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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TABLE I. Routine Titrations of Lansing Virus.

| | No. of titrations | % mortality of mice at following dilutions (log units): −5.2 | −4.7 | −4.2 | −3.7 | −3.2 | −2.7 | Mice per dilution |
|---|---|---|---|---|---|---|---|---|
| Virus pool | 20 | 18 | 25 | 38 | 65 | 75 | 92 | 10 |
| | Routine titrations of Lansing Antisera* | −5 | −4.5 | −4 | −3.5 | −3 | −2.5 | |
| Plasma γ globulin | 9 | | | 76 | 70 | 33 | 6 | 8 |
| Placental γ globulin | 8 | | 88 | 78 | 38 | 4 | | 8 |
| Monkey serum | 12 | 93 | 83 | 51 | 13 | 11 | | 8 |

* Dilution of globulin or serum mixed with equal volumes of virus to give a final virus concentration of $10^{-2.7}$.

TABLE II. Reproducibility of $ED_{50}$'s (Log Units) Estimated by the Probit Method.

| | No. of titrations | Avg $ED_{50}$ | Range of $ED_{50}$'s | Stand. dev. of $ED_{50}$'s | Avg stand. error of individual $ED_{50}$ |
|---|---|---|---|---|---|
| Virus pool | 20 | −4.06 | −4.72 to −3.38 | .35 | .22 |
| Plasma γ globulin | 9 | −3.36 | −3.99 to −2.94 | .36 | .17 |
| Placental globulin | 8 | −3.71 | −3.87 to −3.47 | .14 | .14 |
| Monkey serum | 12 | −3.98 | −4.38 to −3.44 | .32 | .16 |

was prepared from the brain stems and spinal cords of approximately 300 mice inoculated intracerebrally with Lansing virus as previously described(6). The gamma globulins were commercial preparations formerly used by us in protection tests(7) and containing at least 16% gamma globulin; the monkey serum was obtained following a course of intramuscular injections of Lansing virus(8). The virus pool and monkey serum were distributed into screw-capped vials and stored in a carbon dioxide chest; the gamma globulins were stored in packages at 4°C. The gamma globulin and monkey serum were diluted in normal saline for the tests, and held at 4°C overnight. An equal volume of Lansing virus pool, diluted in normal saline, was then added to each serum dilution to give a final concentration of virus of $10^{-2.7}$. The dilutions of the materials used, the number of mice per dilution, and the number of titrations carried out are given in Table I. The mice were inoculated cerebrally with 0.03 ml of each suspension; they were all males, 12 to 16 g in weight obtained from the colony maintained at these Laboratories. The inoculated mice were observed daily for 4 weeks and all deaths recorded. Deaths occurring on the day after inoculation were not regarded as specific. The average percentage mortalities are given in Table I. It will be noted that these percentages did not usually run from 0 to 100%, but that considerable truncation often occurred at one or both extremes. Included among the titrations were several in which the responses were very erratic.

*Statistical methods.* Estimates of the $ED_{50}$'s and their standard errors were calculated for the individual titrations by the probit method(9). Two iterations were carried out in all cases. In addition, the $ED_{50}$'s were calculated by the following procedures: Kärber(10); Reed and Muench(11); and moving average(12). The maximum possible span was used in determining the $ED_{50}$'s by this last procedure.

*Results.* The $ED_{50}$'s obtained by the probit method are summarized in Table II. The variability of these quantities is considerable, the estimates differing among themselves as much as one log unit or more. The standard deviation of the titrations is generally greater than the error of the individual assay, suggesting that titrations carried out on different occasions vary more than those performed at the same time. The average slope is 0.93 probit per log unit for the virus assays and 1.6 probits per log unit for the serum titrations.

TABLE III. Comparison of $ED_{50}$'s Obtained by Various Simple Methods with Those Obtained by the Probit Method.*

| | Kärber | | Reed & Muench | | Moving avg | |
|---|---|---|---|---|---|---|
| | Max diff | Avg diff | Max diff | Avg diff | Max diff | Avg diff |
| Virus pool | 65 | 23 | 63 | 29 | 63 | 30 |
| Plasma γ globulin | 31 | 19 | 72 | 42 | 56 | 31 |
| Placental globulin | 47 | 12 | 110 | 36 | 53 | 23 |
| Monkey serum | 64 | 16 | 64 | 25 | 68 | 27 |

* Differences as percentages of stand. errors of probit method.

A comparison of the estimates obtained by the Kärber, Reed and Muench, and moving average methods with those obtained by the probit procedure is given in Table III. The differences in log units are expressed as percentages of the standard errors of the individual assays, as calculated by the probit method. None of these methods gives widely different results, on the average, from those of the probit method; several of the largest discrepancies occur for titrations that cannot be considered satisfactory. The Kärber method appears to give noticeably better estimates than the others, differing on the average by less than one quarter of a standard error.

A comparison was also made of the standard errors of the individual asays as estimated by the probit and by the Kärber methods. In general, it was found that the agreement was quite satisfactory, providing the truncation was not too great. For titrations with no more than 20% mortality at the one extreme or no less than 80% at the other, the Kärber estimates were found to differ by 12%, on the average, from the probit estimates. In general, they tended to be smaller than the probit estimates, appreciably so if the truncation was over 25%.

*Discussion.* As judged by these results, titers of virus or immune serum based upon 50-60 mice are subject to considerable variation. This variation is greater than that reported by Morgan(1) and Bell(2), and that expected from the results of our individual titrations. For this reason it would be unwise to use the standard error of the individual assay for comparing $ED_{50}$'s determined on different occasions, as advocated by Schwerdt and Merrell(3). While the accuracy of comparisons could be improved by increasing the number of animals, it should be noted that a 4-fold increase in number would be required to reduce the standard error of the individual titration by one half. This, of course, would not affect variation from one time to another. The most accurate comparisons would be made with titrations done on the same occasion. Since the average slope of the dosage response curves of the serum assays is considerably greater than that of the virus, serum titers can be determined a good deal more precisely than virus, *i.e.*, approximately one third the number of animals are required for the same accuracy.

The comparisons tabulated above suggest that the $ED_{50}$'s calculated in routine assays by the Kärber method compare very favourably with those obtained by the probit method. Providing the response runs from 0 to 100%, both $ED_{50}$'s and their standard errors as given by this simple procedure will agree very closely with those of the more involved maximum likelihood method. If, then, care is taken to avoid instances where extreme truncation occurs, particularly those in which the $ED_{50}$ lies far from the center of the dilution series, the estimates of this quantity obtained by the Kärber method should be quite satisfactory, and likely more accurate than those obtained by the Reed and Muench procedure. In addition, the arithmetic of the Kärber method is somewhat simpler than that of the Reed and Muench procedure. It would seem then that the Kärber method is to be preferred in the estimation of $ED_{50}$'s from this type of data.

*Summary.* 1. Over a period of several months, routine titrations in mice were carried out by a single worker of a pool of Lansing virus, and of samples of plasma gamma globulin, placental gamma globulin, and immune monkey serum. Estimates of the $ED_{50}$'s and their standard errors were calculated for the individual titrations by the probit method. In addition $ED_{50}$'s were calculated by the

Kärber, Reed and Muench, and moving average methods. 2. The standard deviation of the titrations was found to be greater than the error of individual assays, suggesting that titrations carried out on different occasions vary more than those performed at the same time. 3. None of the methods mentioned gave widely different estimates of the $ED_{50}$. In particular, the $ED_{50}$'s calculated by the Kärber method compared very favourably with those obtained by the probit method. 4. The Kärber method is proposed as a suitable alternative to the Reed and Muench procedure for the calculation of $ED_{50}$ in routine titrations of virus and antiserum.

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## Effect of X-Ray on Radioactive Phosphorus Turnover and Oxygen Consumption of Brain.*† (19797)

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Studies of the pathological effects of radiation on the central nervous system of adult mammals have shown no clearcut findings. Warren(1) presents evidence which indicates that at least "therapeutic radiation" has little effect on adult nerve tissue. Findings which demonstrate radiation damage to nervous tissue appear to result from effects on vascular components rather than direct damage to neural elements. The earliest experimental observations(2) of the effects of radiation on brain were in experiments where the whole body was irradiated; congestion and hemorrhage were the most consistent pathological findings.

The purpose of the present investigation was to demonstrate the presence or absence of alterations in the phosphorus metabolism of brain after whole-body irradiation with roentgen rays. In order to eliminate at least one variable, vascular circulation effects, studies of oxygen consumption and phosphorylation were done *in vitro* after *in vivo* irradiation of the animals.

*Experimental.* Six- to eight-week-old male albino mice were irradiated with 500 to 800 r and, in another experiment, with 19,000 r in air. A 250 kv General Electric Unit was used with 1 mm Al and 0.5 mm Cu filters at 15 ma output. The smaller dosages were delivered at 1 meter distance and the greater at 7 cm. The mice were killed by severing the spinal cord at various times after irradiation. The brain was excised as rapidly as possible and split along the longitudinal fissure into equal halves. The halves were weighed, minced, and then added to 3.0 cc of McIlwain's buffer(3) containing $P^{32}$ as orthophosphate.‡ The tissues were incubated at 37.5°C for 3 hours

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