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A NOMOGRAM FOR ASSAYS IN RANDOMIZED BLOCKS

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This nomogram results from an extension of the idea presented by Healy (1949) in which the fiducial or confidence limits of an assay were found at the intersections on the branches of a hyperbola of a straight line whose position was determined by the mean potency, slope, and residual error of the assay, the error being estimated from the mean of the ranges within the sets of responses to each dose. The same principle has been extended to cover assays arranged in randomized blocks with the particular case of tuberculin assay in mind (*British Pharmacopoeia*, 1948); some modifications enable a single graph to be applied to assays of different sizes. Error is again estimated from range, and the method presented fulfils the condition (Finney, 1952a) that the deviations for the interactions with blocks of "slope" and "materials" should be eliminated from the error variance estimate. Identical computations on a series of 12 similar assays are presented to show the practical implications of Finney's argument and to test the value of the range in estimating the error of these assays.

The nomogram illustrated (Fig. 1) is constructed for the particular case of the 6-point assay in randomized blocks with a dose ratio of 4:1; this arrangement is commonly used in the assay of tuberculin. The modifications required for other designs are specified in a later section.

Numerical Example

The data in this example (Table I) are part of the results of an assay of two tuberculins, being the diameters in mm. of red weals measured 24 hours after the intradermal injection of tuberculin into sensitized guinea-pigs. The responses to the two materials are entered in columns *a*, *b*, *c* and *g*, *h*, *i* respectively; the other nine columns are filled in with the results of simple operations on these six, the operations being specified at the head of each column. The same operations on the totals of columns *a*, *b*, *c* and *g*, *h*, *i* will check with the algebraic sum of each of the other nine columns.

The statistics required for using the nomogram are *D*, *B*, and *R*, the derivation of each being illustrated in the example. The values of *P*, C_1, C_2 should also be estimated and used to check the validity of the assay. For a valid assay, *P* should not be greater than $u'R$; C_1 and C_2 should not exceed $\sqrt{3}u'R$ (u', for different sizes of assay, has

TABLE I

SPECIMEN COMPUTING SHEET

Block No.	(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)	(*i*)	(*j*)	(*k*)	(*l*)	(*m*)	(*n*)	(*o*)
	Responses to Standard			$a+b+c$	$c-a$	$a+c-2b$	Responses to Unknown			$g+h+i$	$i-g$	$g+i-2h$	$(k-e)$	$(f+l)$	$(f-l)$
	$\frac{1}{1600}$	$\frac{1}{400}$	$\frac{1}{100}$				$\frac{1}{1600}$	$\frac{1}{400}$	$\frac{1}{100}$						
1	8	13	16	37	8	−2	4	8	14	26	10	2	2	0	−4
2	12	15	15	42	3	−3	9	12	16	37	7	1	4	−2	−4
3	15	16	19	50	4	2	12	15	20	47	8	2	4	4	0
4	8	14	17	39	9	−3	10	10	16	36	6	6	−3	3	−9
5	14	14	17	45	3	3	10	14	18	42	8	0	5	3	3
6	9	16	17	42	8	−6	12	12	16	40	4	4	−4	−2	−10
7	9	14	18	41	9	−1	8	14	16	38	8	−4	−1	−5	3
8	6	12	18	36	12	0	4	10	19	33	15	3	3	3	−3
Total	81	114	137	332	56	−10	69	95	135	299	66	14	10	4	−24
												Ranges	9	9	13

From column totals, compute: $D=j-d=-33$. $B=e+k=122$. $M=4D/3B=-0.361$.
From ranges: $R=\sqrt{3m+n+o}=37.6$. $R/B=0.308$.
For validity tests, compute: $P=e-k=-10$. $C_1=f+l=4$. $C_2=f-l=-24$. $Ru'=14.9$. $\sqrt{3}Ru'=25.8$.
Standard deviation (if required) $=R/(6\sqrt{3}.d_N)$.

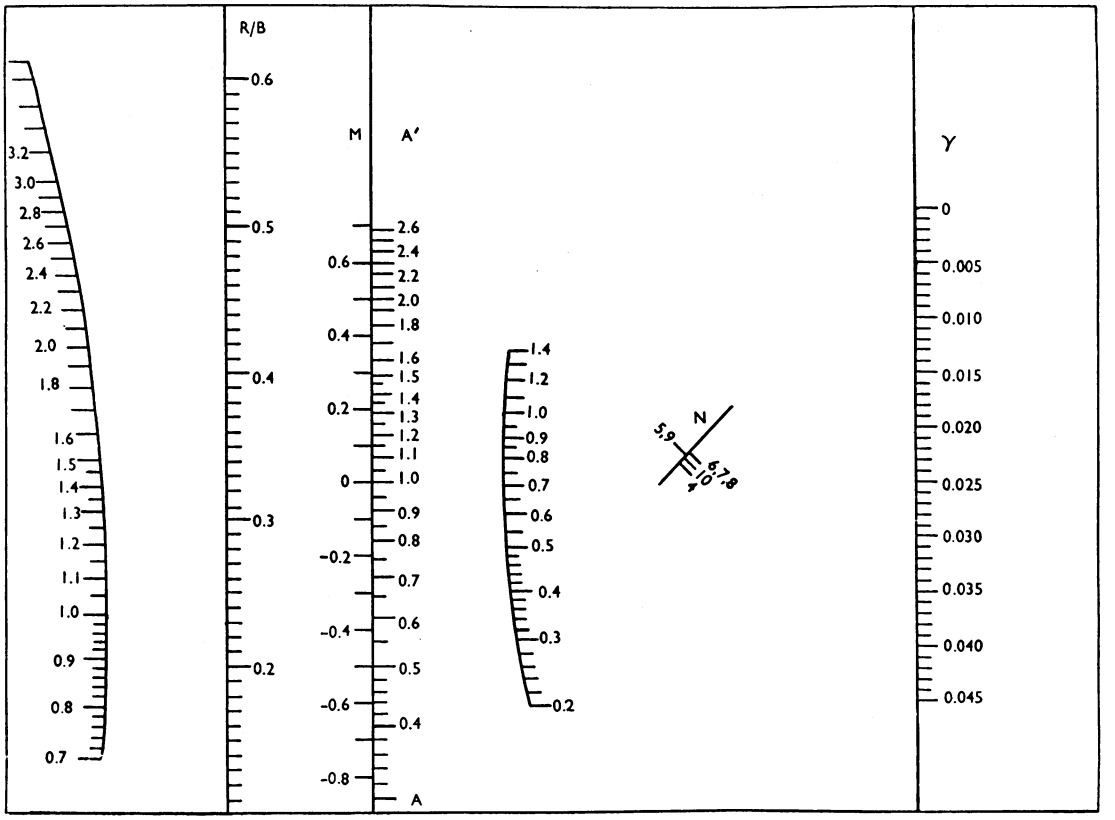

FIG. 1.—Nomogram for a 6-point assay where the dose ratio is 4 : 1

been tabulated (Table II)). If the validity of the assay is not in doubt, calculation of the ratios $4D/3B$ and R/B completes the computations.

TABLE II
TABLE OF u' (5% SIGNIFICANCE LEVEL)

No. of Blocks	6-point Assay	8-point Assay
4	0·415	0·410
5	0·398	0·398
6	0·392	0·396
7	0·393	0·397
8	0·396	0·401
9	0·400	0·408
10	0·405	0·413

Construction of the Nomogram

The nomogram can conveniently be drawn on a paper whose sides are approximately in the proportions 4:3. The horizontal scale (x) is graduated from -20 to $+30$ and the vertical scale (y) is -0.9 to $+1.1$. In the example from which the illustration was prepared (Fig. 1) the unit of x was 0.5 cm. and the unit of y 10 cm. Vertical axes are drawn at $x=0$ and $x=30$. On the former a scale of μ is graduated, covering the range -0.9 to $+0.7$, the units of μ being equal to the units of y. (μ is the quantity estimated by $M=4D/3B$). The other scale (AA') on the axis is one of 4^{μ}, i.e., antilog (μ log 4), which gives the relative potency corresponding to the value of μ graduated. The co-ordinates set out in Table III will enable the curves shown in Fig. 1 to be drawn without further calculation. The values of relative potency are those to be marked when the dose ratio is 4:1. The finer calibrations may be interpolated by eye; if greater precision is desired, there is an easy graphical method (Allcock and Jones, 1950) that obviates further computation. The position of the curves is not affected by alterations in the dose ratio or in the size of the assay. It will be appreciated that it is only necessary to draw the left-hand branch between the values 0.7 and 4.0 and the right hand branch between the values 0.2 and 1.4. For other values of the dose ratio, the curves may be calibrated quite rapidly once the scale AA' has been drawn. Where a line drawn through the point (30, 0.75) and any point on $x=0$ cuts

TABLE III
CO-ORDINATES FOR THE CURVES IN FIG. 1

Relative Potency	Left-hand Curve		Relative Potency	Right-hand Curve	
	x	y		x	y
0·70	−14·82	−0·755	0·20	8·58	−0·614
0·80	−14·65	−0·606	0·22	8·46	−0·573
0·90	−14·57	−0·477	0·24	8·36	−0·534
1·00	−14·55	−0·364	0·26	8·26	−0·498
1·10	−14·57	−0·262	0·28	8·18	−0·463
1·20	−14·62	−0·170	0·30	8·10	−0·431
1·30	−14·70	−0·085	0·32	8·03	−0·401
1·40	−14·79	−0·007	0·34	7·97	−0·372
1·50	−14·90	0·065	0·36	7·91	−0·345
1·60	−15·02	0·133	0·38	7·86	−0·318
1·70	−15·14	0·197	0·40	7·82	−0·293
1·80	−15·28	0·258	0·50	7·64	−0·182
1·90	−15·42	0·315	0·60	7·52	−0·088
2·00	−15·56	0·370	0·70	7·45	−0·007
2·20	−15·86	0·473	0·80	7·41	0·064
2·40	−16·17	0·568	0·90	7·39	0·128
2·60	−16·48	0·656	1·00	7·39	0·185
2·80	−16·79	0·739	1·10	7·39	0·237
3·00	−17·10	0·817	1·20	7·40	0·284
3·20	−17·41	0·891	1·30	7·42	0·328
3·40	−17·72	0·961	1·40	7·45	0·369
3·60	−18·02	1·029			
3·80	−18·32	1·093			
4·00	−18·62	1·155			

the curves, the 3 intersections will bear the same calibration.

A vertical scale on the line $x=-8$ is marked with a suitable range of values of R/B. A scale of γ is marked on the axis $x=30$ from the equation

$$\gamma=(0.75-y)/30$$

On the line joining $R/B=0$ to $\gamma=0$ a short scale of N may be graduated, the calibrations for integral values of N being found at the intersections of lines joining the point $R/B=0.6$ to the points at which γ is numerically equal to $u'/10$ (Table II): the scale of γ is only required for calibrating the scale of N and may then be erased.

Using the Nomogram.—A straight-edge is placed across the appropriate points on the scales of R/B and N (0.308 and 8 respectively in the example) and the intersection of this line on the γ scale marked. The fiducial limits may then be read off at the intersections on the curves of the line joining this point to the observed value of $M(-0.361)$. The relative potency corresponding to the observed M can be read off without further computation from the scale AA'.

THEORY

The fiducial or confidence limits for a ratio derived from a 6-point assay may be expressed as

$$(4D-3B\mu)^2=Nt^2s^2(96+36\mu^2) \quad \ldots\ldots(1)$$

where $\mu=\log$ (potency ratio)/log (dose ratio) and D and B are explained in Table I; s^2 is the error variance of a single observation and t the appropriate deviate of the Student distribution. Since a range estimate of s is used (Table I) in place of the least squares estimate, the statistic u (Lord, 1947) must be substituted for Student's t. Taking m, n, and o (Table I) as the ranges appropriate to the estimation of s, we may write $s=R/(d_N.6\sqrt{3})$, where $R=m\sqrt{3}+n+o$ and $d_{\prime}$ is the expected value of the range in samples of N randomly selected from a normal population with unit standard deviation; this quantity has been tabulated by various writers, the table (Table 5.5) given by Snedecor (1946) being probably the most accessible. If we now write $\gamma=\{R/B\}\ \{u\sqrt{N}/18\sqrt{3}d_N\}$, equation (1) may be written

$$(M-\mu)/\gamma=\pm\sqrt{(96+36\mu^2)} \quad \ldots\ldots(2)$$

This is equivalent to

$$\begin{vmatrix} x_1 & y_1 & 1 \\ \lambda & -\gamma\lambda & 1 \\ 0 & M & 1 \end{vmatrix}=0 \quad \ldots\ldots(3)$$

where λ is an arbitrary constant and

$$x_1=\frac{\pm\lambda\sqrt{(96+36\mu^2)}}{\lambda\pm\sqrt{(96+36\mu^2)}}, \qquad y_1=\frac{\lambda\mu}{\lambda\pm\sqrt{(96+36\mu^2)}}$$

The form of equation (3) is such that the points with co-ordinates (x_1, y_1), $(\lambda, -\gamma\lambda)$, and $(0, M)$, say F, G, and H, are collinear. Owing to the introduction of the constant λ, the locus of G provides a scale of γ at finite distance. The corresponding property of Healy's nomogram is, in our notation, that the points F' and H' with co-ordinates $(\pm\sqrt{(96+36\mu^2)}, \mu)$ and $(0, M)$ lie on a line of gradient $-\gamma$; that is, they are collinear with the point G' given by $x'=\infty$, $y'/x'=-\gamma$. The two schemes are connected by the projective transformation

$$x=\frac{\lambda x'}{\lambda+x'}, \qquad y=\frac{\lambda y'}{\lambda+x'},$$

which converts F', G', H' into F, G, H respectively.

Since only positive values of γ are relevant, it is convenient to shear the right-hand side of the nomogram (including the scale of γ) vertically upwards. This may be effected by adding λ' times all x-co-ordinates to the y-co-ordinates, so that the determinant becomes

$$\begin{vmatrix} \pm\lambda\sqrt{(96+36\mu^2)} & \lambda\{\mu\pm\lambda'\sqrt{(96+36\mu^2)}\} & 1 \\ \lambda\pm\sqrt{(96+36\mu^2)} & \lambda\pm\sqrt{(96+36\mu^2)} & \\ \lambda & \lambda(\lambda'-\gamma) & 1 \\ 0 & M & 1 \end{vmatrix} \quad \ldots\ldots(4)$$

Values of $\lambda=30$ and $\lambda'=0.025$ were found to be convenient; the determinant, with the values substituted leads at once to a nomogram with scales given by the three sets of co-ordinates

(i) $\left(\frac{\pm 30\sqrt{(96+36\mu^2)}}{30\pm\sqrt{(96+36\mu^2)}}, \frac{30\mu\pm 0.75\sqrt{(96+36\mu^2)}}{30\pm\sqrt{(96+36\mu^2)}}\right)$

(ii) $(30,\ 0.75-30\gamma)$

(iii) $(0,\ M)$

The scale of N (the number of blocks) is derived from the scales of γ and R/B from the relationship $\gamma(B/R)=(u\sqrt{N})/(18\sqrt{3d_N})$; here the right-hand side is equal to $u'/6$ in the notation of Table II. Any convenient position and graduation of a vertical scale R/B may be used, provided only that it is graduated in the opposite sense to the scale of γ.

The scale AA' of potency ratios (Fig. 1) is a scale of 4^{μ}, since $\mu=\log$ (potency ratio)/log 4. If the dose ratio is reduced, the scale expands and the nomogram will have to be compressed. Conversely, a wider dose ratio calls for an expansion of the nomogram. These alterations are effected by changing the unit of y. It will usually then be an advantage to alter the degree of shear, increasing λ' when the dose ratio is less than 4:1; alterations of λ' are less necessary if the dose ratio is increased.

Modifications for the 8-point Assay

The estimate of μ is now $M=\frac{5D}{B}$, where D and B are the totals corresponding to the contrasts which Finney (1952b, p. 155) symbolizes as L_p and L_1. The co-ordinates of the hyperbola become

$$\left(\frac{\pm\lambda\sqrt{(5+\mu^2)}}{\lambda\pm\sqrt{(5+\mu^2)}}, \frac{\lambda\{\mu\pm\lambda'\sqrt{(5+\mu^2)}\}}{\lambda\pm\sqrt{(5+\mu^2)}}\right)$$

Suitable values of λ and λ' will have to be chosen for the particular conditions of assay. The value of R can be estimated from 5 ranges, each of which is divided by the square root of the sum of squares of the coefficients (Finney, 1952b) used to evaluate the interaction concerned. If m, n, o, p, and q are respectively the ranges within the contrasts that Finney (1952b, p. 155) symbolizes as L_1', L_2, L_2', L_3, and L_3' and we write

$$R=\sqrt{5}(n+o)+m+p+q,$$

then $s=R/(10\sqrt{10d_N})$, from which it follows that $u'=(u\sqrt{N})/5d_N$ is the appropriate factor for testing the significance of the analogue of P (Table I) and $u'/\sqrt{5}$ is the factor for testing the analogues for C_1 and C_2. (In Finney's notation, P, C_1, and C_2 are respectively L_1', L_2, and L_2'.) A range of values of u' is included in Table II. It should be noted that here we put

$$\gamma(B/R)=(u\sqrt{N})/5d_N=u',$$

so that the values of u' also provide the basis for calibrating the scale of N.

Relative Value of Range and Root-mean-square in Evaluation of Fiducial Limits

Both range and root-mean-square calculations have been applied to the data of 12 assays in which the potencies of the same two tuberculins were compared on sets of 8 guinea-pigs. One set of standard deviations was estimated from range by the method demonstrated in Table I; a second set was derived from least squares, using the same set of degrees of freedom (the error term in Table IV).

TABLE IV

SCHEME FOR THE ANALYSIS OF VARIANCE OF A 6-POINT ASSAY IN 8 RANDOMIZED BLOCKS

	d.f.
Treatments	5
Blocks	7
Materials × blocks	7
Slope × blocks	7
Error	21
Total	47

Five per cent. fiducial limits were calculated from the two sets of standard deviations, using the value of $u(2.07)$ for the range estimates and $t(2.08)$ for the least-squares estimates. The fiducial limits were then expressed as percentages of the mean. It will be seen from Table V that the intervals between the upper and lower percentage limits based on range compare satisfactorily with the corresponding intervals based on the root-mean-square estimate. (The mean interval on the scale of a normal variate is of course $2\times 2.07=4.14$ in units of the standard deviation, when the range estimate is used. For the root-mean-square estimate, since the expected value of $\sqrt{(\chi^2/21)}$ with 21 degrees of

TABLE V

(a) INTERVALS BETWEEN UPPER AND LOWER FIDUCIAL LIMITS WHEN THE LIMITS HAVE BEEN EXPRESSED AS PERCENTAGES OF THE MEAN RELATIVE POTENCY. (b) STANDARD DEVIATIONS

	(a)		(b)	
Assay No.	Range Estimate	Least Squares Estimate	Range Estimate	Least Squares Estimate
1	78	79	1·337	1·357
2	83	84	1·396	1·402
3	84	85	1·322	1·346
4	73	72	1·453	1·439
5	66	62	1·414	1·326
6	55	64	1·236	1·398
7	68	70	1·009	1·028
8	85	82	1·658	1·610
9	87	80	1·492	1·381
10	55	60	1·036	1·129
11	69	70	1·163	1·167
12	95	92	1·743	1·685
Mean ..	74·83	75	1·3541	1·3557

freedom is found to be 0.9882, the corresponding figure is $2 \times 2.08 \times 0.9882 = 4.11$. Thus the observed results are slightly more favourable to the range estimate than can be expected in the long run.)

The analysis of variance (Table IV) is constructed on the principle recently demonstrated by Finney (1952a), that the interactions of " materials " and of " slope " with blocks should be removed from the error sum of squares, because each contains a component that is a function of the block-to-block variation in the slope of the response line, and does not affect the variability of the relative potency estimate.

Bliss (1952) has presented some data in which these interactions were negligible, and surmises that the theoretical argument may have little practical importance; an investigation of the above 12 tuberculin assays revealed an entirely different situation, for in these assays the interactions were often of considerable magnitude; in consequence the errors of the assays were often grossly over-estimated by the earlier procedure. It is also noteworthy that the correct estimate is much more stable from one assay to another than the estimate commonly used which, in our example, would be based on 35 d.f. The separate mean squares with 14 and 21 d.f. are given in Table VI; Bartlett's test for homogeneity gives $\chi^2_{(11)} = 8.60$ for the error, but 32.15 for the pooled interactions.

TABLE VI

ERROR MEAN SQUARE (21 D.F.) AND POOLED INTERACTION MEAN SQUARE (14 D.F.) FROM TWELVE ASSAYS OF THE SAME TUBERCULINS

Assay No.	Error Mean Square	Mean Square for Interactions, of Slope and Materials (with Blocks)
1	1·843	3·843
2	1·966	0·724
3	1·811	0·942
4	2·071	2·167
5	1·758	4·130
6	1·954	4·549
7	1·057	2·563
8	2·593	7·353
9	1·906	1·581
10	1·274	4·975
11	1·363	2·206
12	2·839	4·657
Mean ..	1·8696	3·3075

SUMMARY

1. A scheme, based on a nomogram, has been devised for the routine computation of the 5% fiducial limits of the relative potency of an assay arranged in randomized blocks. An illustration is given of the appropriate method of obtaining a range estimate of error.

2. The theory and construction of the nomogram are discussed; a table of the co-ordinates of the curves is provided to facilitate the construction.

3. Range is shown to be a reliable estimator of the error of tuberculin assays.

4. The magnitude of the interactions of " materials " and " slope " with blocks has been investigated. It is shown that in tuberculin assays they may be large and must therefore be eliminated from the estimate of error.

The data on tuberculin assays were kindly placed at our disposal by Mr. A. B. Paterson, of the Veterinary Laboratory, Weybridge. We wish also to acknowledge helpful discussions with Mr. M. J. R. Healy.

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