NORTHAM, B. E. & NORRIS, F. W. (1952). *J. gen. Microbiol.* 7, 245-256.

A Microbiological Assay of Inositol: its Development and Statistical Analysis

BY B. E. NORTHAM AND F. W. NORRIS

Department of Applied Biochemistry, University of Birmingham*

SUMMARY: The assay of inositol has been developed using *Kloeckera brevis* B 768 and a strain of *Schizosaccharomyces pombe*. In the assay of Difco Bacto yeast extract it was necessary to supplement the basal medium with an inositol-free preparation from yeast extract. A new assay design has been proposed and the results treated statistically.

A microbiological assay of inositol was described by Woolley (1941) using a strain of *Saccharomyces cerevisiae*. This was based on the fact that the rate of growth of the organism is stimulated by inositol; but it has the disadvantage that the organism can grow in the absence of an exogenous supply of inositol. A similar method is given by Williams, Stout, Mitchell & McMahon (1941).

Burkholder, McVeigh & Moyer (1944) described an assay method using *Kloeckera brevis*, and Emery, McLeod & Robinson (1946) have given standard curves for *K. brevis* and *Schizosaccharomyces pombe*. These organisms appeared to be most suitable for further study since they were unable to grow in the absence of inositol even after prolonged incubation.

Although the inositol content of Difco Bacto yeast extract (DYE) was in itself of small importance it was chosen as suitable test material in the development of the assay since it is rich in substances that stimulate the growth of yeast. Hence a reliable method for the assay of yeast extract might also be expected to give reliable results when applied to other complex biological materials.

EXPERIMENTAL

Tube tests. The usual procedure for microbiological assay was followed, but in place of bacteriological tubes, flat-bottomed tubes (Samco), $4 \times \frac{3}{4}$ in., were used. Tubes were selected so that their internal diameter was within the limits 1.66 and 1.72 cm.

Test and standard solutions were added to the tubes from a 5 ml. micro-burette, and the volume made up to 3 ml. with water; 3 ml. of basal medium at double strength was added by means of an automatic pipette, similar in construction to that described by Ridyard (1949).

The tubes containing 6 ml. of test medium and covered with loosely fitting glass caps were sterilized in the autoclave by heating until 10 lb. pressure was reached, at which point the gas was extinguished and the tubes allowed to cool. After inoculation, they were incubated in a water-bath at $25^\circ \pm 0.2^\circ$.

* Formerly Industrial Fermentation.

Estimation of amount of yeast growth

(a) When studying growth rate: the turbidity of the yeast suspension was measured by fitting the tubes into a wooden container placed in the optical beam of the Spekker absorptiometer (Northam & Norris, 1951).

(b) When measuring growth for an assay: the yeast was brought into an even suspension by swirling the medium in the tubes and then pouring into a 1 cm. glass cell. The turbidity was measured in the Spekker absorptiometer fitted with neutral grey-green filters no. H 508.

Dilution unit-turbidity calibration. The drum readings (turbidity) obtained using the Spekker were plotted against the cell concentration of yeast suspensions (dilution units) prepared by the dilution of portions of cultures grown under the usual experimental conditions.

Maintenance of cultures. *K. brevis* and *S. pombe* were subcultured monthly, or more frequently as required. The organisms were carried on malt agar slopes supplemented with a yeast extract, such as Difco Bacto yeast extract, at the rate of 2 g./l.

Inoculum. A loopful of a 24 hr. culture was suspended in 6 ml. of basal medium to give a turbidity in the tubes reading between 0.15 and 0.25 in the Spekker (i.e. approximately 0.2–0.4 mg. dry weight of yeast/6 ml.). For inoculation one drop (20 μ l.) of the suspension was added to each tube from a sterile Pasteur pipette.

Development of the basal medium

Unlike the other B vitamins, inositol is required in relatively large amounts by certain micro-organisms so that in the assay of a test preparation considerable amounts of other substances will be added to the assay medium. Consequently it is important that the basal medium should be as complete as possible with respect to these substances (Woolley, 1947). It was found that the medium of Emery *et al.* (1946) could be improved by increasing the concentration of vitamins and adding casein hydrolysate (CH). Since test substances often have strong buffering effects, it was considered that the basal medium itself should be well buffered. Lactate buffer was found to be satisfactory for this purpose.

The modified basal medium, BMI + CH, corresponds to 'Basal medium III' described by Northam & Norris (1951) with the exceptions that the lactate buffer was adjusted to pH 4.8 and the concentration of the casein hydrolysate was diminished by one half.

Evidence that the basal medium BMI + CH was deficient in certain stimulatory substances required by *K. brevis* is given in Fig. 1*a*. Here the weight of yeast formed after 72 hr. incubation at a level of 12 mg. DYE/6 ml. of medium was approximately 80 % greater than that formed in the presence of an optimal concentration of inositol (curves *A* and *B*). The rate of growth of *K. brevis* was also markedly stimulated by DYE.

In the case of *S. pombe*, DYE had little effect on the rate of growth and increased the final extent of growth by only 10 % (Fig. 1*b*, curves *A* and *B*).

As might be expected from these considerations, the result of the assay of inositol in DYE obtained using *S. pombe* (3.59 mg./g.) was lower than that given by *K. brevis* (3.72 mg./g.) (Table 4). In these circumstances the assay results could not be regarded with confidence, and therefore it was considered desirable to supplement the basal medium with an inositol-free preparation of yeast extract which would supply the unknown stimulatory substances.

Fig. 1. Effect of the supplement on the response of (a) *K. brevis* and (b) *S. pombe* to inositol and DYE. - - - -, BMI + CH; —, BMI Supp.; ×, inositol; ●, DYE.

Preparation of the supplement. As only a limited amount of DYE was available, a yeast extract produced by Norman Evans and Rais Ltd. (NEYE) was used.

Of the methods of fractionation attempted (Northam, 1951) the following was found to be satisfactory:

9.6 g. NEYE were stirred for 10 min. with a mixture of 760 ml. absolute ethanol and 40 ml. 2N-NaOH. After centrifuging, the extract was neutralized with 5N-HCl and filtered through Whatman no. 1 filter-paper. The ethanol was removed by evaporating almost to dryness *in vacuo* at 30°. The residue was taken up in distilled water, adjusted to pH 4.8, filtered on Whatman no. 44 filter-paper, and made up to 100 ml. This supplement was added to the basal medium at the rate of 83 ml./l.

Effect of the supplement on the growth of the organism

At the lower levels of inositol (0.5 μ g./6 ml.) the growth of *K. brevis* reached a limit within 24 hr. in the presence of the supplement compared with 48 hr. in the unsupplemented medium. Moreover, the rate of growth in the presence

of the test preparation (DYE) was the same as that at corresponding levels of the standard preparation (Fig. 2). This suggests that in the presence of the supplement, DYE is acting merely as a dilute solution of inositol, and that accurate assay results could be obtained at any time during the growth of the organism. The effect of the supplement on the final extent of growth of *K. brevis* and *S. pombe* is shown in Fig. 1*a, b* respectively. It was shown that the addition of casein hydrolysate to the supplemented basal medium had no effect on the growth of either organism at various levels of inositol. It was therefore omitted from the basal medium.

Fig. 2. Comparison of the response of *K. brevis* to inositol and corresponding levels of DYE in the supplemented basal medium. —, $A=1.0$; $B=2.0$; $C=3.0$; $D=5.0$; $E=10.0$ μg . inositol/6 ml. - - - -, $A=290$; $B=580$; $C=870$; $D=1450$; $E=2900$ μg . DYE/6 ml.

Design and analysis of the assay

In order to compare assay values for the amount of inositol in Difco Bacto yeast extract (DYE) under the various experimental conditions described it was desirable to ascribe standard errors to these results. The need for the statistical analysis of microbiological assays has recently been emphasized by Finney (1951*a*). Such calculations can only be carried out conveniently when the response, or a metameter of the response, is a linear function of the dose. When the response was measured as the turbidity of the yeast suspension, the dose-response relationship was not linear. Log transformations did not help and other methods were sought.

(1) While there was reason to believe that a linear relationship existed between the weight of yeast formed and the dose, it was impracticable to weigh the yeast directly and a dilution method had to be used. Weight-turbidity transformation curves were constructed by plotting dilution of yeast suspension (grown on a suitable medium) against the turbidities of these dilutions. Certain disadvantages however were inherent in the method:

(a) The weight-turbidity relation of yeast grown at a concentration of, say, 40 μg . inositol/6 ml. was not the same as that grown at 2 μg ./6 ml.

(b) Since the shape of the standard curve varied significantly from one experiment to another, it was not possible to apply a fixed transformation curve to a series of assays.

While this method gave satisfactory results on some occasions, on others there was a significant deviation from linearity which may have been due to fundamental faults in the assay, or to a systematic error in the construction of the transformation curve.

(2) A method of transformation first suggested by Ipsen (1941) and later by Finney (1947*a, b*) avoids the errors inherent in the construction of a weight-turbidity transformation curve. In this method, an accurately determined standard curve is used as the transformation curve and the response in subsequent assays expressed in 'dose-equivalents' by reading off the doses corresponding to those responses from the transformation curve. Thus if a standard curve has the same shape as the transformation curve, then when the response is expressed in dose-equivalents, the regression of dose-equivalents upon dose will be linear and will pass through the origin.

Furthermore, if the test curve has the same shape as the standard curve (and this must be so if the assay is valid; Wood, 1945; Finney, 1945), then it will also become a straight line passing through the origin and the ratio of the slopes of the test and standard lines will give an estimate of the relative potency of the preparation. The deviations from linearity will provide a measure of the validity of the assay. However, the method does not overcome the difficulty that when the shape of the standard curve changes from one experiment to another the transformation curve is no longer applicable.

(3) For this reason, it was considered preferable to prepare a transformation curve with each assay. The usual standard curve can in fact, with suitable modification to the statistical analysis, be used as the transformation curve. If this curve is to be drawn by eye, gross errors must be avoided by arranging that the test and standard responses are near the same level. However, the disadvantages of this procedure may be avoided by having two points (referred to subsequently as a 'Bracket') close together on the standard curve on either side of the test responses. The response may then be expressed in dose-equivalents by simple calculation from the slope of the line joining these two points (the slope is given by y/x in Figs. 3 and 5). The distance between these points is governed by two considerations, viz. (a) they should be sufficiently far apart to permit an accurate determination of the slope between them; and (b) they should be sufficiently close together for a straight line joining the points to deviate but slightly from the true curve.

The transformed data may be analysed according to the method of multiple regression given by Burn, Finney & Goodwin (1950) with certain modifications to the statistical treatment (described below).

It must be emphasized, however, that this design and analysis can only be expected to give a close approximation to the correct result when the

replication is good and the dose-response curve of a regular shape (Finney, 1951, private communication). The method described above for the design and analysis of assays will be referred to as the 'bracket transformation' method.

Worked example of the 'bracket transformation' method

The assay of DYE using *S. pombe* in the supplemented basal medium, BMI Supp., is here described as an example of the application of the bracket transformation method given above. The turbidity was measured in a 1 cm. Spekker cell after 72 hr. incubation. The relationship between dose and response is shown in Table 1 and Fig. 3. The turbidities of the replicates at

Table 1. *Response of S. pombe—turbidity measured in Spekker cell—to various doses of inositol and DYE after 72 hr. incubation in the supplemented basal medium BMI Supp.*

One unit of standard preparation (x_s) = 0.25 μ g. inositol.
One unit of test preparation (x_t) = 75 μ g. DYE.

	Spekker readings						
Units inositol	Standard curve						Mean
3.6	0.172	0.160	0.160	0.169	0.179		0.168
4.4	0.309	0.310	0.307	0.310	0.309		0.309
5.6	0.477	0.485	0.488	0.475	0.479		0.481
6.4	0.568	0.580	0.577	0.589	0.575		0.578
7.6	0.710	0.702	0.718	0.713	0.708		0.710
8.4	0.790	0.787	0.798	0.798	0.782		0.791
Units DYE	Test curve						
4.0	0.261	0.270	0.257	0.290	0.270	0.269	0.269
6.0	0.560	0.563	0.560	0.562	0.565	0.566	0.563
8.0	0.779	0.787	0.775	0.786	0.782	0.786	0.782

each test level were expressed in dose-equivalents by simple calculation from the slopes of the appropriate brackets, and the replicates at each standard level were treated in the same way. Thus a turbidity 'y' may be expressed in dose-equivalents 'Y' from the equation

$$Y = x_1 + (y - y_1) \left(\frac{x_2 - x_1}{y_2 - y_1} \right),$$

where x_1 , y_1 are the co-ordinates of the lower point and x_2 , y_2 are the co-ordinates of the upper point of the standard bracket.

For example, the dose-equivalent corresponding to a turbidity of 0.261 at the lowest test level (Table 1) was given by the equation

$$Y = 3.6 + (0.261 - 0.168) \left(\frac{(4.4 - 3.6)}{(0.309 - 0.168)} \right).$$

The transformed data are given in Table 2 and Fig. 4.

These data were analysed by the method of multiple regression (Burn *et al.* 1950, pp. 97–101) and the symbols used here are the same as those adopted by these authors.

Table 2. Response of *S. pombe* expressed in dose-equivalents

One unit of standard preparation (x_s) = 0.25 μ g. inositol.
One unit of test preparation (x_t) = 75 μ g. DYE.

Standard	x_s	x_s^2	y_s —dose-equivalents						Σy_s	$\Sigma x_s y$
	3.6	12.96	3.62	3.55	3.56	3.61	3.66		18.0	64.8
	4.4	19.36	4.40	4.41	4.38	4.41	4.40		22.0	96.8
	5.6	31.36	5.57	5.63	5.66	5.55	5.59		28.0	156.8
	6.4	40.96	6.32	6.42	6.39	6.49	6.38		32.0	204.8
	7.6	57.76	7.60	7.52	7.68	7.62	7.58		38.0	288.8
	8.4	70.56	8.39	8.36	8.47	8.47	8.31		42.0	352.8
Totals	36.0	232.96	—	—	—	—	—		180.0	1164.8
Test	x_t	x_t^2	y_t —dose-equivalents						Σy_t	$\Sigma x_t y$
	4.0	16.0	4.13	4.18	4.10	4.29	4.18	4.17	25.05	100.20
	6.0	36.0	6.26	6.28	6.25	6.27	6.30	6.31	37.67	226.02
	8.0	64.0	8.28	8.36	8.24	8.35	8.31	8.35	49.89	399.12
Totals	18.0	116.0	—	—	—	—	—	—	112.61	725.34
	$\bar{x}_s = 180$	$\Sigma x_s^2 = 1164.8$	$\Sigma x_s y = 1164.80$						$\Sigma y = 292.61$	
	$\bar{x}_t = 108$	$\Sigma x_t^2 = 696.0$	$\Sigma x_t y = 725.34$						$\Sigma N = 48$

Fig. 3

Fig. 4

Fig. 3. Response of *S. pombe* (turbidity measured in the Spekker cell) to various doses of inositol and DYE after 72 hr. incubation in BMI Supp. $\times$, inositol; $\circ$, DYE.

Fig. 4. Response of *S. pombe* expressed in dose-equivalents. $\times$, inositol; $\circ$, DYE.

Multiple regression. The test and standard lines may be represented by the bivariate equation

$$Y = a + b_s x_s + b_t x_t,$$

where x_s (the dose of the standard preparation) is zero for all doses of the test preparation and x_t (the dose of the test preparation) is zero for all doses of the standard. The regression coefficients may be evaluated from the equations

$$\left. \begin{aligned} b_s S x_s x_s + b_t S x_s x_t &= S x_s y, \\ b_s S x_s x_t + b_t S x_t x_t &= S x_t y, \end{aligned} \right\} \quad (i)$$

where

$$\begin{aligned}
 Sx_sx_s &= \Sigma x_s^2 - (\Sigma x_s)^2/N \\
 &= 1164 \cdot 8 - (180)^2/48 = 489 \cdot 8, \\
 Sx_sx_t &= \Sigma x_sx_t - (\Sigma x_s)(\Sigma x_t)/N \\
 &= 0 - (180)(108)/48 = -405, \\
 Sx_tx_t &= \Sigma x_t^2 - (\Sigma x_t)^2/N \\
 &= 696 \cdot 0 - (108)^2/48 = 453, \\
 Sx_sy &= \Sigma x_sy - (\Sigma x_s)(\Sigma y)/N \\
 &= 1164 \cdot 8 - (180)(292 \cdot 61)/48 = 67 \cdot 5125, \\
 \text{and} \quad Sx_ty &= \Sigma x_ty - (\Sigma x_t)(\Sigma y)/N \\
 &= 725 \cdot 34 - (108)(292 \cdot 61)/48 = 66 \cdot 9675.
 \end{aligned}$$

Substituting these values in equations (i) gives

$$b_s = 0 \cdot 9974177, \quad b_t = 1 \cdot 0395621.$$

The equation for 'a' is given by

$$\begin{aligned}
 a &= \bar{y} - b_sx_s - b_tx_t \\
 &= 292 \cdot 61/48 - (0 \cdot 9974177 \times 3 \cdot 75) - (1 \cdot 0395621 \times 2 \cdot 25) \\
 &= 0 \cdot 0167106.
 \end{aligned}$$

The multiple regression equation therefore becomes

$$Y = 0 \cdot 0167106 + 0 \cdot 9974177 x_s + 1 \cdot 0395621 x_t.$$

Analysis of variance. The regression sum of squares is given by

$$\begin{aligned}
 b_sSx_sy + b_tSx_ty &= 0 \cdot 9974177 \times 67 \cdot 5125 + 1 \cdot 0395621 \times 66 \cdot 9675 \\
 &= 136 \cdot 955037.
 \end{aligned}$$

The total, between doses, and error components are obtained by the usual statistical methods. However, since the dose means of the standard preparation have been forced to be co-linear it is necessary to remove a number of degrees of freedom two less than the number of doses for the standard from the total, between doses, and deviations components of the analysis of variance (Finney, 1951*b*).

The analysis of variance is given in Table 3.

Table 3. *Analysis of variance*

Adjustment for mean		1,783.76275		
Nature of variation	...	D.F.	Sum of squares	Mean square
Regression		2	136.95504	—
Deviations from regression		2	0.00613	0.00306
Between doses		4	136.96117	—
Error		39	0.10038	0.00257
Total		43	137.06155	—

Test of validity. The ratio of the deviations mean square to the error mean square

$$F = \frac{0 \cdot 003064}{0 \cdot 002574} = 1 \cdot 19.$$

With $n_1=2$, $n_2=39$, this is clearly well below the significance level of 3.24, showing that the assay is statistically valid.

Relative potency. Unit dose of the test preparation is equal to R units of the standard, where

$$R = \frac{b_t}{b_s} = 1.0422536.$$

From the definition of the units of the two preparations at the head of Tables 1 and 2, 75 μ g. DYE contains 0.25×1.0422536 μ g. inositol. Hence, 1 g. DYE contains 3.47418 mg. inositol.

Fiducial limits. The approximate variance of the potency ratio R is given by the equation

$$V(R) = \frac{s^2}{b_s^2} \times \frac{Sx_s x_s \times 2RSx_s x_t + R^2 Sx_t x_t}{Sx_s x_s Sx_t x_t - (Sx_s x_s)^2}.$$

This expression is applicable only if the values of g is small (say, less than 0.1) where

$$g = \frac{s^2 t^2 Sx_t x_t}{b_s^2 [Sx_s x_s Sx_t x_t - (Sx_s x_s)^2]}.$$

For good microbiological assays, g will always be negligible (Wood & Finney, 1946), and for this particular assay, $g=0.0001$. Thus:

$$\begin{aligned} V(R) &= \frac{0.002574}{(0.9974177)^2} \times \frac{489.8 - 810 \times 1.0422536 + (1.0422536)^2 \times 453}{489.8 \times 453 - (405)^2} \\ &= 0.0000061566 \\ s_R &= \sqrt{V(R)} = 0.00248 \end{aligned}$$

and

$$R = 1.04225 \pm 0.00248.$$

At 39 degrees of freedom and a probability of 0.05, $t=2.023$ so that the fiducial limits, 2.023×0.00248 on either side of R became 1.03723, 1.04727. The estimated potency, 3.474 mg. inositol/g. yeast extract, was therefore assigned fiducial limits of 3.457, 3.491 mg. inositol/g. Difco Bacto yeast extract.

A similar design and analysis was employed for assays using *K. brevis* (Figs. 5 and 6) with the modification that the dose levels of the standard preparation were 0.75, 1.05; 1.65, 1.95; 2.55, 2.85 μ g. inositol/6 ml. (inositol solution made up at a concentration of 1 μ g./ml.) and the test levels were 270, 540 and 810 μ g./tube (DYE solution at 300 μ g./ml.).

RESULTS

The results for the assay of inositol in DYE under the various conditions discussed are given in Table 4. The assay using *K. brevis* on the unsupplemented medium was computed using a dilution unit-turbidity transformation curve. Since the results were obviously significantly higher than that on the supplemented medium the assay was not repeated using the bracket transformation method.

On the supplemented medium, the assay using *K. brevis* was computed using the bracket transformation method with the six standard doses (i.e. three standard brackets) replicated six times and the three test doses replicated

four times. This was modified to a more efficient design (Finney, 1951*b*) for the *S. pombe* assays with the three standard brackets replicated five times and the three test doses replicated six times.

Fig. 5

Fig. 6

Fig. 5. Response of *K. brevis* (turbidity measured in Spekter cell) to various doses of inositol and DYE after 72 hr. incubation in BMI Supp. ×, inositol; ○, DYE.

Fig. 6. Response of *K. brevis* expressed in dose-equivalents. ×, inositol; ○, DYE.

Table 4. Comparison of assay results

Organism	Basal medium	Period of incubation (hr.)	Potency $\pm$ s.e. Fiducial limits ($P=0.95$)	Test of validity	
				F	P
<i>K. brevis</i>	BMI Supp.	24*	3.441 $\pm$ 0.025 3.39 — 3.49	0.54	> 0.20‡
		72*	3.461 $\pm$ 0.023 3.41 — 3.51	1.32	> 0.20‡
		74†	3.441 $\pm$ 0.021 3.40 — 3.48	0.68	> 0.20‡
	BMI + CH	72†	3.723 $\pm$ 0.019 3.68 — 3.76	8.45	< 0.001
	<i>S. pombe</i>	BMI Supp.	48*	3.462 $\pm$ 0.018 3.42 — 3.50	0.45
70*			3.458 $\pm$ 0.016 3.42 — 3.49	0.77	> 0.20‡
72†			3.474 $\pm$ 0.008 3.46 — 3.49	1.19	> 0.20‡
BMI + CH		73†	3.586 $\pm$ 0.012 3.56 — 3.61	6.61	0.01–0.001

The potency is expressed in mg. inositol/g. DYE.

* Turbidity measured in the tubes.

† Turbidity measured in Spekter cell.

‡ Assay statistically valid.

DISCUSSION

It is evident from Table 4 that addition of the supplement to the basal medium reduced the apparent inositol content of Difco Bacto yeast extract obtained from assays using either *K. brevis* or *S. pombe*. (While estimates of

potency and fiducial limits of statistically invalid assays may not be strictly accurate it is likely that these estimates are sufficiently accurate for the purposes of comparison.) From previous considerations the lower value would be expected to give a truer estimate of the potency. The observation that assay values obtained using *K. brevis* and *S. pombe* in the supplemented medium do not differ significantly provides strong support for the reliability of assays carried out under these conditions.

The choice of organisms for subsequent assays is governed by the following considerations:

(a) Assays using *K. brevis* and *S. pombe* may be completed after 24 and 48 hr. respectively without loss of accuracy.

(b) The 95 % fiducial limits of assays using *S. pombe* were approximately ± 0.5 % as compared with ± 1 % for *K. brevis*.

(c) *S. pombe* requires less inositol than *K. brevis* to produce a given response, and although this does not affect the precision of the assay (Wood, 1946) it may be advantageous in that less test material (and therefore less interfering substances) would be added to the assay medium.

The method is being applied successfully to cereals and cereal products in these laboratories.

The authors are indebted to Dr N. S. Cutts for contributions to the development of the transformation method; to Dr D. J. Finney, Oxford University, for assistance with the statistical matter; and to Dr M. Horwood, of Messrs Norman Evans and Rais Ltd., Wilmslow Road, Cheadle, Cheshire, for samples of yeast extract.

This contribution, carried out under a grant from the Department of Scientific and Industrial Research, formed part of a thesis submitted by one of the authors (B. E. N.) for the degree of Ph.D. (Birm.).

REFERENCES

- BARTON-WRIGHT, E. C. (1946). *Practical Methods for the Microbiological Assay of the Vitamin B Complex and Essential Amino-Acids*. London: Ashe Laboratories.
- BURKHOLDER, P. R., McVEIGH, L. & MOYER, D. (1944). Studies of some growth factors of yeasts. *J. Bact.* **48**, 385.
- BURN, J. H., FINNEY, D. J. & GOODWIN, L. G. (1950). *Biological Standardization*. London: Oxford University Press.
- EMERY, W. B., McLEOD, N. & ROBINSON, F. A. (1946). Comparative microbiological assays of members of the vitamin B complex in yeast and liver extracts. *Biochem. J.* **40**, 426.
- FINNEY, D. J. (1945). The microbiological assay of vitamins: the estimate and its precision. *Quart. J. Pharm.* **18**, 77.
- FINNEY, D. J. (1947*a*). The principles of biological assay. *J. R. statist. Soc. (Suppl.)*, **9**, 46.
- FINNEY, D. J. (1947*b*). Statistical aspects of microbiological assays. *Biochem. J.* **41**, v.
- FINNEY, D. J. (1951*a*). The statistical analysis of slope-ratio assays. *J. gen. Microbiol.* **5**, 223.
- IPSEN, J. (1941). *Contributions to the Theory of Biological Standardization*. Copenhagen: Nyt Nordisk Forlag, Arnold Busck.
- NORTHAM, B. E. (1951). *The Microbiological Assay of Inositol*. Ph.D. thesis, Birmingham University.

- NORTHAM, B. E. & NORRIS, F. W. (1951). Growth requirements of *Schizosaccharomyces octosporus*, a yeast exacting towards adenine. *J. gen. Microbiol.* **5**, 502.
- RIDYARD, H. N. (1949). High accuracy and high speed pipettes and associated apparatus. *Analyst*, **74**, 24.
- WILLIAMS, R. J., STOUT, A. K., MITCHELL, H. K. & MCMAHON, J. R. (1941). Assay method for inositol. *Univ. Texas Publ.* no. 4137, 27.
- WOOD, E. C. (1945). Calculation of the results of microbiological assays. *Nature, Lond.* **155**, 632.
- WOOD, E. C. (1946). The theory of analytical procedures with special reference to microbiological assays. *Analyst*, **71**, 1.
- WOOD, E. C. & FINNEY, D. J. (1946). The design and statistical analysis of microbiological assays. *Quart. J. Pharm.* **19**, 112.
- WOOLLEY, D. W. (1941). A method for the estimation of inositol. *J. biol. Chem.* **140**, 453.
- WOOLLEY, D. W. (1947). The determination of inositol. *Biol. Symp.* **12**, 279.

(Received 19 March 1952)