Failure of "Reticulose" to Reduce Radiation Lethality in Mice and Rats.* (19795)

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In recent reports it was stated that a lipoprotein-nucleic acid complex, Reticulose,† given to mice increased their leucocyte counts and resistance to infection(1), and given to rabbits after whole-body X-irradiation inhibited leukopenia(2). No experiments showing its effect on survival after irradiation were reported. Because of our particular interest in the role of infection in post-irradiation injury, we conducted experiments on mice (with and without added streptomycin) and rats to test this point.

Procedures. Male mice of an inbred NIH strain were used, maintained and irradiated as previously described(3), and caged singly beginning one week prior to irradiation. They were irradiated at the age of approximately 7 weeks and distributed among treatment groups in such a way that litter-mates and radiation groups were represented as equally as possible. Radiation factors were 200 KV, 20 ma., 0.25 mm Cu and 0.51 mm. Al added filtration, 50 cm distance to target, and radiation dose in air 625 r. The rats used were males of the Holtzman strain received at weaning and irradiated 3 weeks later at the age of approximately 7 weeks. They were caged singly from one week prior to irradiation, and were irradiated 12 at a time, factors being the same as with mice except that the distance to the target was 75 cm and radiation dose 570 r. Mice were given daily subcutaneous injections of 0.2 ml. Reticulose diluted 1:1 with sterile saline at the time of injection, beginning the day following irradiation and continued for 16 days. Streptomycin sulfate, 5% in saline, was used as the diluent instead of saline in one group, and its control was given 0.2 ml of 2.5% streptomycin sulfate so that each of these groups received 5 mg per mouse per day for 16 days. Rats were given 1.0 ml of undiluted Reticulose daily for 7 days beginning the day after irradiation. Sterile precautions were observed in making the injections, and only freshly opened ampules of Reticulose were used. Leucocyte counts were made‡ only on the mice, separate groups being set aside for this purpose. Tail blood was used and no mouse was bled oftener than once a week.

Results. In Table I it is seen that Reticulose administration affected neither the percent surviving nor the time of death in mice given 625 r or rats given 570 r. Although streptomycin treatment significantly reduced the mortality in mice (see also Miller *et al.*) (4), Reticulose did not influence this effect in either direction. The uniformity of the results is well within the limits of variability to be expected from chance differences alone.

Table II shows that Reticulose administration did not significantly affect leucocyte counts of mice with or without radiation, with or without streptomycin treatment. Furthermore, while streptomycin treatment resulted in a significant reduction (of about 1/3) in the number of mice showing positive heart blood cultures at autopsy, Reticulose, with or without streptomycin, had no effect on the incidence of infection, which averaged 33 to 34% of the mice irradiated, with or without Reticulose.

Discussion. In the data of Berry and Mitchell(1) concerning resistance of Reticulose-injected mice to infection, on the day when the cumulative survival curves show the widest separation, the value for χ^2 (3.6) indicates a probability somewhat greater than one in 20 that the difference was due to chance alone, and this difference was nullified a few days later. Concerning the effect of Reticulose on leucocyte counts (mean of 7 = 17,350

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‡ We are indebted to Miss Helen Auth of this Laboratory for making the counts.

TABLE I. Mortality and Time to Death of Mice Treated with Reticulose or Reticulose and Streptomycin, and of Rats Treated with Reticulose, Compared to Controls.

Treatment*		No. of animals	% mortality (28 days)	Mean time to death ± S.E.
Mice				
625 r, R	Not bled	29	90	13.2 ± .6
	Bled	40	78	12.1 ± .6
	Total	69	83	12.6 ± .4
625 r, C	Not bled	30	77	12.5 ± .6
	Bled	40	90	12.1 ± .5
	Total	70	84	12.3 ± .4
625 r, S, R	Not bled	29	69	15.8 ± 1.3
	Bled	39	67	13.9 ± .9
	Total	68	68	14.7 ± .8
625 r, S, C	Not bled	30	50	14.2 ± 1.1
	Bled	40	72	14.3 ± .8
	Total	70	63	14.3 ± .6
Rats				
570 r, R	Not bled	36	75	11.8 ± .9
570 r, C	" "	36	75	11.6 ± .9

* R = Reticulose; C = Control; S = Streptomycin.

TABLE II. Leucocyte Counts from Tail Blood of Reticulose-Treated Mice, with or without Irradiation or Streptomycin Treatment, Compared with Controls.

Treatment group*		Leucocytes, thousand/mm³ (Day after beginning Reticulose; † No. of mice)			
625 r	R‡	5.24 ± .37 (0; n = 20)	2.02 ± .15 (2; n = 20)	1.05 ± .07 (6; n = 19)	.77 ± .07 (13; n = 19)
	C	4.91 ± .34 (0; n = 20)	2.60 ± .09 (2; n = 19)	1.40 ± .06 (6; n = 20)	.92 ± .17 (13; n = 15)
	S, R	5.40 ± .27 (1; n = 20)	1.73 ± .08 (3; n = 19)	.61 ± .07 (7; n = 18)	.53 ± .07 (14; n = 23)
	S, C	4.84 ± .36 (1; n = 20)	1.44 ± .08 (3; n = 20)	.42 ± .05 (7; n = 19)	.56 ± .06 (14; n = 18)
No irrad., R		14.58 ± .56 (0; n = 29)		12.49 ± .47 (8; n = 27)	11.24 ± .38 (15; n = 27)

* Reticulose (.1 ml) and streptomycin (5 mg) subcutaneous injections begun the day after irradiation and continued for 16 days.

† Day 0 = 1 day after irradiation of the first four groups, and just prior to the first Reticulose injection.

‡ R = Reticulose; C = Control; S = Streptomycin.

± 1,200 for tail blood the day following injection) there were, unfortunately, no tail-blood counts made on the controls (mean for heart blood = 5,450 ± 850). Wide differences in leucocyte counts from heart and tail blood are generally acknowledged. For example, Feketè(5) reports the average value for tail blood of 8 normal mice to be 21,510 cells/cmm compared with 3,717 for ventricle blood. In the data of Thompson(2) on leucocyte and lymphocyte counts in rabbits the differences appear to be in excess of expected chance variation.

Although it has not been established that an increased number of circulating lymphocytes improves the chance of survival after irradiation (Campbell and Ross)(6), the possibility remained that a very small improvement in the animal's natural defenses might enhance the saving effect of antibiotic treatment. Reticulose treatment did not accomplish this in mice where streptomycin treatment was effective.

It seems somewhat futile to attempt an explanation of the discrepancy between our results and the conclusions drawn by Thompson(2), and Berry and Mitchell(1), but their

procedures differed from ours in several respects. In their rabbits Reticulose was given intramuscularly and in their mice, intraperitoneally; the rabbits were given 2 ml each on days 2 and 3, and the mice were given a single injection of 0.15 ml; and the radiation dose given rabbits (450 r) was in the low lethal range. The response of irradiated rabbits to treatment with Reticulose, which was small though apparently significant, may be different from that of rats or mice.

Whether or not a real difference exists between the experimental results from the laboratories concerned, the fact that survival was not increased in irradiated rats and mice, and that no augmentation of the streptomycin effect occurred in mice, indicates to us that no spectacular results are to be expected from the use of Reticulose following whole body exposure to radiation.

Summary. Reticulose given by subcutaneous injection to mice, with and without streptomycin treatment, failed to alter mortality, leucocyte count, or incidence of blood stream infection after 625 r whole-body X-irradiation. Given to rats it failed to alter mortality after 570 r whole-body X-irradiation.

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Routine Titrations of Lansing Virus and Antibody in Mice: Comparison of Methods of Estimating Endpoints.* (19796)

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The reproducibility of endpoints in titrations of Lansing virus and antiserum has been discussed by Morgan(1) and Bell(2), who present the results of relatively short series of independent titrations from which the ED_{50}'s have been calculated. These estimates seem very consistent, considering the limited numbers of mice on which they are based. Similar results have been obtained by Schwerdt and Merrell(3) in cotton rats. In all 3 reports the ED_{50}'s have been estimated by the Reed and Muench method. The relative advantages of estimating the ED_{50} by this and other methods have been reviewed by various workers, notably Finney(4), who suggests that the Kärber method may be expected to give very satisfactory results when responses run from 0 to 100%. An advantage of the Kärber method under these conditions is that the standard error of the estimate may be readily calculated; in contrast, the procedure outlined by Pizzi(5) for determining the standard error of Reed and Muench estimates seems arbitrary. The purpose of this note is to present some results obtained in these laboratories in the routine titration of Lansing virus suspensions and antisera. The ED_{50}'s of the titrations have been calculated by a number of different methods in order to compare the estimates obtained.

Materials and methods. Over a period of several months, one of us (E.M.C.) carried out routine titrations of a pool of Lansing virus, and of samples of single lots of plasma gamma globulin, placental gamma globulin, and immune monkey serum. The virus pool

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