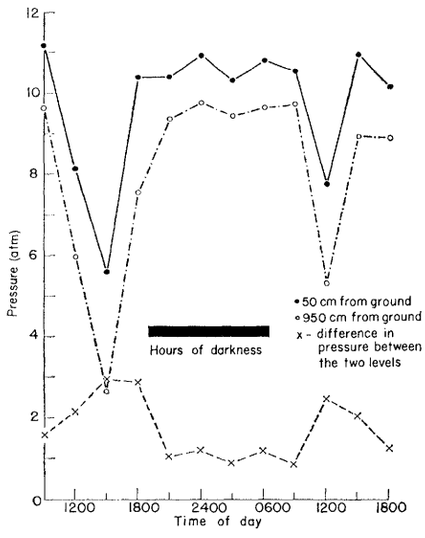

Fig. 1. Diurnal changes in hydrostatic pressure recorded at two heights on the trunk of a tree of *Hevea brasiliensis*.

pressure fell steadily until midday, but recovered again after a heavy rainstorm at 1220. This diurnal fluctuation in pressure has been amply confirmed in several experiments, although it is highly sensitive to weather conditions.

Hydrostatic pressure at the lower level on the tree is always greater than that at the upper position (Fig. 1). We have detected no direct influence of girth size on observed hydrostatic pressure and therefore conclude that this height effect is not an artefact occasioned by the smaller volume of laticiferous tissue at the high level. As a consequence of the "head" of latex, which has a specific gravity close to one, it might be expected that the pressure at the lower point would exceed that at the higher by approximately 1 atmosphere. At night, the observed difference does, in fact, approach this figure. It may be supposed that this hydrostatic pressure difference is counterbalanced by a corresponding difference in osmotic pressure; otherwise water would be lost from the vessels at the base of the trunk, owing to the excess pressure.

During the day, pressure falls more rapidly near the top of the tree than near the ground, so that the pressure difference between the two levels increases. Reduction in turgor pressure of the latex vessel system during the daylight hours is most probably brought about by loss of water to the xylem, although contraction of the wood under transpirational tension may have an effect, by relieving the pressure within the encircling bark (which may be thought to be under a peripheral, elastic, tension at night). In either case, the production of a steeper pressure gradient during the day would seem to reflect a considerable tension gradient in the xylem under conditions of rapid transpiration. The difference in pressure between the two levels reaches a maximum of 2.9 atmospheres; this figure is of the same order as estimates published previously for xylem tension gradients during active transpiration (*6*).

The diurnal variation in turgor pressure suggests a passive reflection of changes in xylem tension. It is of interest to consider what effect such changes in turgor of the phloem tissue might have on translocation. The latex vessel system and sieve tubes are closely associated elements of the phloem tissue in *Hevea* (*7*) and, in the absence of any active mechanism controlling sieve-tube turgor, one might expect the pressures in the two systems to be similar. For a simple form of Münch's pressure-flow hypothesis to operate in sieve tubes, a turgor gradient is required from crown to base in an actively growing tree. At no time does a significant gradient exist in this direction in our measurements. Thus if pressure-flow does operate, sieve tube turgor must be controlled by a separate mechanism.

A number of workers have observed gradients in the concentration of sieve-tube exudate (*5*, *8*), generally in the direction required for pressure-flow, but because of the probable loss of turgor due to transpiration, such determinations cannot be accepted as proof of the existence of turgor-pressure gradients. Direct measurement of sieve-tube turgor might be possible by the use of micromanometers in conjunction with the aphid-stylet technique (*9*), but the technical difficulties involved need no emphasis.

B. R. Buttery
S. G. Boatman
Botanical Division, Rubber Research Institute of Malaya, Kuala Lumpur, Malaysia

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Circadian Rhythmicity in the Sensitivity of Two Strains of Mice to Whole-Body Radiation

Abstract. *When male mice of the Swiss-Webster and C3H strains are maintained on a light-dark cycle in which the light begins at 7 a.m. and ends at 7 p.m., they are more sensitive to whole body x-irradiation (800 to 900 roentgens) given at 2 a.m. than at any other time in the cycle tested.*

The last 10 years have witnessed a considerable renewal of interest in the old observation that organic functions, typically executed once a day in the native state, continue to be executed with a nearly 24 hour rhythmicity in environments of constant light (or dark) and temperature (*1*). Halberg (*2*), and Pittendrigh (*3*, *4*) among others have emphasized the fact that these rhythms, typically assayed by "superficial" phenomena such as locomotory activity or leaf movement, are only reflections of an underlying rhythmicity that pervades the whole metabolic system. Daily—or circadian—rhythmicity, as it is now called, applies to enzyme systems, drug sensitivity, temperature tolerance, sensitivity to ultraviolet light, and other less easily assayed parameters of organisms, as demonstrated by several investigators. It has been emphasized repeatedly that the rhythmicity is an innate, inherent feature of physiological systems (*4*). Thus, circadian rhythmicity not only persists indefinitely with the period of about a day (hence, circadian) in constant conditions of light and temper-

ature, it also develops spontaneously in organisms bred through several generations in such constant conditions (*5*). The role of the light cycle in nature is not to impose rhythmicity on the system; it is merely to entrain it—that is, to regulate the period of the rhythm to precisely 24 hours and bring it into proper phase relative to the earth's rotation and hence to the daily cycle of environmental change. In the laboratory a light cycle can be similarly employed to establish the phase of the rhythm (which would occur in any case in the absence of the light) at any phase angle relative to real local time.

Evidently, it is still not widely appreciated how universal and pervasive this circadian rhythmicity is in physiological systems, and how pertinent it is to all assays of sensitivity. Pizzarello *et al.* (*6*) were certainly not aware of the plausibility of their unexpected and unlooked-for finding that rats subjected to x-rays at morning and evening manifested major differences in radiosensitivity. And it is clear that critics of their paper (especially *7*) found the result even less plausible.

Our earlier observations have now been extended to cover the radiosensitivity of two strains of *Mus musculus* at different times of day. The results, which confirm the implications of the original observation on rats, are reported here and a critical evaluation of the recent papers by Rugh *et al.* (*7*) and Straube (*8*) is included in an attempt to clarify the contradictory positions of our laboratory and theirs, and the compatibility of all the data with our position.

In June and July of 1963 we conducted our first series of experiments. Two strains of mice [Swiss-Webster male mice, delivered by cesarian section, and males of the C$_3$H Strain (*9*)] weighing, on the average, 24 to 29 g, were used in all these experiments. The mice were maintained on a 12-hour light and 12-hour dark cycle (the lights were turned on at 7 a.m.) and given unrestricted access to water and Purina Laboratory Chow. This light-dark cycle is also used by the suppliers of the mice (*9*) so that, except for the period in which the animals were in transit to our laboratories, they had been on this cycle since birth. The rooms in which the animals were housed were maintained at 20°C. No effort was made to control or measure the noise in the building and there was certainly a greater amount of noise from 7 a.m. to 4 p.m. than during the rest of the day. Food and water supplies were replenished at noon each day. The animals were housed in cages of the "hanging-drawer type" with wire mesh floors so that it was not necessary to handle the animals themselves at any time prior to radiation.

The mice were irradiated by means of a high-frequency, deep therapy unit of 280 kv (peak), operating at 20 ma, with added filtration of tin ¼ mm), copper (½ mm), and aluminum (1 mm). The half-value layer of the beam was 1.54 mm copper. The target to midbody distance was 50 cm and doses of 800 and 900 r (measured in air) were given. The dose rates (83 r/min) were checked during each period of irradiation by means of a Victoreen roentgen chamber and meter calibrated by the National Bureau of Standards. The unit was equipped with a dose-rate meter, in the beam, which measured the constancy of the output.

The effect of a given dose of x-rays is measured by the subsequent longevity of the irradiated sample, and is expressed in terms of the number of days elapsing between treatment and 50 percent mortality.

The animals were divided into three groups (two groups of Swiss-Webster mice and one of C$_3$H mice) for x-irradiation with 800 r. The three groups represented three separate shipments and were not irradiated at the same time but on three separate occasions (in subgroups of ten). The animals were held in the laboratory for 14 days after their arrival and before irradiation. After each group had been irradiated, the survival time in days was noted and recorded for each animal within the group, and the next group was not irradiated until all animals in the first group were dead. Radiations scheduled for the animal's dark period (7 p.m. to 7 a.m.) were actually performed in the dark. However, some light was necessary to enable us to remove the animals from their cages and place them in the radiation chamber, as well as to replace them in their cages. Those irradiated in the light period (7 a.m. to 7 p.m.) were in the dark while they were being irradiated since in this series an opaque chamber was used. There were no differences in the handling of the animals prior to irradiation or following it.

In defining treatment times it is important to emphasize that what is being analyzed is a possible cycle of sensitivity (or capacity to recover) whose phase is determined by the prevailing light-cycle acting as an entraining agent (*4*) or Zeitgeber (*10*) for the internal rhythm. Thus it is quite inadequate to define treatment times in terms of local time (say 9 a.m. eastern standard time) without also saying at what hours, local time, the lights go on and off. And in the interests of complete unambiguity it is better to define treatment times on a scale that refers directly to the phase of the entraining light cycle. That scale (Arbitrary Zeitgeber Time, AZT) runs from hour 00 to 24, with hour 00 defined as the onset of the light.

One of the groups of Swiss-Webster mice was divided into subgroups of ten and irradiated with 800 r at AZT: 03, 07, 11, 15, 19, 23; one subgroup being used at each of the times. At 19 AZT the animals appeared to be more sensitive than at any of the other times tested (Fig. 1). The second group of Swiss-Webster mice, also divided into subgroups of ten, were then irradiated at AZT: 15, 17, 19, 21, and 23 to determine whether or not such a sensitive point could be demonstrated again near 19 AZT and whether there might be a time close to 19 AZT at which the animals were even more sensitive (Fig. 1). Finally, C$_3$H mice (in subgroups of ten) were irradiated at AZT: 03, 07, 11, 15, 19, 23 (Fig. 1). Only Swiss-Webster mice were irradiated with 900 r; subgroups of ten were irradiated at AZT: 03, 07, 11, 15, 19, and 23 (Fig. 1). The animals were placed, unanesthetized, on a rotating table (12 rev/min) in a wooden enclosure (15 cm diameter by 2 cm deep) covered by a piece of cardboard, and irradiated. It was determined by using a pane of glass instead of the cardboard in a mock setup that the animals could not climb on top of one another in such an enclosure. The treatment therefore was not complicated by mutual screening. Weights of the animals were checked in the morning, three times weekly, after they had been irradiated, as an additional check on whether or not radiation was received. All groups showed a loss of weight.

The results are presented in Fig. 1 and Table 1. In Fig. 1 the data from all of our experiments as well as those of Rugh *et al.* (*7*) are shown by plotting the day after radiation at which 50 percent of the animals were dead against the time at which they were irradiated, while in Table 1 we have used the mean survival times of our own animals irradiated at different times of day. We have treated the data in Fig. 1 in this way in an attempt to present a meaningful comparison between the results

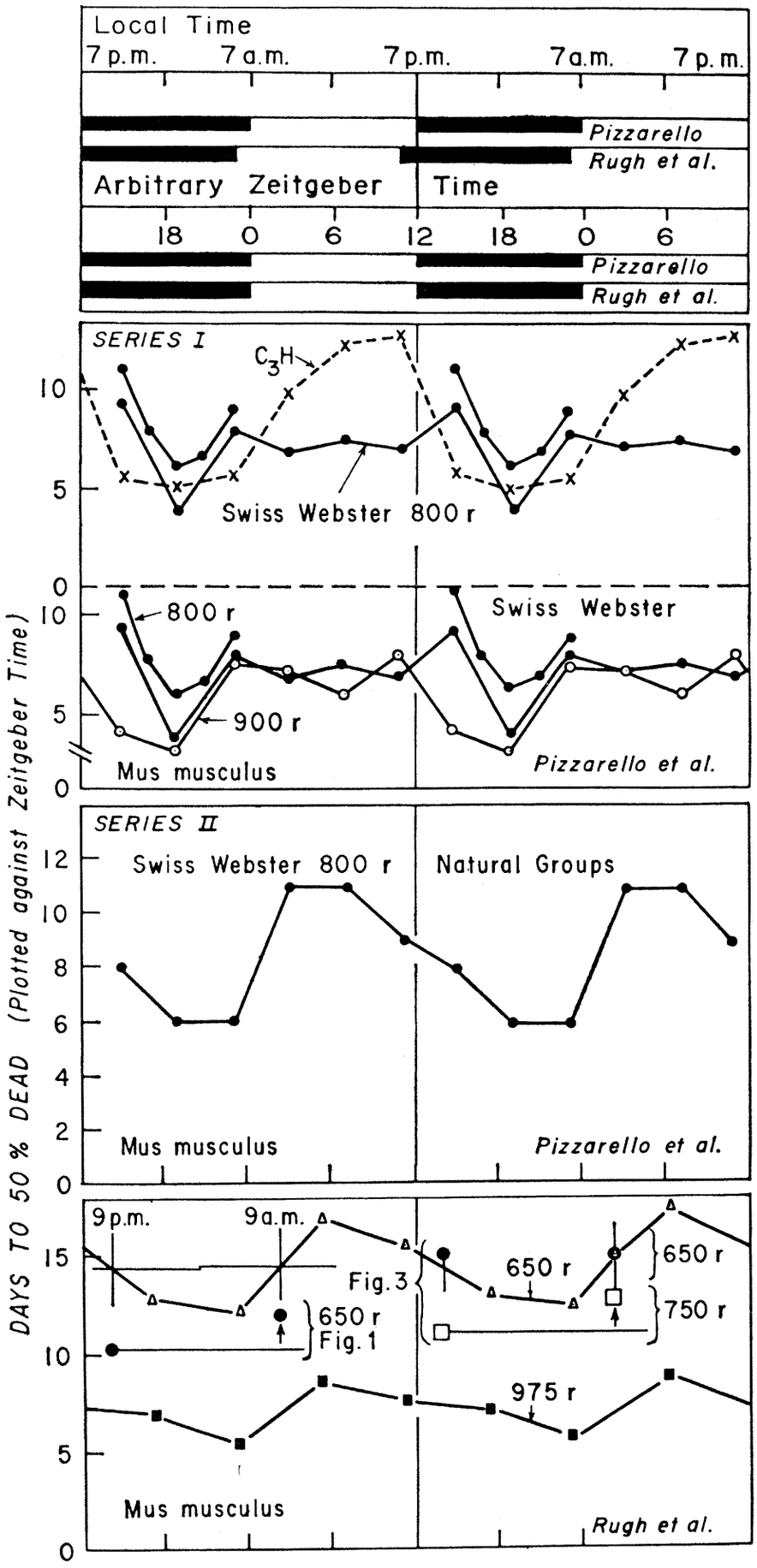

Fig. 1. The data of series 1, a curve summarizing the data of series 2 of this report, and the data of Figs. 1, 2, and 3 of the report of Rugh *et al.* (*6*) are plotted on the same coordinate scale. Two cycles are shown to clarify the "wave-form." The abscissa is Arbitrary Zeitgeber Time (lights on = hour 00 on AZT scale) and local time. Local time for Rugh *et al.* must be assumed since it was not specified. The overall patterns of the two reports are strikingly similar. The two doses given by Rugh *et al.* have produced two curves of nearly identical shape. The curves represented are from Fig. 2 of the Rugh report and the other points are from their Figs. 1 and 3. It should be noted that in choosing 9 p.m. and 9 a.m., Rugh *et al.* chose two points in the cycle, defined by the data in their Fig. 2, that are nearly identical. There is only a *very slight* increase in expected tolerance at 9 a.m.; and, in fact, this increase is found for the treatment of 650 r in their Fig. 1 and the treatment with 750 r in their Fig. 3.

of Rugh *et al.* (*7*) and our own. Since Rugh did not present the survival curves for all of his animals under every condition but ended them with the 30-day survival, it has not been possible for us to obtain the *mean* survival time for each of his experiments, and, thus, we could not compare our data with his in that way. It was possible, however, for us to determine how many days after radiation were required for 50 percent of the animals to die in the Rugh experiments and, since, in our own experience, there has been little or no difference between the time required for 50 percent of the animals to die and the mean survival time after radiation in a given group, we felt that we could compare the data best with this end point. For the statistical testing of our own data, however, the results of which are given in Table 1, it has been necessary for us to obtain and employ the means. The analysis of variance technique and Duncan's new multiple range test (*11*) were used on the data. The results of the analysis of variance performed on each group of animals indicated that the variations among the mean survival times for the various times of day tested would occur by chance alone, less than one time in one thousand, for each of the four analyses. *For both strains of mice irradiated with 800 r, and for Swiss-Webster mice irradiated with 900 r, the shortest survival time after irradiation occurred when the x-rays were delivered at 19.00 Arbitrary Zeitgeber Time* (2 a.m. local time in this case). The results of Duncan's test indicated that the survival time of Swiss-Webster mice after irradiation at 19 AZT with 800 r was significantly dif-

ferent from the survival time after irradiation at AZT: 03, 07, 11, 15, and 23. It cannot, however, be statistically distinguished from 17 and 21. At the 900 r dose-level, 19 AZT is statistically different from 23, 03, 07, and 11, but not from 15. At 800 r, survival time in C3H mice after radiation at 19.00 cannot be distinguished statistically from 15 and 23, but these times are statistically different from 03, 07, and 11 AZT.

Both C3H and Swiss-Webster mice have similar patterns of radiosensitivity (Fig. 1) in that they are more sensitive in the subjective night phase than they are in the subjective day phase. It should be noted, however, that the precise duration and time of onset of the radiosensitive period is different in the two strains.

A second series of experiments was undertaken in March, April, and May of 1964. All the animals used were mice of the Swiss-Webster strain. These were bred, in our laboratory, from animals delivered by caesarian section, obtained from the Charles River Breeding Laboratories, Brookline, Mass. The breeding stock is housed under exceptionally clean conditions, handled by one caretaker dressed in sterile outer garments, face-mask, and cap. Sterile bedding and sterile food are used exclusively, and the drinking water contains sodium hypochloride, 10 parts per million, to prevent growth of, or cross-contamination by, *Pseudomonas* sp. Water bottles are changed three times per week in the early afternoon. The bottles, when not in use, are soaked in sodium hypochloride solution, 100 parts per million. Particular attention is given the nipples, a common breeding ground of *Pseudomonas*. The breeding room is maintained on a 12-hour light, 12-hour dark cycle, lights on at 7 a.m. eastern standard time.

When the animals were ready for use in the experiments they were removed, cages and all, from the breeding room and taken to regular animal rooms. Some remained undisturbed, in the stainless steel cages in which they were housed in the breeding room, but others were transferred to large and small, hanging-drawer wire mesh cages. The cages, then, contained what we have chosen to define as a "natural group," consisting of a mother, father, and litter, all above the age of weaning. Two experiments were conducted upon the "natural groups" remaining in the stainless steel cages (henceforth, regular cages) immediately upon being removed from the breeding room. In three other experiments, those in which the animals were housed in large and small, hanging-drawer wire mesh cages, the groups remained in the animal rooms for one week prior to exposure to radiation. The temperature of these rooms was continuously monitored and was maintained at 20.2° ± 1°C. The only dosage used was 800 r and the physical conditions of delivering the x-rays were the same as described in the previous series of experiments except that the radiation chamber consisted of a lucite chamber, 15 cm in diameter and 2 cm deep (designed exactly as the wooden one described for the first series) with a perforated lucite lid. As a result of using this kind of container, groups irradiated during the light period of the day (7 a.m. to 7 p.m.) were in the light when they were irradiated, and those irradiated during the dark period of the day (7 p.m. to 7 a.m.) were irradiated in the dark. The times chosen for radiation were AZT 03, 07, 11, 15, 19, and 23.

The results of all the experiments in this series are given in Fig. 1 and Table 1. The analysis of variance technique and Duncan's new multiple range test were used on the data. The results of the analysis of variance performed on each group indicated that the variations among the mean survival times after radiation for the various times of day tested would occur by chance alone, less than one time in one thousand, for each of the five analyses. In all of these experiments, as well as in those of the first series, the period of maximum sensitivity or smallest capacity to recover occurred in the dark period of the 24-hour day. And, in four out of the five experiments maximum sensitivity developed by 19.00 AZT (2 a.m. local time, in this case). In these four experiments the animals were no more sensitive to radiation at any other time, but at 23.00 AZT (6 a.m. local time) the animals were, in two cases, just as sensitive as they were at 19.00 AZT. A curve summarizing the five experiments done with natural groups is given as part of Fig. 1. This was constructed by determining the number of days that each animal survived after radiation at a given point in the circadian cycle in each of the five experiments and determining the day at which 50 percent of them were dead. This curve, then, represents the results of irradiating a total of 313 animals, with each point representing between 44 and 56 animals. The results of these experiments clearly demonstrate that radiosensitivity in mice is different at different phase points in the circadian cycle. In both series of experiments the point of maximum sensitivity or minimal capacity to recover occurred at 19.00 AZT, but in the second series, some of the animals appeared to be equally sensitive at 23.00 AZT. The manner of caging did not seem to have any effect on the outcome of the experiments. Since our data were represented graphically as the day on which 50 percent of the animals were dead after radiation at given points in the circadian cycle, we felt that the variations thus indicated should be tested statistically. Therefore, we applied the χ^2 test to the variations observed in the curve summarizing the data obtained in the second series of experiments and presented in Fig. 1. We obtained a χ^2 value of 29.987 and the probability levels for χ^2 (5 degrees of freedom) are as follows: 0.05 = 12.6, 0.01 = 15.1, and 0.005 = 16.7.

These data lack the dramatic impact of the apparently all-or-none effect we encountered earlier in rats (*6*). Actually, the all-or-none aspect (100 percent death in 13 days as opposed to 0 percent death in 130 days) of our earlier results (*6*) is misleading without further comment. After radiation both groups became very sick. The group irradiated at 02 AZT (9 a.m., local time, under the conditions of that experiment) did not, however, get as sick as that irradiated at 14 AZT (9 p.m. local time) and eventually recovered fully (as assayed at 130 days); the group irradiated at AZT:14 simply did not recover. It is clear that, given a cycle of changing radiosensitivity such as our present results make more explicit, overall mortality may well not be different for two treatments unless first, a dose is used which is very near to being lethal and secondly, the treatments are given at just the right phases in the cycle of sensitivity (or capacity to recover). It is evident in view of the data presented here that Pizzarello *et al.* (*6*) were lucky on both counts. In any case it will be better to assay the effects of the treatments not simply by overall mortality, as we were able to do originally, but by its effect on the full time-course of survival.

Rugh *et al.* (*7*) have reported results they regard as an adequate basis for casting doubt on our original claim that radiosensitivity does vary with time in the circadian cycle. Further, they regard their data on whole body irradiation of mice as being adequate for ex-

Table 1. The data for, and results obtained by, the analysis of variance technique and the results of Duncan's new multiple range tests for the two series of experiments. Values for degrees of freedom (df), mean square (MS), F value, and probability (p) are given for analysis of variance showing in all instances that differences between times of radiation are not due to chance alone. Any two times in the Duncan test *not* included in the same column are significantly different. Any two times included in the same column are *not* significantly different.

Series I. Analysis of variance

Item	Swiss-Webster group 1, 800 r				Swiss-Webster group 2, 800 r				C_3H, 800 r				Swiss-Webster, 900 r			
	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p
Between times	5	32.8	15.6	< .001	4	37.4	11	< .001	5	130.5	76.8	< .001	5	20.3	6.2	< .001
Error	54	2.1			45	3.4			54	1.7			54	3.3		
Total	59				49				59				59			

Series I. Duncan's new multiple range test

Swiss-Webster group 1, 800 r	Swiss-Webster group 2, 800 r	C_3H, 800 r	Swiss-Webster, 900 r
2am	2am	2am	2am
10am	12midnight	10am	2pm
6pm	10pm	10pm	10am
2pm	4am	2pm	6pm
6am	6am	6am	6am
	12midnight	6pm	

Series II. Analysis of variance (Swiss-Webster, 800 r)

Item	Regular cage				Regular cage				Large hanging drawer				Large hanging drawer				Small hanging drawer			
	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p
Between times	5	73.8	10.84	< .001	5	223.7	30.3	< .001	5	27.7	6.55	< .001	5	55.2	30.0	< .001	5	60.5	25	< .001
Error	66	6.81			72	7.36			50	4.23			52	1.8			49	2.42		
Total	71				77				53				57				54			

Series II. Duncan's new multiple range test (Swiss-Webster, 800 r)

Regular cage	Regular cage	Large hanging drawer	Large hanging drawer	Small hanging drawer
6am	2am	2am	2am	2am
10am	6pm	6am	2am	6pm
6pm	10pm	10am	10am	10pm
2pm	2pm	2pm	6am	2pm
2am	6am	10pm	10pm	6am
10pm	10am	6pm	10am	10am
			6pm	6pm

trapolation to the radiation of tumors in man and thus presume that radiodiagnosis and radiotherapy may be practiced around the clock without fear or suspicion. We, on the other hand, find their results to be a striking confirmation of our view; namely, that radiosensitivity does vary with time in the circadian cycle. The great discrepancies between (i) their results and their conclusions, and (ii) their conclusions and ours warrant the following critical evaluation of their report. In two experiments reported by Rugh *et al.* in their Fig. 2 (*7*), four groups of mice, irradiated, respectively, at 12:00 noon, 6:00 p.m., 12:00 midnight, and 6:00 a.m., and at two dosages (650 r and 975 r), indicated significant shifts in survival distribution between each of the times at which they were tested by the Kolnozorov-Smirnov goodness-of-fit test (*12*). As stated earlier, Rugh *et al.* did not report the full time course of survival after treatment at each of the stated times but chose to report only those animals which died up to 30 days after irradiation (truncated data). Thus, we have not been able to evaluate their results exactly as we have evaluated our own, namely, by obtaining differences in distribution of mean death following radiation at different times of day. The Kolnozorov-Smirnov goodness-of-fit test makes possible comparison of distributions without determination of means and standard deviation. We have replotted Rugh's data on the same coordinate scales as used in our experiments (Fig. 1). *At each of the two dose-levels used by Rugh* et al. *there is a clear daily pattern, and, furthermore, the pattern is identical for the two dose-levels adding still greater support to the conclusion we derived from the Kolnozorov-Smirnov test, namely, that Rugh* et al. *have, in fact, demonstrated a clear daily rhythm of sensitivity in their strain of mice.* Moreover, although the times at which they x-irradiated their mice are not identical with those used in the experiments described in this report, the general pattern of sensitivity as a function of time of day is the same (Fig. 1): maximum resistance occurs within the subjective day and maximum sensitivity within the subjective night.

In other experiments Rugh, *et al.* tested radiosensitivity at 9 a.m. and 9 p.m. local time and reported that no statistical differences in survival time could be demonstrated after irradiation at those times. In choosing 9 a.m. and 9 p.m. local time to make such comparisons they could scarcely have chosen two points closer to the same ordinate value: inspection of their data in our Fig. 1 shows that it is likely that no difference between these times exists (Fig. 1). If a simple two-point comparison of "day" and "night" was to have been made, it should have been based on two points phase-shifted to the right—that is about noon and midnight local time (Fig. 1).

In two experiments Rugh, *et al.* claimed slightly better survival if radiation was given "in the evening" than if it were given "in the morning." No more precise definition of time is given and no figures were presented to illustrate these experiments. Furthermore, while we must assume that lights were on during the normal, local day-time we are given no information as to the precise light cycle. What was the precise photoperiod? When was dawn? Without answers to these questions, the information on irradiation time specified as, for example, 9 a.m. (presumably eastern standard time) has no real meaning with reference to the precise phase of the cycle of radiosensitivity obtaining at the time of treatment. Since we cannot fix the AZT scale we cannot tell how far into the light period radiation was given "in the morning" or how far in the dark it was given "in the evening." The most sensitive period in mice maintained and irradiated as we have described occurred with only one exception, about 7 hours after the dark period was in progress (that is 19 AZT or 2 a.m. in our case). We have no doubt that, as Rugh *et al.* have described, slightly better, statistically insignificant survival times might occur at *some point* "in the evening" than at *some point* "in the morning." In addition, in one of the experiments of Rugh *et al.* in which sensitivity is described as slightly better in the evening than

in the morning, it is not possible to tell how many animals were used. They reported using a total of 2347 mice in their experiments. Actually, it is possible to account for 2364 mice if the number of animals reported for each experiment is totaled. Even this number of animals does not allow for any animals to be used in one of the series (their series 2) in which it is claimed that survival is better in the evening than in the morning.

The same authors (*7*) have also described experiments in which 12 or 24 hours of either light or dark were added into the "normal" cycle and the animals irradiated at 9 a.m. or 9 p.m. after either 12 or 24 hours of light or dark. This series was presumably designed to shift the phase of the putative sensitivity cycle relative to local time. However, this change was done just once, just before irradiation, and the animals were then returned to the "normal" cycle immediately. As Menaker (*13*) has recently noted, one should not expect the rhythm in a mammal to be greatly shifted immediately following a single change in the light cycle, and this was evidently the case (Rugh *et al.*, Fig. 1). Phase shifts in mammalian rhythms require many days.

Throughout the report of Rugh *et al.* the authors chose, arbitrarily, the number of mice that survived the 30-day test period in computing their statistical test of significance. With this as a test criterion, *few* statistically significant results were obtained; some were, however, found to be statistically significant but the authors chose to ignore them—which is surprising in an argument where the final position is that no such differences exist. That is to say, since the final position that Rugh *et al.* adopt is based on failing to find significant differences, they are under special obligation to explain away the significance they did in fact find.

In any case we note there are clear hazards in selecting any arbitrary cutoff point (such as 30 days) in comparing two effects whose essential features are expressed as distributions (of survival) through time. We find, by means of the Kolnozorov-Smirnov goodness-of-fit test (*12*) that in *all* the experiments, of Rugh *et al.* in which the animals were anaesthetized before x-irradiation, significant shifts in the time course of survival when one group treated at night is compared with the other group treated in the morning: the irradiation always proved more deleterious in the night group.

The work of Straube with rats (*8*) did not show a similar dependence of radiation sensitivity on time of day. However, there are, as Straube himself noted, differences between the methods used in his experiments and those used by us in experiments with rats (*6*), some of which might account for the discrepancies in our results. Most important of these is the fact that Straube restricted his assay to two points in the cycle and used a 12-hour photoperiod as against the 9-hour photoperiod used in our earlier work with rats. His assay of sensitivity in the "night" phase was made 3 hours after the onset of darkness as against the 5 hours in our work on rats and the 7 hours shown here to be the most sensitive point in C_3H and Swiss-Webster mice maintained on a 12-hour photoperiod. There is abundant evidence (see, for example, *3, 5, 14*) that the phase of entrained circadian rhythms shifts relative to any fixed point in the light cycle (like dusk, or lights-off) when the photoperiod is changed. Without direct empirical study of the dependence of phase on photoperiod in the rat one cannot compare sensitivity at the time chosen by Straube with the times chosen by Pizzarello. Moreover, there is evidence (*14*) that the whole "wave-form" or pattern of a circadian oscillation is liable to change with photoperiod.

On several counts, therefore, any further work designed to confirm or reject the view offered earlier (*6*), and further developed here, should avoid argument based on simple two-point comparisons at least until the general wave-form of the circadian cycle in question has been explored, including its dependence on photoperiod. As noted previously, Pizzarello *et al.* (*6*) were simply lucky in their choice of two points for comparison; and Rugh *et al.* (*7*), in failing to note the waveform indicated by their own data, were equally unlucky.

Pizzarello and colleagues were probably lucky, too, in their choice of dosage and strain which, with the times chosen, combined to produce the dramatic difference they encountered originally. The fact that at other dosages, in other species, the circadian rhythm of sensitivity is less spectacular in its "amplitude" does not, however, detract from the significance, both practical and theoretical, of its existence. Nor do the added difficulties for further analysis presented by the differences between strains in the wave-form of the cycle. It is clear that, to the extent that there is a circadian cycle of sensitivity, the radiobiologist is confronted with a new tool: phase correlations between the cycle of sensitivity and the many known circadian cycles of physiological change should provide new leads to the physiological basis of sensitivity. What is now needed is an intensive study, within a single strain, of the circadian system (*3, 4*) as a whole including: (i) concurrent measurements of radiosensitivity and some other circadian rhythm (for example, locomotion) as a "marker"; (ii) dependence of phase and wave-form of both rhythms (sensitivity and locomotion) on photoperiod; and (iii) an ultimate search among the many known physiological rhythms (see *2*) for the basis of the increased sensitivity at a given phase in a given photoperiod.

DONALD J. PIZZARELLO
DANIEL ISAAK*
KIAN ENG CHUA
Department of Radiology
A. LEONARD RHYNE
Department of Preventive Medicine, Bowman Gray School of Medicine, Winston-Salem, North Carolina 27103

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* Present address: Pacific College, Fresno 2, California.

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