Examination of Diurnal Variation in Lethally Irradiated Rats

Abstract. *In anesthetized white rats there is no significant diurnal variation in lethality of an x-ray dose that killed 100 percent of the rats in 30 days.*

The remarkable diurnal variation in radiation lethality of anesthetized white rats reported by Pizzarello *et al.* (*1*) is of great interest. An effect of this magnitude might complicate interpretation of many radiation lethality studies. I have, therefore, attempted to confirm this observation, employing larger numbers of animals. Ninety female Sprague-Dawley rats, weighing 172 ± 3 g, were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally) and irradiated two at a time with 900 r. Radiation was delivered with a GE Maximar 250-kv unit; radiation factors: target distance, 40.6 cm, 0.75 mm Cu + 1.0 mm Al, ma 15, half-value layer = 1.98 mm Cu, 87.29 r/min; or a Norelco Phillips 300-kv unit (Muller MH301) was used; radiation factors: target distance, 48.6 cm, 0.5 mm Cu + 1.0 mm Al, 10 ma, half-value = 1.98 mm Cu, 90.11 r/min. The experimental groups were replicated, 23 being irradiated at 9 A.M. and the same number at 9 P.M. on 8 March 1963, and another 22 at both times on 4 April 1963. Both machines were used in each experiment.

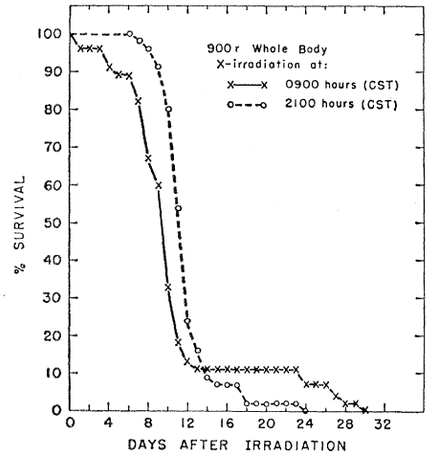

Fig. 1. The mortality response of anesthetized Sprague-Dawley female rats subjected to 900 r of whole-body x-irradiation. $N = 45$ animals for each group.

Table 1. Survival of Sprague-Dawley female rats after 900 r of whole-body x-irradiation.

Days after irradiation	Irradiated at			
	0900 hours		2100 hours	
	S/T*	%	S/T*	%
	Experiment 1			
0	23/23	100	23/23	100
5	20/23	87	23/23	100
10	5/23	22	19/23	83
15	2/23	9	1/23	4
20	2/23	9	1/23	4
25	2/23	9	0/23	0
30	0/23	0		
	Experiment 2			
0	22/22	100	22/22	100
5	20/22	91	22/22	100
10	10/22	45	17/22	77
15	3/22	14	2/22	9
20	3/22	14	0/22	0
25	1/22	4		
30	0/22	0		

* S/T = ratio of survivors to total.

Table 1 shows the mortality responses on the two individual experiments, and from Fig. 1, which summarizes both experiments, there is no significant difference in radiation mortality between the animals irradiated in the morning and those irradiated in the evening. The shapes of the survival curves suggest that the dose used is very close to the LD_{100} for 30 days (*2*).

The discrepancy between these results and those previously reported (*1*) is difficult to explain. The protocol differed from that of the previous investigators in four ways; (i) rats were maintained on a 12-hour on, 12-hour off light cycle (6 A.M. to 6 P.M.), rather than a 9-hour light, 15-hour dark cycle; (ii) prior and subsequent to irradiation the rats were housed five to a cage, rather than individually; (iii) the experiments were performed in March and April rather than June and August; and (iv) Sprague-Dawley rather than Nelson (CFN) strain females were used. Although these factors might conceivably have some influence, it is difficult to see how they could alter a completely lethal to a completely nonlethal response. Mean survival time of the two groups in my study differs by a single day, and this difference is not statistically significant. Thus, no diurnal variation was observed in sensitivity of white rats to x-irradiation in the LD_{100} for 30 days range, in contrast to the work recently reported (*3*).

ROBERT L. STRAUBE
Division of Biological and Medical Research, Argonne National Laboratory, Argonne, Illinois

References and Notes

1. D. J. Pizzarello, R. L. Witcofski, E. A. Lyons, *Science* **139**, 349 (1963).
2. In order to duplicate the conditions of the experiment of Pizzarello *et al.* (*1*), anesthetized rats were used. In this laboratory unanesthetized rats are used routinely; therefore, LD_{50} values for 30 days for anesthetized rats are not available.
3. Performed under the auspices of the U.S. Atomic Energy Commission.

30 August 1963

Lipid-Protein Particles: Isolation from Seeds of Gossypium hirsutum

Abstract. *Subcellular particles were isolated from cottonseed by tanning of the cell contents followed by differential centrifugation. The particles, high in protein content and containing approximately 28 and 44 percent lipids, are thought to be the site of oil storage and lipid synthesis.*

Numerous seeds have a high lipid content, yet the exact location of the oil within the storage tissue is subject to question; one example is the cottonseed, which contains over one-third lipid in the kernel. Leahy (*1*) was unable to see free oil droplets in cottonseed sections and considered the oil "to occur within the cell as a cytoplasmic emulsion." This concept is in general agreement with the views cited by Tharp (*2*). We confirm these observations. When cottonseeds are sectioned under conditions which prevent spreading of oil, droplets of free oil are not observed.

Free-hand sections of cotyledon parenchyma cells of cottonseed show numerous spherical bodies 1 to 4 μ in diameter (Fig. 1*A*). These are similar to protein bodies, described for many seeds (*3*), which have been isolated from peanut (*4*) and pea cotyledons (*5*) and from wheat endosperm (*6*). In no instance, however, have such particles been reported to contain lipids. It was of interest, therefore, to determine whether the bodies observed in the cottonseed might also be the site of oil storage.

The classical methods of isolating subcellular particles were inadequate for isolation of the spherical bodies in cottonseed. Grinding media which ranged from hypotonic to hypertonic were tried, but most of the bodies were destroyed when the seeds were ground. However, pure glycerol as a grinding medium maintained the integrity of the bodies for extended periods. The bodies stained positively with Kiton pure blue V, indicating that