17138. Dosage-Mortality in Rats Given Total Body Roentgen Irradiation.*

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In a previous communication[1] we indicated that certain flavonoid compounds may decrease the mortality of guinea pigs given approximately an LD_{50} of total body roentgen radiation.

In attempts to extend these and other treatments to other species of small laboratory animals, some results have been obtained on dosage-mortality in the rat, which may be of value to others working on the biological effects of ionizing radiation.

We have been unable to find previous published mortality data for the rat other than a note by Potter,[2] who reported 100% death in 14 rats with total body radiation of 800 r. Prosser, *et al.*[3] state that the 30-day M.L.D. for single doses of x-radiation is 600 r† for rats but no particulars were given.

In our studies on the rat, over 500 animals have been used. The purpose of this communication is to report the dosage-mortality data for normal rats.

The rats used were of the Slonaker albino stock, from which the Wistar albino stock originated and which have been inbred in this laboratory since 1929.[4] Their food consisted of Purina laboratory chow available on the meshscreen cage floors and tap water from bottles with glass spouts. In all cases the animals were healthy young males, average body weight 100-140 g.‡ Six animals were caged together. The room temperature was 78° ± 2°F.

The animals to be irradiated were placed in a ventilated 6-partitioned plywood box, 6 at a time. The dimensions of each compartment were 2" wide, 5" long (adjustable) and 3" deep. The partitions were ⅛" plywood and the top was ¼" plywood perforated with holes. The bottoms were ¼" hardware screen and beneath this was a layer of rice 3 inches in depth which was used to provide additional backscatter.§

The X-ray factors were: GE Model KX-3, 220-kv deep therapy unit, 200 kv, 20 MA, ½ mm Cu and 1 mm Al added filtration (HVL, 1.05 mm Cu) 52.5 cm target distance to top of box, 30.4 r/min. in air single exposure total body irradiation. The times were varied to give the desired dosage. The unit is calibrated semi-annually by a registered X-ray physicist. The variation in output over the past year has been a little under 3%. This of course does not ensure accurate control from day to day, but since control animals always were under the beam at the same time as the experimentals, this objection is not so serious.

It was found that if rats survived longer than 30 days they generally survived indefinitely. Hence, 30 days were taken as a

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1 Clark, W. G., Uncapher, R. P., and Jordan, M. L., *Science*, 1948, **108**, 269.

2 Potter, J. C., *Radiology*, 1941, **37**, 724.

3 Prosser, C. L., Painter, E. S., Lisco, H., Brues, A., Jacobson, L. O., and Swift, M. N., *Radiology*, 1947, **49**, 299.

† A. H. Dowdy (personal communication) informed us that the Rochester Unit of the Manhattan District found an LD_{50} of 650 r for Wistar rats, which closely agrees with the results reported below.

4 MacKay, L. L., and MacKay, E. M., *Am. J. Physiol.*, 1927-8, **83**, 179.

‡ A wider weight distribution should not be used, since Naiman[5] has shown a considerable effect of age on susceptibility of rats to total body x-radiation.

5 Naiman, D. N., *Am. J. Roentgenol.*, 1949, **61**, 95.

§ Ellinger[6] has shown in mice that additional backscatter of this nature reduces the steepness of the slope of the sigmoid mortality curve in the LD_{50} range.

6 Ellinger, F., *Radiology*, 1945, **44**, 125.

TABLE I.
X-ray Dosage-Mortality in Rats.

r.	No. rats	50% dead (days)	% mortality (30 days)
1000	8	6	100
800	32	8	90
750	8	8	100
715	8	8	100
670	8	16	53
655	235	15	79
640	83	30	41
625	94	45	62
610	8		0
500	17		6
	501		

convenient endpoint for expressing the data, although in most instances observations were continued for 45-60 days or longer. For all practical purposes the present mortality data include indefinite survival.

Table I lists the results on 501 rats.

The dosages (column 1) were plotted against per cent mortality (column 4) as shown in Fig. 1.

Fig. 2 relates the survival time to per cent mortality, for each dosage. Because of overlapping, it was found most useful to pool the data as indicated.

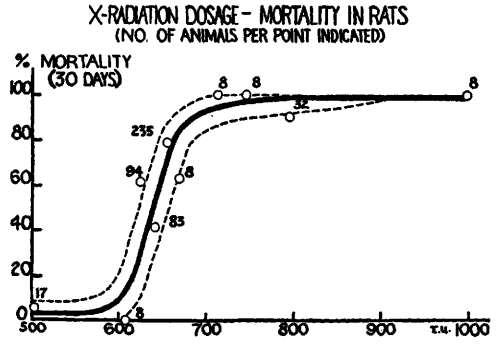

FIG. 1.

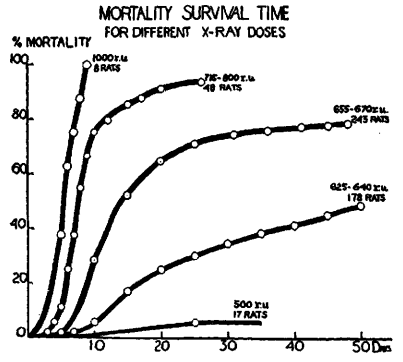

FIG. 2.

The time in days lapsing before 50% of the animals died was plotted against dosage, in Fig. 3. This relationship has been found useful in assessing the effects of drugs on radiation disease.[1]

When the data were plotted by Miller and Tainter's modification[7] of Litchfield and Fertig's method,[8] on logarithmic-probit graph paper, the LD_{50} was approximately 640 $\pm$ 5 r. However, it was difficult to fit the points to a satisfactory straight line by this method because of the variations in the steep part of the curve, and from Fig. 1 it is evident that from 610 to 655 r ($\pm$ 3%), a 0 - 86% mortality may occur. The $\pm$ 3% is the calibrated limit of variation of the X-ray machine, so that any results from 0 to nearly 100% mortality could be expected within the "LD_{50}" range because of the steepness of the slope in this range. Only unless very large numbers of animals were used together with radiation dose variation in the hundredth per cent range, could this portion of the curve be duplicated from one experiment to the next.

Table II lists some calculations from our data on the rat, compared with those of Ellinger[6,9] for goldfish, guinea pigs and mice, and of Tullis, *et al.*[10] for swine; of the rate

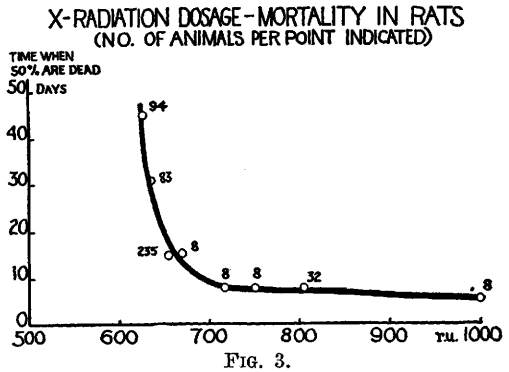

FIG. 3.

7 Miller, L. C., and Tainter, M. L., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 261.

8 Litchfield, J. T., Jr., and Fertig, J. W., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 276.

9 Ellinger, F., *Radiology*, 1940, **35**, 563.

10 Tullis, J. L., Tessmer, C. F., Cronkite, E. P., and Chambers, F. W., *Radiology*, 1949, **52**, 396.

TABLE II.
Mortality Rate per r. Unit—Species Differences.

Species	No. animals used	dx/dy*	Reference
Rats	500	1.2%	Clark and Uncapher
Guinea pigs	50	0.7	Ellinger[6]
Swine	32	0.5	Tullis *et al.*[10]
Mice	119	0.3	Ellinger[6]
Goldfish	774	0.08	Ellinger[9]

* x = % mortality and y = dosage in r, in the LD_{50} range.

of mortality per roentgen unit dose, in the steepest part of the mortality curves. This is an expression of the slope, dx/dy, where x represents unit per cent mortality and y the unit dose.

This means that 1.2% of the rats died per unit increment in dosage of X-rays, where the other species died at a lower rate per dosage increment.

It will be interesting to compare these data with those for the dog, rabbit, monkey, cat and other species.

It occurs to us that in studies directed toward the therapy or protection by pre-treatment of animals given total body ionizing radiation, that if the mortality curves are quite steep, as in the rat, dosages in the "LD_{50}" range may not be permissible, and that dosages should be given which consistently kill 100% of the untreated animals, so that an estimation of protection could be made by both the time required to kill 50% of each group, as shown in Fig. 3, and by the per cent final mortality. If the slope is not so steep (dx/dy is small) and if sufficient animals are used, it would be advisable to include at least 3 dosages, LD_{100}, LD_{50-60} and LD_{20-30}.

Summary. The LD_{50} for total body X-radiation in Wistar rats is 640 ± 5 r with a 30-day endpoint. The time elapsing before 50% mortality occurs at different doses is a more reliable measure of mortality than the dosage-mortality curve or the dosage-survival time curves for different X-ray doses. The dosage-mortality curve of rats has such a steep slope in the LD_{50} range that variations in mortality of from 0 to nearly 100% may occur in this range because of variations in animals and in the X-ray machine output. Therefore in studies directed toward therapy or protection of animals given total body ionizing radiation, if the mortality curves are extremely steep as in the rat, dosages in the LD_{50} range should not be used. Other measures which might be employed are discussed.

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17139. Resemblance of a Strain of Swine Influenza Virus to Human A-prime Strains.*

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In the course of studies in a group of children vaccinated with the Type A PR8 strain of influenza virus, an outbreak of influenza occurred in that population.[1] The children's sera were being tested against different strains of influenza virus, and it was

* This investigation was conducted under the auspices of the Commission on Influenza, Army Epidemiology Board, Office of the Surgeon General, U. S. Army, Washington, D.C.

[1] Quilligan, J. J., Jr., Minuse, Elva, and Francis, Thomas, Jr., *J. Clin. Invest.*, 1949, **27**, 572.