The Principles of Humane Experimental Technique

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CHAPTER 6

REDUCTION

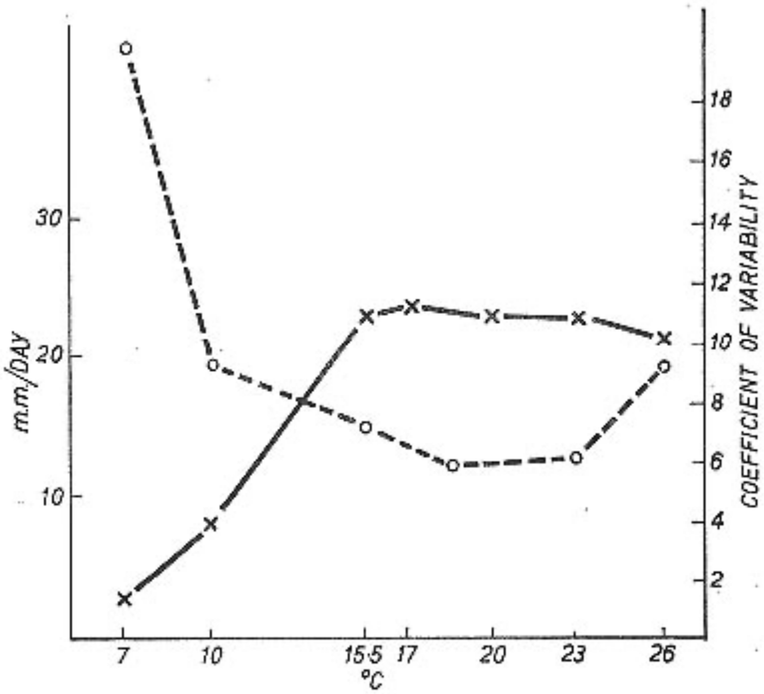
Many laws regulate variation, some few of which can be dimly seen, and will... be briefly discussed.

The Control of the Proximate, Especially Behavioral Environment

Dramatypic Variance and Specific Conditions

So far as the proximate environment is concerned, a simple assumption has been made almost universally until a year or two ago. It is supposed that provided conditions are kept constant (and are not grossly unhealthy) it does not matter what the conditions are: the physiological responses of the animals will tend to be uniform because they are in a uniform environment. This assumption is at variance with the findings referred to by McLaren and Michie in a different context (see last section and Fig. 8), and as an approach to the proximate environment it has now been challenged by Chance (1956b, 1957c). His papers are revolutionary, and we shall devote special attention to them in this section (references are to his 1957c paper unless otherwise specified).

Figure 8. Vigour and Uniformity: The Role of the Environment
(From Michie, 1955, Figure 2)



The double graph shows the results of the work of Went (1953) on peas. The left-hand ordinate refers to the *growth rate* of the peas. This growth rate is plotted as the curve with crosses and solid line. The right-hand ordinate refers to the *coefficient of variability of the growth rate.* This coefficient is plotted as the curve with circles and broken line. The peas were maintained at different *temperatures* for the period of 5 to 13 days after planting; these temperatures are scaled along the abscissa.

It is clear from the curves that those temperature conditions which favored growth also favored a low variability, and vice versa. In general, the better were the environmental conditions, as reflected in increased growth rate, the lower was the variability of the growth rate. Hence conditions which are optimal for the organism favor uniformity.

One of the most obvious features of the proximate environment is the current temperature under which animals are maintained and tested. As Chance has observed, the first studies of the effects of temperature on drug responses concerned only such variables as potency, duration, and rapidity of action. In 1943, Chen and others examined the effect of temperature on these aspects of the response to a variety of drugs. They incidentally provided figures for the standard errors of their observations, and Chance was able to calculate from these that in several responses not only the potency but also the *variance* was affected by the temperature. Sometimes potency and variance were similarly affected; sometimes the two effects were independent.

In 1956 Chance himself published the results of a study of the assay response of immature female rats to serum gonadotrophin. He found that the coefficient of variation in ovary weight (the test response) was affected, independently of the effect on the mean, by a number of different environmental variations. These included changing the animals' cages (this produced different effects according to whether or not littermates were caged together), changes in the social environment (i.e. being caged with stronger rats), variation in the size of the cage, and above all (independent of the last factor) variation in the number of animals caged together. For instance, by caging together a specific number of (female) rats, a predictable coefficient of variation could be obtained, irrespective of the absolute mean response. The optimum condition was found to be that of caging in pairs; the coefficient of variation so obtained was *less than one quarter* of that found when animals were caged singly, and a little more than one quarter of that found when animals were caged in groups of size. Other numbers were tried, and each gave a specific figure for the coefficient of variation.

Under the influence of this discovery, Chance reexamined his own earlier work (1946, 1947) on the toxicity of sympathomimetic amines to mice, and found that here, too, temperature differences affected the mean and variance of the response differentially.

Physiological variability can be affected without any effect on the response mean. Thus, a cyclical change in the variability of histamine excretion by guinea pigs was demonstrated and shown to be unaccompanied by change in the total amount excreted.

Finally, in the gonadotrophin assay, it was found that change in some environmental factors (such as the number of visits to and disturbances of the rat by the experimenter) had no appreciable effect of the *mean* of this response, while others (such as cage change) did.

It follows from all this that constancy of certain conditions (e.g. caging rats by sixes) may still be associated with an avoidably high variance, while change in other conditions (e.g. visits) causes no variation at all in a particular response. Chance was thus led to the important postulate that *"the size of the variance is related to the exact nature of the conditions"*--his italics--"and is sometimes unaffected by differences in the conditions". Hence an environment optimal for uniform response need not be constant (i.e. uniform in time) in all respects, but in certain critical respects it must be not only constant but *right.* To put it in terms we have made familiar by now, in the repetition of experimental conditions *discrimination is more important than fidelity.*

Neither Chen and his colleagues nor anyone else noticed the significance of their (1943) published results on the response variance, until Chance reexamined them in

1957. Moreover, as Chance has had the courage and integrity to point himself, the same applies to *his own* results of 1946-7. From what Chance has now put forward, it is clear that all the necessary knowledge for seeing the significance of the findings was available in the forties. As he puts it,

"... science... is the art of finding out the relevant facts. This means, besides taking advantage of 'lucky breaks' and the opportunities provided by experimental errors, also looking for what is being unconsciously ignored. All awareness is a form of attention and is thus restricted. It is no reflection on any of us, therefore, to find that we wear blinkers half the time."

We shall return to the matter in Chapter 8. But what were the considerations that led Chance to see the importance of a principle everyone else was ignoring, and to search for evidence bearing upon it in sources available to all a decade earlier? (Chance, said Pasteur, favors the mind that is prepared!) These considerations turned out on the application to pharmacological problems of the subject of our first chapter--animal psychosomatics, or behavioral influences on animal physiological responses. It is not too much to say that, in his discussion of this subject (1957c), Chance has opened it up in a new way, and his discussion may have much to contribute to the study of psychosomatics in man. Here we are concerned with the laboratory animal, to which, as already mentioned, the subject had scarcely been applied at all.

The Behavioral Environment and Physiological Responses

In 1953, Lane-Petter (1953a) published a short but important paper about our ignorance of laboratory animal behavior, and the serious consequences this must have in experimentation. There was, as he pointed out, a tendency to disregard this factor altogether. "According to this fallacy, if the animal does not grow the diet is at fault; if it does not breed there is an endocrine disorder; if it will not keep still while it is being inoculated it must be forcibly restrained. Such paralogism is not possible if the animal is regarded"--as of course animals were regarded, decades earlier, in other contexts--"as having its own innate behavior pattern, representing one of the links between the physical environment and the physical response of the animal."

In the same paper, Lane-Petter gave some arresting examples of animal psychosomatics, especially the responses to the behavioral effects of human individuals with whom the animals came into contact. In one guinea pig colony, no deaths had occurred for 5 1/2 months (since it was formed, in fact), until the regular animal technician went on a fortnight's holiday. During the interregnum of another technician, "equally competent and conscientious", four guinea pigs died. Postmortem (including bacteriological) examination gave no clue to the cause of death, and on the return of the original technician the deaths ceased. A less grim and more entertaining observation was that of the surprising slowing of growth of mice at weekends, which could not be correlated with food intake or any fluctuating nonbehavioral factor. It was finally conjectured, plausibly and on the basis of some observation, that the slowing of growth was due to increased activity at weekends, and hence greater consumption of food intake without growth. This, in turn, was thought to depend on the habit of humans of not being in the animal-house at weekends. Human presence depresses murine activity, but when the man's away the mice will play! In this general context, we may also note an observation of Hediger's (1955)--that much work on learning in animals has been vitiated, by failure to take into account the sort of social responses to the human experimenter which the circus trainer ignores at his peril.

No less important for our purposes are homeostatic responses to environmental conditions mediated by the nervous system. Chance remarks that about the only environmental conditions which are taken seriously in bioassay are those of temperature and humidity. Even here, he continues:

"... one gets the impression... that humidity is important to control lest the animal tend to dry up (rather like the crystallization or the deliquescence of a chemical substance), rather than that the alterations in the physiology, which may be made necessary by too humid or too arid an atmosphere, are themselves factors which will distort the animal's response to drugs or various experimental procedures."

Almost all the early work on temperature, for instance, was conceived by its authors as indirect investigation into the action of temperature on enzyme systems in poikilotherms.

Finally, important in their own right and in relation to the other two factors, there remain the responses of animals in various *social* situations, the effects of the social drives of mating, attack, flight and parental behavior, of dominance hierarchies and group relations, and of all circumstances (such as degree of crowding) which influence them. (The study of psychosomatics in man is almost entirely that of social effects upon individual physiology, via the individual's cerebral and other response mechanisms.)

In this connection, Chance (q.v. for references) cites such interesting observations as the following. The incidence of cancer in mice is related to numbers of animals in a cage. (For possible mechanisms of central nervous influence on tumor growth, see Snell and Nicol, 1957, though they eliminate one such mechanism.) Blood eosinophil levels in mice are altered by sounds. Social competition in fighting can induce a slipped disc. Crowding affects the susceptibility of rats to tuberculosis, differentially in the two sexes. (For other examples of animal psychosomatics, see Chapter 1. The work on voles is specially far-going).

Chance emphasizes that we may expect specific physiological states to accompany each behavioral mood, and cites evidence in favor of this.[1] (In the lower vertebrates, where autonomic effects commonly produce visible effects on the surface of the body, which can have social repercussions on other animals, the correlation between behavioral mood and autonomic state has been shown to be perfect--Baerends *et al,* 1955; Morris, 1956a; Russell, in press, c.)

Chance himself has been exploring, for more than ten years, the effects of social factors on bioassay and toxicity responses. In 1946 and 1947 he examined the effects of crowding on the toxicity of amphetamine and other drugs to mice. He was able to work out the chain of behavioral effects which make the same substances so much (up to ten times) more toxic to crowded mice. He also uncovered a number of other factors operating even in single isolated mice. The control of all these factors made possible, for the first time, satisfactory estimates of the toxicity of these drugs (and hence greatly reduced the number of experimental animals exposed to them).

In continuing his exploration, Chance came up against the surprising fact that systematic study of the social behavior of the more common laboratory mammals has scarcely begun--in striking contrast to that of a great many other species (Chance, 1957a; Lane-Petter, 1953a; Russell, 1957b)[2]. The disproportionate neglect of these species is still surprising today. Beniest has just produced an admirable thesis on the parental and fighting behavior of mice (1957). Incidentally, she makes full use of Fisher's exact test thereby doubtless sparing a number of mice experiments on fighting. Among other things, she found that external factors greatly predominate over endocrine ones in determining the reproductive behavior of this species--a relevant finding for our present purposes. (The behavioral sensitivity of mammals to their external environment is notorious, cf. Beach, 1947.) But at this point we may notice her preface. Where both mouse reproductive physiology and mouse maternal behavior are concerned, the remarks, "on découvre avec surprise que les publications sont trés reduites", the mouse is our most common laboratory species!

Chance himself began a determined approach to the study of social behavior in the laboratory rat, and it was on this basis that he obtained his gonadotrophin assay results. From our summary of these, it will be clear that all the effects are behavioral, and such as could only have been detected after sufficient study of normal behavior to pinpoint the likely disturbances. Their other striking feature is the extreme triviality (anthropomorphically speaking) of the variations which could produce such marked effects on this anatomical response. It was, in fact, found that many subtle environmental nuances were significant for endocrine changes other than that in the response itself. The rapier of mild disturbance is replacing the bludgeon of stress. It is a much more humane instrument in itself, but its effects on *reduction* are likely in the long run to be sensational, when these pioneer studies are extended in scale and scope.

The theoretical issues raised are touched on by Chance in his key paper (1957c). They will call, in due course, for profound changes in physiological thought. But the tapping of this rich vein lies in the future, and here we need only point to the importance of such work for reduction by control of variance.

Towards a New Bioassay

In the second part of his (1957c) paper, Chance adumbrates a New Deal for bioassay as a whole. The implications of the gonadotropic assay results are far-reaching. Such factors as cage cleaning, cage changing, introduction of food troughs--in short, any intrusions on the rat's familiar territory (Mead, 1953; Chance and Mead, 1955)--are now seen to be substantially important for assay variance (and the rat is to some extent a bioassay animal).

"Procedures of this kind [writes Chance], are different from laboratory to laboratory, and the days on which they occur also vary. In the same laboratory the timing of these changes may vary from test to test, but what is perhaps less apparent is that in the same laboratory these same factors may interfere differently in supposedly repeat tests, or accidental circumstances may affect one part of a test and not another. A water bottle knocked off and replaced is sufficient interference. A cage found to be defective and replaced will have a profound effect. *Procedures* [our italics] *which would normally go unchallenged must now be carefully controlled and their effects sought after."*

As Chance points out:

"... our lack of knowledge as to what details are important is emphasized by the fact that additional care is taken of particular factors such as light intensity in estrogen assays, for example, when the relevance of these factors is discovered. It should, therefore, be clear that a systematic study should be made of the environmental factors affecting any one procedure."

As a starting point for such a study, Chance provides a systematic classification of environmental factors, and discussion of several of these, such as heat loss in relation to behavior. He notes that rats are tested in conditions which are in many ways not optimal for them. Thus, they are always used (in bioassay) in what is effectively the middle of the night for this nocturnal animal, although their diurnal activity cycle can easily be reversed by suitable environmental control, to make it fit ours. Again, for a variety of reasons, rats in use are often subject to temperatures substantially below their optimum, and this must have manifold behavioral and physiological repercussions. Chance is careful to point out that a correlation between optimum conditions and low variance has yet to be shown in this context, and cannot be assumed *a priori*. However, most of his suggestions would be likely considerably to reduce distress, apart from their advantages for variance control.

Four other general points by Chance deserve notice before we close this chapter. All of them relate to increased control on the experimental animal's physiology and behavior for test purposes.

First, he notes a special possibility--the use of stocks of animals which are free of specific pathogens. Such stocks are now available for laboratory use. Besides removing an obvious source of variance, this is clearly one answer to the problem of contingent mortality.

Second and more general, Chance directs attention to the importance of the *metameter* of a response, a term introduced by Hogben for the variable which is measured as the actual assay response. Many pharmacological papers do not fully specify the nature of a response, the way in which it was measured, and the units in which the measurement was made. Such specification would ideally include complete description of the conditions of testing, but we have seen this to be a goal for the future. Meanwhile, by careful study of the animal used, metameters can be chosen which are standardized, and which minimize variance due to interactions on a short time base between animal and procedure. A very simple improvement of this kind enabled local anesthetics to be assayed at the same level of precision in single tests, instead of in several repeated tests, and by a relatively humane method. This considerably reduced the number of animals needed for the assay (Chance and Lobstein, 1944). (For the special problem of *behavioral* metameters, cf. Russell *et al,* 1954.)

In connection with metameters, we may cite an important comment by Hume (1957c):

"A great many assays depend on the determination of an ED50 or an LD50--that is, on a quantal response which entails the counting of all-or-nothing events (deaths and survivals). That quantal methods are statistically inferior to those which use a continuous variate is recognized by statisticians (Emmens, 1948). It is technically preferable, therefore, to use wherever possible a continuous variate such as body temperature, a reaction-time, the weight of body or organs, the pulse-rate, or an analysis of blood or urine, rather than a discontinuous variate such as a count of deaths and survivals; and meanwhile, from a humane point of view, it is desirable to avoid using death as the endpoint if some more pleasant technique can be found. One cannot help wondering how far the extensive use of the 50% survival test is a hangover due to habit and custom, and whether suitable continuous variates have been sought as diligently as could be desired. Even for testing toxicity with an LD 50, death might not be the only possible endpoint that could be chosen if the phenomena of the moribund state were to be adequately analyzed."

One approach to this problem is currently being tried out under UFAW auspices.

Third, Chance observes that one way of counteracting phenotypic variation is to make animals uniform in particular ways by *training* procedures, thus employing what are normally sources of variance as modes of reducing it[3]. He cites a particularly humane example: the development of tests for mild analgesics by Bonnycastle and Leonard (1950). These workers trained rats to lift their tails away from the source of heat used as a painful stimulus.

"By so doing, [they] obliterated the instinctive variability of response which accounts for one rat squealing, another crouching to a painful stimulus, and a third lifting its tail away from the same painful stimulus. At the same time [they] ensured that the rat was able to behave in such a way that the amount of pain it received was reduced to a minimum. Positive training, therefore, appears to be a possible way of influencing the behavior of animals towards uniformity as well as towards the provision of humane procedures" (Chance).

Finally, Chance himself has noted that variability in innate behavior may include the case of a population which is diethic or *polyethic* (Chance, 1957b), in just the same way as we speak of dimorphism or polymorphism. "We shall undoubtedly have to envisage, therefore, selection of animals from a variable stock, as well as breeding for uniformity, at a later stage of our work" (Chance, 1957c). Selection of this sort is clearly yet another way of controlling variance.

All these brilliant suggestions depend entirely upon the study of laboratory animal behavior, which will provide a new dimension of experimental control. As most of them indicate, this control will bring great rewards in the *refinement* of procedures (see next chapter); here we may conclude that in the study of laboratory animal behavior lie the richest prospects of *reduction.*

[1]In this connection we might note the special care of the blood vessels. Folkow (1955) reviews the cerebral mechanisms controlling vasomotor activity. "Probably any change in psychic activity" he concludes, "is by way of these cortical excitatory and inhibitory areas more or less markedly expressed as an influence on the sympathetic vasomotor fiber discharge". Vasomotor changes are likely to affect profoundly the specific distribution rates of administered substances to different organs, as well as the pattern of the latter's response.
[2]Munn's (1950) *Handbook of Psychological Research on the Rat* contains 474 pages and over 2,500 references. One searches in vain through the mazes of this vast

compilation for more than the sketchiest picture of how rats behave when not solving problems for the experimental psychologist.

[3]This principle may have been "discovered" by natural selection itself. It is possible that some degree of genetic heterogeneity may be countered in the development of behavior by the uniform experiences commonly undergone by members of one species in a circumscribed ecological niche--Russell and Russell, in press. The very general principle underlying such operations has in fact been stated as a theorem by Ashby (1956a, b)--his "Law of Experience".