manner.

CDF[1] male mice, weighing approximately 20 g, were used in all experiments. On day 1, 51 mice received an intraperitoneal injection of 1×10^5 L1210 ascites cells per mouse. On day 6 of tumor growth, six mice received 100 μCi ^{3}H-thymidine (sp. act., 1·9 Ci/m-mole; New England Nuclear, Boston, Mass.) intraperitoneally, and 1 hr later were killed by cervical traction and served as controls. We aspirated ascites tumor from the abdominal cavity and washed the cavity with iced phosphate-buffered 0·85% NaCl, pH 7·4. We then removed the duodenum of each mouse and split it lengthwise on a glass slide and, using the edge of another glass slide, stripped the mucosa from the serosa and muscularis, and agitated and dispersed it in iced phosphate buffer solution. We then removed both femurs of each mouse and expressed the contents through a 25-gauge hypodermic needle and a syringe of iced buffered saline. Smears of control marrows were examined after 6 days of intraperitoneal tumor growth and were found not to be replaced by tumor. Separate samples of ascites tumor, duodenal mucosa and bone marrow from each of the six mice were pooled into two groups of three. These were spun at 2000 *g* for 10 min, the supernatant was discarded, and the button of tissue was frozen for further processing. All samples throughout the study were processed in an identical manner.

Forty-eight mice received 1000 mg/kg of ARA-C, i.p., on day 6 of tumor growth. At 1, 12, 24, 36, 48, 72, 96 and 120 hr thereafter, six mice at each time point received 100 μCi ^{3}H-thymidine, and 1 hr later were killed by cervical traction. Ascites tumor, gastrointestinal mucosa and bone marrow were processed as in the control animals. The DNA content of each of the pooled specimens was extracted by a modification of the Schneider method,[7] and a 0·5-ml aliquot of the final supernatant was added to 15 ml Aquasol (New England Nuclear, Boston, Mass.) and counted in a Packard Tri-Carb liquid scintillation spectrometer. A 0·5-ml aliquot of the final supernatant was used for the determination of DNA by the Burton method.[8] Results were then expressed as counts per min per microgram of DNA.

Results appear in Fig. 1. Each point represents the mean value of the two pooled samples, each containing all the material from three animals. Initially, the L1210 leukemic cells have 7380 counts/min/μg of DNA, compared with a lower value of 189 and 190 counts/min/μg of DNA for the bone marrow and gastrointestinal tract respectively. One hr after the administration of ARA-C, marked inhibition of thymidine incorporation into DNA is evident, and the tumorous ascites has only 16 counts/min/μg of DNA, while the bone marrow and gastrointestinal tract have only 3 counts/min/μg of DNA. Evidence of recovery of the bone marrow and gastrointestinal tract begins by 12 hr, and by 24 hr, thymidine incorporation into DNA from these normal tissues exceeds control levels. This overshoot of thymidine incorporation into DNA is maximal in the gastrointestinal tract at 36 hr and in the marrow at 48 hr, and is followed by a gradual return toward the control, which is complete by 96 hr.

The ascites tumor begins recovery sometime between 1 and 12 hr after therapy. However, it does not return to pretreatment levels until 96 hr. There is, therefore, a period of time from 72 to 96 hr where the bone marrow and gastrointestinal tract have resumed control levels of thymidine incorporation into DNA, but the tumor tissue is much more actively incorporating thymidine and presumably synthesizing DNA. This would appear to be the optimal time for retreatment with an S phase-specific agent, such as ARA-C.

In an attempt to correlate these findings with an effect on survival, we performed a second series of experiments. One hundred and twenty-four mice were injected with 1×10^5 L1210 leukemic cells i.p. Thirty-eight were left as controls, and on day 6 of tumor growth, 86 mice were injected with 1000/mg/kg of ARA-C i.p. Thereafter, in groups of 10 or 11, mice were injected with an additional 1000 mg/kg of ARA-C at intervals of 12, 24, 36, 48, 72, 96 and 120 hr. The animals were then followed for survival, and the results are shown in Fig. 2. A single dose of ARA-C produced a prolongation of survival of 62·5 per cent compared to the untreated controls. Of considerable interest was the finding that the addition of 1000 mg/kg of ARA-C at 12 and 24 hr did not significantly enhance survival over

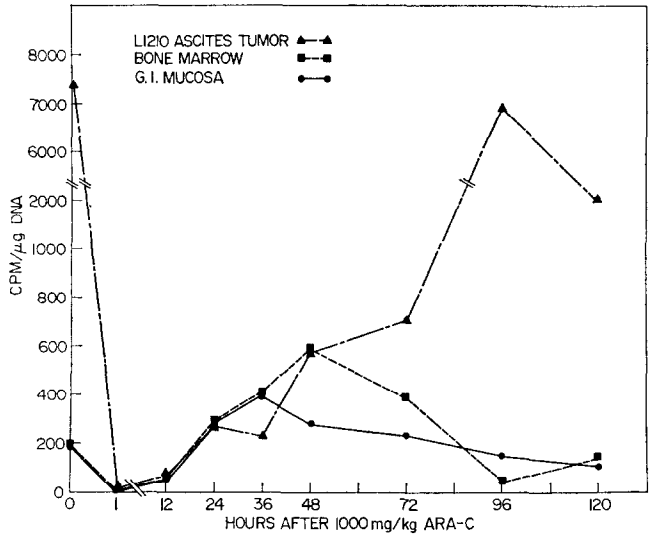

FIG. 1. Effect of a single dose of 1000 mg/kg of ARA-C on the incorporation *in vivo* of ^{3}H-thymidine into DNA in L1210 ascites tumor, normal murine bone marrow and gastrointestinal musocal cells. Each point represents the mean of two pooled groups of three animals each. Values are expressed as counts per min per microgram of DNA for each of the three separate tissues.

that of the single, initial dose. Thereafter, as the interval between doses was prolonged, enhanced antitumor effect was seen and was maximal at 72–96 hr, where there was 125 per cent prolongation of survival. However, if the interval between injections was prolonged to 120 hr, the survival began to decline, as the animals were terminally ill with L1210 leukemia at the time of the second injection.

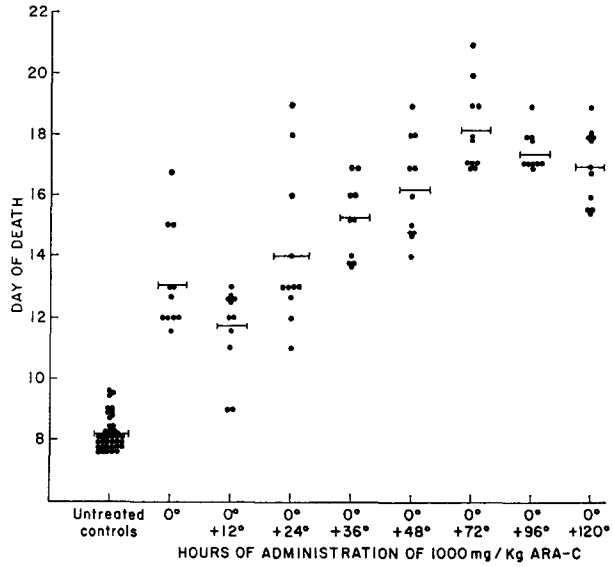

FIG. 2. Survival of mice with L1210 ascites tumor after treatment with various dose schedules of ARA-C. Mean survival for each group is noted with a bar. The 0° animals received a single dose of 1000 mg/kg of ARA-C. Except for controls, all other animals received two doses of ARA-C; the second was given at varying intervals after the first, as noted.

There are two areas on the curves in Fig. 1 which are of particular interest with regard to subsequent chemotherapy. The first is the area from 24 to 48 hr. At this point, the ascites tumor is still suppressed relative to its control level of thymidine incorporation. On the other hand, the normal tissues, bone marrow and gastrointestinal tract, are synthesizing and presumably repairing DNA at a supernormal rate. It would be at this time interval where the administration of an S phase-specific agent, such as ARA-C, would be expected to produce preferentially more damage to normal tissues than to the tumor. In this regard, we studied the effect of various dose schedules of ARA-C on normal CDF_1 mice, as shown in Table 1. One can see that there is enhanced toxicity of schedules where the second dose is administered from 12 to 36 hr after the first dose. There is, however, less toxicity when the second dose is delayed to 96–120 hr.

TABLE 1. TOXICITY OF ARA-C REGIMENS TO NORMAL CDF_1 MICE

Treatment groups*	Maximum wt loss (%)	Day of maximum wt loss	Mortality (%)	Day of recovery to pre-treatment wt
Controls	+2·5		0	
1 Dose,† 0°	—7·9	3	0	8
2 Doses,† 0° + 12°	−23·2	7	50	>10
2 Doses, 0° + 24°	−11·3	4	0	>10
2 Doses, 0° + 36°	−15·5	6	0	10
2 Doses, 0° + 48°	−7·4	5	0	8
2 Doses, 0° + 72°	−8·9	5	0	9
2 Doses, 0° + 96°	−5·2	7	0	9
2 Doses, 0° + 120°	−2·3	6	0	5

* Six mice in each treatment group.
† ARA-C (1000 mg/kg).

The other area of particular interest in the curves of Fig. 1 is the area between 72 and 96 hr. At this time, the tumor has recovered to full control levels of thymidine incorporation, and the normal tissues have returned to their baseline or below. This would be expected to be the period where the differential effect of an S phase-specific drug might favor the killing of more tumor than normal tissue and produce enhanced survival in these animals. This suggestion was supported by the findings described in Fig. 2 and Table 1.

It seems likely that information of this kind obtained in other tumor systems and in man would allow the design of more appropriate drug schedules which enhance the antitumor effect while minimizing toxicity to normal tissues.

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