SPECIAL ARTICLES

THE METHOD OF PROBITS

THE result of an investigtion of the action of a toxic agent upon the mortality of an organism is usually expressed as an asymmetrical S-shaped curve, in which the percentage mortality of each set of individuals is related to the dosage to which it has been exposed (Fig. 1). The effectiveness of a poison used

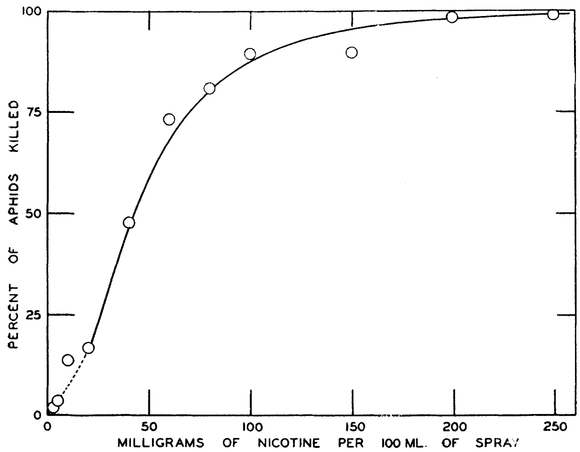

FIG. 1. Net mortality of *Aphis rumicis* L. sprayed in laboratory with different solutions of nicotine; summary of results over 3-year period. Tattersfield and Gimingham.[4] Heavy curve is same as that in Fig. 2 transposed back to original units.

to combat an insect pest is of primary interest to the economic entomologist in the range of dosages approaching 100 per cent. kill. But in this region, the usual type of curve flattens to an asymptote, so that comparisons are commonly based upon dosages which kill only from 25 to 75 per cent. of the organisms. Furthermore, the curve is ordinarily fitted free hand, and in instances where the data are more or less irregular, there is a tendency to adjust the usually asymmetrical S-curve to successive small segments of the data rather than to the observations as a whole. This practise introduces an indeterminate distortion due to the experimental errors and to unconscious bias on the part of the experimenter. It is believed that these and other difficulties can be minimized if percentage kill and dosage are transformed to units which may be plotted as straight lines on ordinary cross-section paper and hence permit fitting by the customary technique of least squares or of the straight-line regression equation.

A survey of the literature revealed that an inherent variability among individuals of a population in their susceptibility is considered to be responsible for the S-shaped character of the curve.[1] With any dosage to which some individuals succumb while others survive, the poison kills not only those which would survive any smaller amount, but also those more susceptible individuals which could be killed with a smaller amount. Because of this inherently cumulative character, the type of curve just discussed has been termed by Shackell[2] the "dose-effect ogive." So many different types of variation in a great variety of organisms have followed the symmetrical normal curve of error that the variation in resistance to poison might be expected to follow suit. Instead of assuming that the observed asymmetry in this case is due to a skewed distribution of errors, an explanation of the asymmetry has been sought in the mode of toxic action. When dosage is plotted directly on an arithmetical scale, the cumulative S-curve could be symmetrical only if equal additions in dosage at all concentrations resulted in equal increments in lethal action. It has been observed in many physiological processes that equal increments in effect are produced only when the stimulus is increased by a constant proportion of the given dosage, rather than by a constant amount. It seems probable that this same rule might hold for toxicological processes, in which case dosage would have to be plotted in logarithmic terms to show a uniform increase in kill or a symmetrical dose-effect ogive.

Of the different methods which might transform a dosage-mortality curve to a straight line, if the above analysis is a valid one, two offer advantages. By the first method, cross-section paper might be so ruled that a relationship involving the two functions, the cumulative curve (as ordinate) and logarithms (as abscissa), would plot as a straight line. Paper with rulings for a symmetrical cumulative curve and logarithms has been devised by Whipple and Hazen,[3] and can be purchased on the market. Because of greater ease in determining the straight line of best fit by the simple regression equation, the present author has found it more convenient to use a second method, to transform the data instead of the paper to the appropriate units. The transformation of dosage presents no difficulties, since tables of logarithms are universally available. For the percentage kill, no equally simple and direct system of transformation was at hand. The nearest approximation was offered by the tables of the probability integral, Nos. I and II in Part I of Pearson's Tables for Statisticians and Biometricians. The principal table (No. I) had the disadvantage of an origin at .50 (or 50 per cent.) and thus involved the use of plus and minus quantities. This difficulty has been avoided by a special table derived from those of Pearson by letting the observed 0.01 per cent. kill equal 0.00 on an arbitrary scale, 50.0 per cent. kill equal 5.00, and 99.99 per cent. kill equal 10.00, and then calculating

[1] S. C. Brooks, *Jour. Gen. Physiol.*, 1: 61, 1918.

[2] L. F. Shackell, *Jour. Pharm. and Exper. Therap.*, 25: 275, 1925.

[3] G. C. Whipple, *Jour. Franklin Inst.*, 182: 205, 1916.

TABLE I

Per cent. kill	Probits	Per cent. kill	Probits	Per cent. kill	Probits	Per cent. kill	Probits
1.0	1.87	50.0	5.00	80.0	6.13	95.0	7.21
5.0	2.79	52.0	5.07	81.0	6.18	96.0	7.35
10.0	3.28	54.0	5.14	82.0	6.23	97.0	7.53
15.0	3.61	56.0	5.20	83.0	6.28	98.0	7.76
20.0	3.87	58.0	5.27	84.0	6.34	98.5	7.92
25.0	4.09	60.0	5.34	85.0	6.39	99.0	8.13
30.0	4.30	62.0	5.41	86.0	6.45	99.1	8.18
34.0	4.44	64.0	5.48	87.0	6.51	99.2	8.24
36.0	4.52	66.0	5.56	88.0	6.58	99.3	8.30
38.0	4.59	68.0	5.63	89.0	6.65	99.4	8.38
40.0	4.66	70.0	5.70	90.0	6.72	99.5	8.46
42.0	4.73	72.0	5.78	91.0	6.80	99.6	8.57
44.0	4.80	74.0	5.86	92.0	6.89	99.7	8.69
46.0	4.86	76.0	5.95	93.0	6.98	99.8	8.87
48.0	4.93	78.0	6.04	94.0	7.09	99.9	9.16

the intermediate values in a symmetrical manner. These arbitrary probability units have been termed "probits" and are given above in an abbreviated table.

The method just described for the analysis of toxicological curves has been applied successfully by the author to a large series of data from the literature, as well as to unpublished records secured by himself or his associates. An example of this transformation is that of the curve in Fig. 1 into that shown in Fig. 2. Aside from an increased accuracy in calculating dosage-mortality curves and in interpolating dosages from such curves over a more extended range of mortalities than has been practicable with the usual asymmetrical S-curve, this type of presentation has led to the following advantages: (1) a test of the proposed theory of toxic action that (a) the variation in susceptibility among individuals is normal, and that (b) the effectiveness of the dose increases as its logarithm; (2) a closer scrutiny of experimental technique to determine if the organisms exposed to each dosage were truly equivalent and if the amounts administered experimentally were uniformly proportional to the effective dosage over the entire range covered by the experiment; (3) the disclosure of change in the mode of lethal action with certain poisons over different sections of the dosage range, indicated by an abrupt change in slope as illustrated in Fig. 2; and (4) a simple method of expressing, in the slope of a straight line, the relative uniformity or diversity between individuals in their susceptibility to a poison.

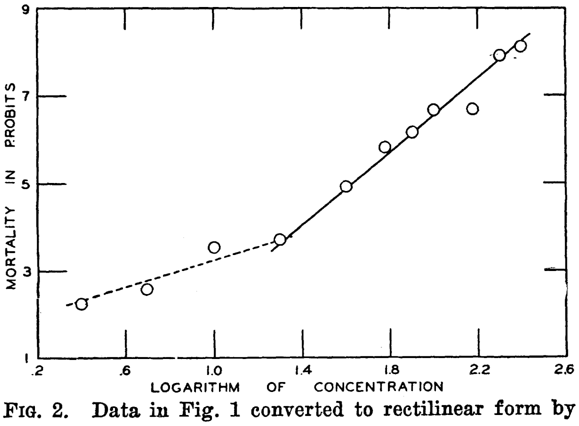

FIG. 2. Data in Fig. 1 converted to rectilinear form by use of logarithms and probits as explained.

The experimental records from the entomological literature to which this theory has been applied successfully include such diverse cases as the action of nicotine sprays upon aphids,[4] of several fumigants upon adult Tribolium,[5] of hot water upon Japanese beetle pupae,[6] of x-rays upon Drosophila eggs,[7] and of acid lead arsenate upon fourth instar silkworm larvae.[8] A more detailed table of probits and a more extended consideration of insect toxicological tests will be presented later.

C. I. BLISS

OXIDATION-REDUCTION REACTIONS BETWEEN NATURAL HYDROCARBONS AND OIL-FILLED WATERS

As far as known to the writers, it was G. S. Rogers who first pointed to certain constant relations between the occurrence of sulphide and sulphate waters on the one hand and of the composition of the associated petroleums on the other.[1] Rogers suggested that this relation might be interpreted as indicating that sulphate waters were reduced to sulphide waters by petroleums with paraffin as a base, the latter at the same time becoming oxidized and polymerized so as to yield naphthene or asphalt bases. Subsequently Bastin and his associates demonstrated that certain bacteria may serve as agents in such or similar changes and it may well be suggested, on the basis of Bastin's experimental evidence, that at moderate temperatures bacterial action is not only a sufficient but a necessary cause.[2]

In 1928 and 1930 Colacurcio and Bengtson,[3] at the

[4] F. Tattersfield and C. T. Gimingham, *Annals Appl. Biol.*, 14: 217, 1927.

[5] A. L. Strand, *Ind. and Eng. Chem.*, Analyt. Ed., 2: 4, 1930.

[6] W. E. Fleming and F. E. Baker, *U. S. Dept. Agr. Techn. Bull.*, 274, 1932.

[7] C. Packard, *Jour. Cancer Res.*, 10: 319, 1926.

[8] F. L. Campbell, *Jour. Econ. Entom.*, 23: 357, 1930.

[1] G. S. Rogers, "The Sunset-Midway Oil Field, California.—Part II, Geochemical Relations of the Oil, Gas, and Water," U. S. Geol. Surv. Prof. Paper 117, pp. 26–31, 1919.

[2] E. S. Bastin and others, "The Problem of the Natural Reduction of Sulphates," Am. Assn. Petrol. Geol., Bull., vol. 10, pp. 1281–1826, *et sequor*, 1926.

[3] M. J. Colacurcio, "Interactions of Hydrocarbons and Sulphate Waters," Univ. of Cincinnati, Unpublished Master's Thesis, 1928. R. A. Bengtson, "Interactions of Hydrocarbons and Sulphate Waters," Univ. of Cincinnati, Unpublished Master's Thesis, 1930.

and auricles but a much shorter head than that occurring in the normal forms. This might seem to indicate that, simultaneous with the non-specific effect of x-rays upon head development, other effects, as upon cell division and growth, are occurring. As regards the non-specific nature of the more immediate effects of x-rays, it is significant that for any one dose of 4, 8 or 12 skin units the range of distribution of types regenerated increases as crowding increases.

No explanation is offered regarding the significance of the disappearance of tissue differentiated during the first two weeks. It seems to be an effect of x-rays on planarian tissue which gains expression at a period after regeneration has proceeded to its limits and which is first apparent in that region of tissue having the highest rate of metabolism.

In all x-rayed forms receiving 4, 8 and 12 skin units, the ultimate effect of x-rays is complete cytolysis. Cytolytic effects first become prominent on the thirty-fourth day after exposure. The rate of cytolysis is not greatly affected by crowding. The results of these experiments regarding the effects of x-rays are in accord with those of Bardeen and Baetjer, who conclude that x-rays affect cell division and cell differentiation and that the effects are probably confined to these two. They cite evidence from which they conclude that cell differentiation is not as much affected as cell division and that the effect upon cell division is not direct.

While both the more immediate effects and the delayed effects of x-rays may be specific upon the protoplasm, it does not necessarily follow that, because head frequency is affected by x-rays, the factors which control head frequency are specific and directly related to the activity of special formative cells. The formative cell theory of Curtis does not recognize the fact that the variation in head forms regenerated are the same type as those produced by other physical and chemical agents. It is no more necessary to assume the selective action of x-rays on formative cells than it is necessary to assume selective action of other physical and chemical agents which alter head frequency. The first apparent effects of x-rays, like various other agents, seems to be not on special formative cells but upon non-specific protoplasmic factors upon which head development depends.

Crowding varies only the rates at which effects of x-rays gain expression, allowing, in some instances, an increase in head frequency and a delay in cytolysis. As for the ways in which crowding alters these effects of x-rays on head frequency and length of life, the following possibilities may be considered: (1) Mechanical stimulation of the group upon each member; (2) a lowering of metabolic rate favoring a delay of cytolysis; (3) whatever these factors favoring group survival are, they are more effective as the dose of x-rays is increased.

The above conclusions were reached after studying the effects on over 800 Planaria.

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THE METHOD OF PROBITS—A CORRECTION

Since submitting the paper which appeared under the above title,[1] my attention has been called to recent papers by Hemmingsen[2] and by Gaddum,[3] in which substantially the same method has been proposed for toxicity tests with mice. Their "normal equivalent deviations" are measured from zero at 50 per cent. kill, taking the standard deviation as the unit, so that the elimination of a change in sign at 50 per cent. kill, as provided by the "probits," seems justified. However, the constant multiplier of 1.344447, used to equilibrate 0 and 10 on the probit scale with 0.01 and 99.99 on the percentage scale, interferes with the conversion from one system to the other. It seems desirable, therefore, to redefine the probit unit as equal to 5 plus (algebraically) the deviate of the normal curve expressed in terms of its standard deviation. As convenient sources of this deviate, either the Sheppard-Galton Table I[4] or the column of x corresponding to p and q in the Kelley-Wood Table[5] may be suggested. At 50 per cent. kill, the probit will be 5.00 as before; below 50 per cent. kill it will

TABLE I

Per cent. kill	Probits	Per cent. kill	Probits	Per cent. kill	Probits	Per cent. kill	Probits
1.0	2.674	50.0	5.000	80.0	5.842	95.0	6.645
5.0	3.355	52.0	5.050	81.0	5.878	96.0	6.751
10.0	3.718	54.0	5.100	82.0	5.915	97.0	6.881
15.0	3.964	56.0	5.151	83.0	5.954	98.0	7.054
20.0	4.158	58.0	5.202	84.0	5.994	98.5	7.170
25.0	4.326	60.0	5.253	85.0	6.036	99.0	7.326
30.0	4.476	62.0	5.306	86.0	6.080	99.1	7.366
34.0	4.588	64.0	5.358	87.0	6.126	99.2	7.409
36.0	4.642	66.0	5.412	88.0	6.175	99.3	7.457
38.0	4.694	68.0	5.468	89.0	6.226	99.4	7.512
40.0	4.747	70.0	5.524	90.0	6.282	99.5	7.576
42.0	4.798	72.0	5.583	91.0	6.341	99.6	7.652
44.0	4.849	74.0	5.643	92.0	6.405	99.7	7.748
46.0	4.900	76.0	5.706	93.0	6.476	99.8	7.878
48.0	4.950	78.0	5.772	94.0	6.555	99.9	8.090

1 Science, 79: 38, January 12, 1934.

2 A. M. Hemmingsen, *Quart. Jour. Pharmacy and Pharmacol.*, 6: 39 and 187, 1933.

3 J. H. Gaddum, Med. Res. Counc. Spec. Rept. 183, His Majesty's Sta. Of., 1933.

4 K. Pearson, "Tables for Statiticians and Biometricians. Part I," Cambridge.

5 T. L. Kelley, "Statistical Method," Macmillan, 1923.

equal 5 minus the deviate read from one of these tables; and above 50 per cent. kill 5 plus the corresponding deviate. For convenience, these corrected probits are shown in Table I for the same percentage kills as before.

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WHO'S WHO IN THE BERI-BERI VITAMIN FIELD

I HAVE been surprised and somewhat overwhelmed by the amount of publicity which our recent work on the antineuritic vitamin has had. The press has perhaps naturally ignored a great deal of other work of equal or greater importance. In the April 6 issue of the *World-Telegram* there was an editorial, the overemphasis of which upon my own achievement I have attempted to correct by writing the editor of that newspaper along the following lines:

Your editorial of April 6th on the beri-beri vitamin is one of a gratifying series of newspaper recognitions of the work of our group, Mr. R. E. Waterman, Mr. John C. Keresztesy, Miss Marion Ammerman and myself. As is probably inevitable, popular taste for a hero being what it is, the press articles have generally accorded me an over-generous share of the credit, to the detriment of this group of loyal collaborators. I wish here publicly to record their substantal part in the undertaking and our debt to Dr. W. H. Eddy of Teachers College whose interest and influence has been indispensable to success.

But I am especially concerned about your comment in that editorial on the work of Eijkman. His was an achievement of first rank. Without the experimental production of the disease in animals progress would have been impossible. While it may seem obvious now that the disease can be produced by feeding animals on polished rice, it was not obvious then. Indeed the disease he produced was not generally accepted as beri-beri for fifteen years after Eijkman's first paper. During this time his conclusions had to be reinforced by supplementary work of Pol, Grijns, Fraser, Strong, Vedder, Andrews and a score or more of others.

Notable names in the subsequent developments include Casimer Funk, a Pole, who while working in London first correctly guessed the general nature of the curative substance, Seidell of Washington, D. C., who invented the use of fullers' earth for adsorbing the vitamin, and Jansen and Donath who working in Eijkman's former laboratory in Java, first isolated small amounts of the substance and described it. Peters of Oxford, England, Ohdake of Japan, Windaus of Germany have also made important advances. I could, however, fill a column of your paper with the names of those who in various ways and in many lands have added their bits to the beri-beri vitamin problem.

Science is international. Science at its best is also a fraternity. As in other fields of endeavor, we must recognize that in reaching for our objectives we stand on the shoulders of our predecessors and companions.

R. R. WILLIAMS

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NEW YORK, N. Y.

"WHEN THE SKY RAINS STONE"

IN the issue of *The Literary Digest* for March 17 there appears an article under the caption, "When the Sky Rains Stone." The article is presented under the name of the present writer. The facts are that the article was written by a professional magazine writer after an interview and was not seen by the present writer until its appearance on March 17.

Unfortunately the article does not in every instance present the views of the man whose name it bears. He wishes to use this opportunity for disclaiming its authorship.

H. H. NININGER

DENVER, COLORADO
MARCH 28, 1934

WHO PAYS REPARATIONS?

From time to time, in the columns of this and other journals, gentle voices of protest have been raised against the prices for scientific books charged by the German publishers. I would like to draw particular attention to the latest flagrant example of "gouging the public."

We are informed that the most recent "supplement" volume to "Beilstein" can be supplied to us at the modest price of $60.55! Since, unfortunately, "Beilstein" still remains the bible of the organic chemist, since these "supplement" volumes come out ever so often, and since one must, after all, keep up-to-date, why not charge any fancy price that you want to charge? Apparently, so argue the Germans.

We in the department of chemistry at the college have decided not to get any further volumes until the Germans cut down these "reparation payments."

BENJAMIN HARROW

CITY COLLEGE, COLLEGE OF
THE CITY OF NEW YORK

REPORTS

GRAVITY STATIONS ON THE NILE DELTA

IN a report on the geodetic work accomplished in Egypt for the years 1930 to 1933, which was presented at the meeting of the International Geodetic Association held in Lisbon, Portugal, in September, 1933, is a brief account of the gravity survey that was made over the Nile Delta.

Many geologists have felt that the earth's crust is