A NOMOGRAM FOR 50 % END-POINT INTERPOLATION

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(With 1 Figure in the Text)

In certain types of titrations the experimental results are obtained as percentages, say of mortality, at different dilutions in geometric progression of the test material. The result of a titration is then expressed as the 50 % end-point which is estimated by interpolation as follows.

If a is the percentage mortality at a dilution $1/A$, b the percentage mortality at a dilution $1/B$, $(a > 50 > b)$, and D $(= B/A)$ the dilution factor, then E, the estimated titre, or the reciprocal of the dilution giving the 50 % end-point, is obtained from the equations

$$E = AD^f,$$

where $f = \dfrac{a-50}{a-b}$; i.e. $\log E = \log A + f \log D.$

The arithmetic calculation of E is a simple matter but may involve much time. Great saving of time with negligible loss of accuracy may be effected by the use of a nomogram, shown in Fig. 1. This enables the 50 % end-point to be estimated rapidly, for a titration in which either twofold or tenfold dilutions are used.

The Z-shaped diagram in the middle of the chart gives the value of f. The scales at the sides give the values of E, that on the right being applicable when the titrations are in twofold dilutions, and that on the left when the titrations are in tenfold dilutions. The two rows of figures heading the columns of the right-hand scale readings are the values of the small intermediate divisions, the first row for use above and the second row for use below point X of the scale.

HOW TO USE THE CHART

The use of the chart is best explained by an example. Suppose that the results of a virus titration show percentage mortalities of 80 and 33 for virus dilutions 1/8 and 1/16 respectively. The percentage figures may be either those actually occurring or those calculated by the method of moving averages (Thompson, 1947). It is required to estimate the 50 % end-point titre of the virus preparation, that is the reciprocal of the virus dilution which would cause 50 % mortality.

Using a Perspex ruler, join the points 80 and 33 on the a and b axes. This line cuts the f axis at 0·64. With the ruler make a horizontal axis from this point to the right-hand scale AB and its columns of scale values. The appropriate scale reading will be in the column of entries extending from 8·0 to 16·0, since the 1/8 and 1/16 dilutions are known to bracket the 50 % end-point. Actually the reading is one small division in excess of 12·4, giving a virus titre of $12{\cdot}4 + 0{\cdot}08 = 12{\cdot}48$. Changing the decimal place does not affect the method. If in the given example the virus dilutions were 1/800 and 1/1600 the virus titre would become 1248 instead of 12·48. In cases where the virus dilutions bracketing the 50 % mortality

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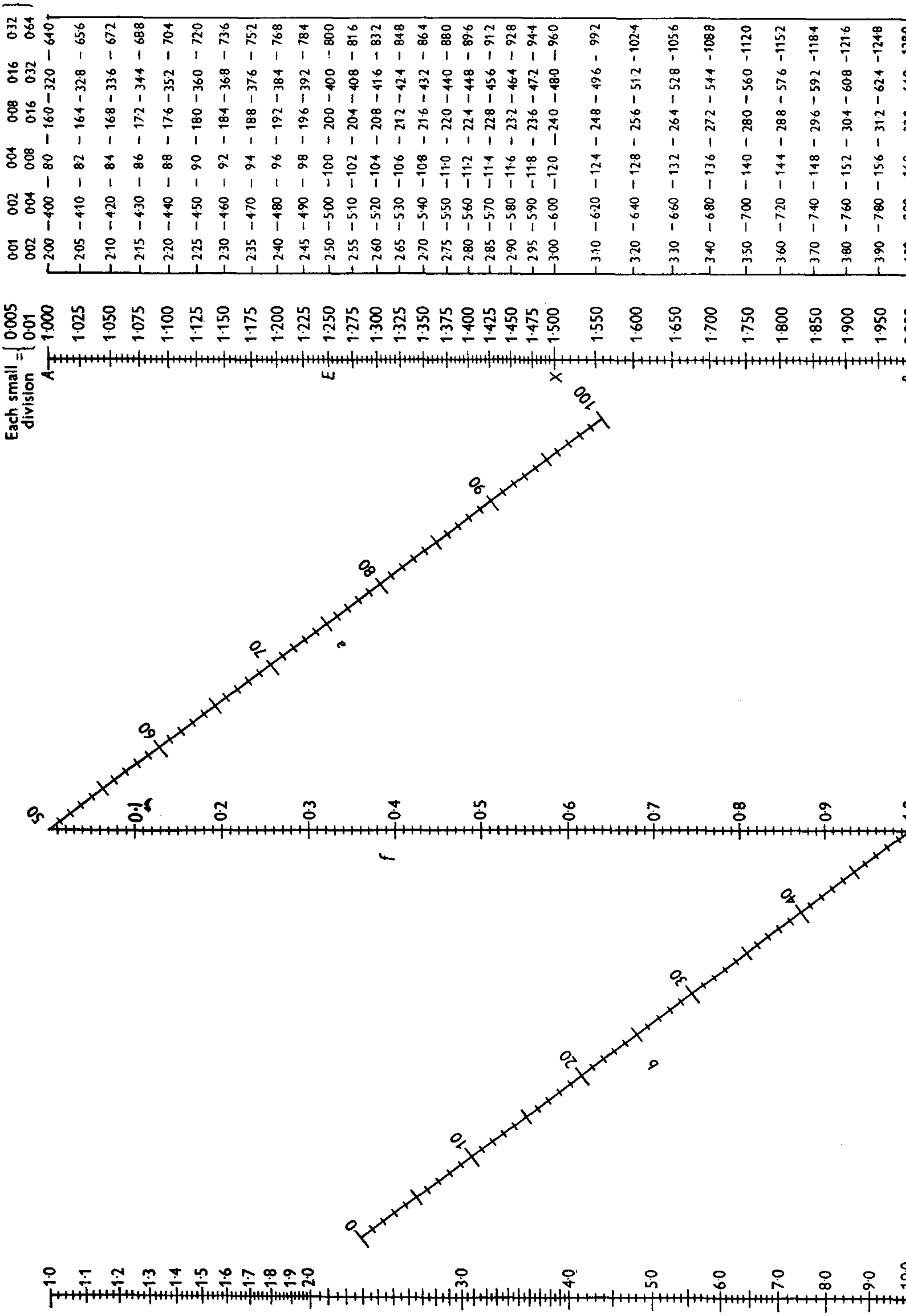

Fig. 1.

end-point lie beyond the scale values shown in the last column of the chart the first column value is multiplied by the reciprocal of the lower dilution. For example, if virus dilutions 1/512 and 1/1024 gave 80 and 33 % mortalities respectively, the virus titre would be $1{\cdot}56 \times 512 = 798{\cdot}7$.

For titrations in which serial tenfold dilutions are tested the reading obtained on the left-hand scale is multiplied by the appropriate power of 10. If by experiment virus dilutions $1/10^3$ and $1/10^4$ give percentage mortalities 65 and 45 respectively, the f-axis reading is 0·75 and the horizontal axis from this point cuts the left-hand scale at 5·6. The virus titre is therefore $5{\cdot}6 \times 10^3 = 5600$.

The chart is subject to the error of all alinement charts. The larger the chart the smaller will be the error involved in its use. A convenient size is one with a 10 in. f-stem. This allows interpolating by eye between the small divisions. The figures given in the examples quoted were obtained from a chart of this size and the arithmetical solutions (namely, 12·45 instead of 12·48, and 5623 instead of 5600) reveal errors of only 0·24 and 0·41 % respectively.

For titrations using dilutions other than twofold or tenfold, the value of f can be obtained from the Z-diagram but $E = AD^f$ must be obtained arithmetically. If, however, many such titrations are contemplated it may be worth while making a new scale of E from the equation

$$x = \frac{\log y}{\log D} S,$$

where x = the distance along the scale of a graduation, y = the value of the graduation, D = the dilution factor, and S = the length of scale. This will give values of E between 1 and D, and if the end-point lies between dilutions $1/D^n$ and $1/D^{n+1}$, E is given by multiplying the scale reading by D^n.

OTHER APPLICATIONS

The chart may be used also for graded responses, where the end-point corresponds to a response of 50 units, provided that the dilutions are in geometric progression. In this laboratory we have used the chart for finding the end-point of haemagglutination titrations by a densitometer method. Various degrees of haemagglutination are indicated by potentiometer readings, and the titre is arbitrarily taken as the reciprocal of the virus dilution which would give a reading of 50 (Belyavin, Westwood, Please & Smith, 1951).

If the end-point of a given test is taken as the reciprocal of a dilution giving a response of say, 20 units, then the chart may be used by adding $50 - 20 = 30$ to all the results. Furthermore, the a and b axes may be extended and scaled in linear units to any useful range.

This nomogram is in daily use in this laboratory and has been found to relieve much of the tedium of interpolation.

REFERENCES

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