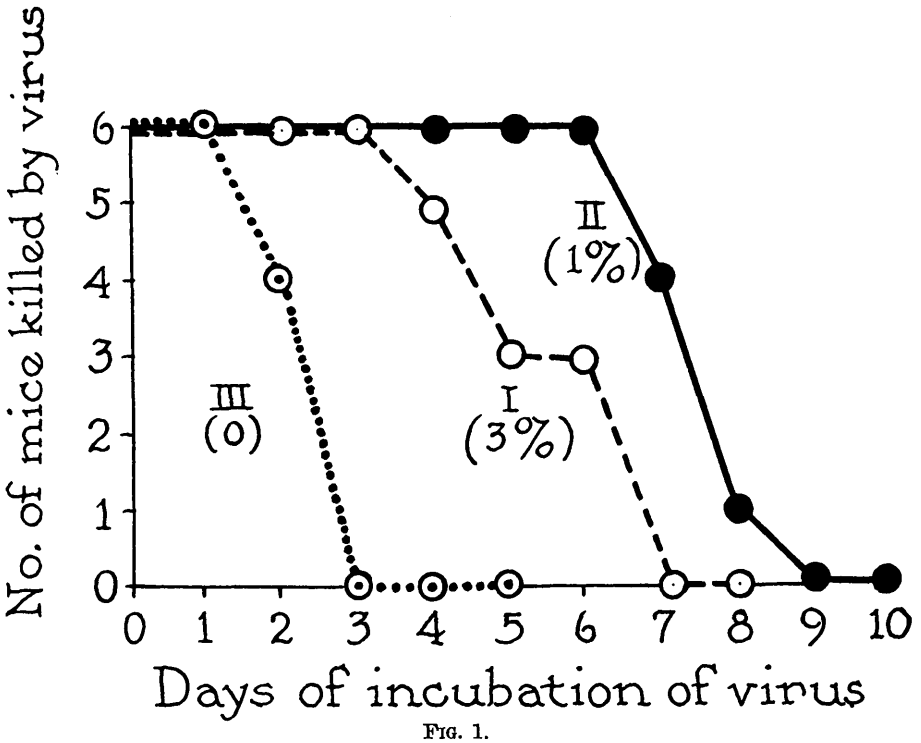

FIG. 1.
Experiment of September 17, 1941. Six mice were injected intracerebrally each day with each mixture. The graphs show the number of mice which died between the fourth and tenth days after injection.

Conclusions. (1) Neurotropic yellow fever virus in serum-saline dilution is protected by the addition of one per cent ammonium sulfate. (2) Much of the preservative effect is due to a shift of the reaction of the medium to the acid side. The optimal reaction appears to be near pH 6.3-6.5. (3) Addition of ammonium sulfate to 1% concentration seems to be the most convenient method of bringing about an optimal reaction.

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Estimation of the ED_{50} and Its Error by Means of Logarithmic-Probit Graph Paper.*

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With the increased emphasis on evaluation of all-or-none data there is need for a sound approximate procedure for arriving at a numerical expression of effect such as is represented by the ED_{50} (the dose affecting 50% of the group treated) and its standard error. The publication of Bliss[1] on the maximum likelihood solution of data based upon small numbers of subjects afforded a rigorous although somewhat tedious procedure which has been generally accepted. Using the latter for comparison, Irwin and Cheeseman[2] con-

[1] Bliss, C. I., *Quart. J. Pharm. Pharmacol.*, 1938, **11**, 192.

[2] Irwin, J. O., and Cheeseman, E. A., *Suppl. J. Roy. Stat. Soc.*, 1939, **6**, 174.

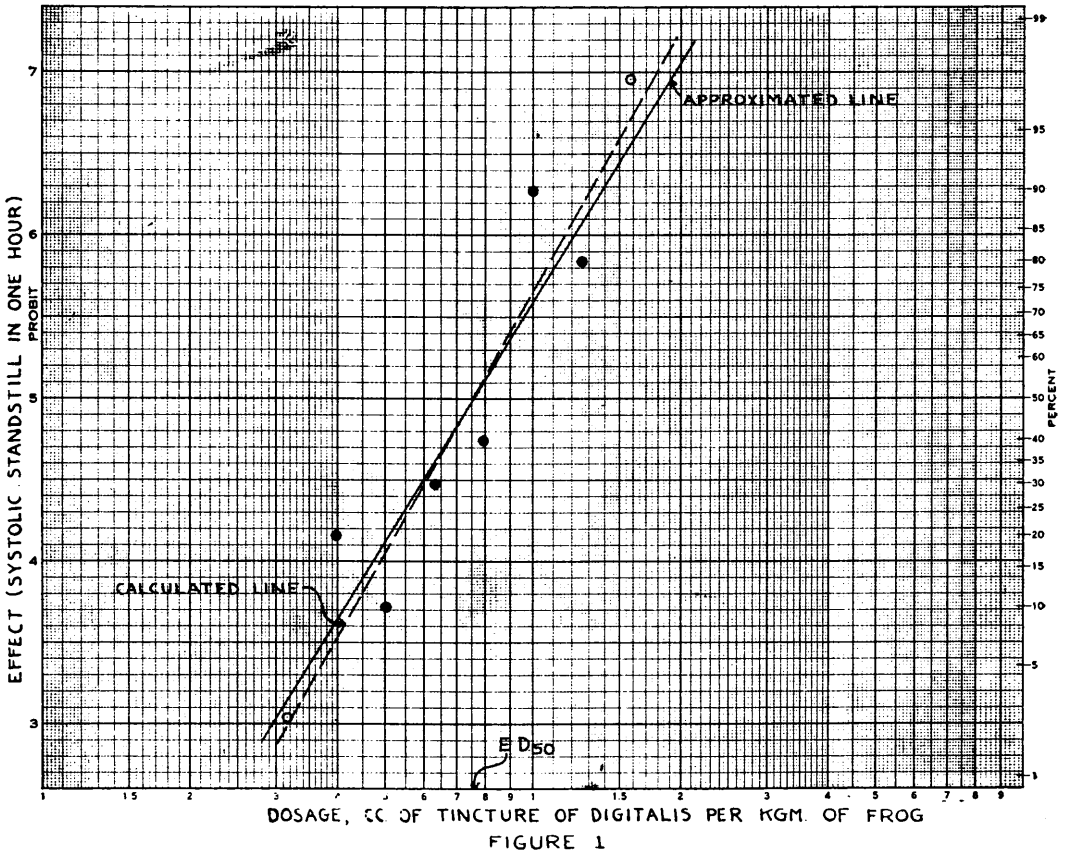

FIGURE 1

Graphic Estimation of the ED_{50} by the One-hour Frog Method.

Group No.	Dose, cc/kg	Effect P	Effect Probit
1	3.16	0/10	(3.04)
2	3.98	2/10	4.16
3	5.01	1/10	3.72
4	6.31	3/10	4.48
5	7.94	4/10	4.75
6	10.00	9/10	6.28
7	12.59	8/10	5.84
8	15.85	10/10	(6.96)

Probit $4 \cong 4.75$

$6 \cong 12.20$

$2s = 12.20 - 4.75 = 7.45$

$$\text{s.e.}_{ED_{50}} = \frac{2s}{\sqrt{2N'}} = \frac{7.45}{\sqrt{2 \times 60}} = \pm 0.68$$

cluded the Kärber method was preferable to the other short-cut methods available at that time. More recently, Litchfield and Fertig[3] have advanced a graphic procedure having much merit, particularly since it provides for the first time a simple reliable means of deriving the standard error of the ED_{50}. The technic proposed here is a simplification and extension of their method.

A logarithmic-probit graph paper (Fig. 1) has been designed to facilitate the estimations.† The paper is 11 x 16½ inches which provides space enough to plot most experi-

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

† This graph paper may be obtained from the Special Chemicals Division, Winthrop Chemical Company, Inc., 170 Varick St., New York 13, N.Y.

mental data on a scale that permits reading the values from the graph with adequate accuracy. The vertical ruling of the paper represents a two-cycle logarithmic scale, whereas the ordinate or probit scale is divided into units of 0.02 extending from 2.60 to 7.40 probits, with heavy lines indicating the main subdivisions. Percentages are indicated on the right-hand ordinate margin, which may be used by those unfamiliar with the probit transformation. A generous margin has been allowed for tabulating the basic data. As may be noted, the range extends slightly beyond the 1% and 99% points, which is quite adequate for most biologic purposes.

In use, the original data, such as those illustrated in Fig. 1, are plotted on the paper either as the percentage effect or as the probit effect obtained by reference to a suitable table‡ without calculating the percentage. Usually each series of toxicological data includes at least one dose low enough so that all subjects survive and one high enough so that none survive. There is no finite probit value corresponding to either result. As a close approximation, Bartlett[4] suggests writing ¼ for 0 in the result obtained with the highest dose showing $0/n$, n being the number in the group on that dose.§ Similarly $n - ¼$ is substituted in the numerator of the result obtained with the lowest dose showing no survivors. This suggestion has been followed in Fig. 1, the probits for the substituted values being in parentheses and the plotted circles left unshaded to indicate the substitution. The line best fitting the points is drawn in by eye with the aid of a transparent straightedge. The estimated ED_{50} is the dosage value at 50% (probit 5.0) and is read directly from the graph paper in original dosage units.

In drawing the best-fitting line a closer approach to the maximum likelihood solution may be reached by bearing in mind that the points farthest from 50% (probit 5.0) have the least weight per animal in fixing the true position of the line.[1,3] Thus with equal numbers of experimental animals, points at probits 4.0 and 6.0 have only approximately two-thirds as much weight as one at probit 5.0 while those at probits 3.0 and 7.0 have only one-fifth as much weight. Allowance for this fact can be made in planning the experiment by increasing the size of the groups receiving the low and high doses, if desirable, or in fitting the line when the data have been plotted.

In estimating the standard error of the ED_{50} the only 2 additional values required may be read directly from the graph, *i.e.*, the dosages corresponding to 16% and 84% (probits 4.0 and 6.0). Subtracting the former from the latter gives $2s$, which is the estimated increment in dosage necessary to increase the effects by two probits in this dosage range. The approximate average standard error is given by the formula

$$\text{Approximate s.e.}_{ED_{50}} = \frac{s}{\sqrt{N'/2}} = \frac{2s}{\sqrt{2N'}}$$

the latter form being more convenient in the present case. In this formula, N' has a slightly different meaning from that assigned by Litchfield and Fertig, since it is the total number of animals in the groups which, from the best-fitting line, would be *expected to show effects* between 6.7 and 93.3% (probits 3.50 and 6.50). In the example given, this takes in groups 2 to 7, inclusive, and $N' = 60$. Occasionally this definition results in there being included in N' one or two groups in which the observed effects are either below 6.7% or above 93.3%. This occurs when the data on all the rest of the doses indicate that if a large number of subjects were used, a result between 6.7 and 93.3% would normally be expected. The reasons for using the expected rather than the observed result in this connection have been given in detail by Bliss.[1] The estimation of the ED_{50} and the calculation of the standard error are clearly illus-

‡ A table giving the probits corresponding to all possible results in groups up to 25 animals is available upon request from the authors; for a complete probit table see Reference 1 or Fisher, R. A., and Yates, M. A., *Statistical Tables for Biological, Agricultural, and Medical Research*, 1938, Oliver & Boyd, London.

[4] Bartlett, M. S., *Suppl. J. Roy. Stat. Soc.*, 1937, **4**, 137.

§ The authors are indebted to Dr. Chester I. Bliss for calling their attention to this reference and for other helpful suggestions.

trated in Fig. 1 from which the values of 7.55 ± 0.68 cc of digitalis tincture/kg of frog are obtained. The corresponding values by the more exact method of maximum likelihood were calculated to be 7.55 ± 0.58 and the equation of the best-fitting line with dosages (X) in logarithmic units, $Y = 5.289X + 0.356$. The line drawn in by eye coincides well with that calculated (the broken line of the figure).

It happens in this example that the graphically determined ED_{50}, obtained in less than 10 minutes, agrees exceedingly well with the maximum likelihood solution arrived at independently after the expenditure of considerably longer time, even with the aid of an automatic calculating machine. Differences between the two procedures will rarely be of practical significance. In this example the standard error is somewhat larger by the graphic procedure due principally to the fact that the line drawn by eye was slightly flatter than the best-fitting line found by the least-squares calculation. While a difference in the estimated standard error of this magnitude would scarcely modify the pharmacologic inferences, it nevertheless emphasizes the approximate nature of the graphic estimation and points up the fact that in crucial cases the standard maximum likelihood solution should be used.

Summary. A log-probit graph paper is described by which a simple graphic estimation of the ED_{50} and its standard error may be quickly made.

14777 P

Cumulative Effects of Repeated Head Trauma of Minimal Intensity.* Observations on Experimental Animals.

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This investigation is concerned with the immediate and delayed effects of repeated, minimal, blunt impacts on the head.

Using as traumatizing agent an apparatus which permitted the strength of the blow to be measured exactly, a mass of 453.6 g dropping with a velocity at the point of impact of 330.9 cm/sec. was found to be just insufficient to produce loss of consciousness or any other immediate or delayed ill effects in a normal animal (subconcussive trauma). It was this blow of minimum intensity that was used in the investigation of cumulative effects of repeated trauma.

One hundred twenty rats of the same strain and of approximately the same weight were used in the experiment. Of these, 60 were given 15 blows in close succession, one minute apart; the other 60 were given the same amount of trauma (15 blows) at longer intervals, from 2 to 6 days apart, in a 50-day period. Half of each group were struck on the stationary head, and the other half were made to strike the rapidly accelerated head against a stationary object.

In all experiments precautions were taken that the head of the animal should be struck at the same region each time and that in each of the two mechanisms all animals should receive a trauma of the same momentum at all times.

Observations. Cumulative effects, as revealed by temporary loss of consciousness, at times by death or by persisting changes in behavior, were clearly shown in all groups of animals as a result of the repeated trauma. The frequency with which ill effects were displayed in the different classes at the end of the experimental period is shown in Table I.

In the great majority of cases there was a certain correspondence between the effects of the trauma during life and the extent of

* Read at the May meeting, 1944, of the Massachusetts Psychiatric Society.