The Principles of Humane Experimental Technique

W.M.S. Russell and R.L. Burch

CHAPTER 6

REDUCTION

Many laws regulate variation, some few of which can be dimly seen, and will... be briefly discussed.

The Problem with Variance

The ideas of the last section are relevant chiefly to research, especially applied research. The remaining aspects of reduction form a compact subject, whose application is quite general but of special importance for bioassay, with which we shall be largely concerned in this chapter. The subject turns on what is perhaps the central fact of biology--animals *vary.*

If every single individual (of a species, say) were absolutely identical in all respects, very few animals would be needed for assay purposes. Dose-response curves could be obtained by using one animal for each plotted point, and the estimate of potency of the unknown preparation would be as accurate as we could possibly make it. The use of standard preparations for comparison could be dispensed with altogether.

In the real world, individual animals do vary. We can, therefore, never measure simply how animals of a given species respond to a given dose of a given substance. We have to take a *sample,* out of a population made up of all the other samples we could have taken at any time, and infer from the mean response of the sample chosen, combined with the variation within it, something about the effect of the treatment on any other sample we might have chosen. Our inference is of only relative accuracy, whose degree depends on the *size of the sample, the extent to which individuals of the species vary in response to the drug,* and *the efficiency in design and analysis of our experiment.* It was in just such situations that statistical methods were developed. These, in turn, gave rise to the first formulation of the technical concept of information. In the terms of communication theory, which was to emerge later, the channel in such experiments is always noisy. (For historical reviews, cf. Russell, in press, b, c.)

In practice, everything in bioassay depends upon the reliable reproducibility of results. The usual parameter employed in assay work is the estimate of the slope of the dose-response curve, which in turn depends on the coefficient of the slope itself and the deviational and residual variance which determines its variability. Variation in the physiological responses of animals affects all these, as well as the sensitivity, or absolute level of the curve on the ordinate axis.

A practical step of great importance was taken when standards were prepared. Differences between animal populations in different laboratories and at different times could now be overcome by regular comparison with batches of a standard preparation made in one place and under closely comparable conditions. The potency was now estimated not in absolute terms but by comparison of the curves for unknown and standard. But the assayist may encounter considerable variation between the animals used for the unknown and those used for the standard, and still greater variation between animals used in successive tests. One of the laboratories surveyed by the L.A.B., in answer to the question: "In what ways do any of the animals you are using fall short of your requirements?", wrote as follows:

"The individual variation shown by all species... is the main disadvantage encountered with experiments involving laboratory animals. This variation is controlled by using *large groups of animals* or using a wide dosage range" (our italics). The experience of this laboratory is typical. Chance (1957c) circulated a number of pharmacological laboratories with inquiries about the variance in their assays. The answers revealed that at least *eleven* different tests still have an undesirable and uncontrolled variance, while two more were regarded as far from satisfactory.

The history of this problem reveals three overlapping phases. We have seen that the size of the sample is one important determinant of the accuracy of estimates. This was the first fact to be recognized (and, as the above quotation shows, it is still allotted considerable weight when all else fails). As a result, in the early days of large-scale experimental biology very large numbers of animals were used for each plotted point. At this early stage (cf. Fisher, 1942), nobody knew the exact relation between the numbers of animals used in an experiment and the precision of its results. "Experienced" workers were apt to shake their heads over research results which did not accord with their preconceptions, and damn them by accusing their author of "inadequate controls"--a phrase of positively diplomatic imprecision. The result, in both research and routine, was a competitive rat race. This must have wasted a very large number of animals, and perhaps it is not entirely over.

The next step was taken in the brilliant series of studies in which Sir Ronald Fisher and others built up the modern techniques of statistical design and analysis (see Fisher, 1938, 1956, and above all 1942). In this second phase, it was accepted that a

large contribution to variance must be expected from factors other than differences in dose of the preparation assayed. But it now became possible to eliminate much of this variance from interference with the desired estimates, by ingenious design of experiments. In such designs, blocks of factors surmised to contribute variance could be isolated in the results; the truly residual error variance ("noise" in communication theory terms) was thus cut down. Most important of all, it now became possible to specify, for a given level of residual variance, the exact relation between the number of animals used and the precision of the estimate. Statistical methods alone, even carried to their ultimate refinements, may still leave us with the necessity for using a certain number of animals, sometimes quite large. But the *minimum* necessary can now be specified.

The third phase has barely begun. As a systematic process, it is a product of the fifties. It is not independent of the second phase, and relies upon statistical methods for the adequate segregation of controlled and uncontrolled variance. But the new approach is a serious attempt to reduce unwanted variance at its source, by *controlling* variation between individual animals, through control of the factors which determine it. The most obvious special application is the attempt to make animals more *uniform* in their responses; but the notion of variance control can be carried much further (cf. Chance, 1957c). If physiological variation between individual animals can be brought largely under our control, and statistical methods used to exploit this control to the full, the number of animals necessary for assay purposes may be dramatically reduced; for the number of animals to be used is roughly an inverse function of the residual or uncontrolled variance. In this chapter we shall briefly examine the problem of variance control.