APPENDIX

STATISTICAL EVALUATION OF RELATIVE POTENCIES OF SELECTED ANTIDEPRESSIVE COMPOUNDS

D. A. WILLIAMS AND A. TODRICK

From the Department of Statistics, University of Edinburgh and the Department of Clinical Research, Crichton Royal Hospital, Dumfries, Scotland

One of the purposes of the investigation was to determine whether there was any empirical correlation of the clinical efficacies of the drugs and their potencies as inhibitors of platelet 5-HT uptake, that is, the external validity of the assay. We must first, however, establish the internal validity of the assay. In this section we therefore discuss the validity of the assumptions made in the analysis of the data and the estimation of the relative potencies.

Previous experience behind the assay has until recently been limited to this and one other laboratory (Cockrill, Somerville & Whittle, 1968) where, in terms of actual work done, it has been fairly extensive. The investigation arose from a chance observation that imipramine causes a fall in blood 5-HT level during therapy. It was extended to structurally related compounds synthesized in the research departments of pharmaceutical firms in the hope that the results might assist in the laboratory assessment of their therapeutic potential. These compounds inevitably became available at irregular intervals and, since their investigation was not the only problem in hand, the data here reported were collected in four phases of concentrated activity lasting 2–3 months and spaced out over a period of 4 years. Towards the middle of each phase the prototype drug, imipramine, was tested to give a standard for comparison. Modifications to the technique during the course of the investigation have been minimal and, of course, have affected the standard as well as the other compounds.

The investigation therefore developed in an unplanned manner and the fundamental condition for validity of any statistical analysis, that of randomization of treatments to experimental units, is absent. Although we proceed with the statistical analysis as if randomization had been used it must be remembered that the estimates of relative potency may be biased.

The data appeared on inspection to possess the properties of parallelism and linearity necessary for the application of the parallel line assay model (Finney, 1964, Chapter 4). The response, that is, the percentage inhibition of uptake of 5-HT by the platelets, appeared to be linearly related to log drug concentration over the ranges of concentration used. The slopes of these lines did not vary greatly from one drug to another.

In testing the assumptions of linearity and parallelism in an analysis of variance we must further assume that the observations are homoscedastic. A plot of the standard deviation of the variation between replicates against the mean response for each treatment suggested that the variation might be greater with treatments which cause less inhibition. This was confirmed by Bartlett's test, which gave a χ^2 of 63·7 with 45 degrees of freedom compared with the tabulated 5% level of 61·7. There is therefore evidence for heteroscedasticity, but the actual magnitude of the slope of the standard deviation/response line is low (0·066/1% change). Since quite a significant degree of heteroscedasticity does not materially affect the validity of the analysis it has been ignored.

The analysis of variance table was calculated; this confirmed that the percentage inhibition/log concentration regressions for the compounds could be assumed linear and parallel ($F = 1·0$ and 0·8 respectively): hence the relative potencies of pairs of compounds may be estimated using the usual formulae (Finney, 1964, § 4.11 and 4.12).

The estimate of the common slope of the regression is 45·85 with standard error $\pm 1·58$. With an error root mean square of 8·320 and $\Sigma Sxx = 27·805$, the value of g for 117 degrees of freedom is negligible (0·0046). The fiducial limits may therefore be determined from the standard errors of the relative potency estimates.

We should next enquire whether the relative potencies can be estimated in the above manner irrespective of the phases in which the compounds were investigated. Our only check on this comes from the pI50 estimates of the imipramine standard which was assayed in each of the four phases. It is found that there are small but nonetheless significant differences between these estimates. No explanation of this in terms of experimental procedure, which would suggest grounds for discarding any estimate or estimates, can be given. The differences must therefore be regarded as real differences between the phases and taken into account in the inter-drug comparisons.

Comparisons between drugs investigated in different phases are therefore made via the standard, assuming that the difference between the pI50 values of any pair of drugs would be the same in all phases. Although this is the simplest model we can assume in calibrating phase differences it is not one that can be tested from our data.

For two compounds P and Q investigated in different phases A and B the relative potency R is then given by

$$M = \log R = m_{P(A)} - m_{Q(B)} - (m_{S(A)} - m_{S(B)})$$

where $m_{P(A)}$ is pI50 of drug P in phase A
$m_{Q(B)}$,, ,, Q ,, B
$m_{S(A)}$,, standard ,, A
$m_{S(B)}$,, ,, ,, B.

Using the notation of Finney, 1964, § 4.11 and 4.12,

$$M = \bar{x}_{P(A)} - \bar{x}_{Q(B)} - (\bar{x}_{S(A)} - \bar{x}_{S(B)}) - \frac{\bar{y}_{P(A)} - \bar{y}_{Q(B)} - (\bar{y}_{S(A)} - \bar{y}_{S(B)})}{b}$$

and Standard Error of M

$$= \frac{s}{b}\left\{\frac{1}{N_{P(A)}} + \frac{1}{N_{Q(B)}} + \frac{1}{N_{S(A)}} + \frac{1}{N_{S(B)}} + \frac{[M - \bar{x}_{P(A)} + \bar{x}_{Q(B)} + (\bar{x}_{S(A)} - \bar{x}_{S(B)})]^2}{\Sigma S_{XX}}\right\}^{\frac{1}{2}}$$

The maximum likelihood estimates and standard errors of the log Relative Potencies have been calculated for all pairings of the thirteen compounds handled in this analysis and are given in Table A. The ranking list given in the results section of the main paper was prepared from this data.

Table A. *Relative potencies of tricyclic antidepressive drugs*
Values tabled are M (= log R) ± s.e.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
3-Chloroimipramine I		0·21 ±0·10 S	0·25 ±0·11 S	0·31 ±0·11 S	0·62 ±0·11 S	0·72 ±0·07 S	0·76 ±0·11 S	0·98 ±0·13 S	1·24 ±0·13 S	1·25 ±0·13 S	1·29 ±0·11 S	1·32 ±0·11 S	1·45 ±0·08 S
Imipramine metho-chloride II			0·04 ±0·07 NS	0·09 ±0·07 NS	0·41 ±0·07 S	0·50 ±0·07 S	0·55 ±0·07 S	0·77 ±0·13 S	1·03 ±0·13 S	1·04 ±0·13 S	1·07 ±0·07 S	1·10 ±0·11 S	1·24 ±0·10 S
3-Methoxyimipramine III				0·06 ±0·07 NS	0·37 ±0·08 S	0·47 ±0·08 S	0·51 ±0·07 S	0·73 ±0·13 S	0·99 ±0·13 S	1·01 ±0·13 S	1·04 ±0·07 S	1·07 ±0·11 S	1·20 ±0·10 S
2 -Methoxyimipramine IV					0·31 ±0·07 S	0·41 ±0·08 S	0·46 ±0·07 S	0·67 ±0·13 S	0·93 ±0·13 S	0·95 ±0·13 S	0·98 ±0·07 S	1·01 ±0·11 S	1·05 ±0·10 S
3-Methylthioimipramine V						0·10 ±0·08 NS	0·14 ±0·07 NS	0·36 ±0·13 S	0·62 ±0·13 S	0·64 ±0·13 S	0·67 ±0·07 S	0·70 ±0·11 S	0·84 ±0·10 S
Imipramine VI							0·05 ±0·08 NS	0·26 ±0·11 S	0·52 ±0·11 S	0·54 ±0·11 S	0·57 ±0·08 S	0·60 ±0·09 S	0·74 ±0·07 S
3-Methylsulphinylimipramine VII								0·22 ±0·13 NS	0·48 ±0·13 S	0·49 ±0·13 S	0·52 ±0·07 S	0·58 ±0·11 S	0·69 ±0·10 S
Amitriptyline VIII									0·26 ±0·11	0·28 ±0·11 S	0·31 ±0·13 S	0·34 ±0·14 S	0·48 ±0·13 S
Desipramine IX										0·02 ±0·10 NS	0·05 ±0·13 NS	0 08 ±0·14 NS	0·22 ±0·13 NS
Nortriptyline X											0·03 ±0·13 NS	0·06 ±0·14 NS	0·20 ±0·13 NS
Protriptyline XI												0·03 ±0·11 NS	0·17 ±0·10 NS
Desdimethylimipramine XII													0·14 ±0·11 NS
Desdimethylamitriptyline XIII													

S = significantly
NS = not significantly
} different at the 5% level of probability ($t_{11?} = 1·9805$).
(These were calculated on values correct to 3 significant figures.)

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