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Healy, M. J. R. and Leech, F. B. 1950. Statistical analysis of results for successive tests on the same organism. *Nature*. (4216), p. 319.

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lower latitudes is a real one, and the question of its presence at higher latitudes is debatable. The fact that the drop in the intensity curve at all elevations occurs at the same thickness of lead near magnetic latitude 22° N. supports the suggestion of Swann and Morris<sup>6</sup> that it is concerned with something happening in the lead and does not depend on the energy of the rays. It is intended to investigate this point in more detail in these latitudes.

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<sup>1</sup> Clay, J., *Physica*, **3**, 332 (1936).

<sup>2</sup> Clay, J., Gemert and Wiersma, *Physica*, **3**, 627 (1937).

<sup>3</sup> Clay, J., Venema and Jonker, *Physica*, **7**, 673 (1940).

<sup>4</sup> Aliya, Chandrashekar S. V., *Nature*, **153**, 375 (1944).

<sup>5</sup> Swann, W. F. G., and Morris, P. A., *Phys. Rev.*, **72**, 1262 (1947).

<sup>6</sup> George, E. P., and Appapillai, V., *Nature*, **155**, 726 (1945).

<sup>7</sup> Fenyves, E., and Haiman, O., *Nature*, **165**, 244 (1950).

### Statistical Analysis of Results for Successive Tests on the same Organism

In a recent letter<sup>1</sup>, Leech has pointed out an error of method which sometimes occurs in the analysis of experimental data; this error "arises from regarding successive tests on the same animal as independent". With this criticism I am in complete agreement. However, as an example of this error, he refers, *inter alia*, to a paper<sup>2</sup> for the statistical analysis in which I am responsible. Bailey also, in a review<sup>3</sup> of this paper, has made a similar criticism. The purpose of this note is to explain the method of analysis used, to indicate how it may be employed where the independence of successive observations cannot be assumed, and to point out that the criticisms of Bailey and Leech do not apply in this particular case.

In the experiment under consideration, the animals were divided into two groups, one treated and one untreated. The treatment was continued over four experimental periods, during each of which measurements of various physical characters were taken for each animal. In determining the treatment effect, the relevant comparison is not the main treatment comparison, but the interaction of treatments and periods. The interaction measures the progressive change, due to the treatment, of the difference between the two groups. On the hypothesis of no-treatment effect, the mean squares for the treatment-period interaction and the cow-period interaction can be shown to have the same expectation, whether or not successive observations on the same animal are independent. Consequently, a comparison of the two mean squares provides a valid test of significance for the relevant treatment effects.

In the published paper<sup>3</sup>, only the bare essentials of the statistical analysis have been presented, and this may have led to some misunderstanding. The mean squares compared in Table 1 are those for treatment-period interaction and cow-period interaction, which, as already explained, give a valid test of significance.

Finally, with reference to a criticism of Bailey<sup>3</sup>, the standard errors quoted for measurements taken at the beginning and end of the experiment were not intended for use in testing significance, but only to indicate the variability of the results. All the significance judgments are based on the analyses of variance. When measurements are taken at autopsy only, the standard errors are then, of course, appro-

priate for testing the significance of the corresponding differences.

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<sup>1</sup> Leech, F. B., *Nature*, **165**, 323 (1950).

<sup>2</sup> McQuillan, M. T., Trikojus, V. M., Campbell, A. D., and Turner, A. W., *Brit. J. Exp. Path.*, **29**, 93 (1948).

<sup>3</sup> Bailey, G. L., *Vet. Bull.*, **19**, 421 (1949).

In the paper referred to, Mr. Williams has avoided the error of method originally attacked, and his analysis has undoubtedly led to correct inferences being made. A theoretical criticism which may be of consequence when the experimental results are less definite can be illustrated by a simplified example.

Suppose that twelve animals are allocated at random to two equal groups receiving different treatments, and that measurements are made on each animal in three successive periods. It is convenient to replace the series of measurements on each animal by its mean and linear and quadratic components. This leads to the following analysis of variance:

		Degrees of freedom
Item 1	Periods	2
2	Treatments	1
3	Treatments × periods—linear	1
4	—quadratic	1
5	Animals	10
6	Animals × periods —linear	10
7	—quadratic	10
Total		35

The average effects of treatment on the mean measurements and on the linear and quadratic components can be estimated, and tests of significance which are separately valid can be made by comparing items 2, 3 and 4 with items 5, 6 and 7 respectively, the latter three items also providing estimates of the standard errors appropriate to these effects. If items 3 and 4 are pooled and compared with a pooled error taken from items 6 and 7, the resulting variance ratio will have an expected value of 1 on the null hypothesis; but in the situation which often occurs in animal experimentation, when item 6 is substantially larger than item 7, the ratio will not follow the *F*-distribution with 2 and 20 degrees of freedom. This procedure may also conceal a significant linear effect by pooling it with a negligible quadratic term.

The problem of obtaining the most efficient estimate of treatment differences when, for example, these differences increase steadily as the experiment progresses, is more complicated; in essence, the required estimate in this case will be a linear function of the mean and linear components, with coefficients depending on the variances and covariance of these components. Once the coefficients have been determined, the linear function can be calculated for each animal separately, and standard errors and tests of significance can be obtained by carrying out an ordinary analysis of variance on these values.

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