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NOMOGRAMS FOR MULTIPLE SLOPE-RATIO ASSAYS ARRANGED IN BLOCKS, AND IMPROVED NOMOGRAMS FOR ASSAYS WITHOUT BLOCKS

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It is commonly advantageous with multiple assays, which involve several test preparations at a time, to incorporate in the design some form of local control, usually by blocking in complete replications. In a microbiological assay, for example, blocks might consist of different racks or different days. The ordinary methods of analysis can of course be used in such cases, but repeated applications become tedious, particularly for slope-ratio assays, in which the responses are approximately linearly related to the doses. It is the intention of this paper to present graphical methods for the rapid analysis of results of multiple slope-ratio assays arranged in blocks, and tables will be given from which the nomogram for a particular design may easily be drawn up.

The methods have been developed in general terms, but only the results for the most usual designs will be given here. Attention will thus be restricted to assays in which there are two non-zero doses of each preparation, with a common zero dose, and with the number of replications for each dose-level of the standard preparation and for the zero dose greater by a factor of approximately $\sqrt{(v-1)}$ than the number of replications for the other treatments, where v is the number of preparations, including the standard. The reduction of the general method for cases when there is no blocking results in simpler methods than those previously presented (Clarke and Hosking, 1953) both for multiple assays and for simple assays, that is, assays of only one test preparation at a time, and the results for simple assays will also be described here. Numerical examples will illustrate the construction and use of the nomograms.

Estimation of Error Variance.—For slope-ratio assays arranged in blocks the appropriate estimate of error variance is given, as well as by the within-block replication mean square, by the interaction of treatments with blocks after the elimination of the interaction with blocks of the multiple regression on doses (Clarke, 1955). Hence the error variance estimate may be derived from the interactions of blocks with what have been described as "blanks" and "intersections" (Finney, 1951; Clarke, 1952).

This method of estimating the error variance has been used in deriving the nomograms presented here for multiple assays arranged in blocks; as usual for mainly graphical methods, the estimates are made from ranges so as to shorten the calculations. Assays which are not arranged in blocks afford an easy estimate of the appropriate error variance from the ranges of observations on the same dose-level and preparation.

NUMERICAL EXAMPLES

Multiple Slope-ratio Assay Arranged in Randomized Blocks

The data used in this example are taken from the results of a microbiological assay of cyanocobalamin in samples of cows' milk, taken at different stages of lactation. The complete experiment involved 16 milk samples and a standard, each at three equally spaced dose-levels, with a common zero dose, but it is sufficient to consider here only six of the samples and the two lower dose-levels, and in each block two tubes for each dose-level of the standard and for the zero dose. A complete replication of the tests was carried out on each of two days, so that there were two blocks. The results of the assay and the numerical computations required are presented in Table I.

The upper dose of each preparation is taken to be 1 unit, so that with equal spacing the lower dose is then $\frac{1}{2}$ unit. The individual results are listed as shown in the first two rows (a and b), and totals are calculated where there are replicates within a block, as for the standard and zero dose. The remaining calculations are as follows.

1. Block differences (Block 1 − Block 2), for each column.
2. $\delta_H = 2\{$value in column (c)$\} - \{$value in column (d)$\}$, calculated from block differences, for each preparation.
3. r = range of values of δ_H, using *mean* for standard preparation.
4. Block totals (Block 1 + Block 2), for each column.
5. $H = 2\{$value in column (c)$\} - \{$value in column (d)$\}$, calculated from block totals, for each preparation.
6. Q, calculated for each preparation as shown underneath Table I.
7. $S(Q)$ = total of values of Q.
8. $S(Q)/Q$, for each preparation (a slide-rule gives sufficient accuracy).

Thus for sample 1, for example

$$\delta_H = (2 \times 15) - 12 = 18,$$
$$H = (2 \times 71) - 110 = 32,$$
$$Q = 18\{71 + (2 \times 110)\} - 3690 = 1548,$$
$$S(Q)/Q = 5526/1548 = 3.57.$$

Before proceeding to estimate relative potencies, tests of the validity of the assay are made, and the form of these tests in this particular example is found from the lower part of Table III. Corresponding to the test for " blanks " (Clarke, 1952) we have the criterion (i) that $S(H) - 4T_0$ should not be greater than $3.47r$. The coefficient of r here depends on the critical probability level to be used: in Table III the values appropriate to a 5% level have been presented. The test for " intersections " (Clarke, 1952) is conveniently split up into two parts: (ii) $S(H) - 4H_S$ should not be greater than $4.47r$, and (iii) range (H_T), the range of the values of H for the test preparations only, should not be greater than $2.14r$. As may be seen from the bottom of Table I, the tests for validity are satisfied in this example.

It is now possible to proceed immediately to estimate the relative potencies and their fiducial limits from Fig. 1. The linear parts of the diagram are substantially the same as the preliminary chart described in the earlier paper (Clarke and Hosking, 1953), but use of the exact fiducial limits enables curves for finding the limits to be superimposed on the same chart, and the limits are found with only one alignment.

The value of b_S, the slope of the dose-response line for the standard preparation, is first found by joining $S(Q)/Q_S = 1.71$ on AA′ to $S(Q) = 5526$ on BB′, and reading off $b_S = 49$ on CC′, whence b_S/r is calculated as 1.88. The point corresponding to $S(Q)/Q_S$ on AA′ is joined to the point corresponding to $S(Q)/Q_T$ on DD′, for each test preparation in

TABLE I

COMPUTING SHEET FOR A MULTIPLE SLOPE-RATIO ASSAY

The responses are readings on a photo-electric absorptiometer, multiplied by 10

Preparation	Control	Standard	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Total	Range
Dose (Units)	0	½ (c) 1 (d)	½ (c) 1 (d)	½ (c) 1 (d)	½ (c) 1 (d)	½ (c) 1 (d)	½ (c) 1 (d)	½ (c) 1 (d)		
Responses:										
Block 1 .. (a)	7 9 → 16	35 38 → 73; 63 66 → 129	43 61	37 62	25 44	19 35	23 43	33 54	697	
Block 2 .. (b)	10 9 → 19	25 30 → 55; 51 51 → 102	28 49	30 51	19 31	17 23	17 26	24 42	533	
Block differences:										
(a) − (b) ..	−3	18 27	15 12	7 11	6 13	2 12	6 17	9 12	164	
$\delta_H = 2(c) - (d)$ ..		9 (mean = 4·5)	18	3	−1	−8	−5	6		26 = r
Block totals:										
(a) + (b) ..	T_0 35	S_1 128; S_2 231	T_{11} T_{12} 71 110	T_{21} T_{22} 67 113	T_{31} T_{32} 44 75	T_{41} T_{42} 36 58	T_{51} T_{52} 40 69	T_{61} T_{62} 57 96	1,230 = G	
$H = 2S_1 - S_2$ ..		25	32	21	13	14	11	18	134 = $S(H)$	
Q ..		3,240	1,548	1,584	−198	−954	−486	792	5,526 = $S(Q)$	
$S(Q)/Q$ ..		1·71	3·57	3·67	−27·9	−5·80	−11·37	6·98		
From chart:										
Relative potency			0·98	0·99	0·58	0·41	0·52	0·81		
5% fiducial limits			0·87; 1·11	0·88; 1·12	0·48; 0·70	0·30; 0·52	0·41; 0·63	0·70; 0·93		
Calculated values:										
Relative potency			0·984	0·992	0·585	0·413	0·520	0·811		
5% fiducial limits			0·882; 1·092	0·890; 1·101	0·489; 0·682	0·315; 0·509	0·423; 0·616	0·713; 0·913		

$Q_S = 18(S_1 + 2S_2) - 6G$; $Q_{Ti} = 18(T_{i1} + 2T_{i2}) - 3G$; $b_S = 49$; $b_S/r = 1{\cdot}88$

$6G = 7{,}380$ $\quad 3G = 3{,}690$

Validity tests: $S(H) - 4T_0 = -6$; $t_1 r = 90$; $S(H) - 4H_S = 34$; $t_2 r = 116$; Range (H_T) = 21; $t_3 r = 56$ } (5% probability level)

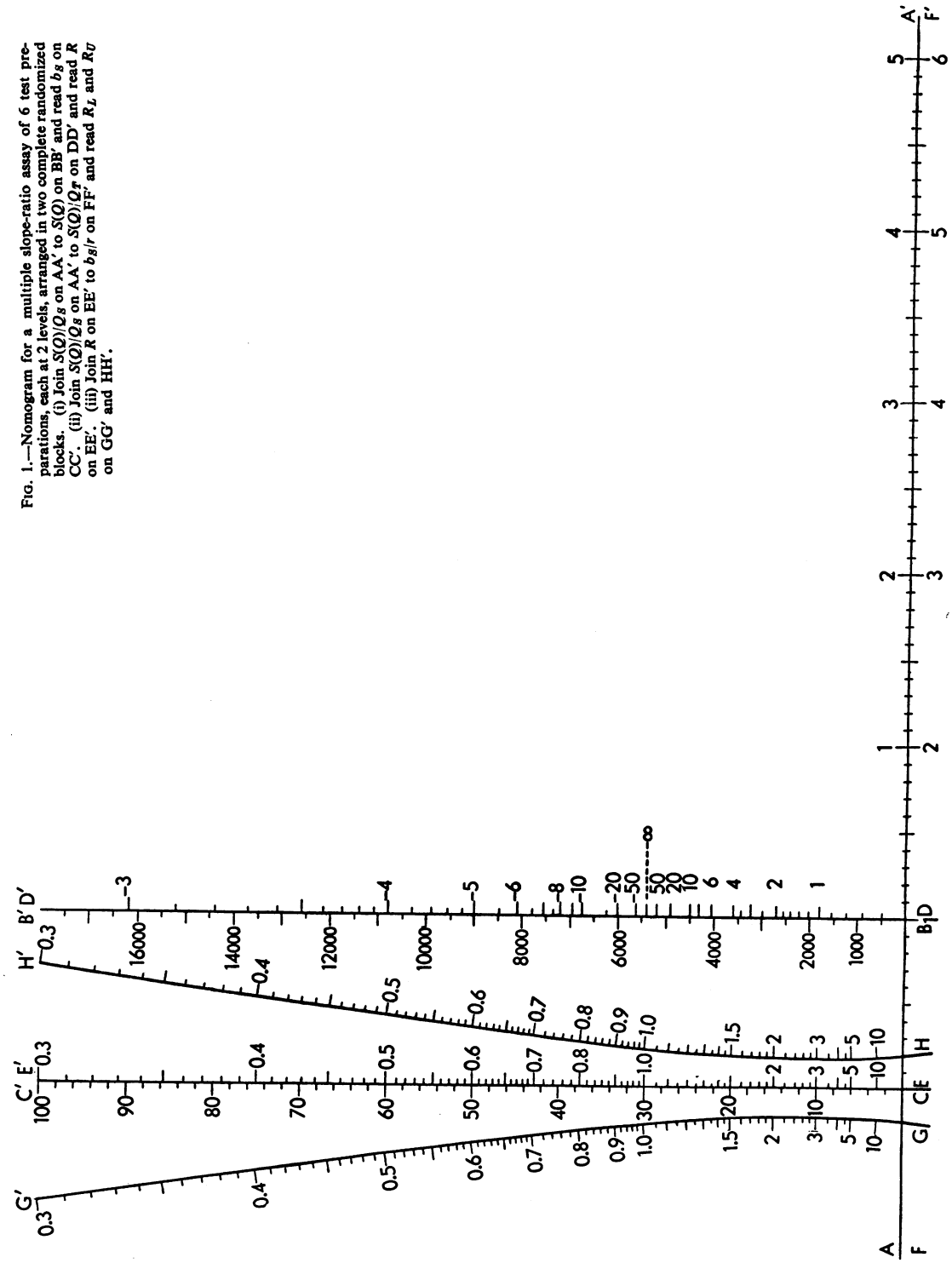

FIG. 1.—Nomogram for a multiple slope-ratio assay of 6 test preparations, each at 2 levels, arranged in two complete randomized blocks. (i) Join $S(Q)/Q_S$ on AA' to $S(Q)$ on BB' and read b_S on CC'. (ii) Join $S(Q)/Q_S$ on AA' to $S(Q)/Q_R$ on DD' and read R on EE'. (iii) Join R on EE' to b_S/r on FF' and read R_L and R_U on GG' and HH'.

turn, and the relative potency (R) is read off on EE'. This point on EE' is then joined to the point for b_S/r on FF'; the intersections with the curves GG' and HH' give the lower and upper 5% fiducial limits. The vertical scales for these curves are the same as for the line EE'. Thus for sample 1, when the point $S(Q)/Q_S=1.71$ is joined to the point $S(Q)/Q_T=3.57$ on DD', the relative potency R is found from EE' to be 0.98, and joining this point on EE' to the point $b_S/r=1.88$ on FF' the intersections with GG' and HH' give 5% fiducial limits of 0.87 and 1.11. The calculated values of the relative potencies and their fiducial limits are given for comparison in Table I, and it may be seen that the approximate estimates of the limits obtained by using the range estimate of error are within 0.02 of the calculated values. The fact that the approximate fiducial interval is wider than the calculated interval is due to the discrepancy between the range estimate of error variance (9.99) and the mean square estimate (7.85).

Simple Slope-ratio Assay, without Blocks

Table II shows the results and computations for another assay of cyanocobalamin, this time a simple assay of only one milk sample, with a common zero dose and two non-zero doses of each preparation. There were four replications in this 5-point assay, and there were no blocks in the design.

TABLE II

COMPUTING SHEET FOR A SIMPLE SLOPE-RATIO ASSAY

Preparation	Control	Standard		Test		Total
Dose (units)	0	½	1	½	1	
Response ..	0·8	3·9	7·0	2·3	3·9	
	0·8	3·8	6·8	2·6	4·0	
	0·9	3·7	7·2	2·2	4·0	
	1·0	3·8	6·6	2·2	4·2	
	T_0	S_1	S_2	T_1	T_2	
Total ..	3·5	15·2	27·6	9·3	16·1	$71·7=G$
Range ..	0·2	0·2	0·6	0·4	0·3	$1·7=r$
Validity tests						
$H=2S_1-S_2$, etc.	2·8			2·5		$5·3=S(H)$
$S(H)-2T_0=-1·7$; $t_1r=2·60$ } (5% probability level)						
$H_S-H_T=0·3$; $t_2r=2·21$ }						
Estimation of relative potency and fiducial limits						
$Q=5(S_1+2S_2)-3G$, etc.	136·9			−7·6		
Q_T/Q_S	—			−0·0555		
Q_S/r	80·5			—		
From chart:						
R (relative potency) ..	—			0·52		
5% fiducial limits ..	—			0·49	0·55	
Calculated values:						
R	—			0·523		
5% fiducial limits ..	—			0·492	0·555	

The preliminary calculations required are indicated in the table; they are the same as those given in the earlier paper (Clarke and Hosking, 1953). The estimate of error is obtained from the sum (r) of the ranges for each treatment, and validity tests are made as described in the 1953 paper. The change to be described here is in the form of the chart, which is illustrated in Fig. 2. This, besides giving the exact fiducial limits instead of the approximate ones, is more convenient for use than the chart published earlier, since the upper and lower fiducial limits are found with one alignment. It is also possible to design one chart for any number of replications, provided the number of doses of each preparation remains constant.

In this example, since $S(H)-2T_0$ is not greater than t_1r and H_S-H_T is not greater than t_2r, where t_1 and t_2 are found, from the bottom of Table V, to have the values 1.53 and 1.30 respectively, the validity tests are satisfied, and we may proceed to estimate the relative potency and its fiducial limits. The relative potency, R, is read off immediately on the opposite side of the scale (AA') for Q_T/Q_S: the value, −0.0555, of Q_T/Q_S, corresponds to a relative potency of 0.52. To find the fiducial limits, a line through the point corresponding to $Q_S/r=80.5$ on CC' is produced through the point for $n=4$ (n is the number of replications) on DD' to meet EE' at a point X, say. This point X is then joined to the point corresponding to $Q_T/Q_S=-0.0555$ on AA' and the 5% fiducial limits, 0.49 and 0.55, are given by the intersections with the curves FF' and GG'. The scales for these curves are linear in the vertical direction, and are the same as that for R. The scale marks on EE' are needed only for the construction of the chart.

The range estimate of error variance in this example (0.0272) is very close to the direct estimate (0.0263), and consequently the approximate fiducial interval agrees well with the calculated interval.

If n is unaltered from assay to assay, it may be more convenient to draw up a chart for the fixed value of n. This is done as explained in the later section on the construction of the chart: briefly, the scales CC' and DD' are omitted, and a scale for Q_S/r is made on the line EE'.

METHOD OF CONSTRUCTION OF CHARTS

Nomograms suitable for a wide range of the most usual types of assay designs may be easily constructed using the coordinates and scale factors presented in Tables III, IV, and V. The tables have been drawn up so as to give charts fitting a sheet of paper 20 units high and 30 units wide. Adjustment

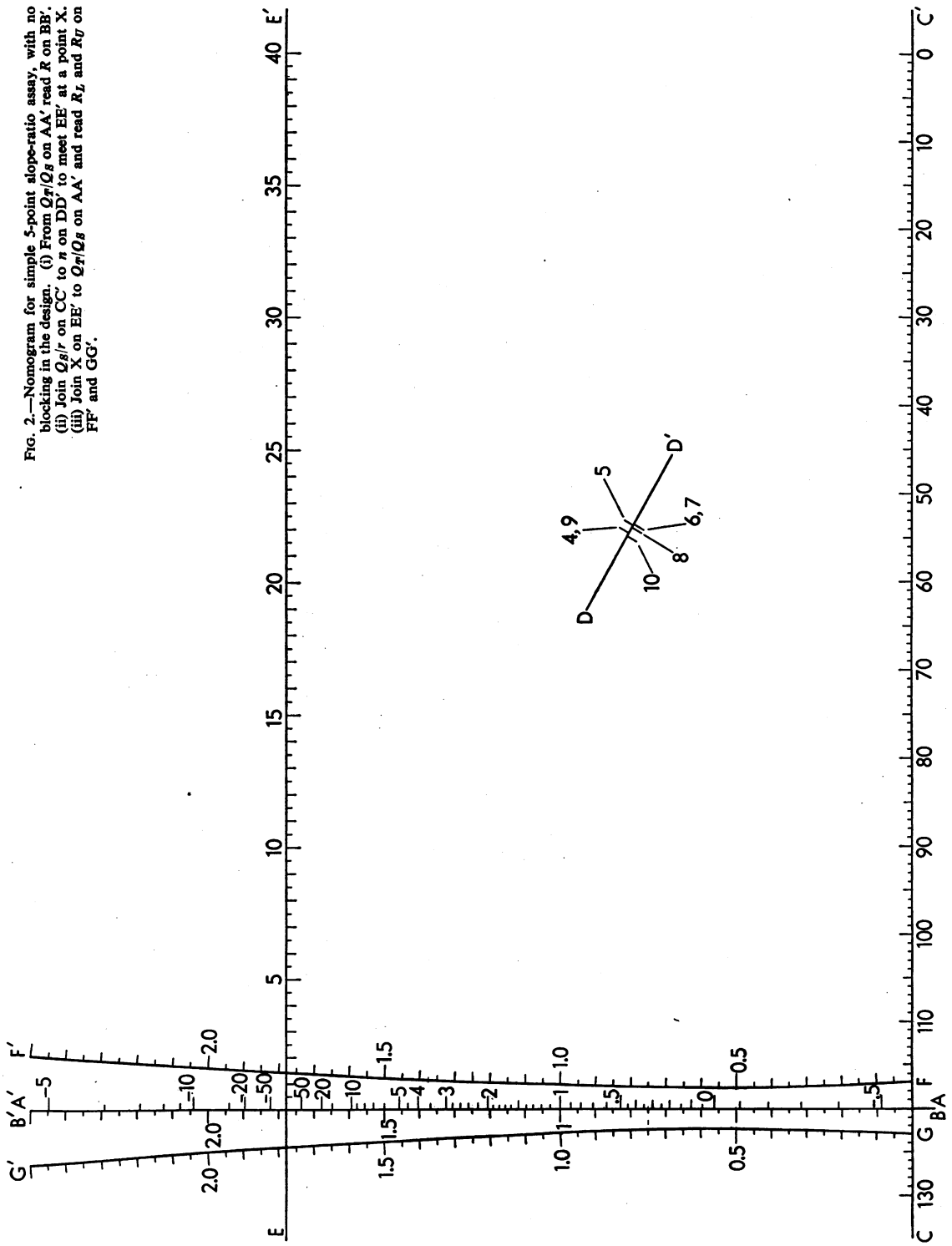

FIG. 2.—Nomogram for simple 5-point slope-ratio assay, with no blocking in the design. (i) From Q_T/Q_S on AA′ read R on BB′. (ii) Join Q_S/r on CC′ to n on DD′ to meet EE′ at a point X. (iii) Join X on EE′ to Q_T/Q_S on AA′ and read R_L and R_U on FF′ and GG′.

to suit other dimensions is made by multiplication of all vertical or horizontal scales by some suitable conversion factor.

Multiple Slope-ratio Assay, arranged in Blocks

The type of design to be discussed is illustrated in the first numerical example. There is a common zero dose, and each preparation is represented at two dose-levels; the doses are equally spaced on an arithmetic scale, so that they may be regarded as 0, $\frac{1}{2}$, and 1 units. Using the notation of earlier papers, let v denote the number of preparations, including the standard: the tables cover values of v from 3 to 16 inclusive. The assay is arranged in two blocks, and in each block there is one observation at each level of each test preparation and $(p+1)$ observations at the zero dose and at each level of the standard preparation, where $(p+1)$ is taken approximately equal to $\sqrt{(v-1)}$, as recommended for multiple assays (Finney, 1952).

The method of calculation of the quantity r required for using the nomogram has been described in the example. The only other basic quantities required are values of Q for each preparation, from which the total $S(Q)$ and ratios $S(Q)/Q$ are calculated. These values of Q are given by the equations

$Q_S=(3p+2v+1)(S_1+2S_2)-3(p+1)G$, for the standard and $Q_{Ti}=(3p+2v+1)(T_{i1}+2T_{i2})-3G$, for the ith test preparation ($i=1, 2, .., v-1$),

TABLE IV

RELATIVE POTENCY SCALE FOR MULTIPLE SLOPE-RATIO ASSAY NOMOGRAMS

R	y	R	y	R	y	R	y
0·30	20·00	0·50	12·00	0·70	8·57	1·25	4·80
0·31	19·35	0·51	11·76	0·72	8·33	1·30	4·62
0·32	18·75	0·52	11·54	0·74	8·11	1·35	4·44
0·33	18·18	0·53	11·32	0·76	7·89	1·40	4·29
0·34	17·65	0·54	11·11	0·78	7·69	1·45	4·14
0·35	17·14	0·55	10·91	0·80	7·50	1·50	4·00
0·36	16·67	0·56	10·71	0·82	7·32	1·60	3·75
0·37	16·22	0·57	10·53	0·84	7·14	1·70	3·53
0·38	15·79	0·58	10·34	0·86	6·98	1·80	3·33
0·39	15·38	0·59	10·17	0·88	6·82	1·90	3·16
0·40	15·00	0·60	10·00	0·90	6·67	2·00	3·00
0·41	14·63	0·61	9·84	0·92	6·52	2·20	2·73
0·42	14·29	0·62	9·68	0·94	6·38	2·40	2·50
0·43	13·95	0·63	9·52	0·96	6·25	2·60	2·31
0·44	13·64	0·64	9·38	0·98	6·12	2·80	2·14
0·45	13·33	0·65	9·23	1·00	6·00	3·00	2·00
0·46	13·04	0·66	9·09	1·05	5·71	3·50	1·71
0·47	12·77	0·67	8·96	1·10	5·45	4·00	1·50
0·48	12·50	0·68	8·82	1·15	5·22	4·50	1·33
0·49	12·24	0·69	8·70	1·20	5·00	5·00	1·20
						10·00	0·60

where S_1, S_2 are the totals for the lower and upper doses respectively of the standard preparation, T_{i1} and T_{i2} are the corresponding totals for the ith test preparation and G is the total of all observations.

Rectangular coordinate axes are drawn through an origin on the base-line of the chart and 6 units from the left-hand edge. All subsequent lines and scale marks, summarized in Tables III and IV, will be described by reference to these axes.

Two scales are marked along the x axis. The first, along the lower side, is a linear scale for b_S/r,

TABLE III

COORDINATES AND SCALE FACTORS FOR MULTIPLE SLOPE-RATIO ASSAY NOMOGRAMS

v (No. of preparations including standard)	3	4	5	6	7	8	9	10	11	12	13	14	15	16
$p+1$ (No. of replications, per block, of standard and blank)	1	2	2	2	2	3	3	3	3	3	3	4	4	4
Scale for b_S/r: linear scale along x-axis from $x=0$														
Scale factor	2	3	3	4	4	4	4	4	4	5	5	5	5	5
Scale for $S(Q)/Q_S$: linear scale along x-axis from $x=X$														
X	4·44	5·00	4·44	3·78	4·00	4·63	3·85	4·00	4·15	3·22	3·33	4·11	4·22	3·25
Scale factor	5	6	5	4	4	5	4	4	4	3	3	4	4	3
Scale for R: inverse scale along y-axis; scale marks given in Table IV.														
Scale for b_S: linear scale along y-axis; scale factor θ chosen to suit data.														
Scale for $S(Q)$: linear scale along vertical line $x=X$ (see values of X above).														
Scale factor	$\theta\times$0·03214	0·01000	0·00804	0·0062	0·00556	0·00277	0·00247	0·00222	0·00200	0·00182	0·00166	0·00128	0·00118	0·00110
Scale for $S(Q)/Q_T$: non-linear scale along vertical line $x=X$. Scale given by $y=\{6S(Q)/Q_T\}/\{\psi+S(Q)/Q_T\}$														
ψ	0·$\dot{8}$	1·$\dot{6}$	1·$\dot{7}$	1·$\dot{8}$	2·0	2·$\dot{7}$	2·$\dot{8}$	3·0	3·$\dot{1}$	3·$\dot{2}$	3·$\dot{3}$	4·$\dot{1}$	4·$\dot{2}$	4·$\dot{3}$
Curves for R_L and R_U: symmetrical curves about y-axis. Scale marks as for R.														
Coordinates of points on curves														
$y=$ 0, $\pm x=$	1·32	0·96	0·74	0·83	0·73	0·57	0·52	0·49	0·47	0·55	0·53	0·46	0·44	0·43
$y=$ 1·2, $\pm x=$	1·20	0·88	0·68	0·77	0·68	0·53	0·49	0·46	0·44	0·52	0·50	0·44	0·42	0·41
$y=$ 3·0, $\pm x=$	1·12	0·87	0·68	0·77	0·68	0·56	0·52	0·49	0·47	0·56	0·54	0·49	0·48	0·46
$y=$ 6·0, $\pm x=$	1·28	1·13	0·88	1·00	0·88	0·79	0·73	0·70	0·66	0·80	0·77	0·73	0·71	0·69
$y=$10·0, $\pm x=$	1·87	1·73	1·34	1·54	1·37	1·23	1·15	1·09	1·04	1·25	1·21	1·15	1·12	1·10
$y=$15·0, $\pm x=$	2·82	2·62	2·02	2·30	2·05	1·85	1·72	1·63	1·56	1·87	1·80	1·73	1·68	1·64
$y=$20·0, $\pm x=$	3·86	3·54	2·74	3·11	2·77	2·49	2·32	2·20	2·09	2·51	2·42	2·32	2·26	2·20
Coordinates of the turning-point														
y	3·18	2·25	2·16	2·08	2·00	1·59	1·54	1·50	1·46	1·42	1·38	1·17	1·15	1·12
$\pm x$	1·12	0·86	0·66	0·75	0·67	0·53	0·49	0·46	0·44	0·52	0·50	0·44	0·42	0·41
Validity tests	(i) $\lvert S(H)-AT_\theta \rvert \leqslant t_1 r$; (ii) $\lvert S(H)-AH_S \rvert \leqslant t_2 r$; (iii) Range $(H_T)\leqslant t_3 r$													
A	3·0	2·5	3·0	3·5	4·0	3·$\dot{3}$	3·6	4·0	4·3	4·$\dot{6}$	5·0	4·25	4·50	4·75
t_1	4·94	4·20	3·69	3·53	3·47	3·47	3·47	3·50	3·55	3·60	3·67	3·65	3·72	3·78
t_2	5·53	4·20	4·13	4·28	4·47	4·11	4·30	4·52	4·74	4·95	5·20	4·84	5·04	5·24
t_3	5·00	3·09	2·54	2·33	2·14	2·03	1·95	1·88	1·83	1·79	1·75	1·72	1·70	1·68

with scale factor as shown in Table III. Thus, for 6 test preparations and $p=1$, the scale factor is 4, so that the marks for $b_S/r=1, 2, 3, \ldots$ fall at $x=4$, 8, 12, .. respectively, and convenient intermediate scale marks are easily located. Along the upper side of the x-axis is a linear scale for $S(Q)/Q_S$, which starts, not at the origin, but at $x=X$, where X and the appropriate scale factor are given in Table III. Taking the same example, we have $X=4.00$ and the scale factor is 4, so that the marks for $S(Q)/Q_S=0$, 0.5, 1.0, 1.5, .. fall at $x=4.00$, 6.00, 8.00, 10.00, .. respectively.

There are also two scales along the y-axis. On the right-hand side is an inverse scale for R, the relative potency estimate, and the positions of the marks for this scale, which are unaltered for different values of v and p, are given in the separate Table IV. A linear scale for b_S, the slope of the dose-response line for the standard preparation, is marked along the left-hand side of the y-axis, and here the scale factor is chosen to suit the data. The scale factor θ may conveniently be chosen as a round number such that, if Δ is the maximum difference expected between the mean for the highest dose of the standard and the mean for the zero dose in any one assay, then θ is approximately equal to $20/\Delta$. The choice of the value of θ clearly depends on the units of observation. Thus if the maximum mean difference expected was about 100, the value of θ would be taken to be 0.2, as in the numerical example in this paper.

A vertical line is drawn through the point $x=X$, $y=0$ previously located as the starting point of the scale for $S(Q)/Q_S$. A linear scale for $S(Q)$ is marked on its left-hand side; the scale factor depends on the value chosen for θ, and the factors listed in Table III have to be multiplied by θ. Thus if $v=7$ and $p=1$, and if θ is taken as 0.2, the scale factor for $S(Q)$ is 0.2×0.00556, i.e. 0.00112, so that the marks for $S(Q)=0$, 1,000, 2,000, .. fall at $y=0$, 1.12, 2.24, .. respectively. Space does not permit the listing of the coordinates of the scale points for $S(Q)/Q_T$, which are marked on the right-hand side of this vertical line; they may be easily computed from the formula $y=\dfrac{6S(Q)/Q_T}{\psi+S(Q)/Q_T}$ where the value of ψ for any particular case is given in Table III. Taking the same example, ψ is seen to be equal to 2, so that the scale marks for $S(Q)/Q_T=0$, 1, 10, ∞, -10 fall at $y=0$, 2.00, 5.00, 6.00, 7.50 respectively.

It remains only to draw the curves for R_L and R_U, the lower and upper fiducial limits, here determined, as usual, for the 5% probability level. These curves are symmetrical about the y-axis, and since they are smooth they may be adequately defined by only a few coordinates. The values of x for seven particular values of y are listed in Table III, and in addition the coordinates are given of the point, for each curve, at which the horizontal distance from the y-axis is a minimum (the turning-point). From these values it should be possible to draw the curves with sufficient accuracy, and marking them with scales is an easy matter, since the scales for R_L and R_U are the same, in the vertical direction, as the scale for R.

The nomogram is then complete, and the method of use is illustrated in the numerical example and summarized in the legend to Fig. 1.

Simple Slope-ratio Assay, not arranged in Blocks

When there is only one test preparation, the resulting simplification of the general method makes it possible to draw up a single nomogram for use with any number of replications. As mentioned earlier, the cases to be considered here are of 5-point assays not arranged in blocks, the usual design for simple microbiological assays.

The quantities Q required for entering the nomogram are calculated as for multiple assays, taking $p=0$ and $v=2$, so that for the standard preparation, for example, $Q_S=5(S_1+2S_2)-3G$. The method of calculating r is changed, since there are no blocks: r is given by the sum of the 5 ranges of n observations within each treatment, where n is the number of replications.

Assuming, again, a sheet approximately 20 by 30 units in size, the origin is taken on the base line, 4 units from the left-hand edge. Rectangular axes are drawn through the origin, and a horizontal line is also drawn at $y=14.22$.

The y-axis bears, on the left-hand side, a linear scale for R, with scale factor 8, and on the right-hand side, a scale for Q_T/Q_S given by $y=\dfrac{8(9+16Q_T/Q_S)}{16+9Q_T/Q_S}$. A number of scale marks for Q_T/Q_S have been tabulated in Table V.

Coordinates sufficient to draw the curves for R_L and R_U, which are symmetrical about the y-axis, are also given in Table V, and include the coordinates of the turning-point. The curves so defined refer to the 5% probability level. The scale marks are simply located by the same vertical scale as that for R.

Any convenient linear scale is marked off for Q_S/r on the x-axis, running in the negative direction as shown in Fig. 2, and starting at $x=24$. The upper horizontal line is graduated with a linear scale for an intermediate variable, β, starting at $x=0$, and with a scale factor 0.6.

TABLE V

COORDINATES AND SCALE FACTORS FOR SIMPLE SLOPE-RATIO ASSAY NOMOGRAMS

Scale for R: linear scale along y-axis, with scale factor 8.
Scale for Q_T/Q_S: non-linear scale along y-axis. Scale marks given below.

Q_T/Q_S	y	Q_T/Q_S	y	Q_T/Q_S	y	Q_T/Q_S	y
−0·5	0·70	0·50	6·63	2·0	9·65	15	13·19
−0·4	1·68	0·6	6·95	2·2	9·88	20	13·43
−0·3	2·53	0·7	7·25	2·4	10·09	50	13·89
−0·2	3·27	0·8	7·52	2·6	10·27	∞	14·22
−0·1	3·92	0·9	7·77	2·8	10·45	−50	14·58
0·0	4·50	1·0	8·00	3·0	10·60	−20	15·17
0·05	4·77	1·1	8·22	3·5	10·95	−15	15·53
0·10	5·02	1·2	8·42	4·0	11·23	−10	16·32
0·15	5·26	1·3	8·61	4·5	11·47	−9	16·62
0·20	5·51	1·4	8·78	5	11·67	−8	17·00
0·25	5·70	1·5	8·95	6	12·00	−7	17·53
0·30	5·90	1·6	9·11	7	12·25	−6	18·32
0·35	6·10	1·7	9·25	8	12·45	−5	19·59
0·40	6·29	1·8	9·39	9	12·62		
0·45	6·46	1·9	9·52	10	12·75		

Curves for R_L and R_U: symmetrical curves about y-axis. Scale marks as for R.
Coordinates of points on curves:

y	4·50 Turning-	0·00	2·00	6·00	8·00	12·00	16·00	20·00
x	0·48 point	0·60	0·53	0·51	0·56	0·75	0·99	1·26

Scale for Q_S/r: linear scale along x-axis in negative direction, starting at $x=24$.
Scale factor φ chosen for convenience.
Scale for β: linear scale along horizontal line $y=14{\cdot}22$, with scale factor 0·6.
Scale for n: intersections of line joining $Q_S/r=0$ and $\beta=0$ with line joining points $x=10$, $y=0$ and $x=\lambda/\varphi$, $y=14{\cdot}22$. Values of λ given below.

n:	4	5	6	7	8	9	10
λ:	3·367	3·467	3·485	3·475	3·442	3·383	3·340

Validity tests: (i) $|S(H)-2T_0| \leqslant t_1 r$; (ii) $|H_S-H_T| \leqslant t_2 r$

t_1:	1·53	1·49	1·48	1·49	1·50	1·53	1·55
t_2:	1·30	1·26	1·25	1·26	1·27	1·29	1·31

The nomogram is completed by marking off a scale for n, the number of replications, on a straight line joining the points corresponding to $Q_S/r=0$ and $\beta=0$. The scale marks are made at the intersections of this line with a straight line joining the point $x=10$ on the x-axis to the point on the upper horizontal line with x-coordinate equal to λ/φ, where φ is the scale factor chosen for Q_S/r and λ is given in Table V.

This nomogram is applicable to any value of n. If it is only required to make a chart suitable for one particular value of n, the scale on the x-axis may be omitted, and a linear scale for Q_S/r substituted for the β-scale on the horizontal line at $y=14.22$. The scale factor is then taken as $\lambda/14$ where λ is given in Table V.

The method of use of the nomogram is explained in the legend to Fig. 2 and is illustrated in the second example.

VALIDITY TESTS

The validity tests are made in much the same way as described in the earlier paper (Clarke and Hosking, 1953), except that it is necessary to replace Student's t by Lord's u (Lord, 1947) in the tests since ranges have been used to estimate the error variance. Unequal replication and arrangement in blocks, in the case of the multiple assay, introduce a few complications, but the application of the tests has been simplified here by listing the critical values which arise in the tests. These values have been calculated for the 5% level of probability. For an explanation of the tests for "blanks" and "intersections" the reader is referred to Finney (1952).

Multiple Slope-ratio Assays

As a preliminary step, the quantity H is calculated from the treatment totals for each preparation, where, for example,

$$H_S=2S_1-S_2.$$

The test for "blanks" requires that $S(H)-AT_0$ should not be numerically greater than $t_1 r$, where A and t_1 are given in Table III, $S(H)$ is the sum, over preparations, of the values of H, and T_0 is the sum of the observations for the zero dose.

The test for intersections may be divided into two parts, first comparing the standard preparation with the mean of the test preparations and next making comparisons among the test preparations. Thus $S(H)-AH_S$ should not be numerically greater than $t_2 r$, and the range of the values of H for the test preparations only should not be greater than $t_3 r$: the values of t_2 and t_3 are given in Table III.

Simple Slope-ratio Assays

The quantity H for each preparation is calculated in the same way as for multiple assays. The tests for "blanks" and "intersections" then require, respectively, that $S(H)-2T_0$ should not be numerically greater than $t_1 r$ and that H_S-H_T should not be numerically greater than $t_2 r$ where t_1 and t_2 are given in Table V.

OTHER DESIGNS

The general forms of the nomograms for slope-ratio assays are unaltered by differences in design. The range function used to estimate the error variance depends on whether or not the assay is arranged in blocks, and this correspondingly affects the scale for Q_S/r (multiple assays) or n (simple assays). In a simple assay, the error variance may be easily estimated from ranges for any number of blocks, and a multiple assay without blocks may also be simply dealt with regardless of the number of replications. Another type of design to which range methods are easily applicable is that of multiple assays arranged in more than two blocks and with more than two non-zero dose-levels; for only two dose-levels and more than two blocks, however, range methods become cumbersome, and it may often be simpler to calculate the error mean square from an analysis of variance, although a nomogram will still be found helpful for estimating fiducial

limits, particularly if there is a large number of preparations.

Using similar methods as for multiple slope-ratio assays, nomograms may be developed for multiple parallel-line assays, in which the response is linearly related to the logarithm of the dose. The basic formulae may be obtained as described by, for example, Finney (1952), and used to adapt the nomogram presented by Leech and Grundy (1953). When the assay is arranged in blocks, the estimation of the error variance from ranges is complicated if the number of dose-levels per preparation exceeds 2, unless only the deviations from linearity of the dose-response curves are used to estimate the variance. With multiple assays, it is not in general possible to make one nomogram to serve for several values of n, as with the simple assay, and so the scale for γ (Leech and Grundy, 1953) is replaced by a scale for a function analogous to R/B in that paper.

Summary

Graphical methods for the evaluation of results of multiple slope-ratio assays arranged in randomized blocks are presented, and the development of similar methods for simple slope-ratio assays arranged in blocks and for multiple parallel-line assays is discussed. Improved nomograms for simple assays without blocks are also described, and the modifications for multiple assays without blocks are outlined. The methods for multiple assays allow for increased replication of the standard preparation, and validity tests are given for use in all cases considered. Numerical examples illustrate the procedures.

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