ON SUGGESTED FORMULAE CONNECTING DOSAGE AND DEATH TIME.

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(With Four Charts.)

Several attempts have been made at various times to obtain a mathematical expression of the relation between dosage, body weight and death time. The subject has recently gained importance through the interesting publications of Dreyer and Walker, who have put forward such a formula, and have illustrated its application to toxicity determinations for a number of substances, and, in particular, for different samples of diphtheria toxin—a matter of considerable practical importance. I propose to examine the theoretical basis of their formula, and its relation to formulae which other workers have suggested, and to criticise the evidence with which they have illustrated its application to experimental material.

The formula proposed by Dreyer and Walker[1,2] to connect the dose of any toxic substance with the lethal time is

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k\,(T_0 - T_1),$$

where D_0 and D_1 are "surface doses" corresponding to the times T_0 and T_1 in which the death of the animal takes place, and a and k are constants for the particular toxin and species of animal used. The surface dose is calculated from the formula $D = \frac{d}{W^{0.72}}$, where d is the actual dose administered, and W the weight of the animal.

[1] *Lancet*, April 11th, 1914, p. 1023.
[2] *Biochemische Zeitschrift*, 1914, vol. lx. p. 112

https://doi.org/10.1017/S002217240000591X Published online by Cambridge University Press

The formula consists essentially of two parts, (*a*) the surface dose, (*b*) the connexion between dose and lethal time.

(*a*) *Surface dose.* Dreyer and Walker[1] suggest that the blood volume in warm blooded animals is a function of the body surface. From this they deduce that dosage should be calculated in terms of body surface instead of body weight, on the assumption that the activity of a toxic substance depends upon the concentration in the blood. This assumption may be justified in the case when an acutely acting poison is injected directly into the blood stream. On the other hand, more slowly acting poisons, such as bacterial toxins, are probably fixed rapidly by the tissues, and it is even conceivable that they may have practically disappeared from the blood, before the toxic action is manifest. In such cases the maximum concentration in the blood cannot bear a strict relation to the magnitude of a dose given hypodermically. It is of interest to note that dosage according to surface was recommended some years ago by B. Moore[2] from quite different theoretical considerations. It is possible, therefore, that Dreyer and Walker's dosage in relation to surface may be correct, although deduced from a doubtful assumption. Within the limits of a single species, and with animals of a sufficiently uniform age and condition to exclude other unknown factors of variation, the correction made by substituting calculated surface for weight will often be within the limits of accuracy of toxicity experiments. The use of $W^{\frac{2}{3}}$ as suggested by Moore and previously by Dreyer and Roy[3] will probably yield as accurate results as $W^{0 \cdot 72}$ determined by Dreyer and Walker, and is much easier to use in dealing with large numbers of experiments. If the doses for two animals are being compared, and the weight of one animal is double that of the other, the difference between the results obtained by the two formulae is less than 4 per cent. If the ratio between the weights of animals is greater than 2 : 1, the comparison between the dosage must be a very rough one, because it is found in practice that there is considerable variation, due to age and condition of the animals, and either formula would give an equally good rough approximation.

(*b*) *Connexion between dose and lethal time.* Dreyer and Walker, having deduced their equation from theoretical considerations, illustrate its application to a number of data from their own and others'

[1] *Proc. Roy. Soc.* B. vol. LXXXVII. 1914, p. 319.

[2] *Biochemical Journal*, 1909, vol. IV. p. 323.

[3] *Journ. of Path. and Bact.* 1909, vol. XIII. p. 344.

experiments, and claim that it fits the results with great precision. I propose to examine both the theoretical basis of their equation, and their claims to have established it experimentally, the theoretical discussion of the equation being dealt with under the following headings:

1. General criticism of the equation.
2. The theoretical basis of the equation.
3. The value of the constants.
4. Comparison with other suggested equations.

1. *General criticism of the equation.* Dreyer and Walker start from the difficulty of comparing two toxins on the basis of their lethal doses for a standard time, since they find that, with different times, different ratios of activity are obtained for the same toxins. They point out, therefore, that a true measure of toxicity cannot be obtained from the dose that kills in an arbitrarily fixed time. To overcome this difficulty they have suggested the equation

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k(T_0 - T_1),$$

in which a is the non-effective dose and k a constant which, they state, is the true toxic value of the toxin. Now this statement involves several assumptions.

(*a*) It is assumed that k and a bear some single relation to one another; otherwise two toxins could exist for which k was the same, but a differed.

(*b*) It is assumed that if two toxins have the same k and a they kill at the same rate. The equation does not necessarily involve this. It is conceivable that two toxins giving different results may have the same values for the constants; for this to be the case, it is necessary that the lethal time for each dose of one toxin should differ always by a constant amount from the lethal time for the same dose of the other toxin. For instance, for doses D_0, D_1, etc. the lethal times for one toxin being T_0, T_1, etc., those of the other toxin might be $T_0 + x$, $T_1 + x$, etc., and the formula would still hold good in each case; for if it is true that

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k(T_0 - T_1),$$

it is equally true that

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k\{(T_0 + x) - (T_1 + x)\}.$$

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In other words, the knowledge of the value of the constants a and k can only give the difference between the lethal times for two different doses, and not the actual lethal times.

This point can be made clearer by giving figures for two such toxins. In the following table the lethal times are given for various doses of toxins X and Y. Toxin X corresponds to the theoretical value given by Dreyer and Walker for their toxin C, and toxin Y is an imaginary toxin. In both cases $k = 665 \times 10^{-7}$, $a = 2200$.

TABLE I.

Dose (D)	*Toxin X* Lethal time (T)	*Toxin Y* Lethal time (T)
2496	76·0	69·7
2659	57·8	51·5
2788	51·0	44·7
2953	45·0	38·7
3039	43·3	37·0
3283	39·0	32·7
3555	36·3	30·0

The connexion between dose and lethal time for these two toxins is shown graphically on Chart I.

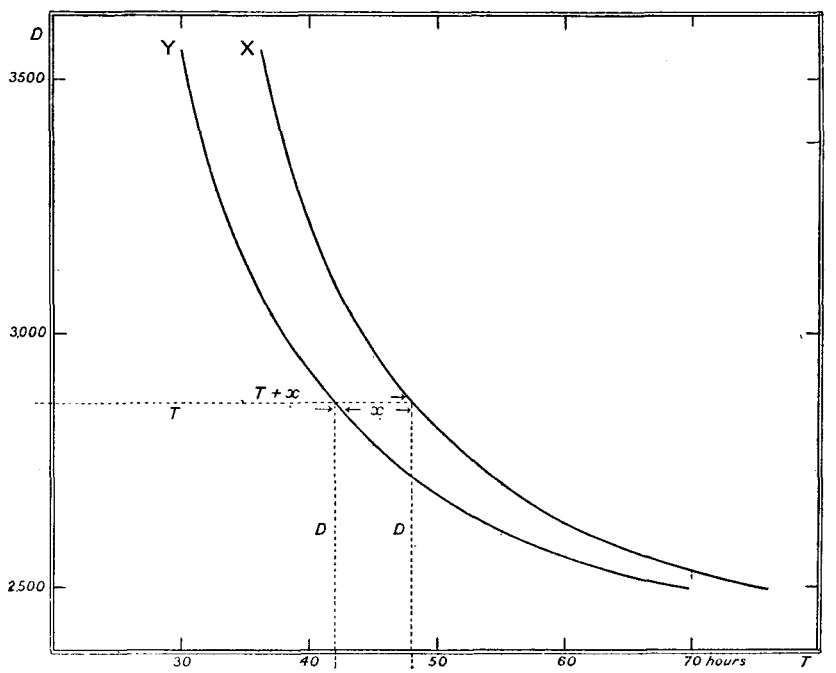

Chart I.

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It is, of course, possible that, owing to some inherent characteristic of the action of toxin, no two such toxins as we have supposed can exist, but this has yet to be shown. Dreyer and Walker have applied their formula to diphtheria toxin, and have compared three different toxins by the ratio of the values of the constant k. A consideration of the complex nature of diphtheria toxin, containing, as it does, toxin, toxoids, toxones, etc., leads one to doubt the possibility of a single expression giving a measure of the relative strength of a toxin.

The formula $\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k(T_0 - T_1)$ cannot fix the strength of any particular toxin, unless, in addition to the constants a and k, the value of T for some value of D is known. A reference to Chart I makes this obvious—if dose be plotted against time the points will lie upon a curve at a constant distance from either of the curves shown on the chart, if the values for a and k are the same. The knowledge of one point upon the chart is necessary before the curve can be drawn representing the connexion between dose and lethal time of the toxin. It follows that, in comparing two different samples of toxin, three values must be known, a, k, and the value of T for a certain value of D. The choice, therefore, of the value of k for comparing the strengths of different samples of toxin would appear an arbitrary one. It might be pointed out here that the necessity of knowing three values for a toxin corresponds with the present method of recording the strength of diphtheria toxin by means of the m.l.d., the L_0 dose and the L_+ dose. It is probably the presence of toxoids and toxones that complicates the comparison of the toxicity of two samples of toxin by means of the ratio of the doses that kill in a stated time. It is possible that in pure poisons, such as inorganic substances, the ratio of the lethal dose remains constant, whatever standard death time be chosen. Dreyer and Walker have shown that this is not the case when the lethal times for male and female *Gammarus* in various concentrations of salt are compared. In this case, however, it must be remembered that the same poison is here compared on two sexes of the same species having different resistance, in place of comparing different toxins on animals of the same sex and species.

2. *The theoretical basis of the equation.* The simplest formula to connect dose with lethal time is $(D \times T) =$ a constant.

This formula as it stands cannot be true, and needs two modifications.

(a) If D is made infinitely large, T must become 0 for this formula

to hold. For no toxin can this be true. In the case of a quickly acting poison (such as hydrocyanic acid or cobra venom), injected intravenously, some short time must elapse between the injection and distribution in the blood stream. In practice, of course, this short time is negligible, but must be taken into consideration when dealing with the subject theoretically. In the case of slowly acting poisons injected subcutaneously, the minimal lethal time is more prolonged, and may reach 12 hours or more for some samples of diphtheria toxin. The value for T, therefore, cannot become 0, but as D is increased so T approaches nearer to the minimal lethal time. It follows that $(T - b)$ must be substituted in the formula for T, where b denotes the minimal lethal time.

(*b*) If in the formula T is made infinite D would vanish. This again is not true, because, for all toxins, there is a dose that fails to kill. As the lethal time increases, so the dose necessary is decreased, until it reaches the dose that may be termed the theoretical minimal lethal dose, *i.e.* the dose that kills in an infinite time. It is necessary, then, in the formula to subtract this dose from D, in order that this part of the formula may vanish when T is made infinite. It follows that $(D - a)$ must be substituted in the formula in place of D, where a denotes the theoretical minimal lethal dose.

The formula now reads $(D - a)(T - b) = C$. This, as will be shown later, is an alternative expression for the Dreyer-Walker formula.

The connexion between dose and lethal time may not, however, be a simple linear function, but the time may vary inversely as some function of the dose, such as the dose raised to a power or as the logarithm of the dose. In the first case the general formula would read

$$(D - a)^m (T - b) = C.$$

If, however, the lethal time varies as the logarithm of the dose, the constitution of the formula must be slightly altered, so that the logarithm of the expression for the effective dose will be equal to 0 when the lethal time becomes infinite. The formula then reads

$$\log \frac{D}{a} = \frac{K}{(T - b)}.$$

This formula has a reasonable foundation, for if we consider $\frac{D}{a}$ (*i.e.* the number of theoretic minimal lethal doses in the dose injected) to be the stimulus, and the reciprocal of $T - b$ (*i.e.* the delay in lethal time) to be the true measure of the effect, then this formula corresponds with the Weber-Fechner law.

3. *The value of the constants.* The theoretical minimal lethal dose a is the dose that just gives the required effect, *i.e.* causes death; consequently this dose can be considered as the only satisfactory lethal unit. In the case of a pure toxin it seems reasonable to assume that the lethal effect of a dose depends entirely upon the number of lethal units present. We have already seen that the effective dose must be measured as $(D - a)$, and a is the lethal unit, so the lethal effect of any dose D may be expressed as $\frac{D-a}{a}$, where a is the lethal unit or theoretical minimal lethal dose. If we are dealing with a pure toxin whose action is uncomplicated by the presence of toxoids, etc., it appears probable that, apart from variation in rapidity of absorption due to variation in concentration of the solutions injected, equal numbers of lethal units of different samples of toxin would kill in equal times. Since b is the lethal time for an infinite number of lethal units, b must be constant if we can neglect the rate of absorption. It follows therefore that $T - b$ is constant for a given number of lethal units $\left(\frac{D-a}{a}\right)$ of any sample of a pure toxin, so that for pure toxins $\left\{\frac{D-a}{a}\right\}(T - b)$ has a constant value independent of the sample of toxin under investigation. The modified form of the Dreyer-Walker equation reads

$$(D - a)(T - b) = C;$$

it follows, therefore, that $\frac{C}{a}$ has a definite value for each species of pure toxin. Later we show that C in the modified form is the reciprocal of k in the original formula. If, therefore, we accept Dreyer and Walker's equation, $a \times k$ must have a constant value for all samples of one species of pure toxin, provided that any variation in the rate of absorption of different samples of toxin can be ignored, as in the case of intravenous injections.

In subcutaneous injections, the rate of absorption of different samples of toxin probably varies sufficiently to make some slight variation in the values of the constants. When very large doses of toxin are injected subcutaneously, a large number of lethal units must be absorbed very rapidly, and as the dose is increased, so the number of lethal units almost immediately absorbed must be increased, so that theoretically infinite doses, injected subcutaneously, should kill in almost the same time as massive doses injected intravenously, the difference

in time being that which elapses before any of the toxin injected subcutaneously passes into the blood stream. Thus it follows that b, the lethal time for infinitely large doses, is very nearly the same for a toxin whether injected subcutaneously or intravenously. This should yield a simple means for testing whether the suggested values for the constant, calculated for a given toxin, are admissible or not.

Considering the case of more complex toxins, we do not know to what extent toxoids retard or accelerate the action of toxin. If toxoids have any effect then $\frac{c}{a}$ (or $a \times k$) cannot have a constant value, because the same number of lethal units of two toxins, having a different toxin to toxoid ratio, would not kill in the same time.

4. *Comparison with other suggested equations.* Warren's[1] formula for the lethal time of small crustaceans immersed in solutions of various salts is $\frac{1}{T} = k(c - n)$, where k and n are constants, and c the concentration of salt. Warren's formula cannot then be applied to the injection of toxic substances into animals, because, for most toxins, death is not instantaneous when massive doses are injected, and so the formula must be modified to $\frac{1}{T - b} = k(c - n)$, which is the same as

$$(D - a)(T - b) = C.$$

Again Ostwald and Danoscheck's[2] formula $\frac{1}{T} = k(c - n)^m$ would need modification to $\frac{1}{T - b} = k(c - n)^m$, or using our own notation

$$(D - a)^m (T - b) = C.$$

The formula in this form is a generalisation of all the other suggested formulae. Warren's formula makes $b = 0$, $m = 1$; Ostwald and Danoscheck's $b = 0$; Dreyer and Walker's $m = 1$. Craw and Dean[3] have suggested that

$$\text{Lethal Time} \times \sqrt[n]{\text{Lethal Dose}} = \text{Constant, } i.e.\ D^{\frac{1}{n}}T = C,$$

which corresponds to the general formula with $a = 0$, $b = 0$.

[1] *Journal of Microscopical Science*, 1900, vol. XLV. p. 199.
[2] *Zeitschrift für Chemie und Industrie Kolloide*, 1910, vol. VI. p. 297.
[3] *Journal of Hygiene*, 1907, vol. VII. p. 512.

The connexion between the general formula given above and that of Dreyer and Walker can be shown as follows :

The formula $$\frac{1}{(D_0 - a)} - \frac{1}{(D_1 - a)} = k(T_0 - T_1)$$

can be rewritten $$\frac{1}{(D_0 - a)} - kT_0 = \frac{1}{(D_1 - a)} - kT_1,$$

or $$\frac{1}{(D - a)} - kT \text{ is a constant.}$$

Further re-arrangement brings us to the formula

$$(D - a)(T - b) = C,$$

where a has the same value as in the original equation, C is the reciprocal of k, and b is a new constant equal to the minimal lethal time. Expressed in words, Dreyer and Walker's formula states that equal increments in lethal time correspond to equal increments in the reciprocal of the effective dose. The modified form of the same equation states that the product of the effective dose (or increase in dose over the theoretical minimal lethal dose) and the delay in lethal time beyond the minimal lethal time is a constant. Dreyer and Walker were probably fully aware of this modification of the equation, but preferred their own form, although the modification of the equation appears preferable for the following reasons : (1) it can more easily be compared with other suggested formulae, (2) any sample of toxin can be compared with another by means of the values of three constants, instead of two constants together with the value of T for a certain D, (3) in testing the formula upon experimental data the calculations are less involved.

The more general form of Dreyer and Walker's equation to correspond to $(D - a)^m (T - b) = C$ would read

$$\frac{1}{(D_0 - a)^m} - \frac{1}{(D_1 - a)^m} = k(T_0 - T_1),$$

and we have now to consider the experimental evidence given in support of their formula, and whether such evidence is sufficient to warrant the assumption that $m = 1$ if the general formula is correct.

Statistical Evidence.

(*a*) *Diphtheria toxin.* Tables II, III and IV give the results of Dreyer and Walker's own experiments upon diphtheria toxin. There

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is obviously a misprint in their table for toxin A[1], as the values given for D for each guinea-pig do not agree with the values calculated from the dose and weight in each case. In Table II given below these values have been calculated afresh from $D = \frac{d}{W^{0.72}}$, and the values agree with the average values for D for each group given by the authors.

TABLE II. (From *Biochemische Zeitschrift*, vol. LX. p. 120.)

Number	D	T (in hours)	D (average)	T (average)	T (calculated)
1	1291	220	1291	220	223
2	1315	84			
3	1333	170	1359	161	161
4	1350	192			
5	1443	192			
6	1618	125	1640	83	83
7	1677	45			

TABLE III. (From *Biochemische Zeitschrift*, vol. LX. p. 119; *Lancet*, April, 1914, p. 1023.)

Number	D	T (in hours)	D (average)	T (average)	T (calculated)
1	2370	30			
2	2552	118	2496	76·0	76·0
3	2565	80			
4	2640	100			
5	2640	38	2659	57·8	58·0
6	2661	55			
7	2696	38			
8	2745	64			
9	2800	38	2788	51·0	50·8
10	2820	51			
11	2920	40			
12	2940	42	2953	45·0	45·2
13	3000	53			
14	3015	44			
15	3020	38	3039	43·3	43·1
16	3040	44			
17	3080	47			
18	3220	44			
19	3230	49	3283	39·0	39·1
20	3258	32			
21	3424	31			
22	3525	49			
23	3560	30	3555	36·3	36·3
24	3580	30			

[1] Dr Ainley Walker has kindly pointed out to me that this misprint was corrected in the next number of the *Biochemische Zeitschrift*.

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TABLE IV. (From *Biochemische Zeitschrift*, vol. LX. p. 121.)

Number	*D*	*T* (in hours)	*D* (average)	*T* (average)	*T* (calculated)
1	1123	180			
2	1311	180	1268	148	160
3	1371	84			
4	1391	60			
5	1404	132			
6	1404	180	1415	107	107
7	1404	96			
8	1427	65			
9	1461	108			
10	1461	72			
11	1461	72			
12	1466	180	1514	89	89
13	1498	62			
14	1591	84			
15	1604	65			
16	1686	64			
17	1781	72	1739	67	67
18	1781	64			

In dealing with the results of inoculation of toxin into animals it must be remembered that individual variation is very great, and little reliance can be placed upon single results.

If we consider the results given in Table II, it will be seen that the number of experiments performed (7) is small, and the agreement between observed and calculated times of death has been based in one case upon only one observation, and in another case upon the average of two observations of animals dying in 45 and 125 hours respectively. From two such discordant results no satisfactory averages can be taken. The other group consists of four observations, one of which (guinea-pig No. 2) dies earlier than four out of five other guinea-pigs injected with larger doses. The three other animals in the same group survived over twice as long as No. 2. The presence of this guinea-pig in the group lowers the average time of death so that the three out of four guinea-pigs in the group die later than the time taken to represent the death time for the group. The averages for the three groups in Table II are plotted below in Chart II, and the actual points from which the averages were taken are marked. It is obvious that, however carefully the scattered points are grouped, the coincidence of their averages upon the curve representing any formula is worthless. If one discrepant point (No. 2) be ignored, the averages for the three groups lie upon a straight line.

Turning to Table III, we find that, where the results are grouped in a certain way, the average of the observed death times for seven

different groups agrees with the calculated death times to within 12 minutes. At first sight this would appear to be strong confirmation of the accuracy of the formula. Considering the table in detail, we find that 76 hours is taken as the average death time for the first group of three observations, with death times of 30, 80, and 118 hours. There can be little reason to suppose that 76 hours represents the true average (within 12 minutes) for that group. Guinea-pig No. 1 can scarcely be included as a true result, as, with a dose represented by the figure 2370, it dies in 30 hours, whereas out of 23 other guinea-pigs, with doses ranging

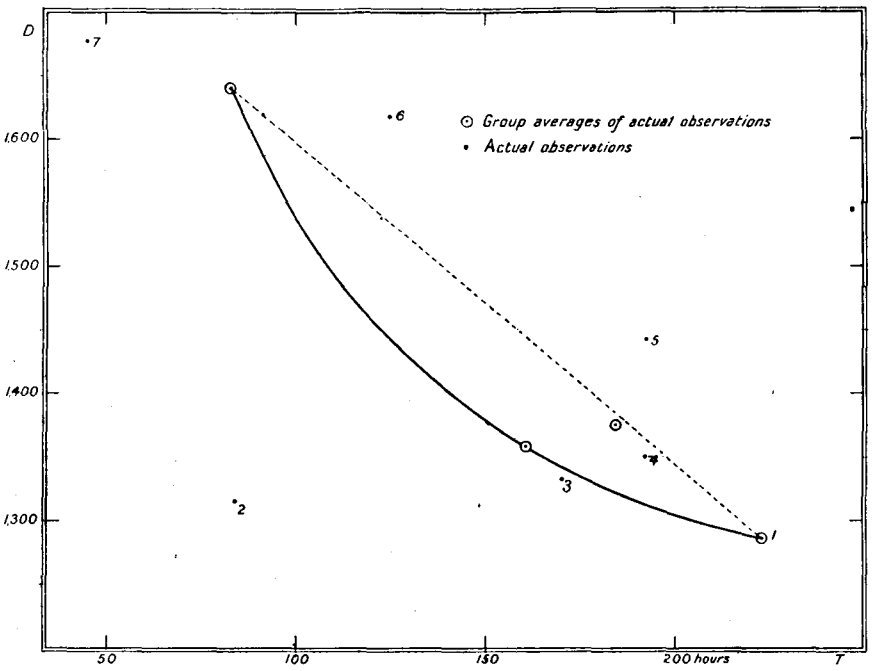

Chart II.

from 2552 to 3580, only two with the largest doses die in so short a time. Again, if we consider No. 22, we find that this animal dies in 49 hours with a dose of 3525; only one other guinea-pig, out of 11 injected with a greater dose than 3000, survives for this length of time. The absence of these two observations would make a very great difference to the average observed death time for the first and last groups, and, as will be seen later, these figures are of special importance.

It is only when a special choice of groups is made that any regularity

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of results is obtained. If the results are grouped into divisions so that the extreme value for D does not vary more than 200 (well under 10 per cent.) in each division, we obtain averages as in Table V, which vary considerably from the calculated figures according to Dreyer and Walker's formula, if we accept the value of constants given by them for the particular toxin.

TABLE V.

Numbers	Range of value of D	Average value of D	Average value of T	Calculated value for T
1	2370	—	30	—
2— 8	2552—2745	2642	70·4	57·6
9—13	2800—3000	2896	44·8	45·2
14—17	3015—3080	3039	43·3	41·5
18—20	3220—3258	3236	41·6	38·1
21—24	3424—3580	3522	35·0	35·0

In Table VI death times are given calculated by substituting various values for a in Dreyer and Walker's formula. It will be seen that with a range of a from 1200 to 2400 and corresponding values for $k \times 10^7$ from 150 to 1126, the calculated death times for the last five groups are very near the observed times, and even for the first two groups they are almost all within the limit of variation. It will be seen that it is only with the lower doses that the value for a makes any appreciable difference to the calculated death times. It is probable that many of the theoretical values of a given in the table could be shown to be inadmissible, if further experiments were made with lower doses. When we consider that a represents the dose that would kill in infinite time, the value for a must be greater than the dose that invariably fails to kill, and allows the animals to recover in weight. If use is made of the modified formula, other values may be discarded as giving an inadmissible value for b which can easily be ascertained by a few experiments upon the lethal time for very large doses.

TABLE VI.

				Calculated lethal times for various values of a				
Group	Average D	Variation in lethal time	Average lethal time observed	a=1200 $k\times10^7$= 150 b= 7·1	1600 241 12·4	2000 450 22·2	2200 665 25·2	2400 1126 28·9
1	2496	30—118	76·0	58·3	60·6	66·4	76·0	121·4
2	2659	38—100	57·8	52·6	53·5	55·3	58·0	63·2
3	2788	38— 64	51·0	48·9	49·2	49·8	50·8	51·8
4	2953	40— 53	45·0	45·0	45·0	45·0	45·2	45·0
5	3039	38— 47	43·3	43·2	43·2	43·1	43·1	42·8
6	3283	31— 49	39·0	39·0	39·0	39·0	39·1	39·0
7	3555	30— 49	36·3	35·3	35·6	36·1	36·3	36·6

The figures given in Table VI show that unless a long range of tests be made no very definite value can be assigned to k which, it is claimed, should be taken as a measure of the strength of a toxin.

On Chart III where values for D are plotted against T the result of each observation is marked together with the average for each group lying on the curve marked D. Considering the distance apart of the various points, any curve, giving points within a few hours of the group averages, must be taken as giving a reasonable formula. Curves B, D, E represent Dreyer and Walker's formula

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k(T_0 - T_1),$$

with a equal to 1200, 2200 and 2400 respectively (see Table VI). The other two curves A and C represent $(D - a)^m (T - b) = C$ where $m = 2$ and $\frac{1}{2}$, $a = 1200$, 2400; $b = 15$ in each case and $C = 92 \times 10^6$ and 705 respectively. Curve B cannot be considered as giving a true interpretation of the points, but is depicted with the object of showing how important the end points are, for between the doses of 2800 and 3200 all the curves shown coincide within a limit of two hours, a difference which must be taken as negligible, when we consider the individual variation between the separate observations. The chart helps to show graphically how discrepant the results of observations 1 and 22 are from the others. Without these two points the average for the upper group would lie much further to the left, and that of the lower group further to the right. From Chart III it will be seen that curves C and E and possibly A fit the experimental results as satisfactorily as D. We have here a series of 24 observations and find it quite impossible to choose between the formulae

$$\frac{1}{D_0 - 2200} = \frac{1}{D_1 - 2200} = \frac{665\,(T_0 - T_1)}{10^7},$$

$$i.e.\ (D - 2200)(T - 25) = \frac{10^7}{665},$$

$$\frac{1}{D_0 - 2400} = \frac{1}{D_1 - 2400} = \frac{1126\,(T_0 - T_1)}{10^7},$$

$$i.e.\ (D - 2400)(T - 29) = \frac{10^7}{1126},$$

$$\frac{1}{\sqrt{D_0 - 2400}} = \frac{1}{\sqrt{D_1 - 2400}} = \frac{1}{705}(T_0 - T_1),$$

$$i.e.\ (D - 2400)^{\frac{1}{2}}(T - 15) = 705.$$

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If a number of further observations were made at each end of the scale, so that the curve could be considerably extended towards the asymptotes, the choice of formulae would be more limited. The curves depicted upon the chart by no means exhaust the possible equations, but are merely given for the purpose of showing that, with a very limited portion of a curve, it is possible to apply almost any equation by careful choice of the constants. For example, the logarithmic formula $\log \frac{D}{a} = \frac{k}{T-b}$, if plotted on the chart would be indistinguishable from curve D except at the two end points, and even here the difference is very slight.

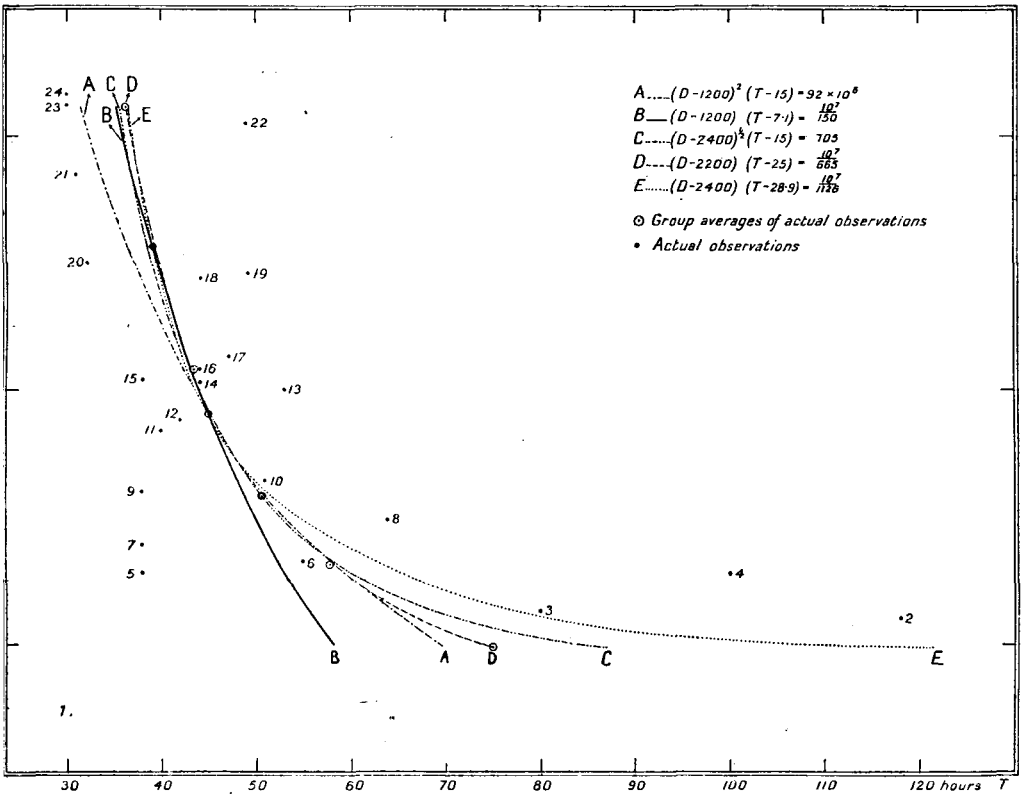

Chart III.

Toxin C (Table IV) again shows careful selection in grouping observations to form averages. It will be seen that three guinea-pigs received the same dose (1461), yet one, dying later than the others, is included in group 2, and the others in group 3. This table also shows the fallacy

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of averaging lethal times; in a series of experiments one animal may die considerably later than all the others, and, when a simple average is taken, a much higher figure is obtained than corresponds to the majority of the experiments. In the third group of Table IV five guinea-pigs die between 62 and 84 hours, yet, owing to the presence in the group of one pig (No. 12) with a delayed death, the average lethal time has been taken as 89 hours, which is later than the observed death time for five out of six of the animals in the group, and 18 hours later than the average of the five animals that give consistent results.

Chart IV gives the curves for the following formulae:

$$\text{(A)} \quad \frac{1}{D_0 - 1045} - \frac{1}{D_1 - 1045} = \frac{316\,(T_0 - T_1)}{10^7}$$

$$\text{or} \quad (D - 1045)\,(T - 21{\cdot}5) = \frac{10^7}{316},$$

$$\text{(B)} \quad \frac{1}{(D_0 - 600)^2} - \frac{1}{(D_1 - 620)^2} = \frac{(T_0 - T_1)}{545 \times 10^5}$$

$$\text{or} \quad (D - 600)^2\,(T - 25) = 545 \times 10^5,$$

$$\text{(C)} \quad \frac{1}{\sqrt{D_0 - 1200}} - \frac{1}{\sqrt{D_1 - 1200}} = \frac{(T_0 - T_1)}{1223}$$

$$\text{or} \quad (D - 1200)^{\frac{1}{2}}\,(T - 20) = 1223.$$

Further observations on the toxin would doubtless show the value of a in curve B to be inadmissible. Upon the chart are also marked the individual observations, and it must be agreed that any of the three curves depicted can represent these points equally well. The presence of a number of other points representing the lethal times for much larger and smaller doses would greatly assist in deciding the shape of the curve. The curves marked on the chart are not intended seriously to represent the true results of this toxin, but are arranged to fit in with the average of the groups as arranged by Dreyer and Walker, and it will be seen that, if these averages were truly correct, and obtained from numerous observations, the various curves chosen fit almost equally well. It is obvious that before any formula can be demonstrated to connect dose with lethal time in the case of diphtheria toxin in guinea-pigs, it is essential that a much larger number of observations be made upon each toxin, and that these observations be spread over a much wider range of dosage.

(*b*) *Other toxic substances.* Dreyer and Walker have also tested their formula upon the results of Schultz for the injection of synthetic adrenalin into mice, and their figures are given in Table VII. It will

be seen again that the coincidence of observed and calculated lethal time depends upon the grouping of the results; observation No. 22 with a dose of 2530 belongs rather to group 5 with doses of 2538, 2663, and 2700 than to group 4 with other doses ranging from 2234 to 2420. This slight, but obviously rational, adjustment alters the average for the groups to D 2323, T 20·6 and D 2608 and T 13·2, bringing

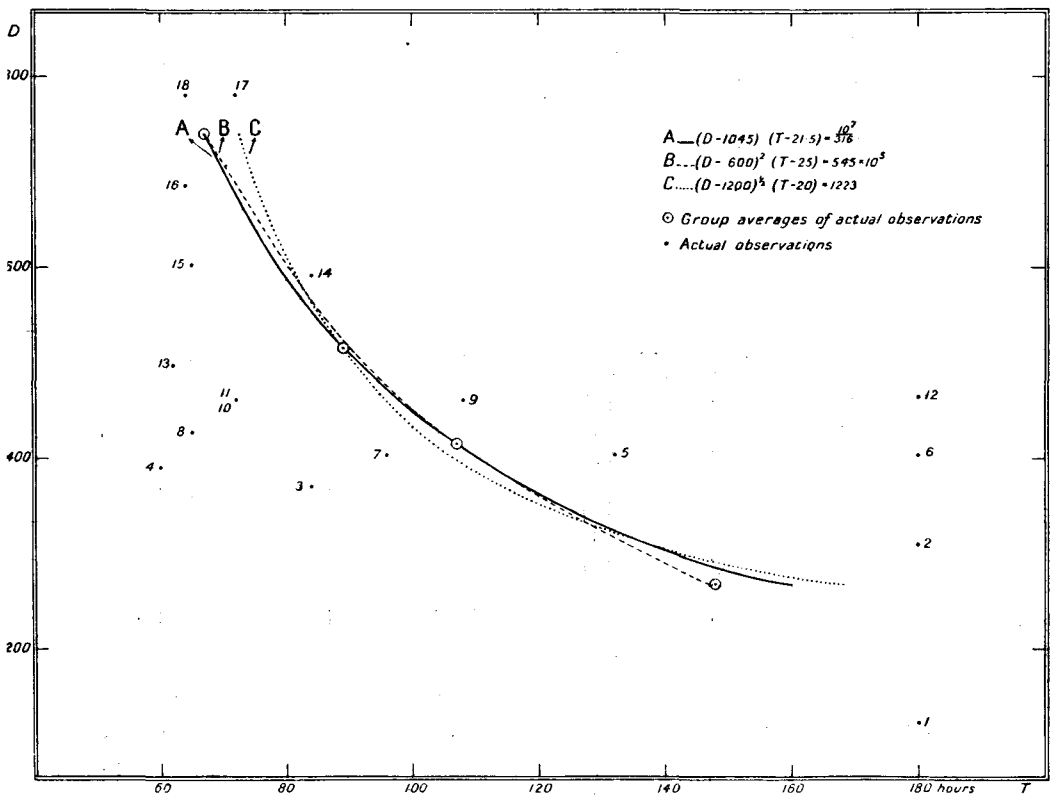

Chart IV.

the observed results still further from the calculated ones. The average for group 1 cannot be considered as reasonable, as it is taken from a group of six observations with a range of death times varying from four minutes to 92 minutes. In addition to this, a reference to Schultz's original paper shows that three mice, that should be included in this group, have been overlooked. These mice (Nos. 170, 258, 262) were all injected within the period of time chosen by Dreyer and Walker with doses

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1741, 1622, and 1637, and all lived. We must conclude, therefore, that the result of the second and third groups can only be considered, and it is obvious that there is a great choice of formulae that would fit these figures.

TABLE VII. (*From Biochemische Zeitschrift*, vol. LX. p. 122.)

Number	*D*	*T*	Average *D*	Average *T*	Calculated *T*
1	1622	35			
2	1632	92			
3	1641	11	1677	30·7	30·7
4	1699	10			
5	1717	32			
6	1749	4			
7	1800	17			
8	1803	14			
9	1919	41	1886	25·0	25·0
10	1940	33			
11	1970	20			
12	2002	24			
13	2035	23			
14	2045	22	2051	22·0	22·0
15	2075	23			
16	2100	18			
17	2235	19			
18	2278	27			
19	2320	7	2357	18·8	18·2
20	2360	12			
21	2420	38			
22	2530	10			
23	2538	19			
24	2663	11	2634	14·3	15·9
25	2700	13			

Two tables have been published[1] giving the results of individual guinea-pigs injected with cobra venom; these show great variation among themselves and in one case the observed lethal times for six different pigs are compared with the calculated times and the percentage errors are -2, $+16$, -13, -24, $+65$ and -11 per cent.; in the other case eight observations are compared and the percentage errors are -1, 0, 0, -24, $+66$, -1, -7, and $+5$ per cent. The variation is considerable, as would be expected with observations on single animals, and from what we have seen from the curves given above for diphtheria toxin, it is probable that other formulae would fit equally well.

The conclusions drawn from the comparison of Dreyer and Walker's

[1] *Biochemische Zeitschrift*, 1914, vol. LX. p. 124.

formula with that of Ostwald and Danoscheck applied to *Gammarus* are instructive. Two tables are given[1] with 10 observations upon the male *Gammarus* and 11 on the female, and the observed lethal times are compared with those calculated according to the formulae

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k(T_0 - T_1)$$

and

$$\frac{1}{T} = k(c - n)^m.$$

The difference in the formulae is better seen from the comparison

$$(D - a)(T - b) = C$$

and

$$(D - a)^m \times T = C.$$

Both formulae fit equally well, and the average errors in the two tables are 4·50 and 4·42 minutes for Dreyer and Walker's formula, and 4·48 and 5·94 minutes for Ostwald and Danoscheck's. This shows again how alternative formulae can be applied equally well. In this instance Dreyer and Walker have shown that Ostwald and Danoscheck's figures are inadmissible, in that they have to choose a lower value for the non-effective dose for the more resistant animal than for the less resistant.

A similar objection may be urged against Dreyer and Walker's figures. They state elsewhere in their paper that in " toxic substances of the same quality the weaker toxin has a larger k in the formula." It seems reasonable to suppose that if the formula is applied equally to testing one and the same toxic substances upon unequally resistant strains of a single species, and also to testing different samples of a toxin upon animals of equal resistance, then the toxic substance in the first case behaves to the more resistant strains as a weaker toxin in the second case. It follows that the more resistant male *Gammarus* should give a higher value for k, but we find that the values given by Dreyer and Walker are 841×10^{-6} for the male, and 1055×10^{-6} for the female.

Practical application of the formula. Dreyer and Walker claim that by use of their formula "a great saving both of time and animal material is introduced," and that " the results obtained will also possess a greater validity and a wider application than it has been possible to attain by the use of an arbitrary death time and a fixed standard weight of experimental animals."

[1] *Biochemische Zeitschrift*, 1914, vol. LX. pp. 127 and 128.

It is difficult to see upon what this claim is based. The advantages claimed for the equation may be divided as follows :

(*a*) the use of surface doses,

(*b*) the connexion between dose and lethal time,

(*c*) comparison of toxins by means of their constants.

We will consider these points more particularly in connexion with the testing of diphtheria toxin on guinea-pigs.

(*a*) *Surface dose.* In the form of $D = \frac{d}{W^{\frac{2}{3}}}$, a formula connecting dose and body weight has been in use for a number of years, but in practice the advantages of using guinea-pigs of a standard weight (from say 240–280 grammes) exceed the disadvantages of selecting animals within a short range of weight. By always using animals of approximately the same weight, the size of local reaction, and the change in weight occurring within two days of the injection, are extremely useful in giving early information of the ultimate result of the test. If the size and weight of animals in constant use is spread over a wide range, interpretation of early results is far more difficult, owing to the variation in standard of measurement. The use of animals of different weights is limited to a range of about 230 grammes to 500 grammes or even less. Outside this range it is found in practice that the individual variation in animals is very great.

(*b*) *Connexion between dose and lethal time.* It is customary to accept, as the minimal lethal dose of a toxin, that dose that kills say five out of six guinea-pigs within 24 hours on either side of the standard time limit. Very few animals are needed to obtain rough limits within which the required dose must fall, and then about three sets of three, six or more animals (according to the accuracy required), are injected with graduated doses within these limits. To apply Dreyer and Walker's formula, a large number of animals are required to obtain the value of the constants, and the lethal times for at least three doses over a long range must be accurately ascertained. The use of Dreyer and Walker's equation cannot increase the reliability that can be placed upon individual results. In the normal method of testing, isolated results among the orientating tests give rough indications of the dose to be tested ; the actual minimal lethal dose, being tested by direct experiment, prevents any misconception of the strength of the toxin, due to any inaccuracy in such individual results. In the Dreyer and Walker method such individual results may give misleading values for the constants, unless each result is confirmed by a number of others. From

this it appears clear that no saving in animals is effected; nor is there any saving in time, because, if it is required urgently to know the dose that will kill in a certain time, then, to avoid waiting for the results of orientating experiments, a number of guinea-pigs at each of three or four doses may be injected, but in place of solving the equation for the values of the constants, and again substituting in the equation to obtain the dose for a required lethal time, all that is necessary is to plot the observed times and doses, and obtain the required dose by interpolation upon the curve so obtained.

(*c*) *Comparison of toxins by means of their constants.* It is difficult to understand any practical need for a theoretical ratio between the toxicity of two samples of toxin. It is usually required either to know the ratio of the dose lethal in a given time, or, more frequently, the comparative immunising values of toxin. In the former case the present method of testing the minimal lethal dose is satisfactory, and, in the latter case, the comparison needed is not between the toxicity of two specimens, but between the binding unit contents. This comparison is made by means of the Lo dose, which can be determined with sufficient accuracy upon far fewer animals than are needed for the determination of the minimal lethal dose.

In Dreyer and Walker's experiments upon three samples of diphtheria toxin, k appears to bear a fairly constant relation to a—for the three toxins A, B and C $\frac{k \times 10^7}{a} = 3{\cdot}45$, $3{\cdot}308$, and $3{\cdot}307$; if this is a true relation for all samples of diphtheria toxin, then the suggestion that the strength of a toxin should be determined by the value of the constant k, resolves into a suggestion of recording the dose that barely kills as a true measure of the toxicity of any sample of toxin, and this appears theoretically to be the true standard to adopt. If, on the other hand, no true relation exists between a and k in complex toxins, then we must again consider the value of each constant a, k and b when recording the value of a toxin, and the basis of comparison made between the toxins must depend upon the result required.

We have already seen, under the heading "The value of the Constants," that in the case of pure toxins injected intravenously it is probable that, if Dreyer and Walker's equation be accepted, $k \times a$ is a constant so that it seems reasonable to conclude that the agreement in the value of the expression $\frac{k}{a}$ for the three samples of diphtheria toxin is purely adventitious.

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General Conclusions. Theoretical consideration does not show Dreyer and Walker's equation to be inadmissible.

On the other hand, the experimental evidence published in support of their formula is by no means conclusive, and could be applied equally well in support of other formulae—all suggested formulae and modifications of the general formula

$$(D - a)^m (T - b) = c.$$

Dreyer and Walker's choice of a formula (making $m = 1$ in the general formula) can only be justified if supported by a far wider range of experiments.

I am now testing the formula by data, obtained experimentally, of the lethal times for a wide range of doses of diphtheria toxin.