with morphine and challenged with saline; or rats given a single large dose of morphine and challenged with naloxone.

Typical results from an experiment using 181 animals are shown in Table 1. In the group of rats withdrawn from chronic morphine the incidence of jumping and diarrhoea was statistically significant from controls ($P<0{\cdot}01$).

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REFERENCES

BUCKETT, W. R. (1964). A new test for morphine-like physical dependence (addiction liability in rats). *Psychopharmacologia*, **6**, 410–416.

LORENZETTI, O. J. & SANCILIO, L. F. (1970). Morphine dependent rats as a model for evaluating potential addiction liability of analgesic compounds. *Archs int. Pharmacodyn. Ther.*, **183**, 391–402.

MAGGIOLO, C. & HUIDOBRO, F. (1961). Administration of pellets of morphine to mice; abstinence syndrome. *Acta physiol. Latinoam.*, **11**, 70–78.

MARSHALL, I. (1970). Laboratory experiments suitable for practical classes in psychopharmacology. *Br. J. Pharmac.*, **39**, 246–247 *P*.

MARSHALL, I. & WEINSTOCK, M. (1969). A quantitative method for the assessment of physical dependence on narcotic analgesics in mice. *Br. J. Pharmac.*, **37**, 505–506 *P*.

WAY, E. L., LOH, H. H. & SHEN, F. H. (1968). Quantitative assessment of morphine tolerance and physical dependence. *Pharmacologist*, **10**, 188.

Some multivariate statistical techniques applied to pharmacological research

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When the effects of a drug are statistically evaluated, it is possible to measure several responses (such as heart rate and blood pressure) at the same time, or the same response at several times after treatment. In these circumstances the multiple responses can be correlated with one another, and the methods used in statistical analysis of the results should take such correlation into account.

Smart, Sneddon & Turner (1967) described the application of a multivariate technique to psychopharmacological data. The method has now been modified using Hotelling's T^2 statistic (Hotelling, 1931; Anderson, 1958), to test the significance of differences between treatments and of individual regressor variables.

If there are p regressor variables $x_1 \ldots . x_p$ and q response variables $z_1 \ldots . z_q$ the error, sums of squares and products (SSP) matrix $\mathbf{E}$ [based on n degrees of freedom (DF)] can be written as a partitioned matrix

$$\left[\begin{array}{c|c} \mathbf{X} & \mathbf{Y} \\ \hline \mathbf{Y}' & \mathbf{Z} \end{array}\right]$$

where $\mathbf{X}$ is the $p \times p$ matrix of SSP for the regressors, $\mathbf{Y}$ is the $p \times q$ matrix of sums of products of the regressors with the response variables, $\mathbf{Y}'$ is the transpose of $\mathbf{Y}$, and $\mathbf{Z}$ is the $q \times q$ SSP matrix for the response variables. Then the matrix of partial regression coefficients of the response variables on the regressors is defined as

$$\mathbf{B} = \mathbf{Y}' \mathbf{X}^{-1}$$

where $\mathbf{X}^{-1}$ is the inverse of $\mathbf{X}$. The matrix of SSP for regression is $\mathbf{R}=\mathbf{BY}$, and we define the adjusted variance-covariance matrix of the response variables as

$$\mathbf{S} = \frac{1}{(n-p)}\left[\mathbf{Z}-\mathbf{R}\right]$$

The matrix B can be written in the form $\mathbf{b}_1 \ldots \mathbf{b}_p$ where $\mathbf{b}_i$ is the column vector of partial regression coefficients of all the response variables on x_i, the i-th regressor. The variance-covariance matrix of $\mathbf{b}_i$ is $\mathbf{V}_i = x^{ii}\mathbf{S}$, where x^{ii} is the element in the i-th row and column of $\mathbf{X}^{-1}$. Then we have $T_i^2 = \mathbf{b}_i'\mathbf{V}^{-1}\mathbf{b}_i$, and the significance of the regression can be tested by taking the quantity $\frac{(n-q+1)\,T^2}{nq}$ as a variance ratio with q and $(n-q+1)$ DF

If none of the regressors is found to be significant, then no regression adjustments are necessary. If the regressors $x_1 \ldots x_s$ $(s \leqq p)$ are significant, the correction of the test of significance for any contrast between the treatment means is made as follows:

Let $\bar{\mathbf{x}}$ be the $k \times s$ (assuming k treatment groups) matrix of means of the significant regressors, let $\bar{\mathbf{z}}$ be the $k \times q$ matrix of means of the response variables, let $\mathbf{U}$ be the variance-covariance matrix of the significant regressors ($\mathbf{U}$ is derived by eliminating from $\mathbf{X}$ any rows and columns corresponding to non-significant regressors, and dividing the resultant $s \times s$ matrix by n) and let $\boldsymbol{\theta}'$ be a *row* vector of factorial coefficients corresponding to any orthogonal contrast among the treatment means. Now let $\mathbf{d}' = \boldsymbol{\theta}'\bar{\mathbf{z}}$ be the row vector of differences for the contrast in question among the response variables, and let $\boldsymbol{\delta}' = \boldsymbol{\theta}'\bar{\mathbf{x}}$ be the corresponding vector for the regressors. Define the quantities

$$\text{LJ}_0 = \boldsymbol{\delta}'\mathbf{U}^{-1}\boldsymbol{\delta},\ \text{LJ} = \mathbf{d}'\mathbf{S}^{-1}\mathbf{d},\quad \text{f} = \frac{1}{\sum_i \frac{\theta_i}{n_i} + \text{LJ}_0}$$

then $T^2 = f\text{LJ}$, and the T^2 can be tested as a variance ratio with q and $(n-q+1)$ DF as described above.

REFERENCES

ANDERSON, T. W. (1958). *Introduction to Multivariate Statistical Analysis.* London: Wiley.

HOTELLING, H. (1931). The generalization of Student's ratio. *Ann. Math. Stats.*, **2**, 360–378.

SMART, J. V., SNEDDON, J. M. & TURNER, P. (1967). A comparison of the effects of chlorphentermine, diethylpropion and phenmetrazone on critical flicker frequency. *Br. J. Pharmac. Chemother.*, **30**, 307–316.

Effect of five β-adrenoceptor antagonists on the effects of isoprenaline and acetylcholine on human isolated smooth muscle

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The effects of propranolol, practolol, alprenolol, oxprenolol and prinodolol (LB46) were studied on the responses to isoprenaline and acetylcholine of human isolated smooth muscle. The tissues were obtained from operation specimens and were prepared as previously described (Coupar & Turner, 1969). Equilibrium pA_2 values were determined by the method of Schild (1947). pA_2 determinations at 2 min were carried out according to Lockett & Bartlett (1956). A time course for each antagonist