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A useful transformation for comparing dose-response curves

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Several mathematical equations have been tested for their ability to fit pharmacological dose-response curves over the range 5 to 95% of the maximal response. It has been shown that one such equation, which will be called the L-transformation, adequately fits sets of dose-response data obtained from a number of different tissues. The graphical application of this equation has been suggested previously by other workers but has been used only to a very limited extent.

Techniques have now been developed which enable the L-transformation to be fitted to single sets of dose-response data, and to pairs of sets of data simultaneously. By itself the L-transformation cannot adequately fit *all* dose-response curves. However if another preliminary transformation is carried out on the measured responses then the L-transformation can usefully be applied to dose-response curves of widely different shapes and slopes. Various applications of these techniques are discussed. When the L-transformation is applied to pairs of dose-response curves obtained, for example, from a single piece of isolated tissue, it can be used to calculate potency ratios, with standard errors and fiducial limits. When combined with the occupation theory of drug action and use of the null method, the L-transformation can be applied to suitable pairs of dose-response curves to provide estimates of the affinity constants (with their standard errors) of drugs for their receptors. The techniques can be extended to other models of drug-receptor interaction. Computer programs are available which greatly facilitate the application of these curve fitting methods to the types of problem outlined above.

The results obtained from pharmacological experiments frequently can be summarized in the form of dose-response or log dose-response curves which are then used to obtain information about changes in the properties of the biological system or about the drug being studied. Such information is usually derived from pairs of log dose-response curves by graphical methods, unless the curves are parallel. In the latter case the slopes and intercepts of the linear region of the log dose-response curves may be calculated directly from suitable data to yield information about changes in tissue sensitivity, potency ratios of full agonists or affinity constants of competitive antagonists.

However the linear log dose transformation usually can be used only to describe dose-response data over a limited range of response (e.g. 30–70% of maximal). Parker & Waud (1971) used a logistic function to fit theoretical curves over a much wider range of responses. They have emphasised the weaknesses of simple graphical techniques for analysing sets of dose-response data and have employed the logistic function to estimate affinity constants of agonists, partial agonists and competitive antagonists for their receptors (Parker & Waud, 1971; Waud & Parker, 1971).

In the present paper an alternative method is presented for the analysis of dose-

response data, which seems to be simpler in principle than that used by Parker and Waud and is based on the use of a different type of transformation. The most useful example of this type of transformation seems to be that suggested by Kirschner & Stone (1951) and subsequently used, in a slightly modified form, by Ariëns, van Rossum & Koopman (1960).

THEORETICAL BASIS AND USE OF THE L-TRANSFORMATION

1. *The choice of transformation*

Several transformations of the general form

$$\frac{1}{F(r)} = a + \frac{b}{C_r} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad 1$$

have been tested for their ability to fit theoretical curves to sets of dose-response data. In equation 1, F(r) is a function of the response r, C_r is the concentration of agonist which produces the response and a and b are adjustable constants. This type of transformation was chosen because it has special properties which will become clear later. The functions F(r) which have been tested are log (1 + Kr), (r + L) and r^N where K, L and N are also adjustable constants. Of these three transformations, that with F(r) equal to (r + L) has been found to be the most flexible. This transformation, which for the sake of brevity will be called the L-transformation, will therefore be the only one discussed.

2. *Special properties of the L-transformation*

It will be shown in this section that if, by appropriate choice of the adjustable constants a, b and L, the L-transformation adequately fits one set of dose-response data then under appropriate conditions it would also be expected to fit other sets of dose-response data obtained on the *same* piece of isolated tissue. Although the values of a and b required to obtain a good fit for these other sets of data may differ from those required for the first set, the values of L required should not be significantly different.

(a) *Relative potencies*

Suppose that the equation $1/(r + L) = a_1 + b_1/(A)_r$ has been shown to fit adequately a set of dose-response data obtained for an agonist acting on a suitable biological system. $(A)_r$ is the concentration of agonist A which produces response r. If another agonist B is x times as potent as A then the concentration of A required to produce any chosen response is x times the concentration of B required to produce the same response. The above equation may then be written in terms of the concentration of B as

$$1/(r + L) = a_1 + b_1/[x(B)_r]$$
$$= a_2 + b_2/(B)_r$$

where $a_1 = a_2$, $b_1/x = b_2$ and L is unchanged. The relative potency of the two agonists is $x = b_1/b_2$.

Estimation of relative potency by use of the L-transformation therefore has the characteristics of a slope-ratio assay (see Finney, 1964). The variance of x and its fiducial limits can be estimated from the variances and covariances of b_1 and b_2. The

condition that the values of a and L should not differ significantly for the two sets of dose-response data is the counterpart of the condition of parallelism normally applied in parallel line assays.

(b) *The null method and occupation theory*

The L-transformation, and other transformations of the same general form, can be combined with the standard equations derived by applying the null method to the occupation theory of drug action. The various assumptions made in deriving these standard equations have been discussed in reviews by Mackay (1966) and Waud (1968). The nomenclature used here will be mainly that of the former reference.

Suppose again that the L-transformation adequately fits a set of dose-response data obtained for an agonist A acting, for example, on a piece of isolated tissue. A second dose-response curve might then be obtained on the same piece of tissue using, for example:

(i) a different agonist B,

(ii) the same agonist A, but in the presence of either a competitive or non-competitive reversible antagonist,

(iii) the same agonist A in the presence of a pseudo-irreversible antagonist,

or (iv) the same agonist A after irreversibly inactivating some of the receptors in the tissue.

In each of these examples application of the null method to occupation theory, with various assumptions, yields an equation of the general form

$$\frac{1}{C_{1r}} = \alpha + \frac{\beta}{C_2} \quad .. \quad .. \quad .. \quad .. \quad 2$$

where C_{1r} and C_{2r} are the concentrations of agonist required to produce the same response r during the estimation of the first and second sets of dose-response data respectively. Depending on which of the examples (i) to (iv) is being studied these two sets of dose-response data may be obtained using a single agonist or using two different agonists. The meaning of the constants α and β also varies with the example being considered.

According to occupation theory the two sets of dose-response data are interrelated. For the first set we have for any chosen value of the response,

$$\frac{1}{r + L} = a_1 + \frac{b_1}{C_{1r}}$$

$$= a_1 + b_1\left(\alpha + \frac{\beta}{C_{2r}}\right), \text{ from equation 2}$$

$$= a_2 + \frac{b_2}{C_{2r}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad 3$$

where $a_2 = a_1 + b_1\alpha$, ... 3a

$b_2 = b_1\beta$, ... 3b

and the value of L is unchanged.

It may therefore be stated that if the L-transformation adequately fits the first set of dose-response data then it should also adequately fit the second set of data with the same value of L.

If the above statement is found to be incorrect then either the basic model is not applicable to the data or some of the assumptions are invalid. For example the stimulus-response relation may have changed during the experiment. (The concept of such a relation was introduced by Stephenson (1956). For a general discussion of these ideas see e.g. Mackay (1966).)

The above principles can be applied to examples (i) to (iv) outlined above so as to derive information from the values of a_1, b_1, a_2, b_2 and L estimated from suitable pairs of sets of data. For example, for competitive antagonism equation 2 takes the form

$$\frac{1}{(A)_r} = \frac{1}{(A)'_r}[1 + K_I(I)] \qquad .. \quad .. \quad .. \quad 4$$

where $(A)_r$ and $(A)'_r$ are the concentrations of agonist A required to produce the same response r from a single piece of tissue in the absence and in the presence of a concentration (I) of the competitive antagonist. K_I is the affinity constant of the antagonist for its receptors. Comparison of equations 2 and 4 shows that for this example $\alpha = 0$ and $\beta = [1 + K_I(I)]$, so that from equations 3a and 3b $a_2 = a_1$ and $b_2 = b_1[1 + K_I(I)]$. Therefore if an antagonist is competitive and if the L-transformation adequately fits the dose-response data for the agonist alone then

(a) the L-transformation should also adequately fit the dose-response data for the agonist in the presence of a constant concentration of the antagonist,

(b) neither the values of L nor the values of a from the two sets of data should be significantly different,

(c) $K_I = \left[\frac{b_2}{b_1} - 1\right]/(I)$,

(d) the variance of K_I may be estimated from the variances and covariance of b_1 and b_2.

The appropriate equations for calculating affinity constants (and related quantities) of agonists, partial agonists and antagonists for their receptors, from values of a_1, b_1, a_2 and b_2, can be obtained in a similar way and are presented in Table 1. Since the value of L should not be significantly different for any two sets of dose-response data which are being compared, the L-transformation should first be fitted to each set of data and then simultaneously to the two sets of data with a common value of L. The variances and standard errors of the affinity constants and related quantities can then be obtained from the variances and covariances of a_1, b_1, a_2 and b_2 using standard statistical methods (see Appendix 2).

3. *Method of fitting the L-transformation to a single dose-response curve*

The mathematical basis of the curve fitting technique is given in Appendix 1. A computer program (SLFIT) has been prepared which estimates those values of a, b, and L which minimize the sum of the squares of the deviations of the theoretical responses from the observed responses. The standard errors (s.e.'s) of a, b and L are also calculated.

The program has been written for sets of results which consist of equal numbers (NR) of responses measured at each concentration of agonist. If NR is greater than 1 then the program prints out the mean sum of squared deviations within groups and the mean sum of squared deviations about regression. A variance ratio test can then be used to test the goodness of fit. Facilities are also included for removal of variance due to changes in tissue sensitivity with time, if the doses of agonist are applied in a random block or Latin square design. Such facilities are selected by inserting appropriate code numbers along with the experimental data.

4. *Method of fitting the L-transformation simultaneously to two dose-response curves, with a common value of L*

The mathematical basis of this curve-fitting procedure is given in Appendix 2, and is closely related to that used for fitting the L-transformation to a single set of dose-response data. The appropriate computer program, named DLFIT, estimates those values of the adjustable constants a_1, b_1, a_2, b_2 and L which produce a minimum value for the sum of squared deviations of the theoretical responses from the observed responses. The theoretical responses are given by the equations

$$\frac{1}{r+L} = a_1 + \frac{b_1}{C_{1r}} \text{ for the first set of data,}$$

$$\frac{1}{r+L} = a_2 + \frac{b_2}{C_{2r}} \text{ for the second set of data.}$$

The summation of the squared deviations is over both sets of dose-response data. The program DLFIT also provides estimates of the variances and covariances of these adjustable constants. Since a common value of L is fitted to both sets of dose-response data the values of the adjustable constants will usually differ from those obtained by fitting the L-transformation to each individual set of data.

As in the case of SLFIT, the program DLFIT provides facilities for the removal of variance arising from variation of tissue sensitivity with time, provided that the experimental design justifies such a procedure.

5. *Limitations of the L-transformation, and methods of extending its use*

The L-transformation has been shown to fit experimental data obtained from a number of tissues including the ileum, vas deferens and atrium of the guinea-pig. It has also been shown to fit published dose-response curves obtained for contraction of the rectus abdominis muscle of the frog, and for depolarization of the electroplax of the electric eel. However the L-transformation does not fit *all* types of dose-response curves. For example dose-response curves obtained with guinea-pig trachea have been too steep, and there may be other dose-response curves which are too shallow.

If L is set equal to zero then the L-transformation takes the same form as the Michaelis-Menten equation, and can be rearranged to give

$$\frac{r}{r_{MAX}} = \frac{1}{1 + \frac{1}{C_r/C50}} \quad .. \quad .. \quad .. \quad 5$$

where r_{MAX} is the maximal response produced by the drug and C50 is the concentration

of the drug which produces half of the maximal response. If r/r_{MAX} is plotted against log $[C_r/C50]$ then a single standard curve is obtained as shown in Fig. 1a. The adjustable constant L in the L-transformation may also have positive or negative values, which when added to the observed responses lead to a curve similar to that shown in Fig. 1a. Thus changing the sign of L has the effect of cutting off or adding to the lower part of the standard S-shaped curve as shown in Fig. 1b. It is therefore clear why adjusting the value of L allows the transformation to be applied to somewhat distorted S-shaped log dose-response curves with slopes lying *only within a limited range.*

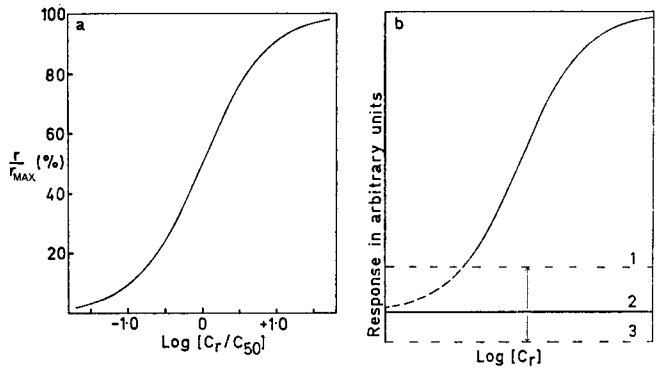

FIG. 1a. The equation for the L-transformation reduces to $r/r_{MAX} = 1/[1 + 1/(C_r/C50)]$ if the value of L is set equal to zero. In this special case a plot of r/r_{MAX} versus log $[C_r/C50]$ gives the symmetrical S-shaped curve shown above. r is the observed response and r_{MAX} is the maximal observed response.

1b. The symmetrical S-shaped curve, with the zero-response axis at position 2, corresponds to the special case when the value of L, in the equation for the L-transformation, is zero. When L is not zero the effect on the appearance of a plot of observed response versus log $[C_r]$ is as though the zero-response axis had been moved, for example, upwards to position 1 (L positive) or downwards to position 3 (L negative) as indicated by the arrows.

Fortunately this limitation of the L-transformation is not as serious as might appear at first sight. The derivation of useful information from comparison of dose-response curves depends on the use of the null method, whether the data is being used to estimate a potency ratio or for the study of drug-receptor interactions. The essential point about equations derived by use of the null method is that they can be applied either to undistorted responses, or to distorted responses provided that both sets of responses being compared have been subjected to the same distortion. The situation is essentially the same as if a single non-linear recording system was used to measure both sets of responses.

The idea of a distortion function can therefore be introduced, the purpose of this function being deliberately to change the shape of an observed dose-response curve. Such a distortion function may be of the general form

$$r/r_{MAX} = s + t(r_{OBS}/r_{MAX}) + u(r_{OBS}/r_{MAX})^2 + v(r_{OBS}/r_{MAX})^3 + \quad .. \quad .. \quad 6$$

where r is the new distorted or modified response, r_{OBS} is the original measured response, and s, t, u, v etc. are adjustable constants. In order to extend the application of the L-transformation it is desirable that the distorted responses should approximately fit equation 5, so that they can then be adequately fitted by the L-transformation.

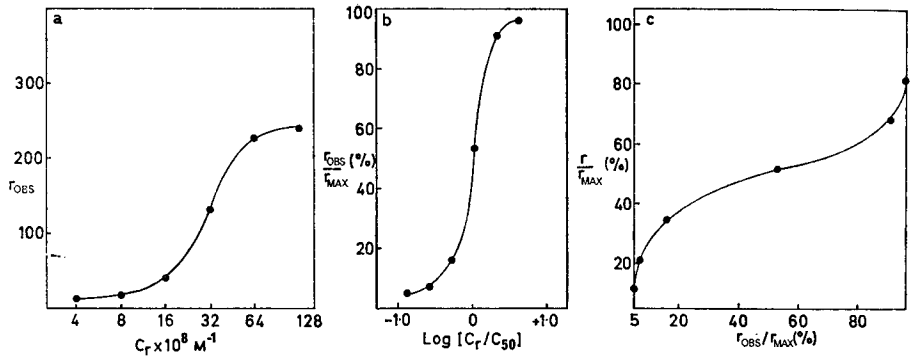

FIG. 2a. The dose-response curve shown above is assumed to have been obtained from the action of a full agonist on an isolated tissue. The responses are in arbitrary units.

2b. In the above graph the results shown in Fig. 2a have been plotted in the standardized form r_{OBS}/r_{MAX} versus $\log_{10}[C_r/C50]$.

2c. For chosen values of $[C_r/C50]$, values of r/r_{MAX} and of r_{OBS}/r_{MAX} have been read from Figs 1a and 2b respectively, and plotted one against the other as shown above.

Suppose that the single set of dose-response data shown in Fig. 2a has been obtained from the action of a full agonist on an isolated tissue. If r_{MAX} is known or can be estimated with reasonable accuracy then C50 can also be estimated and the observed responses, as percentages of r_{MAX}, can be plotted against $\log_{10}[C_r/C50]$ (see Fig. 2b). It is then possible to read from the standard curve (Fig. 1a) those values of r/r_{MAX} and from Fig. 2b those values of r_{OBS}/r_{MAX} which correspond to the experimental values of $C_r/C50$. A plot of r/r_{MAX} versus r_{OBS}/r_{MAX} then gives a curve (Fig. 2c) which corresponds to the distortion function. By fitting equation 6 to the results shown in Fig. 2c the values of the adjustable constants s, t, u and v can be obtained. If the L-transformation is applied to the dose-response data using the modified responses in place of the observed responses then ideally a good fit should be obtained with a value of L not significantly different from zero. In practice the estimates of r_{MAX} and of C50 may be inaccurate, but even then the L-transformation should have a sufficient degree of flexibility to fit the set of modified dose-response data, with a non-zero value of L.

The complete process described above is very nearly the same as fitting a transformation of the form

$$\frac{1}{g + hr + ir^2 + jr^3 \ldots \text{etc}} = a + \frac{b}{(A)_r} \qquad \ldots \quad \ldots \qquad 7$$

to the unmodified experimental data. Were this to be done for each set of dose-response data then a large number of concentrations of drug would have to be used in each experiment.

If one log dose-response curve, obtained from a chosen full agonist acting on receptors in a single piece of isolated tissue, is either too steep or too shallow adequately to be fitted by the L-transformation then it is likely that the same situation will arise with other samples of the same tissue, the various log dose-response curves having fairly similar shapes when the responses are expressed as percentages of r_{MAX}. The implicit assumption behind such a statement is that the stimulus-response relations for the samples of tissues are likely to be of the same general form. In such a case a single distortion function should be determined from a detailed set of dose-response data

obtained for a full agonist acting on an "average" piece of the tissue. A full agonist is chosen because it will cover the widest possible range of responses. This distortion function may then be applied to modify the responses obtained when agonists act on the same receptors in other samples of the tissue. Such a distortion function estimated from one piece of tissue might be that shown in Fig. 2c. Suppose now that two sets of dose-response data to be compared have been estimated on a second piece of the same tissue from the same or from a different animal. An example is shown in Fig. 3a. These responses are then converted to percentages of r_{MAX} which is the

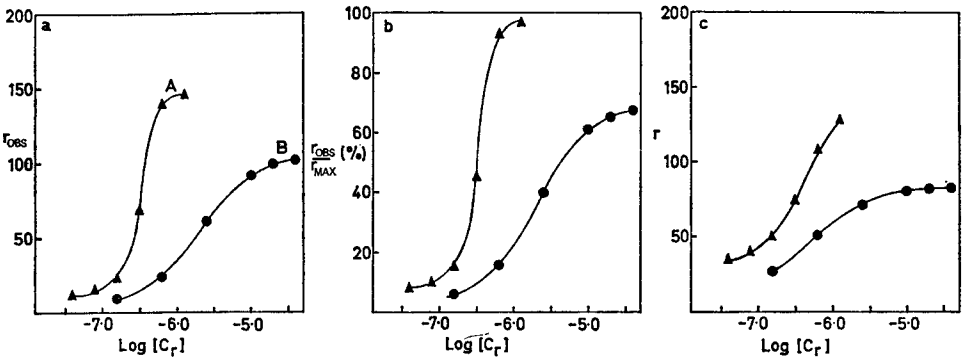

FIG. 3a. The two log dose-response curves shown in this figure are assumed to have been obtained using two drugs A and B on a sample of tissue of the same type as that used to obtain the data shown in Fig. 2c.

3b. The curves shown in Fig. 3a have been re-plotted so that the responses are expressed as percentages of the maximal response to a full agonist.

3c. For each value of r_{OBS}/r_{MAX} corresponding to an experimental value of log [C_r] a corresponding value of r/r_{MAX} has been read from Fig. 2c and converted to a value of r, knowing r_{MAX} for the tissue. In this figure the values of these modified responses, r, have been plotted versus $\log_{10}[C_r]$. Compare this figure with Fig. 3a.

maximal response obtained when a *full* agonist acts on the same receptors in the tissue. The estimation of r_{MAX} must be carried out in the absence of any other drugs and before treatment with any drugs which may produce irreversible changes in the tissue. The resulting plot of r_{OBS}/r_{MAX} versus log C_r is shown in Fig. 3b. From the distortion function (Fig. 2c) values of r/r_{MAX} are read off for each value of r_{OBS}/r_{MAX}, and converted to values of r. A plot of the modified response versus log C_r is shown in Fig. 3c. These sets of distorted responses can then be fitted by means of the L-transformation and analysed in the same way as for unmodified sets of dose-response data.

In practice the procedures described in this section are carried out by a computer program named RMOD, which can be used either to obtain a suitable distortion function from a single set of dose-response data or to obtain modified responses from observed responses if the distortion function is supplied. Of course the same distortion function and the same value of r_{MAX} must be used for each set of the pair of sets of dose-response data being compared.

6. *Extension of the general principle of the L-transformation to other situations*

The general principles of the techniques outlined in the preceding sections can be applied to any adequate transformation of the type given in equation 1 provided that F(r) is a function only of r with adjustable constants (cf. equation 7). An important assumption made in the derivation of the null equations presented in Table 1 is that only one molecule of drug interacts with each receptor. If n molecules of drug inter-

Table 1. *Relation between equations obtained by application of the null method to occupation theory, and the adjustable constants of the L-transformation.*

Nature of experimental results	Null equation	Relation between the required experimental quantity and the adjustable constants of the L-transformation
(i) Dose-response data for two agonists on the same piece of tissue	$\frac{1}{(A)_r} = I_{AB} + \psi_{AB} \cdot \frac{1}{(B)_r}$	$\psi_{AB} = b_2/b_1$
	where $I_{AB} = K_A[\beta_{AB} - 1]$,	$I_{AB} = [a_2 - a_1]/b_1$
	$\psi_{AB} = \frac{K_A}{K_B}\beta_{AB}$	Also $K_B = [a_2 - a_1]/b_2$ if $\beta_{AB} \gg 1$.
	If A and B are full agonists then $I_{AB} = 0$.	
(ii) Dose-response data for an agonist in the absence and in the presence of a competitive antagonist	$\frac{1}{(A)_r} = \frac{1}{(A)_r'} \cdot [1 + K_I(I)]$	$K_I = \left[\frac{b_2}{b_1} - 1\right]/(I)$;
(iii) Dose-response data for an agonist in the absence and in the presence of a non-competitive or pseudo-irreversible antagonist	$\frac{1}{(A)_r} = \frac{1}{(A)_r'}[1 + K_I(I)] + K_A K_I(I)$	$K_I = \left[\frac{b_2}{b_1} - 1\right]/(I)$
		$K_A = [a_2 - a_1]/[b_2 - b_1]$.
(iv) Dose-response data for an agonist before and after treatment of a tissue with an irreversible or pseudo-irreversible antagonist	$\frac{1}{(A)_r} = \frac{1}{(A)_r'} \cdot \frac{1}{[1 - y_I]} + K_A \cdot \left[\frac{y_I}{1 - y_I}\right]$	$K_A = [a_2 - a_1]/[b_2 - b_1]$
		$y_I = 1 - b_1/b_2$.

Footnote: in the above table K_A and K_B are the affinity constants of agonists A and B, for their receptors. β_{AB} is the ratio of the intrinsic efficacy of drug A to that of drug B. K is the affinity constant of an antagonist for its receptor and y_I is the fraction of receptors occupied by antagonist. a_1, b_1, a_2 and b_2 are adjustable constants obtained by fitting the L-transformation simultaneously to the two sets of dose-response data being compared.

act with each receptor in a highly cooperative way then application of the null method to such a modified occupation model would lead to an equation of the same form as equation 2 but with concentrations raised to the power n. If there were good reasons to assume that such a model might be valid then one could look for a suitable transformation of the type shown in equation 1 but with C_r raised to the power n. This could then be combined with the modified version of equation 2 to yield information in a way analogous to that described above.

A model of drug-receptor interaction which is of particular interest as an alternative to occupation theory, is the allosteric 2-state receptor model proposed by Karlin (1967) and by Changeux, Thiéry & others (1967). The null method has been applied to this model by Thron (1973) and by Colquhoun (1973). The resulting null equations are in most cases indistinguishable in form from those derived on the basis of the occupation model. This conclusion can also be reached concerning several other alternative models of drug-receptor interaction. The L-transformation should therefore be useful for deriving information from dose-response data, provided that any one of these models describes the interaction of a drug with its pharmacological receptors.

7. *Use of the computer programs based on the principles set out in the previous sections*

A brief summary of the input, function and output of each program is given in Table 2. Since each program can carry out several alternative processes these have to be specified for each set of dose-response data by means of code numbers. For example, one code number indicates whether a set of input data for program FINCALC is to be used to estimate a potency ratio, or to estimate the affinity constant of an agonist or antagonist.

Table 2. *Outline of technique employed when applying the computer programs to analyse experimental data.*

Input [usually on cards, tape, or in storage file].	Program applied to input	Function of program	Output 1 Detailed output [either printed or put into storage file]	Output 2 Condensed output [into storage file]
Code numbers followed by a theoretical value of t, antagonist concentration, maximal response, agonist concentrations, responses, and either (a) C50, or (b) details of the distortion function.	RMOD	Either (a) to estimate the adjustable constants of a suitable distortion function or (b) to calculate the corresponding modified responses, if the distortion function is supplied	Depending on the code numbers, either (a) best values of the adjustable constants of the distortion function or (b) suitably modified responses	(a) None or (b) output in same form as input to SLFIT
Code numbers followed by a theoretical value of t, antagonist concentration, approximate value of maximal response, agonist concentrations and responses, for each set of dose-response data	SLFIT	To estimate those values of the adjustable constants, a, b and L which give the best fit of the L-transformation for each set of dose-response data	Details of the various steps of the fitting procedure, together with the final results	Output in same form as input to DLFIT
Code numbers followed by a theoretical value of t, antagonist concentration, values of a, b and L, agonist concentrations and responses for each set of dose-response data	DLFIT	To estimate, for each pair of sets of dose-response data, those values of a_1, b_1, a_2, b_2 and L which give the best fit of the L-transformation	Details of the various steps of the fitting procedure, together with the final results	Output in same form as input to FINCALC
Code numbers, followed by a theoretical value of *t*, antagonist concentration, values of a_1, b_1, a_2 and b_2 together with their variances and covariances	FIN-CALC	To estimate the final quantities required from comparison of each pair of sets of dose-response data	Depending on the code numbers used: (a) values and s.e.'s of ψ_{AB} and I_{AB}. Also the value and s.e. of K_B, on the assumption that β_{AB} is much greater than one. (b) values and s.e.'s of K_A and of y_I. (c) values and s.e.'s of K_I. Also the values and s.e.'s of K_A, if the antagonism is pseudo-irreversible. (d) value of the potency ratio, with upper and lower fiducial limits—provided that the proper theoretical value of *t* has been entered.	None

Footnote: *t* represents Students *t*-factor and
C50 is the concentration of agonist which produces a half-maximal response.
For the meanings of the other symbols see footnote to Table 1.

Each program, except FINCALC, has two outputs. One of these gives information about the results of various steps carried out by the program, together with a brief statement of the final results. The other output is merely a collection of numerical data in a form which is suitable for use as input for the next program. Each program may be applied individually to sets of data, but the more usual technique is for the input data to be entered once only and processed in turn by each of the programs. The actual form of the input data will of course depend on whether the data are suitable for direct application of program SLFIT or require initial modification by use of the program RMOD. Another point worth noting is that some quantities in the data input may be irrelevant. For example a value for the concentration of antagonist is required in the input in spite of the fact that in some examples no antagonist may have been used! In such cases any "dummy" number may be entered since if the correct code numbers are inserted the computer will read the number but will not use it. The

same comment applies to the theoretical value of t which is required only for the estimation of the fiducial limits of a potency ratio.

An example of results obtained by the use of these programs is given in Appendix 3.

DISCUSSION

The basic principles of the L-transformation have been discussed in the previous sections. It can be used to fit curves to individual sets of dose-response data obtained on different pieces of tissue, and therefore to summarise such data. However its main use is to compare pairs of sets of dose-response data obtained on the same piece of tissue, so as to obtain useful information such as potency ratios or apparent affinity constants. Estimates of standard errors or of fiducial limits of these quantities can also be made. It has been shown that if two sets of dose-response data have been obtained for drugs acting on the same receptors in the same piece of tissue then the values of L should not differ significantly if the principles, on which the use of the L-transformation is based, are correct. It would be possible to fit the L-transformation simultaneously to *more* than two sets of data with a single common value of L. However this does not seem to be worthwhile since the stimulus-response relation of the tissue is likely to change with time and this is likely to invalidate the comparison of sets of data obtained on a single piece of tissue at widely different times.

One point which has not so far been mentioned is that in fitting the L-transformation to sets of dose-response data it has been assumed that the variance of response is independent of the magnitude of the response (i.e. the responses are homoscedastic). The same assumption was made by Parker & Waud (1971). Small deviations from homoscedasticity are unlikely to modify the results obtained to any appreciable extent, especially if the L-transformation fits the experimental results fairly well. The preliminary use of a distortion function may either increase or decrease any deviation from homoscedasticity but this too is likely to be of little practical importance.

The production of very steep log dose-response curves by drugs acting on some tissues may be due to the existence of a complex relation between the pharmacological stimulus and the response of the tissue. An alternative explanation could be that more than one molecule of agonist interacts with each receptor site. Only detailed experiments can decide between these two possibilities for any chosen example of response, tissue and drug.

A major advantage of the techniques outlined here is that the basic principles are fairly simple and widely applicable. Also the application of the programs requires only that suitable instructions be fed into the computer along with the appropriate code numbers and experimental data. The output from the computer should be examined to see whether, for any single set of data, the mean sum of squares about regression differs significantly from the mean sum of squares within groups. It should also be examined to see whether the individual values of a and L, obtained for each set of a pair of sets of data, differ significantly. Besides these tests, the magnitude of the standard error of each of the final quantities estimated provides a guide to the value of the result. If several estimates are made of some quantity, such as an affinity constant, then the individual values and their standard errors enable one to decide whether the results from different tissue samples are significantly different. If they are not, then suitably weighted mean values and standard errors can be estimated.

The main aim of the authors, when preparing the programs described here, has been

to make them functional. Very little attention has been paid to the efficiency of the programs in terms of computation time. As a rough guide the entire series of calculations shown in Table 2 requires about 5 s for each pair of dose-response curves on the I.C.L. 1906A computer. Copies of these programs (written in 1900 series ALGOL) will be made available on request.

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Appendix 1

The L-transformation can be written as

$$y = a + bx$$

where $y = 1/[r + L]$ and $x = [1/C_r]$.

For any chosen value of L, a weighted linear regression of y on x gives the corresponding best values of a and b. The weighting factor for such a regression can be shown to be $[r + L]^4$. The value of L is initially set equal to zero and moved stepwise in that direction which results in a reduction of the sum of squared deviations about regression, until an approximate minimum sum has been reached. This trial and error method gives only approximate values of a, b and L which can be further improved.

Since the L-transformation can be arranged so that r is a function of the adjustable constants a, b and L,

$$r = \frac{1}{a + \frac{b}{(A)}} - L \qquad \text{A1,}$$

the best values of the adjustable constants and estimates of their variances and covariances can be obtained by using the general method for fitting non-linear regressions, described by Snedecor & Cochran (1971). This method involves the application of Taylor's theorem to equation A1 and iterative modification of the initial values of the adjustable constants.

Appendix 2

The technique described in Appendix 1 can be extended to obtain the best values of the adjustable constants required to fit the L-transformation simultaneously to two (or more) sets of data, with a common value of L.

Application of the program SLFIT to each set of experimental data gives the values of a, b and L for each set. The trial and error method described in appendix 1 is again used to obtain approximate values of a_1, b_1, a_2, b_2 and L which produce an approximate minimum sum of squared deviations of observed responses from the theoretical responses. In this case the summation is over both sets of data and the initial value of L is taken as the mean of the two values of L obtained for the separate sets of data. These approximate values of a_1, b_1, a_2, b_2 and L can then be improved by use of Taylor's theorem and iterative modification.

The relation between the adjustable constants (obtained by fitting the L-transformation simultaneously to the two sets of dose-response data) and the various derived quantities which may be required from the data, are summarized in Table 1. Approximate estimates of the variances of these derived quantities are obtained by applying the standard statistical formula which relates the variance of a function to the variances and covariances of its components.

Appendix 3

Parker & Waud (1971) used their logistic method to estimate the dissociation constant of heptyltrimethylammonium from the following data:

Set (a) (before treatment of the tissue with dibenamine)						
Agonist concentrations (μM)	4	10	20	40	100	200
responses	3	5	8	11	15	14
Set (b) (after treatment of the tissue with dibenamine)						
Agonist concentrations (μM)	10	20	40	100	200	
responses	2	4	7	8·5	10	

The experimental data shown above was fed into the series of programs described in Table 2 of this paper, without use of the preliminary RMOD program, and gave the following results:

$$K_A = 1{\cdot}585 \times 10^4 \text{ litre mol}^{-1}\text{; s.e.} = 1{\cdot}250 \times 10^4 \text{ litre mol}^{-1}$$

Fraction of receptors occupied by the irreversible antagonist

$$= 0{\cdot}573\text{; s.e.} = 0{\cdot}117.$$

These values may be compared with those estimated from the same data by Parker and Waud, which were

$$K_A = 1/58{\cdot}15\ \mu\text{M} = 1{\cdot}720 \times 10^4 \text{ litre mol}^{-1}$$

and fraction of receptors occupied by the irreversible antagonist = 0·564.

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