

The Principles of Humane Experimental Technique

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Addendum

The year since our text was written has seen much activity in several parts of the field, and we shall notice a few events and publications in this brief addendum. We have also taken the opportunity of mentioning a few earlier sources omitted in our text, and of bringing some of the historical aspects up to date.

The Work of UFAW

Croft, at the Department of Physiology, Royal Veterinary College, London, has been extending and generalizing her earlier findings. She is specially concerned with methods of assessing consciousness in curarized animals, and has been studying several relaxant drugs used both in veterinary practice and in animal experiment.

At Birmingham, in the University Department of Pharmacology and the Ethology Laboratory, Uffculme Clinic, Grant and Mackintosh continue to work for UFAW under Chance's direction. Their program includes three linked studies: the social behavior of the commoner experimental animals, the control of the dramatope, and the perfection of a sensitive toxicity test performed on mice under general anesthesia. Progress has been made with all three studies, and the first behavioral result has already been published--a method of establishing, without experimental interference, the social rank order in a caged group of rats, and a study of the conditions under which stable rank orders can develop (Grant and Chance, 1958). UFAW is now planning to establish an Experimental Unit for Humane and Refined Technique.

The introductory paper of the UFAW Symposium has now been published in full (Hume, 1958).

Experimental Design

It is noteworthy that an essentially sequential method has been worked out for pyrogen tests and presented in the *British Pharmacopoeia* as a convenient formula. This method appeared first in the Addendum published in 1955, and is retained in the current edition of 1958.

Variance Control

An important study has been made of the contribution of environmental factors to the variance of insulin assays of mice. This study, first reported at the L.A.C. Congress of March 1958, will be published this year (Seller and Smart, 1959--we are grateful to the authors for advance notice of the paper). Reduction is a high priority in this assay, and this work is specially to be welcomed.

Mention has been made of the work of Went on peas, which showed that temperature conditions unfavorable for growth also increased the variability of the growth rate. A similar result has now been obtained in mice, where the variability is increased by extreme environmental temperatures, hot or cold (Ashoub *et al* 1958).

This and other recent findings (including Chance's) have been discussed in an excellent review of the variance control problems (Biggers *et al*, 1958). The terminology differs slightly from ours (our "phenotype" = their "constitution", our "proximate environment" = their "environmental factors with transient effects"); but the conclusions reached are essentially those of our Chapter 6 (q.v.). The authors state as a general principle that variance reduction must be achieved not by uniform conditions as such but by the *right* specific conditions. They apply this principle to the genotype, the developmental environment, and the proximate environment. The paper provides tabulated summaries of all the available experiments (on naturally cross-breeding animals) in which inbreds, crossbreds and random-breds have been compared in respect of variability. Two other results may be mentioned (See Biggers *et al* for references). Ovariectomized mice were classified into subgroups based on the response of the vagina to locally applied estrone. The relative dramatic uniformity of these sub-groups persisted for ten weeks, but was specific for this particular treatment. Thus it is possible to select groups of individuals specially uniform in respect of certain components of their *phenotype*. Second, "administration of methyl-4-thiouracil approximately halved the variance of the response to oestrone, thus acting as a kind of 'uniformity drug'"--this obviously raises new possibilities of control.

The work of Chance has underlined the importance of relatively mild states of discomfort in animals, and there is no reason why we should not now aim, on humane and practical grounds, at removing discomfort as well as severe distress. The subject of comfort in animals has received little systematic treatment. An attempt has now been made to sketch the main outlines of this subject, as a basis for further investigation (Russell, 1959).

Substitution and Replacement (cf. Chapter 5)

The comparative substitution of lower for higher animals raises difficult issues. But where great severity is concerned, as in the study and assay of natural venoms, we must be glad to see lower forms substituted for mammals. Professor Findlay E. Russell and his colleagues at Los Angeles have been working on just this problem for many years, seeking in *Drosophila*, molluscs, fishes (and also microorganisms) an adequate substitute for the mouse in venom assay. In 1958, when Professor Russell was working on weever fish poison at the Marine Biological Association's Laboratory, Plymouth, W.M.S.R. suggested through the director (F.S. Russell, C.B.E., F.R.S.) that prawns might be suitable assay subjects. Professor Russell obtained encouraging results, and, although subsequent work has been limited by difficulties of supply, he has recently written that "the work we have with the prawn as a test animal for certain drugs and animal venoms shows promise" (in a valuable letter to W.M.S.R.). For general venom assay purposes, most lower species seem to present difficulties of correlation; but marked success has already been achieved with the chick, limited by definite gain (Russell and Emery, in press)¹. It is pleasant to know that this problem is being pursued with vigor.

Comparative substitution is more welcome when it does not entail severe treatment of the substitute. Witt (1952) has studied the effects of a number of neurotropic drugs (including lysergic acid) on web-spinning spiders. The drug-specific effect is objectively and quantitatively recorded in the shape and pattern of the spun *web*. This technique may be worth further study in the context of experimental psychiatry.

As a pendant to our discussion of absolute replacement techniques, we may mention an interesting paper by Thomas *et al*, on the use of fungi as models for higher animals, especially in cancer research, for which they offer a number of special advantages.

Historical (cf. Chapter 3)

At the end of 1957, the L.A.B. became the *Laboratory Animals Center*. It has moved its premises and expanded its activities, especially its own experimental research, which is concerned mainly with infections of laboratory animals, variance control and general problems of breeding. In 1958, it published another important volume of *Collected Papers*, concerned with the Organization of an Animal Division, which includes American and French as well as British contributions. Lane-Petter remains its director.

The Animal Technicians' Association continues to make progress, and much attention is now being paid to the problems of status and training (Lane-Petter, 1957; Lane-Petter and Lockett, 1958).

As a supplement to our brief historical survey, reference may be made to an extremely interesting paper by Jones and Wood (1949); this paper focused attention on many of the key factors which we have seen to be important for humane technique.

Mention was made of the formation in 1952 of the Institute of Animal Resources in the United States; while bringing other information up to date, we may mention that in 1956 this became the Institute of Laboratory Animal Resources.

ICLA, of which Lane-Petter is now secretary-general, is supported by the International Union of Physiological Sciences as well as the other organizations mentioned. It continues to increase its activity, now publishes regular bulletins, and held a successful international symposium in Paris in October 1958. This symposium, on Living Animal Material for Biological Research, will be published in 1959.

We have repeatedly stated that in this book we have concentrated upon the United Kingdom, and we have given no account of the laws and regulations of other countries, or the national organizations in Japan, the United States, France, Holland and (since 1955) in other countries, or such important organizations of private origin as the Animal Care Panel which arose in the United States in 1950. Since the problem of humane technique is international, this restriction perhaps needs further explanation. We felt we had to start somewhere, and have aimed at providing as much background as possible for the analysis of the (then) L.A.B. survey of 1952. We hope this rather full treatment of the situation in one country has provided enough general principles to make it useful to anyone; and we are encouraged by the increasing evidence that the situations in different countries are, in fact, rather uniform than otherwise. A much longer book on this subject will no doubt be desirable before long. Meanwhile, we can take advantage of this addendum to end with a brief extrapolation in space and time. Five of the ICLA Surveys of the year 1956 have now been published, and we shall now take a glance at the results (see Lane-Petter and Howell, ed., 1958).

The ICLA Surveys

The surveys now available (India, Italy, Japan, Switzerland, United Kingdom) were planned in a coordinated way by ICLA and UNESCO, and represent a triumph of international cooperation. They include a great deal of information about the administration of experiment, animal care and nutrition, housing, infections, procurement and breeding policies, and especially the strains of animals used: for the problem of *specifying* animal material is rapidly becoming the overriding international issue (Lane-Petter, 1958a, b).

"The needs of research workers are the same in any country; that could be foreseen. But the extent to which those needs are met in each country... depends on the number and distribution of universities and research institutes; the development of pharmaceutical research and industry, which are the biggest numerical consumers of animals; the presence of commercial suppliers of animals; the country's geography, which often governs the pattern of supply; and the financial and other resources available for effecting improvements" (Lane-Petter, 1958a).

Every attempt was made to standardize the surveys on the basis of the first British one (Lane-Petter *et al*, 1955); but the classification of laboratories inevitable differed in the different countries. Direct comparison is, therefore, not easy here.

The other two dimensions which specially concern us are the relative numbers of different species and the purposes for which animals are used. Unfortunately the information about the purposes in this survey is inadequate for comparative purposes. In four of the countries, the returns showed too much overlap and uncertainty for quantitative analysis. In the United Kingdom, unambiguous classification was provided, but only into six major categories. These are not perfectly equivalent to either our own categories or those of Lane-Petter *et al* (1955) for the year 1952. It does, however, appear that the numerically important species were distributed among purposes in much the same way in the last two years--with one important exception. From our own analysis (Table 8) it appears that about 62% of the guinea pigs used in 1952 were used for diagnosis. In 1956, the figure in the (probably equivalent) category of diagnosis came to 49%. It does not appear that a correspondingly greater proportion of guinea pigs were used for *research* in 1956; and this accords with prediction. There is some indication that in other countries guinea pigs are also much less widely used for diagnosis than in Britain in 1952, but this and other impressions must await confirmation.

The relative usage of different species is, however, accurately recorded, and turns out, on the whole, to be surprisingly uniform in different countries. Due account must be taken of the great adaptability of rats and mice (Lane-Petter, 1958b); but conservation may also be involved and perhaps diffusion from the countries "older" in experimentation. The different countries differ in respect of the number of species they use. Japan is the most versatile user, then the United Kingdom, then Switzerland; India and Italy use only a dozen or so species apiece (roughly the same ones). But in all five countries mammals overwhelmingly predominate (India: 87.1; Italy: 95.7; Japan: 92.7; Switzerland: 98.0; United Kingdom: 93.6%). The following shows how uniform are the proportions of the numerically important species (or larger groups)--those with a total usage each (in at least one country) of over 10,000:

**The Numbers of the Major Species Used in 1956
(Modified from Table 4 of Lane-Petter and Howell, ed., 1958)**

India Italy JapanSwitzerland United Kingdom

	India	Italy	Japan	Switzerland	United Kingdom
Mice	204,965	149,790	1,275,442	510,145	1,722,534
Rats	32,530	90,920	160,047	186,049	328,357
Guinea pigs	16,220	33,825	67,561	59,085	190,677
Rabbits	9,560	19,392	71,726	24,775	38,157
Hamsters	670	20,507	3,505	710	8,124
Cats	600	100	10,154	5,488	8,341
Dogs	890	18	27,489	183	3,999
Chickens	17,516	2,600	22,549	2,520	896,561
Amphibia	16,670	10,450	96,200	13,210	52,005
TOTAL ANIMALS (including other species)	306,598	309,305	1,750,579	802,977	2,428,291

The differences present (e.g. the popularity of rats in Italy and dogs in Japan) are overshadowed by the general agreement of the ratios.

The survey returns of all five countries probably represented a very high percentage of the total numbers of animals in use. In the United Kingdom, this percentage was estimated as 89% (an improvement of on the 82% return in the 1952 survey). By comparison with the Home Office returns for the two years, it was possible to apply a correction by means of which the two years could be quantitatively compared. This gave rise to the following table, which quantifies the spectacular relative fall in the number of guinea pigs and rise of those of amphibia, the main changes to be observed between 1952 and 1956:

Numbers of Some Species Used in 1956 Expressed as a Percentage Increment or Decrement of Those Used in 1952

(corrected by comparison with Home Office Returns)
(From Lane-Petter and Howell, ed., 1958, Table 52)

percent	
All species	+ 32
Guinea pigs	- 12
Mice	+ 34
Rabbits	+ 18
Rats	+ 21
Chickens	+ 115
Amphibia	+ 382

February, 1959

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¹In many ways, the chick seems to be a better test animal than the mouse, so eventually there may also be a gain in terms of reduction.