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The Clark plot: a semi-historical case study

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A study has been made of the origins of the quantitative theory of simple competitive antagonism as manifest in the papers of Gaddum and of Clark. The classical data of Clark on the antagonism of acetylcholine by atropine were reanalysed by recently developed computer-based methods. It was seen how close Clark came to initiating a method having the following significant advantages over the Schild plot: (i) symmetrical treatment with respect to the control (zero antagonist) data; (ii) absence of difficulty with the cases where an estimated dose-ratio is less than unity; (iii) straightforward calculation of approximate standard errors for departure from overall simple competitivity at each antagonist level including control.

The concept of *simple competitive antagonism* (Gaddum 1957) is thought to play an important role in the chemical stimulation and control of many biological responses. Two types of chemical agent or drug are involved: *agonist* and *antagonist*. These interact by their separate chemical combination with receptors located at sites on the surfaces of particular cells. The agonist combines reversibly with the receptors with active consequences that determine the response level for the response metameter concerned. The effect of antagonist is to combine reversibly with a fraction of the receptors, so that the agonist is excluded from those receptors occupied by antagonist. The response level is then supposed to be determined by the fraction of all receptor sites that are combined with agonist.

Under these assumptions,[1] standard chemical kinetics imply that when equilibrium is achieved, the response level is determined by the ratio

$$q = \frac{A}{K_B + B} \quad \cdots \quad (1)$$

where A and B are the free molar concentrations of agonist and antagonist respectively and K_B is the dissociation constant of the reaction in which antagonist combines with receptor.

Experimental studies, designed to provide estimates of K_B, are important to pharmacologists because such estimates, especially if they are precise, can be used to classify the type of receptor involved and hence help to elucidate the biochemical basis of responses such as heart activity, stomach acidity and mental depression.

Unfortunately, theory provides very little guidance as to the form of the relationship between response level and the response determining ratio q, except encouragement for the belief that, in the absence of experimental variation, the relationship should be monotone. In practice, the experimental design has to ensure that the form of the relationship can be determined with sufficient accuracy to support valid estimation of K_B.

[1] Colquhoun (1973) has discussed the assumptions under which q determines the responses.

CLARK'S DATA AND ANALYSES

For the agonist acetylcholine and the antagonist atropine, Clark (1926) plotted the response metameter

$$R = \log\left(\frac{P}{100 - P}\right)$$

against $\log A$ where P was the percentage reduction in isometric contractive force of an isolated ventricular strip of frog's heart. Clark made 55 observations of R at a variety of values of A, B. The values of ($\log A$, R) have been read from Clark's graph as listed below*. Clark did not state his

* $B = 0$: (−7·29, −1·08) (−7·29, −1·04) (−6·62, −0·80) (−6·69, −0·72) (−6·62, −0·67) (−6·62, −0·58) (−6·28, −0·43) (−6·62, −0·36) (−6·28, −0·32) (−6·28, −0·06) (−5·80, 0·06) (−5·60, 0·31) (−5·60, 0·43) (−5·60, 0·48) (−5·60, 0·64) (−5·28, 0·78) (−5·28, 0·84) (−5·28, 1·12) (−4·61, 1·70) (−4·30, 1·70)
$B = 10^{-8}$: (−6·29, −0·48) (−5·73, −0·14) (−5·60, 0·06) (−5·60, 0·24) (−5·23, 0·72) (−4·61, 1·37)
$B = 10^{-7}$: (−6·29, −1·06) (−5·60, −0·28) (−5·60, −0·14) (−5·32, 0·08) (−5·31, 0·16) (−4·61, 1·06)
$B = 10^{-6}$: (−5·31, −0·81) (−4·61, 0·00) (−4·26, 0·31) (−4·26, 0·35) (−3·62, 1·06)
$B = 10^{-5}$: (−4·62, −1·02) (−4·32, −1·02) (−3·62, −0·20) (−3·33, −0·07) (−3·33, 0·12) (−3·33, 0·41) (−2·62, 1·12)
$B = 10^{-4}$: (−3·30, −1·33) (−3·30, −0·83) (−3·30, −0·77) (−2·62, −0·62) (−2·62, −0·40) (−1·89, 1·00)
$B = 10^{-3}$: (−2·42, −1·28) (−2·61, −1·06) (−1·90, −0·75) (−1·30, 0·15) (−1·00, 0·27).

experimental design; it is possible that all 55 readings were made on just one ventricular strip.

It appears that Clark fitted a straight line by eye to the data at each antagonist level, with independent slopes, and read off the values of log A at which the lines gave $R = 0$, corresponding to a 50% reduction in isometric contraction force. (In his own later re-analysis of the data, Clark 1937, quoted the corresponding values of $A \times 10^6$ as 1, 1·6, 3·6, 25, 300, 3500, 47 000 in order of increasing antagonist level.) Clark (1926) plotted his estimate of log A for $R = 0$ against log B and tentatively fitted, as in Fig. 1, a line that was straight with unit slope for A values exceeding 10^{-6}.

His admitted difficulty with the necessary flattening of the relationship at the lower end would have been resolvable if he had exploited the mathematical relationship stated by Gaddum (1926) for the case of the agonist adrenaline and the antagonist ergotamine, acting jointly on rabbit uterus. Gaddum utilized an experimental design of the type that is now widely adopted for antagonist studies. Each piece of uterus was tested at different values of A, firstly in the absence of antagonist ($B = 0$) to establish a 'control' graph of response against log A and then at just one positive level of B to establish a second graph. Gaddum either fitted parallel curves to each such pair of graphs or used a bracketing method (from his account it is not clear which), in order to estimate what he called the 'adrenalin proportion' but which would now be called the *dose-ratio, r, for equal response.* Gaddum's discovery was that 'When similar pieces of the same uterus were tested with varying concentrations of ergotamine, it was found that over as wide a range as was convenient the adrenalin proportions have a linear relation to the concentration of ergotamine.'

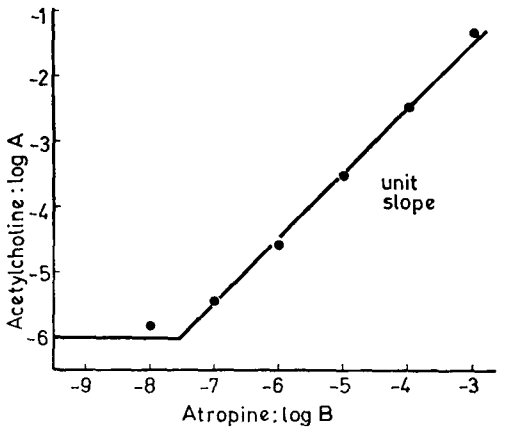

FIG. 1. The isobol tentatively fitted by Clark (1926).

Despite his reference to 'similar results' obtained by earlier pharmacologists, it is possible that Gaddum's verbal statement, which has received little recognition, is the first formulation of the dose-ratio version of the law of simple competitive antagonism:

$$r = 1 + \frac{B}{K_B} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Paton, 1970, referred to other aspects of Gaddum's paper but almost suggests that Gaddum did not formulate (2) until 1957.

It seems that Clark independently discovered how to deal with his fitting problem. In an appendix to Mendez (1928), he noted that Fig. 1 is better fitted by the formula

$$\frac{A - A_0}{B} = \text{constant} \quad .. \quad .. \quad .. \quad (3)$$

where A, A_0 are the estimates of A giving $R = 0$ for level B and control respectively. Apart from differences with respect to the design and analysis, equations (2) and (3) are equivalent if the constant in (3) is taken to be A_0/K_B. Later, Clark (1933, p. 235) fitted the generalized equation

$$\frac{A - A_0}{B^n} = \text{const.} \quad .. \quad .. \quad .. \quad .. \quad (4)$$

to the data of Fig. 1 and estimated the value of n to be 1·1.

Ironically, a whole decade after the initial discoveries of Gaddum and Clark, a peculiar contretemps arose between them concerning the formulation of these empirical equations. Gaddum (1937) objected to (4), apparently on the grounds that it appears to rule out constancy of $r = A/A_0$ for constant B. Clark (1937) accepted the objection and then, paradoxically, used Gaddum's absolutely equivalent version of (4) with $n = 1$ to derive (3) in the guise:

$$K_1A = 1 + K_2B \quad .. \quad .. \quad .. \quad .. \quad (5)$$

where K_1 and K_2 are constants.

He fitted (5) directly to his 1926 data to obtain the estimates $K_1 = 10^6\text{M}^{-1}$ and $K_2 = 3 \times 10^7\text{M}^{-1}$. The latter is equivalent to $K_B = 10^{-7.5}\text{M}$.

It appears likely that, if Clark in 1926 had seen, appreciated and exploited Gaddum's verbal statement of (2), he would have been led to the presently ubiquitous Schild plot (Schild 1957). This is because the very wide range of B values, from 10^{-8} to 10^{-3}

would probably have induced him to write (2) in the logarithmic form

$$\log (r - 1) = -\log K_B + \log B \quad .. \quad .. \quad (6)$$

in preparing a graphical plot. Equation (6) defines the Schild plot.

ILLUSTRATIVE REANALYSIS OF CLARK'S DATA: THE 'CLARK PLOT'

The appendix contains the details of a computer-based reanalysis of Clark's 1926 data, using the GLIM[2] system and the methods developed by Stone & Angus (1978). These follow the general approach of Waud (1975) in employing an iterative non-linear least-squares procedure that fits curves simultaneously to the whole data set. The facilities of the GLIM system are, however, exploited to give greater flexibility in the choice of dose response function, as well as to allow linear adjustments for covariates and experimental design variables. The approach requires determination, by trial and error if necessary, of a suitable empirical equation to fit the dose-response curves. Although Clark (1926) may well have been influenced by some current theory in his choice of dose-response function, our findings are that the choice was in fact acceptable on purely empirical grounds; it fitted his observations.

The residual sum of squares for fitting *common slope* straight lines (of R against log A) is 1·644 with 47 degrees of freedom, while the residual sum of squares for fitting *independent* slope straight lines (as Clark did) is 1·464 with 41 degrees of freedom. The F value for a test of parallelism is $\{(1{\cdot}644-1{\cdot}464)/(47-41)\}/(1{\cdot}464/41) = 0{\cdot}84$, supporting the acceptability of parallelism which is an essential ingredient of simple competitivity. The output of the common slope fit was used for the Schild plot in Fig. 2: for example, the dose ratio (r) for the highest level (7) of antagonist is 10 to the power of (difference in intercept/slope) which can be seen to be 5·068/1·073 = 4·723, giving r = 52,900 (cf. Clark's 47 000). If we were to use Fig. 2 to estimate K_B, this would be done by fitting a straight line with unit slope and taking K_B to be the value of B at which the ordinate is zero. The problem, here of little consequence but potentially quite troublesome for other Schild plots, would be what weight should be attached to the different points, especially the lowest. Because of the proximity of the fitted line for $B = 10^{-8}$ M to the

[2] Purchasable from the GLIM Coordinator, Numerical Algorithms Group Ltd., 7 Banbury Road, Oxford, OX2 6NN. In 1979, the PDP11/Release 3 version cost about £100.

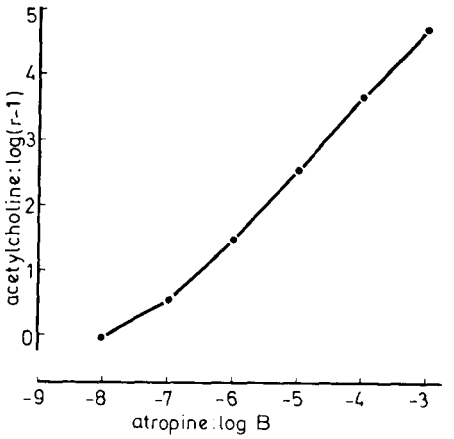

FIG. 2. The Schild plot for Clark's 1926 data.

control line, it is clear that the corresponding point must have appreciably greater variance than the others. Instead of going into the statistical analysis needed to suggest an appropriate weighting for log $(r - 1)$, it will be preferable to introduce the remainder of the calculations detailed in the Appendix and to allow the reader to assess the merits of so doing. Using the special macros for the straight line dose-response function, the least squares estimate of K_B, in the fitting of the single competitivity equation

$$R = a + b \log\left(\frac{A}{K_B + B}\right) \quad .. \quad .. \quad (7)$$

simultaneously to all 55 observations, was given as $2{\cdot}60 \times 10^{-8}$ M with estimated standard error $0{\cdot}32 \times 10^{-8}$ M. This was obtained in just 2 cycles of an iterative fitting procedure starting with estimates of the common slope b and of K_B from the previous fitting of the common slope straight lines. The F value for a test of whether the spacings between these common slope straight lines are consistent with simple competitivity is given by $\{(2{\cdot}022-1{\cdot}644)/(52-47)\}/(1{\cdot}644/47) = 2{\cdot}2$ which is not significant at the 5% level. So we have further support for the simple competivity model and its associated K_B estimate of $2{\cdot}60 \times 10^{-8}$ M. The associated 95% confidence limits for $pK_B = -\log K_B$ are $7{\cdot}59 \pm 0{\cdot}11$ (cf. Clark's pK_B of 7·5!).

The Appendix gives the output of the quantities necessary to determine the position on the Clark plot of the control point; the calculations are precisely the same for the other points, since the Clark plot does not give the control data any special status as does the Schild plot. For $B = 0$, the ordinate is $\log A_B = \log (K_B + B) + \Delta_B = -7{\cdot}58-0{\cdot}02122 = -7{\cdot}60$. Fol-

lowing the method of Appendix 2 of Stone & Angus (1978), the standard error of Δ_B (which is the deviation from the 'Unit line' $A_B = K_B + B$ i.e. $q = 1$) is estimated as

$$\left(2{\cdot}652 \times \left(\frac{2{\cdot}022}{52}\right)\right)^{\frac{1}{2}} \Big/ \; (20 \times 0{\cdot}4678 \times \ln 10) = 0{\cdot}015.$$

Note that for this control point, Δ_B (which would be zero for perfect agreement with simple competitivity) is therefore 0·021/0·015 = 1·4 standard deviations from zero. This compares with the larger value of 1·8 standard deviations for the Δ_B for B = 10^{-8} M. The final Clark plot is given in Fig. 3.

It should be clear that we do not estimate K_B from the plot, as is the common practice for the Schild plot. Rather, it is necessary to have K_B already estimated in order to construct the Clark plot which is for graphical display only.

Finally, the Appendix shows how tests of 'power departure' and 'quadratic departure' from the simple competitivity equation (7) are performed. (These departures replace the B in (7) by B^n and $B(1 + nB/K_B)$ respectively). The first n corresponds to the Schild slope and is found to have 95% confidence limits 1·06 ± 0·06. The second n has limits 0·13 × 10^{-4} ± 0·17 × 10^{-4}. Both findings give further credence to the simple competitivity model for these data. The acceptability of the fit of the model may also be judged from Fig. 4.

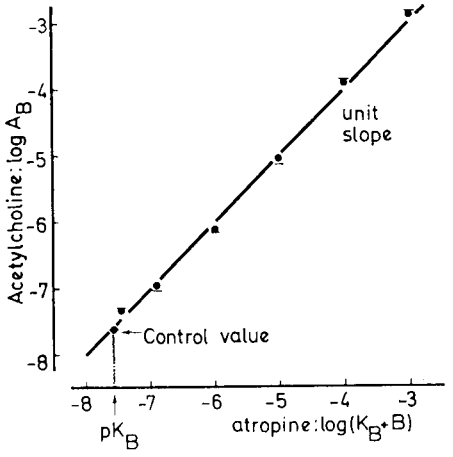

FIG. 3. The 'Clark plot' for Clark's 1926 data. A_B denotes the estimate of the acetylcholine concentration for a particular constant level of response, when the atropine concentration is B. The points therefore lie on an estimated isobol. The straight line of unit slope gives the true isobol for competitive antagonism in the absence of experimental error; the isobol selected corresponds to the same level of response, which is given when $A = K_B + B$. The bars give estimated 2 s.e. limits for the deviations of the individual points from the true isobol due to experimental error.

DISCUSSION

In relation to the historical element in this paper, especially to Clark's use of equation (5), what our 'Clark plot' does is to use an alternatively derived estimate of $K_2 (= 1/K_B)$ and plot log A against log $(K_B + B)$ for a level of response such that K_1 is effectively unity.

The widespread use of the Schild plot is probably due to:

(a) its link with dose-ratio, which is a basic concept for simple competitivity in view of its independence of response level and which can be regarded as a datum for within-preparation designs involving control and one level of antagonist;

(b) its linearity on convenient logarithmic scales facilitating speedy estimation of n and, if a slope of unity can be acceptably imposed, of the pK_B value as an intercept.

However when parameter estimation is now done so easily by computer, we can devote more attention to the matter of informative display of the degree to which the spacings of the dose-response lines fit the theory of simple competitive antagonism. The Clark plot gives no special status to the control value, which makes its appearance on the plot along with the points for non-zero antagonist concentrations; it accepts the arbitrary element in the choice of isobol (that is, equi-response level) in order to preserve the symmetry of presentation. This is in contrast to the Schild plot where, as was found to be the case for the histamine/metiamide data of Angus et al (1978), the plotting technique can even result in the further

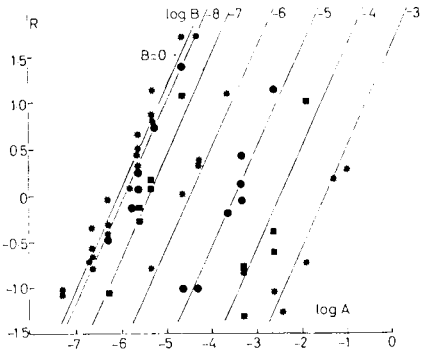

FIG. 4. The Clark data and their simultaneous fitting by a simple competitivity model.

necessary omission of values where $r < 1$, with associated impoverishment of the graphical display. Furthermore the 'Clark plotter' is never faced with the dilemma of a 'Schild plotter' who finds that he has a deviant control value and would like to display the degree to which the non-control points conform to simple competitivity among themselves. Finally, it is possible to calculate easily standard errors for the Clark plot, as illustrated in Fig. 3, whereas such calculation is less straightforward for the Schild plot.

It appears that Clark developed his isobol method for plotting data (of which our Clark plot is but a trivial modification) concurrently but independently of Loewe (1926). Later Loewe (1957) drew attention to what may be the first isobol for antagonism, that of Fraser (1870–71).

Acknowledgements

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APPENDIX: GLIM PROCEDURES FOR THE CLARK DATA

GLIM 3·10 (C) 1977 ROYAL STATISTICAL SOCIETY, LONDON	
$UNITS 55 $DATA LEVB LOGA R	Input of the 55 data points.
$READ 1 −7·29 −1·08	The first column, LEVB, denotes the 'level of *B*' from 1 (control) to 7 (10^{-3} M).
.. .. ..	
7 −1·00 0·27	
$PRINT LEVB LOGA R	Prints inputted data for checking.
$PLOT R LOGA	Graphs R against log A for inspection.
$CAL B=0*%EQ(LEVB,1)+10**(LEVB-2)*%NE(LEVB,1)	Gives B in 10^{-8} M units.
: A=10**LOGA	Converts LOGA into A in M units.
$FAC LEVB 7	Makes LEVB a factor coding for analysis of variance.
$YVAR R	Declares *R* as the '*y* variable' for statistical analysis.
$FIT LEVB+LOGA $DIS E	To fit *common* slope straight lines against log *A* at each level of *B* and to display estimates.
CYCLE DEVIANCE DF	
1 1·644 47	The 'deviance' of 1·644 is the residual sum of squares.
ESTIMATE S.E. PARAMETER	S.E. denotes the estimated standard error.
*1 6·515 0·2343 %GM	The intercept at logA=0 for the 'control' line.
2 −0·3062 0·8889E–01 LEVB(2)	Changes in the intercept for the 'experimental' lines: basis of Schild plot.
.. ..	
*7 −5·068 0·1848 LEVB(7)	
*8 1·073 0·3858E–01 LOGA	The least squares estimate of the common slope.
$FIT LEVB+LEVB*LOGA $	To fit straight lines with *independent* slopes.
CYCLE DEVIANCE DF	
1 1·464 41	Note the *slight* reduction in deviance.
$INPUT 10 NLM1 FL FLP FLQ CLARKPLOT CFL	Input of special macros required.
$ARG M1 FL	Selects the FL macro for the linear dose-response function.
$CAL %B=0·5 : %K=2	Initial guesses of common slope and K_B value from lines * above. Slope value changed for natural logarithms lnA.
$USE NL $FIT BETA+KB $DIS E	With FL, defines competitivity model.
CYCLE DEVIANCE DF	
2 2·022 52	The deviance, 2·022, compares with 1·644 above to give test of competitivity (see text).

```
      ESTIMATE      S.E.        PARAMETER
        1  6·961      0·2650          %GM
        2  0·4678     0·1713E-01      BETA

        3  2·597      0·3193          KB
$CAL RES=%YV-%FV
$PRINT RES
$ARG CLARKPLOT CFL
$CAL %E=1 $USE CLARKPLOT      $

        1     20     -0·2122E-01
    CYCLE     DEVIANCE        DF
        1     2·652           52
        ..

$YVAR R $ARG M1 FLP
$CAL %N=1
$USE NL $FIT BETA+KB+N $DIS E
    CYCLE     DEVIANCE        DF
        3     1·884           51
              ESTIMATE  S.E.          PARAMETER
        1     7·062     0·2641        %GM
        2     0·4622    0·1695E-01    BETA
        3     3·990     0·9616        KB
        4     1·055     0·2788E-01    N
$ARG M1 FLQ
$CAL %B=0·4678 : %K=2·597 : %N=0

$USE NL $FIT BETA+KB+N $DIS E
    CYCLE     DEVIANCE        DF
        2     1·905           51
          ESTIMATE    S.E.          PARAMETER
        1 6·949       0·2601        %GM
        2 0·4644      0·1691E-01    BETA
        3 2·839       0·3804        KB
        4 0·1312E-04  0·8694E-05    N
```

Estimate of common slope under competitivity model.
The K_B estimate is 2·6 with s.e. 0·3 (10^{-8} M units).
Calculates residuals (y values minus fitted values).
Prints residuals for assessment.
Prepares for Clarkplot calculations.
LEVB has replaced B in the CLARKPLOT macro and %E refers to the level of B.
Level of B, no observations, Δ_B.

Deviance is 'Deviance$_B$'.
Calculations repeated for the remaining 6 levels of B.
Prepares for fitting the power departure model.
Sets initial guess for 'Schild slope'.
To fit 'power departure' model

Least squares estimate of Schild slope.
Prepares for fitting the 'quadratic departure' model.
Sets initial guesses: N is now the quadratic parameter.