across the section lines or else by drawing scenes in which none of the objects would extend across the section lines.
8. S. Sternberg, *Acta Psychol.* **30**, 276 (1969).
9. Reaction times could be measured by providing the subject with a target object before the scene was presented. When the scene and cue are presented, the subject would respond, "Yes," as quickly as possible if the cued object was the target, and, "No," otherwise.
10. The logic of the additive factors method (AFM) holds that if two factors, for instance jumbling and probability, are affecting the duration of separate and independent information-processing stages, then their combined effects on reaction time (RT) should be additive. That is, if jumbling adds 50 msec to the average RT and probability adds 25 msec, then the RT for a low-probability target in a jumbled scene should be, on the average, 75 msec longer than that for a high-probability target in a coherent scene. The AFM may also be applicable to error probabilities, but, in that case, if factors are influencing different information-processing stages, the logarithms of the errors should add.
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Increased Tolerance of Leukemic Mice to Arabinosyl Cytosine with Schedule Adjusted to Circadian System

Abstract. *Mice (BDF_1) inoculated with L1210 leukemia survive for a statistically significantly longer span when four courses of arabinosyl cytosine are administered at 4-day intervals—not in courses consisting of eight equal doses at 3-hour intervals, but in sinusoidally increasing and decreasing 24-hour courses, the largest amount being given at previously mapped circadian and circannual times of peak host resistance to the drug. This finding relates to the many therapeutic situations involving rhythmic, and thus predictable, cycles in the host's tolerance of undesired effects from the agent used.*

The mortality of BDF_1 mice receiving arabinosyl cytosine (ara-C) at the same "time of day" for five consecutive days was previously shown to depend on the circadian system phase of the animals at the time the drug was administered (*1*). In mice kept on a regimen of 12 hours light and 12 hours dark ($LD_{12:12}$), with light from 06^{00} to 18^{00} and dark from 18^{00} to 06^{00}, a circadian cycle of susceptibility and resistance to ara-C was found; a superimposed, possibly circannual, variation was found for the circadian acrophase, that is, for the time of highest values—determined as the lag (from local 00^{00}, phase reference) of the crest time of a cosine function fitted to approximate the rhythm. During January and February 1971, the susceptibility acrophase occurred in mice treated during the first half of the dark span (at 18^{00} and at 23^{00}), the lowest mortality rate being found in animals treated during the light span (at 08^{30} and at 13^{00}) of the $LD_{12:12}$ synchronizer cycle (*1*).

The capacity of ara-C, 5-fluorouracil, or actinomycin D to inhibit mitotic activity in the cornea of rats in single doses that are compatible with survival of the animals depends, to a statistically significant degree, on the time of treatment (*2*). This finding was subsequently extended to include doses of dexamethasone (*3*). Isoproterenol altered the circadian rhythm of mitoses in mouse cornea; it was suggested that the response to this drug also is time-dependent (*4*). The scope of circadian rhythms in susceptibility to physical, chemical, and bacterial agents in experimental animals and in man (*5, 6*) was thus extended to include certain drugs used in the clinical chemotherapy of malignancies.

Earlier experiments (*1*) measured the effects and the toxicity of ara-C after administration as single injections or after a short course of treatment during only one circadian system phase. We therefore studied the rhythm in susceptibility to ara-C for the longer course schedules that are currently regarded as optimal for the treatment of experimental leukemia (*7*).

Without using any reported chronobiologic consideration, precaution, or method, such as an explicit control of the lighting regimen in the animal room (*6*), Skipper *et al.* (*7*) inoculated L 1210 mouse leukemia cells in BDF_1 mice, and then eradicated consistently 10^5 leukemia cells (and in some cases 10^6 cells) without animal deaths caused by drug toxicity; this important result was accomplished by giving four treatments of 120 mg of ara-C per kilogram of body weight in eight equal doses spaced at 3-hour intervals over a 24-hour span. Courses of 240 mg of ara-C per kilogram, given by the same schedule, were toxic, reportedly killing all animals, and were not used for treating leukemia from inocula containing more than 10^6 L 1210 cells.

By applying chronobiologic methods and concepts, we attempted to improve the tolerance of BDF_1 mice to the reportedly toxic four-daily courses of ara-C of 240 mg/kg. We adjusted the administration schedule to the circadian susceptibility rhythm previously mapped on similar animals under standardized conditions (*6*); we compared this sinusoidal treatment (*5*) with a reference treatment (R) used earlier by others (*7*) (Table 1).

Male BDF_1 mice, 4 to 6 weeks of age, were singly housed in sound-deadened rooms on a lighting regimen of $LD_{12:12}$ with light from 06^{00} to 18^{00}. The temperature in the rooms was 24° ± 1°C; Purina Laboratory Chow and tap water were freely available to the animals. The mice were kept on this regimen for 7 days, and were then given, by intraperitoneal injection, L 1210 leukemia cells obtained from ascitic fluid of DBA_2 mice carrying the leukemia. In our first two experiments we injected 10^6 cells suspended in Eagles minimal essential medium. Skipper *et al.* (*7*) had been able to achieve a 40 to 60 percent "cure rate" of this inoculum with ara-C (four courses of 120 mg/kg per day) on the schedule outlined as reference treatment below (R). In our third experiment, we injected 10^7 leukemic cells. With this increase of inoculum, no "cures" of the leukemia had previously been achieved (*7*).

In the studies reported here, the first course of chemotherapy with ara-C was begun approximately 44 to 48 hours after inoculation of the malignant cells. Each animal received eight intraperitoneal injections of ara-C in saline at 3-hour intervals over a 24-hour span, the total dose being consistently 240 mg/kg in 24 hours (Table 1). The duration of each series of injections was less than 90 minutes for the entire group, with the direct involvement of each individual animal limited to less than 3 minutes. A total of four courses of eight injections each was given with a 3-day interval free of therapy between the treatments.

One group of mice in each experiment received eight equal doses of ara-C every 3 hours for 24 hours, every fourth day. This schedule had been suggested (*7*) as the optimal daily dose regimen in the treatment of L 1210 leukemia by ara-C. This treatment is referred to as the reference treatment (R). It

should be emphasized that, in our studies of tolerance, the total daily amounts of drug represent overdoses rather than doses.

Another group of mice received the same total dosage according to a so-called sinusoidal treatment schedule (S). Our earlier work on circadian rhythms in mortality from single doses (*1*) served to locate the placement in time of varying amounts of ara-C in this multiple-daily-dose treatment schedule. We assumed that the expected cycle of sensitivity and resistance of the animals to the multiple doses would be similar to that established earlier from data on separate groups of mice, each treated only once daily at different but fixed circadian times (*1*). The time of high circadian resistance to the drug as established for single daily doses (*1*) was selected as the point for the greatest amounts of a sinusoidal drug administration pattern with multiple daily doses; this S-schedule involved ara-C administration in increasing and then decreasing doses every 3 hours for 24 hours every fourth day (Table 1). Moreover, we had found earlier that the lethal effect of ara-C administered as a single daily dose exhibited a low-frequency variation, possibly circannual, as well as a circadian one. The highest dose of the sinusoidal treatment, therefore, was administered 12 hours from the mortality acrophase for the corresponding month of the year as determined by administration of ara-C as a single dose (*1*).

The dose range administered by this sinusoidal pattern extended from 7.5 mg/kg at the predicted time of lowest resistance, to 67.5 mg/kg at the predicted time of highest resistance of the host, corresponding to a ratio of 1 : 9. The total amount of ara-C given as reference treatment in eight equal doses of 30 mg/kg each, and the total amount of ara-C given on the sinusoidal schedule, were the same (240 mg/kg in 24 hours) (Table 1).

Daily checks of the animals revealed that those mice dying of intraperitoneally injected L 1210 leukemia showed a slightly hemorrhagic ascites, with the ascitic fluid containing large numbers of tumor cells. The early mortality after ara-C treatment was invariably due to drug toxicity with no gross evidence of leukemia found at the obligatory autopsy. This does not imply that the animals were cured of the transplanted malignancy; the cure rates are to be determined after prolonged follow-up observations. The survival time, during and shortly after completion of a four-course series of ara-C treatment, is shown in Table 2.

Table 1. Comparison of individual doses of ara-C in reference treatment (R) and in sinusoidal schedule (S).

Treatment	Dose (mg/kg) at injection time (midpoint of group)								24-hour total
	08^{00}	11^{00}	14^{00}	17^{00}	20^{00}	23^{00}	02^{00}	05^{00}	
R	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	240.0 (960)*
S	30.0	67.5	67.5	30.0	15.0	7.5	7.5	15.0	240.0 (960)*

* Total dose given in four 24-hour courses.

The data indicate that treatment adjusted for periodicity significantly improves tolerance to ara-C in leukemic BDF_1 mice, as determined by survival time. In order to evaluate this survival time we included data from all animals. The survival time of the animals that did not die was measured from the time of injection of the leukemia to the (admittedly arbitrary) time of writing this report.

Agents, such as ara-C, presently used in the clinical treatment of malignancies must be given in at least borderline toxic doses in order to achieve a therapeutic result. Therefore, an attempt to improve drug tolerance in patients by timing drug administration appears to be urgent.

The application of the concept of cycles of susceptibility to chemo- and radiotherapy (*8*) of malignancies in man will depend upon the characterization of such rhythms in patients actually receiving relatively toxic doses of these agents as treatment for their tumors. It must be emphasized, however, that the circadian changes in susceptibility to noxious agents do not represent "time-of-day effects" and that attempts to schedule treatment in man will have to be based on individual physiological times that have been determined and monitored by appropriate reference functions (*9*). An appropriate reference function to guide clinical treatment is not presently known; it is reasonable to assume that in the case of agents leading to damage of hematopoietic elements, cycles related directly or indirectly to bone marrow function are most likely to be pertinent.

Therapy timed to defined circadian system phases may improve the tolerance of the host to potentially damaging chemotherapeutic or physical agents used in the treatment of cancer. An improved tolerance may allow more intensive therapy and may thus be the difference between success or failure of a given treatment.

In addition to circadian changes in host resistance, variations with other frequencies—ultradian, circaseptan, circavigintan, circatrigintan (*10*), and circannual—may influence resistance to noxious agents and alter one or several of the characteristics—waveform, acrophase, amplitude, and level—of the circadian cycle of susceptibility.

The tumor itself may exhibit cyclic changes with several frequencies (*8*). Information on such rhythms, insofar

Table 2. Difference in mean survival time of male BDF_1 mice at several (arbitrary) times after leukemia (L 1210) inoculation and ara-C treatment. Six-week-old mice (study 1) were inoculated with leukemia on 13 December 1971, 5-week-old mice (study 2) on 29 December 1971, and 7-week-old mice (study 3) on 10 January 1972. (The role of age differences is beyond the scope of this paper.) The results are compiled from data available up to and including 5 February 1972. If a mouse was still alive on 5 February 1972, the number of days up to that date is taken as its survival time; R, adjusted to putative leukemia cell cycle (*7*); S, adjusted to susceptibility rhythms of host mapped in tests of single daily doses (*1*). Treatments started 44 to 48 hours after leukemia inoculation [in studies 1 and 2, 10^6 cells; in study 3, 10^7 cells suspended in Eagle minimum essential medium, Spinner modified (Difco)]. Twelve mice inoculated with leukemia but not treated with ara-C died within 11 days. Twelve mice given saline, without a leukemia inoculum or ara-C treatment, all survived 38 days after injection.

Study No.	Days after inoculation	Mice per study*	Survival time (days)		(S − R)	t†	P
			R	S			
1	54	20	14.0	28.8	14.8	1.90	.04
2	38	60	18.1	33.3	15.2	5.15	.0005
3	26	28	9.1	17.5	8.4	2.40	.01
1, 2, 3		104	15.0	28.4	13.4	5.45	.0005

* Equally divided per group. † One-sided test.

as they characterize the tumor's sensitivity to the drug, may be invaluable in the search for added benefits from chronotherapy based upon the chronobiology of the tumor as well as that of the host.

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Neural Attenuation of Responses to Emitted Sounds in Echolocating Bats

Abstract. *Bats of the family Vespertilionidae emit strong ultrasonic pulses for echolocation. If such sounds directly stimulate their ears, the detection of echoes from short distances would be impaired. The responses of lateral lemniscal neurons to emitted sounds were found to be much smaller than those to playback sounds, even when the response of the auditory nerve was the same to both types of sounds. Thus, responses to self-vocalized sounds were attenuated between the cochlear nerve and the inferior colliculus. The mean attenuation was 25 decibels. This neural attenuating mechanism is probably a part of the mechanisms for effective echo detection.*

Bats of the family Vespertilionidae emit short ultrasonic pulses for echolocation. The amplitude of these sounds is about 110 to 120 db SPL (sound pressure level referred to 0.0002 dyne/cm^2 root-mean-square) at 3 to 10 cm from the mouth (*1*). If such strong outgoing sounds directly stimulate the ears, echoes from short distances would be difficult to detect, because the response is very poor to a sound that immediately follows another (*2–4*). Bats have the following mechanisms to reduce the degree of selfstimulation. (i) The inner ear is loosely attached to the skull, so that the amount of bone conduction is minimized (*5*). (ii) The middle ear muscles contract synchronously with vocalization and attenuate the amount of self-stimulation by as much as 15 to 20 db [*Tadarida brasiliensis* (*6*)]. (iii) The sensitivity of the auditory systems of *Rhinolophus ferrum equinum* and *Chilonycteris parnellii parnellii* is low for the sound frequencies emitted during flight, but sensitivity is high for sounds a few kilohertz higher than the emitted sounds, such as an echo shifted by the Doppler effect (*7*). In addition, there may be a neural mechanism by which responses of auditory neurons to outgoing sounds are reduced. We report evidence for the presence of such a neural attenuating mechanism in the brain of bats of the genus *Myotis*, which emit short frequency-modulated (FM) sounds for echolocation.

Nails 1.8 cm long were mounted with dental cement on the skulls of 16 gray bats (*Myotis grisescens*) under ether anesthesia. In the following days, the awake animal was placed on a plastic ball floating on water in a soundproof room. The nail was tightly held by a metal rod mounted on a micromanipulator. Without anesthetic, three small holes were made in the skull, and a pair of sharpened steel electrodes and two tungsten electrodes were inserted. The steel electrodes were used to stimulate vocal areas in the midbrain with a train of short elecrtic pulses delivered 2 to 3 sec^{-1} (*8*). One of the tungsten electrodes was inserted into the auditory nerve (or cochlear nucleus) in order to record N_1, the summated activity of the auditory nerve fibers. The other was inserted into the nucleus of the lateral lemniscus on the contralateral side in order to record LL, the summated activity of the laternal lemniscus (*9*). Thirty-two different sounds emitted by every bat were monitored with a ¼-inch microphone (Brüel & Kjaer 4135) placed 5 to 8 cm in front of the bat's mouth and were recorded with a tape recorder with a frequency response of 50 to 150,000 hertz (Ampex FR-100). The N_1 and LL evoked by these 32 emitted sounds were averaged by a computer (Nicolet 1070) and plotted with an X-Y recorder (Fig. 1A); these are called "the self-evoked N_1 and LL." The same 32 sounds were immediately played back at different amplitudes through a loudspeaker 68 cm in front of the bat's head. The pressure level of these sounds was monitored with the ¼-inch microphone, placed near the bat's ear. The N_1 and LL evoked by these playback signals were also averaged and plotted; these are called "the playback N_1 and LL." The self-evoked and playback LL were compared in order to determine whether their amplitudes were different when N_1 was the same for the emitted and playback sounds. Since the computer was synchronized to the onset of either the emitted or the playback sounds, fluctuations in time were always present