INTRODUCTORY PAPERS

# PRACTICAL ASPECTS OF STRAIN VARIATION IN RELATION TO PHARMACOLOGICAL TESTING

BY D. M. BROWN AND B. O. HUGHES

*Department of Pharmacology and Chemotherapy, Beecham Research Laboratories, Brockham Park, Betchworth, Surrey*

THE control of variation in animal experimentation particularly in the field of bioassay and pharmacological screening is perhaps one of the principal problems confronting the industrial pharmacologist. The application of statistical procedures and design to problems of toxicity and bioassay by Trevan (1927) and Gaddum (1933) pointed the way to refinements in experimental method thereby improving the accuracy of the tests. Further advances in bioassay procedure resulted from the classical research of Fisher who used statistical methods to determine precisely the variation due to different factors within the experiment and then by appropriate design, considering each factor, limited the variation to a minimum. With a view to reducing variation still further a number of industrial laboratories concentrated on the production of inbred lines in the hope that more constant and uniform responses would be obtained. This largely became established practice although little experimental evidence was brought forward to support the belief. Mather (1946) subsequently observed that $F_1$ hybrids gave more uniform responses and were generally more vigorous than either of the parent inbred strains. These observations were largely overlooked until McLaren and Michie (1954) and Biggers and Claringbold (1954) independently re-discovered this phenomenon. Biggers, McLaren and Michie (1961) feel in general that the $F_1$ hybrid is the most satisfactory laboratory animal. Chai (1960) has put forward an opposing viewpoint stating that "For the assay of a given substance, the choice of assay animal—either inbred or an $F_1$ hybrid—cannot be made in advance; it has to be based on existing data or determined experimentally". Brown (1961a, b) has lent additional experimental evidence to support this.

Environmental conditions, however, also play a vital part in biological assay. Chance has carried out extensive investigations into the effects of altering the environmental conditions. These investigations have been admirably summarised by Russell and Burch (1959).

Finally in this respect mention must be made of the work of the Laboratory Animals Centre under the direction of Dr. Lane-Petter and of the Animal Technicians Association who have done so much to improve the general quality and health of the laboratory animals. Without a healthy robust animal all experimental work involving animals, whether pharmacological or bacteriological, can be rendered virtually worthless.

Differences in the physiological response of various strains of mice have been clearly recognised. Elizabeth Russell (1955) in an excellent review of the "Significance of Physiological Pattern of Animal Strains in

Biological Research", has drawn attention to the considerable differences that occur between mice to disease susceptibility, nature of disease produced by a given pathogen, survival time of infected individuals; capacity for antibody production, cold tolerance, reaction to specific toxins; sensitivity to and content of various hormones and reaction to endocrine extirpation; differences in normal blood-cell levels, life-expectancy and pathological pattern. The pharmacologist has been perhaps rather slower to give consideration to strain variation and it is the purpose of this paper to draw attention to the considerable variations which may exist and to indicate how, by correct selection of strain the quality of pharmacological assays may be improved.

Siegmund, Cadmus and Lu (1957) have developed a screening technique for mild analgesic drugs which has gained considerable acceptance among industrial pharmacologists. In this test phenylquinone is injected intraperitoneally to mice and a typical response is produced which can be inhibited by analgesic drugs. Phenylquinone produces a characteristically biphasic response in a number of strains of mice—intermittent contraction of the abdomen, rubbing the abdomen on the floor of the cage and stretching of the hind limbs. Unless an adequate degree of this writhing response is produced, the test gives erratic and non-reproducible results (Hendershot and Forsaith, 1959). Similarly in our own laboratory we were unable to get satisfactory responses and we therefore decided to examine as many strains as possible in order to select the one which gave best writhing response. We obtained eight strains through the courtesy of Dr. Lane-Petter of the Laboratory Animals Centre, Carshalton, and three other strains from commercial sources all of which were examined for their writhing response.

## Methods

Random samples of 6 male mice, 5 to 7 weeks old, from each strain were injected intraperitoneally with 2 mg./kg. phenylquinone and the individual reaction of each mouse was recorded for a period of 30 min., noting the cumulative number of writhes at 2 min. intervals. Two mice from each strain were tested on each of 3 days, the strains being randomised throughout the test days. From these results the frequency of writhing during each 2 min. period was computed. The phenylquinone was injected as an 0·2 mg./ml. solution in 5 per cent ethanol and was maintained at 37°, the solution being just below saturation at this temperature. In all tests the technician was unaware of the strain being used. In tests where aspirin was administered it was given orally 50 min. before the phenylquinone.

*Strains used*:

| Pure inbred strains | | Random-bred albino strains (nominal designation) |
|---|---|---|
| CBA | A2G | |
| C57BL/6 | C57L | ALB1 |
| DBA/2 | $C_3H$ | ALB2 |
| C57Br/cd | CE | ALB3 |

## RESULTS

The frequency of writhing in eleven strains of mice is illustrated in Fig. 1, and the total number of writhes per mouse in a 30 min. period following administration of the phenylquinone is shown in Table I. The mice can be divided into three groups: those which show a large number of writhes

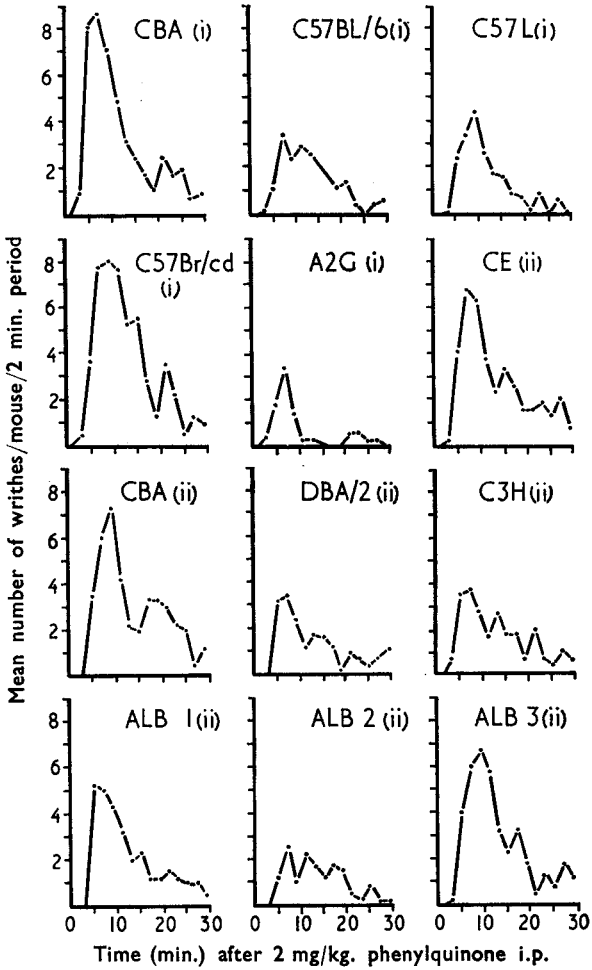


FIG. 1. Frequency of writhing of 11 strains of mice.

CBA, C57Br/cd, CE and ALB3; those which are intermediate in responses C57BL/6, C57L, $C_3H$ and ALB1, and those which give poor responses A2G, ALB2 and DBA/2. The characteristics of the writhes also differ between those groups. In the strain which give a large number of writhes, the syndrome is quite distinct but in the others the phases are less clearly seen, and in the poorly responding animals a writhe may only be detected as a slight contraction of the abdomen or stretch of the limbs.

TABLE I

NUMBER OF WRITHES PER MOUSE IN 30 MIN. FOR EIGHT INBRED AND THREE COMMERCIAL MOUSE STRAINS FOLLOWING INTRAPERITONEAL ADMINISTRATION OF 2 MG./KG. PHENYLQUINONE

| Experiment | Strain | Weight range (g.) | Writhes per mouse | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | |
| (i) | CBA | 14–17 | 38 | 60 | 16 | 90 | 41 | 38 | 47 |
| | C57BL/6 | 12–15 | 3 | 16 | 49 | 19 | 8 | 22 | 19 |
| | C57L | 21–25 | 22 | 21 | 32 | 20 | 1 | 30 | 21 |
| | C57Br/cd | 15–18 | 47 | 17 | 53 | 65 | 49 | 75 | 51 |
| | A2G | 15–18 | 8 | 16 | 9 | 9 | 0 | 10 | 9 |
| (ii) | CE | 14–19 | 36 | 39 | 0 | 49 | 75 | 34 | 39 |
| | CE* | 13–15 | 43 | 40 | 34 | 25 | 33 | | 35 |
| | CBA | 21–24 | 24 | 54 | 34 | 28 | 65 | 39 | 41 |
| | DBA/2 | 8–14 | 7 | 1 | 20 | 36 | 43 | 14 | 20 |
| | $C_3H$ | 20–23 | 12 | 27 | 55 | 20 | 10 | 21 | 24 |
| | ALB1 | 20–23 | 40 | 62 | 5 | 25 | 13 | 32 | 29 |
| | ALB2 | 17–19 | 17 | 12 | 7 | 18 | 16 | 19 | 15 |
| | ALB3 | 15–19 | 39 | 18 | 33 | 77 | 43 | 24 | 39 |

* Litter mates.

Individual variation in the number of writhes within groups is also high even in mice which respond well but this does not detract from using the phenylquinone test to give a graded response with analgesic drugs. Records of the writhing frequencies of batches of five mice from one strain (ALB3) show that 15 mice will give a mean number of writhes of 129 $\pm$ 11·0 S.E.M. For a more accurate test the use of litter mates would obviously reduce this error still further (Table II) but this would generally be impracticable in a screening programme.

TABLE II

MEAN NUMBER OF WRITHES AND A COMPARISON OF THE COEFFICIENTS OF VARIABILITY AT 20 MIN. AND 30 MIN. IN RESPONSE TO INTRAPERITONEAL ADMINISTRATION OF 2 MG./KG. PHENYLQUINONE, IN DIFFERENT MOUSE STRAINS

| Experiment | Strain | 20 min. | | 30 min. | |
|---|---|---|---|---|---|
| | | Mean no. of writhes | Coefficient of variation | Mean no. of writhes | Coefficient of variation |
| (i) | CBA | 39·0 | 51 | 47·2 | 53 |
| | C57BL/6 | 16·3 | 85 | 19·5 | 83 |
| | C57L | 18·7 | 58 | 21·0 | 55 |
| | C57Br/cd | 42·7 | 42 | 51·0 | 39 |
| | A2G | 7·3 | 54 | 8·7 | 59 |
| (ii) | CE | 31·7 | 62 | 38·8 | 63 |
| | CE* | 28·8 | 22 | 35·0 | 20 |
| | CBA | 31·8 | 38 | 40·7 | 39 |
| | DBA/2 | 15·8 | 79 | 20·2 | 81 |
| | $C_3H$ | 19·3 | 65 | 24·2 | 68 |
| | ALB1 | 24·3 | 73 | 29·5 | 69 |
| | ALB2 | 12·8 | 34 | 14·8 | 31 |
| | ALB3 | 33·3 | 51 | 39·0 | 54 |

* Litter mates.

The phase of most frequent writhing is complete in 20 min. although the mice continue to writhe for some time afterwards but at a much reduced rate. No significant difference was found between the estimates of the variability of the number of writhes in 20 min. and 30 min. periods, therefore it is unnecessary to count for more than 20 min.

In order to verify that mice obtained from one source would still retain their writhing characteristics even when reared in a different laboratory, breeding pairs of CBA and C57BL/6 were obtained from the Laboratory Animals Centre. The first strain gave a good writhing response and the second an intermediate response. We raised sufficient numbers to repeat the tests, the results of which are shown in Fig. 2. The curves of the second test overlap precisely the initial curves obtained with both strains.

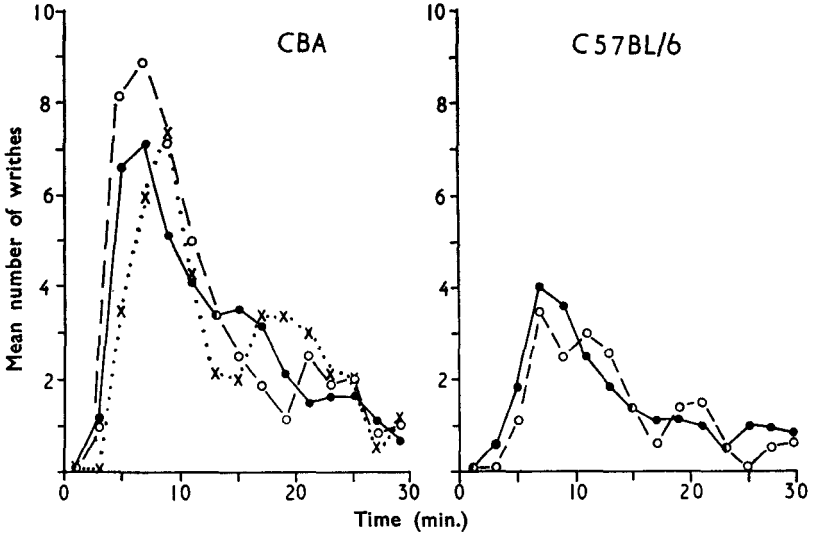


FIG. 2. Frequency of writhing of two strains of mice ○ – – ○ March, 1961 (6 mice); × – – × April, 1961 (6 mice); ●—● November, 1961 (30 mice).

Finally having shown that a mouse strain writhes consistently and then having selected a strain which gives an adequate degree of writhing, it is also necessary to confirm that the animals continue to give the same response to the analgesic drugs (Fig. 3). The dose response line obtained

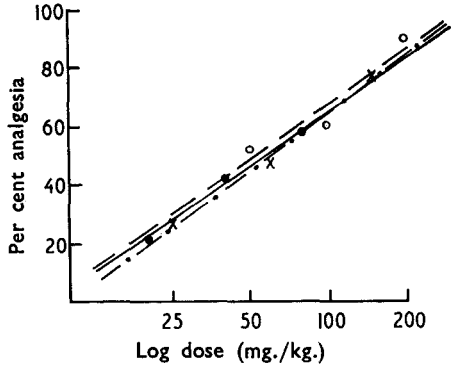


FIG. 3. Single day analgesic assays of aspirin, 15 mice/dose. ●—● 9.12.1960; ○ – – ○ 6.12.1961; × – – × 11.12.1961.

on 9/12/60 for the mean writhing response to graded doses of aspirin for the 20 min. period following phenylquinone was compared with the line obtained exactly a year later (6/12/61) using the same strain of mouse. The two lines were found to be in very close alignment. A further dose response line prepared 5 days after the last test (11/12/61) gave a slope which was also virtually identical to the other two. The ED50 values for each test were respectively 58, 54 and 60 mg./kg. Mice which responded poorly to the phenylquinone failed to give a linear response to aspirin.

## Discussion

By consideration of the genetical, physiological and environmental background of the strain of animal used in assay and screening procedures the quality of a test can be considerably improved with a corresponding reduction in error variance. The sensitivity and accuracy of tests can be enhanced further by the selection of the strain of animal which gives the best pharmacological response. It is not uncommon that many pharmacological testing procedures are acceptable to one laboratory and not to another. The reason for these discrepancies can largely be found in the strains of animal used in the different laboratories. The results with phenylquinone writhing response indicate that there is a large variation between mouse strains, probably much larger than hitherto suspected, and that some strains give such poor responses that it is impossible to get a quantitative response to mild analgesic drugs. The expediency of using a selected strain which gives a high degree of writhing in the phenylquinone test is demonstrated by the consistency in the dose response lines obtained with aspirin and in the comparatively low doses of mild analgesic drugs required to achieve a pronounced and consistent reduction in the frequency of writhing. Additional support to this is provided by Dr. P. F. D'Arcy who tells us that, by using a similar strain of mouse and employing identical assay procedure, he has obtained ED50 values, mean 52 mg./kg. for aspirin. These figures which were obtained independently agree remarkably with our own.

Whilst it is not generally practicable to screen a wide selection of strains before performing every pharmacological test, this would be advantageous when establishing a particular long term programme for the screen or assay of a particular series of drugs. In any pharmacology laboratory, it would be useful to have available two to three strains of mouse of widely differing characteristics (possibly one or two home strains supplemented by commerical stocks), so that should any test appear not to be satisfactory, although other workers have found it to be so, the test could be performed in different strains. Certainly we would advocate that coloured mice be more widely used in pharmacology as well as albino mice instead of the present almost exclusive use of the latter.

With some species, the choice of strain is naturally more limited than with the mouse and it would not be practicable, let alone economical, to screen a variety of strains. Nevertheless, strain variation should be borne in mind as of practical importance to an experiment rather than simply theoretical, particularly when wide discrepancies of results appear between

work performed in different laboratories. It should not, however, become the scapegoat for all unsatisfactory results.

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