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 Phenotype

The suggestions and controversies of the past few years have centered around the use of inbred, cross-bred, and random-bred animals for either uniformity or special suitability or both[1]. At first glance, or when some of the main points are summarized, a faint aura of unreality surrounds the dispute. We learn that the principle of using inbred strains as the most uniform populations was first adopted by accident and then recommended widely on the basis of a misplaced inference about the uniformity of homozygous animals (i.e. animals with pairs of identical alleles at many or most of the loci on their chromosomes), without a single valid experimental trial and without the advice of the geneticists, who already almost certainly knew better (see Biggers and Claringbold, 1954). We learn next that in spite of this (at any rate in 1952) very few laboratories have been using inbred lines at all, as strictly defined (Lane-Petter, 1953b; Lane-Petter *et al*, 1955). We learn finally that even strictly inbred lines are not in fact anything like completely homozygous (Gruneberg, 1954; cf. Billingham *et al*, 1954; Cock and Clough, 1956). What, one might well ask, is all the fuss about?

This bold summary does not, of course, do the situation justice. It is true that relatively few *laboratories* were using strictly inbred animals in 1952. But four were using inbred mice for "applied pharmacology" or bioassay, and twelve were using inbred rats for these purposes (Lane-Petter *et al*, 1955). It is probable that large numbers of *animals* were involved. The question whether these animals are more or less uniform than others is, therefore, of real importance.

Nobody denies that, for specific pure and applied research purposes, *particular* inbred strains are a convenience or (as in cancer research) a necessity. (Eleven laboratories were using inbred strains of mice for cancer research in 1952.) Elizabeth Russell

(1955), in an extensive review, has shown that inbred strains of animals are available with characteristic properties of the greatest variety--susceptibility to specific infections, immunological, endocrine, hematological properties, and many others. She argues cogently the great value of all this material for many research purposes. As we have seen, strains specifically equipped with certain characteristics may have their uses in bioassay and especially toxicity testing. It has recently been shown that this sort of isolable variation in test animals is also valuable for human diagnostic purposes (Young, 1957). We shall see that this special advantage need not be offset by loss of uniformity.

But the great development has been the discovery that the most uniform animals are often obtained by making crosses between two inbred strains and using the first (f1) generation. Biggers and Claringbold (1954), in their intriguing historical survey of this aspect of bioassay, have described the spread of the belief that inbred lines are physiologically uniform. As they have shown, the *only* reported experimental comparison in support of this view was made in 1927, when statistical techniques had not advanced far enough for its accurate analysis. Their own reanalysis shows the result to be without significance. They did not find a single published investigation of the response to a treatment in which inbred lines had been shown experimentally to be more satisfactory than random-bred animals. They ascribe the inbred-line fallacy and its rapid propagation in textbooks and discussions to several factors. Among these factors was the introduction of Wistar rats (popular initially on account of relative *morphological* uniformity compared with random-bred animals--a treacherous guide to performance in physiological responses [McLaren and Michie, 1956; Falconer, 1952; Lerner, 1954]). Another contributory cause was a false inference from the true premise that intra-litter is less than inter-litter variability (members of the same litter obviously share a broadly similar developmental *environment).* In 1939, in the first reliable study, Emmens found "to his surprise" that the CBA mouse strain was more variable in estrogen assay response than random-bred colonies. This discovery seems to have evoked no comment, and the subject was only reopened in 1954, when McLaren and Michie in London (1954) and Claringbold Biggers in Sydney (published in full in 1955) independently reported that, in nembutal and estrogen assay respectively, the F1 progeny of crosses between inbred strains were more uniform than random-bred animals, and the latter more uniform than either inbred parent strain.

At almost exactly the same time, interest in the subject of hybrid uniformity was coming to a head among geneticists and farm animal eugenicists. By 1954, several theoretical models had already been advanced to account for a number of observations (chiefly in plants) on the relative uniformity of hybrids (e.g. Robertson and Reeves, 1952; Lewis, 1953). In the *annis mirabilis,* 1954, there also appeared the important book of Lerner, in which he reviewed many similar observations, including some on animals, and put forward a theory of great generality to account for hybrid uniformity and vigor and for some striking properties of genetical populations as wholes. The observed facts of hybrid vigor and hybrid uniformity are believed to be related, and to arise in common from an increased physiological or metabolic *versatility* of hybrids compared with relatively homozygous stock (and inbred lines are *relatively* homozygous). This hypothesis may be put in the simple form of ascribing greater biochemical versatility to a locus with two different alleles, or in more general term of versatility of the gene complex as a whole. This greater flexibility not only makes the hybrids viable in a wider range of environmental conditions, but *buffers* them in the course of development (cf. Waddington, 1953), in such a way that despite environmental differences they are able to reach a given common phenotypic endpoint. From an evolutionary point of view, the exquisite beauty of this mechanism lies in the fact that both conserves genetic variance and secures phenotypic uniformity in any one generation. It thus permits *both* efficient specialization for one environmental range *and* the capacity to tolerate new ones (see Fig. 7).

Figure 7.

Figure Hybridity and Developmental Buffering
(From Lerner, 1954, Figure 6)

These two diagrams of Lerner are intended to show how differences in developmental potentialities could make hybrid animals *either* less *or* more uniform than inbred animals.

"In each case the point of departure is a zygote of a given genetic constitution, largely homozygous for the inbred, largely heterozygous for crosses between inbreds individuals. In both instances *populations* of such zygotes are genetically homogeneous" (our italics). "The curves originating from each zygote represent courses of development of individuals, the variation between them being naturally of environmental nature" (Lerner).

The left-hand model suggests that the hybrid animals will be *less* uniform than the inbreds. This could rise through their capacity to tolerate a wider variety of environmental conditions in development than the inbred animals. Inbreds reared in the extreme conditions would thus die, while hybrids would survive. At the point in the figure where the dashed lines begin, phenotypics differences can be actually measured. By the time this stage is reached, the hybrids will be more varied, for the *surviving* inbreds will be those reared within a narrow range of environmental variation, and will thus be more uniform.

The right-hand model suggests that the hybrid animals will be *more* uniform than the inbreds. On this model, the heterozygousity of the hybrids, by conferring biochemical versatility, *buffers* them against the effects of environmental variation. They can reach the same phenotypic endpoint in many different environmental conditions, just as any mechanism with corrective feedback can attain a fixed goal despite fluctuations in the environment. They thus remain within the unshaded area, and resist environmental pressures which would divert them out of this phenotypic range. The inbreds, lacking this versatility, may be forced by environmental pressures to take pathways of development outside as well as inside the unshaded area. As the figures show, they will probably diverge in different directions, according to the alleles for which they are homozygous. But the end-result will be a greater phenotypic variability than that of the hybrids.

Lerner himself, on the available evidence, prefers the right-hand model. It serves to provide a convincing explanation of those cases, now numerous, where hybrid uniformity is an established fact. We may suggest, however, that both models might apply, according to the relative severity of the environmental pressures. This would mean that in a stable and comfortable environment heterozygous animals are more perfectly specialized, while in a changing and threatening one they can deploy more overt variation. The evolutionary advantages would be impressive.

It must not be concluded that one particular breeding policy is now established at the expense of all others. On the contrary, each bioassay problem must be tackled on its own merits (McLaren and Michie, 1956; and especially Gruneberg, 1955). There are several reasons for this. In general, hybridity is advantageous quite apart from its effect on uniformity, because it confers increased "vigor"--better viability and general health and toughness under all conditions. Even this advantage may be cancelled in practice if it is accompanied by *behavioral* vigor to an extent that makes handling difficult. In general, any breeding policy is bound to have effects on characters other than those primarily envisaged, and these sideeffects may be sources of trouble in practice.

More important, the relative contributions of genotypic and environmental variation differ in different characters (cf. e.g. Falconer, 1952). Hence the finding that hybrids are more uniform than relatively homozygous material is by no means universal for all characters (as reviewed by Lerner). As has been clearly tabulated by McLaren and Michie (1956), our policy must depend upon the relative importance of two components of variance in the character with which we are concerned. The genetic contribution is in effect twofold. *Both* genetic heterogeneity between individuals *and* the relative homozygosity of each individual contribute to phenotypic variance. The former factor acquires weight with characters whose variation is mainly genetically, the latter with those whose variation is mainly environmentally, determined. For the former kind of character, inbreds and F1 crossbreds will tend to be more uniform, for the latter random-breds and F1 crossbreds. The F1 cross is more generally advantageous because it combines the best of both worlds, its individuals being relatively heterozygous and relatively genetically homogeneous. However, the exact relations will differ for each character.

There is, moreover, the fact that the luxury of breeding inbred lines (necessary for obtaining *both* inbreds *and* crossbreds) is costly. Lane-Petter (1952, 1953b) has warned that this cost must be set against that saved by reducing numbers of animals used; he gives some instructive examples. He advises that attempts should be made to attain uniformity by environmental control before toying with genes. The general application here is clear. It is always desirable to know, for any given response, how much numerical advantage will be afforded by use of either inbreds or crossbreds over random-bred animals. If this advantage is considerable, it may still be worthwhile.

A physiological response, or, as we now call it, a dramatypic character, stands in no one-to-one relation with a phenotype character. Usually it will be determined by several. The problem at this stage seems, therefore, to be one for empirical *ad hoc* solution. But to make this a general policy would be a council of despair. There is one general principle to guide us, based on the correlation between hybrid uniformity and hybrid vigor.

Lerner explicitly states that his hypothesis will apply in particular to those characters specially important for the general fitness of the organism. There is, of course, no such thing as a character of no significance for natural selection (Fisher, 1930, 1954). But although in this sense all characters are equal, some characters (to paraphrase Orwell) are more equal than others. The sort to which Lerner refers have been defined by Clarke and Maynard Smith (1955; see also Maynard Smith *et al* 1955, for complicating factors), as those "which are properties of the organism as a whole rather than of one of its parts, and which confer selective advantage in a wide range of environmental conditions". The general principle, largely borne out by observations to date, is that such characters are specially sensitive to homozygosity, and hence liable to be more uniform in crossbred and random-bred animals. (Conversely, coat color, for instance, is notoriously uniform in inbred lines--Falconer, 1952; McLaren and Michie, 1956. It is less easy to understand why inbred mice should be twice as variable as their F1 hybrids in respect to the number of lumbar vertebrae--McLaren and Michie, 1955; much doubt remains to be learned about "vigor".) In general, physiological responses tend to hinge on "vigor" characters as defined by Clarke and Maynard Smith, so there is some *a priori* ground for favoring hybridity for bioassay purposes. It is also worth noting (though not specifically investigated yet, to our knowledge) that resistance to infection must come under this heading. This may have practical implications for diagnosis and bacteriological research.

In 1955 the L.A.B., with its usual alertness, summoned a symposium (published the same year) to discuss the whole problem of laboratory animal breeding (cf., for summary, review by Russell, 1956b). Much of this symposium centered on the problem of uniformity. The only contributor still markedly favoring inbred strains as such as Bacharach (1955b); he concentrated on rebutting the charge of lack of vigor so far as laboratory animals are concerned. Gruneberg (1955) stressed the danger of adopting hasty generalizations either way. Michie (1955) gave a clear account of the advantages and limitations of F1 hybrids. There was no disagreement on the great advantage of using litter-mate controls (i.e. distributing treatments between the members of one litter), a practice advocated by both Bacharach and Michie and supported at length by Mandl (1955). This is, of course, only possible under conditions of controlled breeding in the laboratory.

In practical terms, nearly all authors in stressing that inbred lines are worth having if only to provide crosses (e.g. Michie, 1955; Biggers and Claringbold, 1954; Elizabeth Russell, 1955). Biggers and Claringbold cite the recommendation of Fisher (1949) for livestock and plant improvement, that many inbred lines should be maintained to provide suitable crosses for special environments and purposes. They suggest exploitation of this idea in the laboratory. Subject only to Lane-Petter's (1952-1953b) caution about costing, there is general agreement that special breeding methods, whichever is adopted in each particular instance, are almost certain to promote uniformity. Breeding on the spot also makes possible the use of litter-mate controls, and even with all its contributions to uniformity its humanitarian advantages are not exhausted, for it eliminates contingent inhumanity in transport and assists the training, interest, and morale of animal technicians--a point made by seven laboratories in the L.A.B. returns as a reason for breeding their own animals. There is, indeed, one danger in the maintenance in many different laboratories of sub-lines from one parent inbred strain. The sub-lines may begin to diverge after quite few generations (Lane-Petter and Bloom, 1957; they ascribe this to Sewall Wright's disputable principle of random drift--it could well result from natural selection in the very slightly different environments of different laboratories; cf. e.g. Sheppard, 1954. They may be right, however, in supposing these laboratory populations, unlike any found in nature, to be small enough for the Sewall Wright sampling effect to become appreciable). As a result of such divergence, two laboratories using sub-lines of the same strain might be using physiologically different material under the impression that it was identical. To cope with this problem, the Bureau is trying out an ingenious new production scheme, whereby user laboratories, after a few generations, return to the original source for a new supply of breeding stock (for details, see Lane-Petter and Bloom, 1957).

In the furtherance of phenotypic uniformity, breeding methods, however excellent, will not avail unless supported by control of the developmental environment. This is, of course, what is meant by husbandry. For all practical aspects of this, reference may be made to the *UFAW Handbook* (Worden, 1947; Worden and Lane-Petter, 1957). But there is one important general point about this aspect of the phenotype, derived essentially from the principles of Lerner, and first put forward coherently by Michie (1955; see also McLaren and Michie, 1956).

"Just as the adverse genetic conditions represented by homozygosis find expression in diminished viability, growth rate, and fertility, etc., so do adverse environmental conditions during development result in individuals constitutionally impaired in these same properties. Can the analogy be pushed further? If so, we may expect not only a decrease in vigor to result from adverse conditions of rearing, but also *an increase in variability* (their italics) in traits correlated with fitness" (McLaren and Michie, 1956).

This contention is in fact supported by references to work on rats, mice and plants, where adverse environmental conditions have actually been found to increase phenotypic variability, and optimal conditions (independently assessed) to diminish it (see Fig. 8). It follows that physiological uniformity is likely to be one of the rewards of good husbandry. "In fact we may not be far wrong if we say that whatever conditions, both genetic and environmental, are best for the health of the individual animals are also best for promoting the biological uniformity of the colony" (Michie, 1955). The humanitarian implications are clear, and a very general principle emerges, which has been independently put forward in another context by Chance. It is to his work that we now turn, as the chief approach to the final problem of controlling the proximate, and especially behavioral environment.