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RECOVERY FROM RADIATION INJURY IN MICE AND ITS EFFECT ON

LD₅₀ FOR DURATIONS OF EXPOSURE UP TO SEVERAL WEEKS

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RECOVERY FROM RADIATION INJURY IN MICE AND ITS EFFECT ON LD₅₀
FOR DURATIONS OF EXPOSURE UP TO SEVERAL WEEKS

Abstract

It is concluded that the lethality of X and γ irradiation applied for various durations from short times up to a few weeks is representable by an appropriate solution of the differential equation

$$\frac{dI}{dt} = A\gamma - \beta(I - \alpha\gamma t)$$

in which I is injury, γ the dose rate, t the time of exposure and A, β , and α are constants. The condition for LD₅₀ is that $I = S_0 = A\sigma$ in which S_0 is the median life expectancy of the sample of animals and σ is the LD₅₀ for the acute dose. The equation is tested on two sets of data on mice. Solutions of the same equation were shown previously (UR-206, UR-207, and UR-274) to represent data on chronic irradiation. The only known inadequacy of this formulation is its failure to predict that life shortening from single large sublethal doses is greater than from divided doses of the same total magnitude.

RECOVERY FROM RADIATION INJURY IN MICE AND ITS EFFECT ON LD₅₀

FOR DURATIONS OF EXPOSURE UP TO SEVERAL WEEKS

It has been shown in previous reports (1) (2) (3) that the data on shortening of life span by ionizing radiation conform closely to formulae derived from the following hypotheses:

1. The primary or latent injury from such radiation is produced in proportion to the dose.
2. Repair takes place in proportion to the level of injury present except that a fraction of all the injury produced is irreparable. This irreparable fraction therefore accumulates in proportion to the total dose.
3. Injury is measurable directly in terms of life span and the animal dies when the total injury, reparable plus irreparable, becomes equivalent to his remaining life expectancy.

These hypotheses are represented by the following differential equation and its solutions

$$\frac{dI}{dt} = A\gamma - \beta(I - \alpha\gamma t) \quad (1)$$

in which I is injury, γ is the rate of irradiation, t is duration of irradiation, and A , β , and α are constants.

For a single dose of duration t

$$I = \frac{\gamma}{\beta}(A - \alpha)(1 - e^{-\beta t}) + \alpha\gamma t \quad (2)$$

According to the third hypothesis above, I may be measured in life span. Therefore,

$$S_0 - S = I \quad (3)$$

in which S_0 is the life expectancy of the animal at the beginning of irradiation and S is his survival time under irradiation.

If t is short, $1 - e^{-\beta t}$ may be written βt and equation 2 reduces to

$$I = A\gamma t = A\sigma = S_0 \quad (4)$$

σ being γt the total dose. This equation is appropriate for the acute lethal

dose delivered within a few hours. S may be neglected because the animals survival time is negligible compared to his life expectancy S_0 and, in particular, because he is virtually dead at the end of the exposure.

When t is very long as in chronic irradiation until death, $e^{-\beta t}$ may be neglected so that for the chronic lethal dose

$$S_0 - S = \frac{\gamma(A - \alpha)}{\beta} + \alpha\gamma S \quad (5)$$

t being replaced by S .

If t is of durations up to 2 or 3 weeks, S may again be neglected in animals whose life expectancies are considerably greater than this time. Therefore, the lethal dose for such exposures is given from equations 3 and 4 by

$$S_0 = A\sigma = \frac{\gamma(A - \alpha)(1 - e^{-\beta t})}{\beta} + \alpha\gamma t \quad (6)$$

σ being the acute lethal dose.

Following a sub lethal dose σ the injury by equation 1 will subside according to

$$\frac{dI}{dt} = -\beta(I - \sigma) \quad (7)$$

but, because with X-rays the irreparable fraction $\alpha\sigma$ is a small fraction of the whole injury, it may be neglected in experiments in which the effect of an initial dose is assayed by means of a second dose at various intervals, the two doses together being the LD_{50} . Therefore, in close approximation, the effect of an initial dose will subside according to

$$I = I_0 e^{-\beta t} \text{ or } R = R_0 e^{-\beta t} \quad (8)$$

R_0 being the applied dose of radiation and R the effective dose remaining at time t .

Data by Paterson et al (4) in Table 1 and by Thomson et al (5) in Table 2 permit testing the validity of equation 6. The former are more complete in that they contain measurements of paired doses suitable for deriving β directly by

means of equation 8.

Paterson et al gave an initial dose of 260 r which is about one-half the LD₅₀ of 518 r. After 2 days an additional dose of 303 r had to be given for LD₅₀. Therefore, the equivalent of $518 - 303 = 215$ r remained of the 260 r. β may be determined by plotting $\ln 260/215$, etc., which according to equation 8 should be linear with the interval t to give a line of slope β . Using the week as the unit of time, β determined in this way is 0.7, and, therefore, the time for half recovery $0.69/0.7 = 1$ week approximately. This is about the same value as that calculated by Paterson et al (4) who concluded also that the recovery was exponential.

In Table 1 are given, "Second Dose Observed", which is the LD₅₀ recorded minus 260 r, while, "Second Dose Calculated", is $518 \text{ r} - 260e^{-.7t}$. In Figure 1 are plotted the values of $260e^{-.7t}$ to form the lower curve and $260e^{-.7t}$ plus the observed second doses which are the stated LD₅₀ doses less 260 r. The sums of these two quantities should equal 518 r, the acute lethal dose, provided the recovery is in fact exponential. It will be seen that the agreement is within the experimental error. This indicates that the irreparable component α of equation 7 is not easily detectible in an experiment of this kind. This is not true, however, if daily doses are given for many days. Data of this type by Paterson et al will be considered now.

These data, given in the lower part of Table 1, are determinations of LD₅₀ as the sum of equal doses administered daily. Consequently, there is recovery following each dose except the last. It can easily be calculated by equation 8 using $\beta = 0.7$, what the total dose given would be equivalent to providing recovery took place between doses as it did in the paired dose experiments. Under the heading, "LD₅₀ Exponential Recovery Only", the results of these calculations are given. It will be seen that the values are progressively more divergent as the number of doses becomes larger. Consequently,

Table 1. Data by Paterson et al (4) on the X-irradiation of mice of the "strong A" strain. Actual exposure rate 45 r per minute with 250 kv X-rays, H. V. L. 1.6 mm Cu.

The upper set of data are for LD₅₀ 30 days with paired doses, the first dose in each case being 260 r. Therefore, the second dose is LD₅₀ observed minus 260 r. The column $260e^{-0.7t}$ gives the predicted value of the effective remainder of the initial dose using the recovery constant $\beta = 0.7$. This added to the second dose observed should equal 518 r, the acute lethal dose. The difference between the second doses observed and calculated measures failure to do so.

The lower set of data are observed values of total LD₅₀ 30 days expressed as the sum of equal brief daily doses. The last daily dose in each case is not subject to recovery but the previous doses are. The column headed, "LD₅₀ Calculated" is obtained using the constants $\beta = 0.7$ and $\alpha/A = 0.08$. The column headed, "LD₅₀ Exponential Recovery Only", gives those doses which would have been required if exponential recovery of the whole injury had taken place rather than of only a part of it.

TABLE 1

No. of Animals	Dosage	LD ₅₀ r Obs.	Interval		Second Dose		$260e^{-0.7t}$
			Days	Weeks	Obs.	Calc.	
364	Single	518					
100	2 Doses the	563 $\pm$ 19	2	— 0.29	303	— 305	213
88	First Being	695 $\pm$ 20	10	— 1.43	435	— 422	96
108	260 r	736 $\pm$ 14	20	— 2.86	476	— 483	35
No. of Animals	Dosage	LD ₅₀ r		LD ₅₀ Exponential			
		Obs.	Calc.	Recovery Only			
40	5 in 4 days	596 $\pm$ 29	594	627			
35	10 in 9 days	764 $\pm$ 24	726	813			
55	21 in 20 days	945 $\pm$ 16	1029	1178			

Figure 1. The lower curve is $260e^{-.7t}$ which is the calculated effective remainder of the initial dose 260 r after the intervals given. The vertical broken line marks the time for half recovery which is almost 1 week. The upper horizontal line is at 518 r, the LD₅₀ 30 days. The observed second doses, when added to the curve, should fall on this line. It will be seen that they do so in close approximation, substantiating the hypothesis that recovery of radiation injury is exponential.

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it must be concluded that irreparable injury is of sufficient importance to be taken into account in these experiments. This may be done as follows.

Using equation 6 and the 4 day case.

$$518 A = \frac{596(A - \alpha)(1 - e^{-.7 \times \frac{4}{7}})}{.7} + 477 \alpha + 119 A$$

The first number on the right is the dose rate per week, 0.7 is β , 119 A is the final dose on the 5th day for which there is no recovery. On solving this equation the ratio of α to A is 0.07. Treating the 20 day case in the same way the ratio is 0.13. The 9 day case gives a small negative result which is obviously in error. It should be noted in this regard that equation 6 is appropriate to continuous dosage whereas the experiment was done with brief daily doses at the beginning of each day. Consequently, there is more recovery in a given time than if the same dose were spread throughout the day. This effect will become negligible as the series of doses becomes long. If the 9 day case is calculated as a series of daily doses, the ratio of $\alpha/A = 0.02$. The average value of α/A was taken therefore as 0.08.

Using the average value of α/A , 0.08, α may be eliminated from equation 6 and the dose calculated as follows for the 4 day case.

$$518 A = \gamma \times .92 A \frac{(1 - e^{-.4})}{.7} + .08 A \gamma + A \gamma \times \frac{1}{7}$$

From this γ , the dose rate is 831 per week. Therefore, the total dose in 5 days is 594 r. Table 1 gives the results of similar calculations for the 9 day and 20 day exposures. The agreement is probably as good as can be expected in this type of determination and the use of different constants would not alter appreciably the results. The observed and calculated values are shown in Figure 2.

The data of Paterson et al are the only ones known which contain the results both of paired dose and of multiple dose experiments ranging up to 3 weeks. Data

Figure 2. The circles are the observed values of LD_{50} 30 days of Paterson et al, for series of equal daily doses. The squares are calculated values derived by means of equation 6 and the recovery constant $\beta = 0.7$ from the paired dose experiments along with $\alpha/A = 0.08$ derived in the text.

by Thomson et al (5), however, give the results of continuous exposure for several weeks to gamma rays which should also be representable by equation 6 according to the present hypotheses. The data are reproduced in Table 2 and in Figure 3.

Application of equation 6 is difficult without additional data to determine the constants, β , but because the approximate value of β is known it can be determined by trial. Using 1.25 as β the data of the 1.64 weeks and 3.43 weeks exposure give

$$785 A = \frac{1008(A - \alpha)}{1.25} (1 - e^{-1.25 \times 1.64}) + 1658 \alpha$$

and

$$785 A = \frac{806(A - \alpha)}{1.25} (1 - e^{-1.25 \times 3.43}) + 2760 \alpha$$

from which, $\alpha/A = 0.09$ and 0.07 , respectively.

Using $\alpha/A = 0.08$ and $\beta = 1.25$ the dose rate and the LD_{50} are calculated as before and are listed in Table 2. The acute LD_{50} is taken as 785 r which is approximately the mean value for the three briefest exposures.

It will be seen that the calculated values agree fairly well except for the 11.6 and 31.9 hour exposures for which the observed values are decidedly higher. The two lowest lines in Table 2 are data (6) on the same strain but in a different experiment. These data were determined in a different way, the irradiation being continued until death rather than stopped in expectation of 30 day LD_{50} . Consequently the observed doses are probably slightly higher than necessary for LD_{50} 30 days, but not much higher, because, in animals irradiated in this way, the latent period between the end of irradiation and death is short. It will be seen that these data agree moderately well with the predicted values although not as well as if α is taken as 0.07. Figure 3 shows the observed values and those calculated using $\alpha = 0.07$.

Table 2. Excepting the lower two lines the column, LD_{50} observed, gives data obtained by Thomson et al (5) of LD_{50} 30 days for continuous gamma irradiation of Carworth CF-1 female mice using Co 60 and Ta 162 sources. The two columns of calculated doses are derived from equation 6 using $\beta = 1.25$ for both but $\alpha = 0.08$ and $\alpha = 0.07$ for the left and right columns respectively. For the acute lethal dose 785 r is assumed because it is the average of the three shortest exposures which are essentially equal. The two lower lines are also data on female Carworth CF-1 mice by Thomson et al (6) but in a different experiment using a Co 60 source and irradiating the animals until death rather than stopping in expectation of death within 30 days.

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TABLE 2

No. of Animals	Dose Rate			Duration of Exposure		LD ₅₀ Observed	LD ₅₀ Calculated	
	Observed		Calculated				$\alpha = 0.08$	$\alpha = 0.07$
	r/hr.	r/wk.	r/wk.	Hours	Weeks	r	r	r
254	2530			0.305		772		
80	896			0.88		785	785	785
140	240			3.33		800		
120	87	14616	12270	11.6	.066	1010	810	810
96	37	6216	4645	31.9	.19	1180*	883	
100	6	1008	1019	276	1.64	1658	1671	1694
60	4.8	806	785	576	3.43	2760	2692	2775
18		805	683		5.3	4230	3620	3747
100		630	595		7.3	4640	4344	4584

*Probable misprint in original paper which gives 1081 r.

Figure 3. The circles are observed values of LD_{50} for continuous gamma irradiation and the squares calculated values using equation 6 and $\beta = 1.25$, $\alpha/A = 0.07$ and acute LD_{50} 785 r. The limits for $p = 0.05$ are marked by broken lines for the 11.6 and 31.9 hour exposures. It is thought that these two data are variants for reasons given in the text.

Placing significance in the observed doses at 11.6 and 31.9 hours Thomson et al (5) analysed these data in a different way concluding that they were accounted for by rapid exponential recovery with half time of 25 hours while they attributed the marked divergence from that formulation of the 276 and 576 hour data to the increasing importance, with increasing duration of exposure, of a second recovery process about 10 times slower. The alternative explanation that the 11.6 and 31.9 hour data are simply experimental variants is preferred for the following reasons.

1. There is no evidence from divided dose experiments of the type of Paterson et al considered above, of recovery in mice with half-recovery time less than 0.45 weeks (Table 3) while the recoveries in the divided dose and prolonged dosage experiments of Paterson et al are the same.
2. There is no convincing experimental evidence that the LD_{50} increases, within the usual experimental error of such observations more than a few percent with times of irradiation ranging from minutes up to about 1 day. With the mice of Paterson et al, for example, the LD_{50} would be 544 r for exposure over 1 day instead of 518 r for actual exposure time of 15 minutes. This is a difference often exceeded in measurements of two samples of the same strain using the same duration of exposure and would be detectable with certainty only by using considerable care in dosage measurement and large numbers of animals.
3. There is considerable evidence that a single recovery process compatible with that derived from paired dose experiments is operative throughout chronic irradiation experiments covering periods up to nearly the whole normal life expectancy of the animal. (1) (2) (Table 3).

The Constants β and α/A :

It will be noted that in Figure 2 the LD_{50} is scarcely doubled with 3 weeks exposure while in Figure 3 it is more than tripled. This results from the difference in recovery rates in the two strains of mice. The recovery rate in the mice of Paterson et al is the slowest which has been determined as will be seen in Table 3 where are collected all the constants for irradiation of mice which have been calculated thus far.

The highest and lowest values of β in the table, 1.54 and 0.70, respectively, were determined directly from paired dose experiments while the remainder were calculated from chronic or multiple dose experiments. There appears to be no

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TABLE 3

Constants for Irradiation of Several Strains of Mice

Observer	Strain	Radiation	Life Span Weeks	$\frac{(A-\alpha) \times 10^3}{\beta}$	$A \times 10^3$	$\alpha \times 10^3$	β	$\frac{.69}{\beta}$ Weeks	$\frac{A \times 10^3}{S_0}$	$\frac{\alpha}{A}$	$\frac{\alpha \times 10^3}{S_0}$
Paterson et al (4)	A	X		<u>Mouse</u>			0.70	0.98		.08	
Kaplan and Brown (7)	C57	X					1.54	0.45			
Henshaw (8)	C3H	X	46	81	131.5	6.75			3.08	.05	0.147
Sacher (12)	ABC	X	77	144	127	12.4	.80	0.86	1.65	.10	0.161
Thomson et al (5)	CF ₁	γ					1.25	0.55		.07	
Henshaw (9)	CF ₁	γ	63	43	71	5.37			1.13	.08	0.085
Lorenz (10)	LAF ₁	γ	101	204.5	310.7	4.2			3.08	.014	0.042
Evans (11)	Swiss	Neutron*	68	480	790	180	1.28	0.54			
Henshaw (9)	CF ₁	Neutron**				<265					

*Fast from cyclotron

**Fast from pile

Life span measured from beginning of Irradiation.

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dependence on the nature of the radiation because recovery from neutron injury is similar to that from X and γ rays. However, it does appear likely that the strain difference is real and this is not unexpected because of the differences between species (2). These differences which depend perhaps on metabolic rate or some such factor make it apparent that extrapolation from mouse to man is not permissible owing to the likelihood that recovery in man is much slower than in the mouse and therefore he will be unable to tolerate as high dose rates without sustaining accumulation of reparable injury to a lethal level.

The constant α/A is the fraction which is irreparable of all the injury produced. The values obtained with the data considered here are approximately equal and the average value for the mouse is 0.066 or approximately 7%, according to Table 3, for X and γ irradiation.

It may be noted that in the paired dose experiments of Paterson et al a residual injury of 7% is equivalent to 7% of 260 r or 18 r. Consequently the relation which should have been tested is

$$242 e^{-\beta t} + \text{second dose} = 518 - 18$$

However, if this equation is solved for β for the data of the first interval, for example, the same value 0.7 is obtained as before. Consequently, as was stated previously, it is difficult to detect the residual injury in experiments of this kind.

Summary and Conclusions:

Hypotheses previously applied to the data on chronic irradiation are shown to represent data on the LD_{50} for mice irradiated for various periods up to a few weeks with X-rays and γ -rays. These hypotheses are that radiation injury is produced in proportion to the dose but that it is reparable in part and irreparable in part. The reparable part subsides exponentially and the irreparable part accumulates in proportion to the total dose. The animals life span is shortened in proportion to the sum of the reparable and irreparable injuries at the time of

death. Formulations of these hypotheses appears to represent all of the data on the lethal effects of radiation in mammals except those on the life shortening consequent to large sublethal single doses. These doses shorten life several times as much as divided doses of the same magnitude.

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