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VARIATION IN RADIOSENSITIVITY OF MICE WITH TIME OF DAY

by

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For centuries there has been interest expressed in the variations of biological sensitivity with the time of day. As early as 1797, HUFELAND of London published a monograph entitled *The Art of Prolonging Life*, relating the 24-hour period and the regular revolution of our earth and how this affects the physical economy of man. In 1938, JORES, mentor of the Society for the Study of Biological Rhythms, compiled a review of the voluminous literature on the physiology and the pathology of 24-hour rhythms. In 1951, SMITH et coll. of the United States Public Health Service reported on the effects of hibernation in the marmot on survival time following whole body radiation. His numbers were small, nine marmots in each group, and he used two different doses, delivering a higher dose to the hibernating animal than to the non-hibernating animal. His experiments showed that there was a delay in the occurrence of death following irradiation in the hibernating versus the non-hibernating animal. This phenomenon may be explained by the lower body temperature of the hibernating animal with an accompanying reduced metabolic rate. The data

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indicates that there is no significant decrease in radiation lethality when the animal is in hibernation, and that reduced metabolic activity only produces a delay in the response and does not significantly alter the sensitivity.

There have been several articles on the variation of sensitivity to ionizing radiation with age, one of the most extensive studies being that of STORER (1957, 1962). It has also been pointed out by EVANS et coll. (1962, 1964) that there is an alteration in the radiosensitivity of mice using anesthesia. RUGH et coll. (1963) published data on a total of 2 347 mice of two different strains checking the survival time of the animals up to thirty days post radiation. Whole body exposure was delivered to the mice using 184 kV roentgen rays with half value layer of 0.6 mm Cu. In his study he was unable to obtain a statistically significant variation in the sensitivity. STRAUBE (1963) exposed ninety female Sprague-Dawley rats to 900 R using 280 kV roentgen rays with half value layer of 2.03 mm Cu to duplicate PIZARRELLO's (1964) experiment. He was unable to confirm the dramatic results of the early paper of PIZARRELLO et coll. (1963).

Although in the literature one does find such things as variation in sensitivity with temperature, age and anesthesia, there have been very few reports demonstrating the phenomenon of a diurnal variation in sensitivity until recently. In 1963, we investigated this phenomenon in 468 mice. Three different strains of female Jackson mice were used, C57BR, C57BL and CBA. At that time, a significant difference in the radiation response as a function of the time of day was not demonstrated. This may be explained in that the light-dark cycle was controlled by manually turning the lights on and off five days a week, and also perhaps the estrus cycle caused us to show no significant variation in sensitivity.

In August 1963, PILGRIM of Germany published a report, and, in 1963 also, PIZARRELLO presented data on forty rats, which showed a variation in survival time after whole body radiation at two times of the day. HALBERG (1959, 1963), particularly in his articles on the 24-hour cycle, showed that there was a variation following parenterally administered endotoxin. In 1964, PIZARRELLO performed a new experiment to study the 24-hour cycle in mice to determine their variation in sensitivity to whole body radiation. For ease in comparing results relative to irradiation time, PIZARRELLO put his data in terms of 'Arbitrary Zeitgeber Time' (AZT), where 0000 equals 7 a.m., which is the beginning of the light cycle. He was able to observe that the shortest survival time post radiation, occurred with the group irradiated at 2 a.m. local time or 1 900 AZT. He also replotted RUGH's data in terms of days when 50 % of the population were dead as a function of the time of day, and he points out a remarkable coincidence of their two data.

Table

Results obtained in the Swiss Webster mouse experiments

Group	AZT	LD_{15}*	LD_{30}*	TBW_o**	TBW_d*	$\frac{TBWD}{TBWT_o}$
1	0100	73.33 %	83.33 %	31.63	22.062	0.697
2	0300	73.33	80.00	31.11	21.841	0.707
3	0500	76.67	90.00	30.57	20.711	0.688
4	0700	73.33	73.33	29.29	20.444	0.693
5	0900	70.00	90.00	29.84	19.356	0.649
6	1100	70.00	90.00	30.18	19.955	0.662
7	1300	83.33	96.67	29.91	18.778	0.618
8	1500	80.00	100.00	29.43	19.400	0.660
9	1700	70.00	83.33	29.38	19.025	0.636
10	1900	86.67	100.00	30.34	18.994	0.617
11	2100	83.33	96.67	31.13	18.928	0.632
12	2300	70.00	96.67	30.57	19.325	0.655
Controls	—	—	—	29.14	29.746	1.027

* Percent mice dead at fifteen and thirty days post radiation, respectively.

** Total body weights one day pre-irradiation and at death, respectively.

Because of the indications both pro and con diurnal variations, a new study was undertaken to determine whether such 'circadian' variations in susceptibility to radiation injury did exist.

Method

In order to reduce the number of variables encountered in trying to detect diurnal variations, the following steps were taken: (1) each group within the series contained a statistically significant number of animals; (2) the mice were all from the same breeder farm; (3) the age range of the mice was $\pm$ 1 day; (4) the mice were of the same weight $\pm$ 1 gram; (5) the mice were acclimatized and conditioned for a period of at least 14 days prior to irradiation; (6) only male mice were used to eliminate the possibility of the estrus cycle overshadowing any variation in diurnal sensitivity; (7) the mice were irradiated during one 24-hour period.

Four hundred male Swiss Webster mice, 6 weeks old were used in our experiment. The mice were placed in individual cages in a room with no other animals. The weights of the animals were checked every 7 days. The animals were divided into 13 groups, the 12 radiation groups had 30 mice each and the control group had 40. The windows and the door in the animal room were covered so that no light could enter the room. The lighting cycle was controlled

by an automatic clock and the lights remained on 7 days a week from 7 a.m. to 7 p. m. and off from 7 p. m. to 7 a. m. The mice were conditioned in this manner until they were 14 weeks of age or 8 weeks after they arrived at this institution. On the 8th week plus 1 day, the 12 groups were irradiated. The twelve groups were irradiated at 2-hour intervals. For purpose of comparison, the times of irradiation relative to the day-night cycle are expressed in terms of 'Arbitrary Zeitgeber Time' (AZT). Therefore, the mice were exposed beginning at 0100 AZT, which was 8 a. m.

The animals were irradiated during a single 24-hour period so as to lessen the possibility of age and stock becoming another variable. The animals were irradiated bilaterally using two Picker Vanguard 280 kV X-ray machines operating at HVL 2.0 mm Cu with an exposure rate of 42 R/min. A special jig was designed to hold thirty animals and to give a uniform dose to all animals. Exposure was determined using a Victoreen electrometer with a chamber that had been calibrated at the National Bureau of Standards. A check on the output was made after every third run during the experiment and it was found that the output did not vary more than $\pm$ 0.2 R/min. For purposes of comparing our data with PIZARRELLO's, the 12 groups of mice were exposed to 800 R. Commencing at 0100 AZT, the animals were brought to the roentgen room on racks, placed in the radiation holder and exposed. The daytime group presented no problem at all. However, the night group starting at 1 300 AZT had to be kept always in the dark. This was accomplished by having an opaque cover put over the animal rack when it was brought to and from the radiation room. The animals were inserted into the radiation holder under essentially dark conditions except for a 15-watt dark red bulb.

Starting with day-1 post-radiation, the animals were checked twice a day for lethality. At death, their total body-weight was determined. The weights of the animals were not taken at any time post-radiation except at death because of the possible added trauma which might alter the death rate.

Statistical analysis

As can be seen from the Table, there appeared to be a definite trend of a greater lethality for the night group than the day group. It also appeared that groups 4 and 9 had a lower mortality rate.

A chi squared test was done to compare the lethality of the total day group with the total night group. The $\chi_T^2 = 11.14$ with one degree of freedom gave a significant differences of $0.001 > P$.

A comparison between the day low-mortality group 4 with group 3 just preceding it, gave $\chi_T^2 = 1.78$, and with 1 degree of freedom was $0.25 > P > 0.10$.

Then groups 3 and 5 were compared to group 4. This gave $x^2 = 3.05$, and with 1 degree of freedom was $0.10 > P > 0.05$. This calculation was done to determine if group 4 was significantly different from the other day groups.

The same comparison was done for the night group, 9 compared to 10, χ_T^2 equaled 3.50, and with 1 degree of freedom was $0.10 > P > 0.05$. However, when groups 8 and 10 were compared to 9, $\chi_T^2 = 7.63$, and with 1 degree of freedom the result was significant $0.10 > P > 0.005$.

All the values of the day group were individually compared and the variations from one group to another proved that it could be random.

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SUMMARY

Using 400 Swiss Webster male mice, 14 weeks old, an investigation was undertaken to determine their variation in sensitivity to radiation as a function of the time of day as measured by their death rate. Twelve groups were exposed to 800 R with 280 kV roentgen rays HVL 2.0 mm Cu, at two-hour intervals. Using chi square tests, the night group as a whole appeared more sensitive than the day group as a whole, 0.001 P. The variations within the day and night groups were not considered to be significant.

ZUSAMMENFASSUNG

Es wurde an 400 vierzehn-Wochen alten männlichen Mäusen untersucht, inwieweit zu verschiedenen Tageszeiten eine Verschiedenheit der Strahlenempfindlichkeit besteht. Dies wurde durch die Tötlichkeitsrate ausgedrückt. Zwölf Gruppen erhielten eine Strahlendosis von 800 R mit 280 kV, HVL 2,0 Cu mit zweistündigen Interwall. Unter Verwendung von „chi square tests" ergab sich, dass die ganze Nachtgruppe strahlensensitiver als die Tagesgruppe war (0.001 P). Die Unterschiede innerhalb der Tages- und Nachtgruppen waren nicht signifikant.

RÉSUMÉ

L'auteur a étudié sur 400 souris mâles Swiss Webster âgées de 14 jours les variations de leur radiosensibilité en fonction du moment de la journée, mesurée par leur taux de mortalité. Douze groupes ont été exposés à 800 R de rayons roentgen de 280 kV et de C.D.A. 2 mm Cu, à des intervalles de deux heures. Les tests chi^2 ont montré que l'ensemble des groupes irradiés la nuit est plus radiosensible que l'ensemble des groupes irradiés le jour, avec une probabilité de 0,001. Les variations à l'intérieur des groupes de jour et de nuit n'ont pas été considérées comme significatives.

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