The Principles of Humane Experimental Technique

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CHAPTER 5

REPLACEMENT

With respect to the "analogical" ... resemblances between organic beings ...

Modes of Absolute and Relative Replacement

Progress in replacement could be classified in two ways. We could divide up the field in terms of the techniques replaced, using the classification of Chapters 3 and 4. Or we can make our division in terms of the replacing techniques. This is, in fact, much more convenient, and we shall start with an analysis of this kind. Full cross-classification will no doubt eventually be valuable, and in the sections that follow we have been obliged several times to consider particular replacing techniques under several different fields of application. At this stage, we can only list most of the main types of replacing technique, and touch on the relations between them.

We may first distinguish relative and absolute replacing techniques (Russell, 1957a). In relative replacement, animals are still required, though in actual experiment they are exposed, probably or certainly, to no distress at all. In absolute replacement, animals are not required at all at any stage. It follows from what has been said earlier that absolute replacement may be regarded as the absolute ideal. But where relative replacement is combined with great reduction--as in the use of tissue culture in virology--it may be very welcome indeed, and such developments are among the most important in the whole progress of humane technique. Absolute replacement, and relative replacement + reduction, are thus the two optimal developments of all we shall consider in this and the next two chapters.

We may begin with relative replacement. First, there is the case of non-recovery experiments on living and intact but completely anesthetized animals. Provided the anesthesia is general and sufficiently deep, and its time-course properly synchronized with the treatment itself, of course, important (cf. Croft, 1952a, 1957a, d, e and Chapter 2), in relation to contingent inhumanity. Provided the qualification is met, even recovery experiments may fairly be included in this category if they involve, for

instance, the injection of a drug with transient effect which does not outlast the anesthesia. Certain biological assays are performed on anesthetized mammals, e.g. that for pressor activity of posterior pituitary preparations (Waring and Landgrebe, 1950) and the cat and guinea pig methods for the assay of digitalis preparations (British Pharmacopoeia, 7th edn., 1948, p. 821--the latest edition available in 1952). The possibility of imperfect anesthesia means that still further improvement may be possible on such techniques.

Second, we may consider experiments in which animals are still required, but only to furnish preparations after being painlessly killed. This already constitutes a further advance. Provided the euthanasia is satisfactory, and provided there is substantial reduction in numbers, as such experiments are beyond reproach.

They may in turn be subdivided. First, there are experiments on animals deprived of enough of their central nervous system to be reliably regarded as insentient, such as spinal and decerebrate preparations. Such material formed the basis of the work of Sherrington and his associates (Sherrington, 1906; Creed et al, 1932); spinal animals still account for a substantial proportion of all neurophysiological research (cf. CIBA Symposium, 1953). Franklin (1951) has observed that, in consequence of the 1876 Act "and of other, more economic factors", the two main objects of study in physiology teaching courses have been the pithed frog "or some constituent part of it" and the student himself--"for a number of investigations, particularly in the field of respiration and metabolism, are well or even best carried out in man". The third object has been the body of the decerebrated or decapitated mammal, which formed the basis of Sherrington's famous course of practical exercises. Franklin deplores the prohibition by the 1876 Act of non-recovery experiments by students on intact anesthetized animals, "because the excellent object of the act was to avoid the infliction of unnecessary pain, and none such is involved in anesthetizing, and keeping anesthetized, a rat or other mammal during the course of a non-survival experiment". We do not intend to discuss the Act of 1876 or the question of its review, but there does seem a need for rationalization here. Of the two relative replacing techniques just considered, one is permitted and one is prohibited to students (who cannot hold licences or certificates). The difference between them in respect of contingent inhumanity is certainly a real one. But against this we must weigh what is perhaps a more important fact. The acquisition of technical skill in non-recovery experiments would benefit not only human and animal patients, but also (as Major Hume has pointed out to us) animals used later for experimental purposes by the licensed graduate.

The remaining types of relative replacement involve work on the isolated cells, tissues, or organs of vertebrates. The tissues may be maintained for short periods in vitro for acute experiments; the commonest examples are contractile organs of

mammals and amphibia (heart, gut, uterus, etc.) and isolated nerve and nerve-muscle preparations. The latter account for a further large proportion of neurophysiological research, the former for a number of bioassays (notably the adrenal amines, histamine, and oxytocic activity) and much pharmacological research. The use of such acute isolated preparations has depended on the development of suitable perfusion fluids--an important chapter in the history of the biological sciences. A further branch of this type is the study of biochemical reactions of isolated tissues in vitro.

These techniques are all relatively old. The use of chronic, growing preparations of isolated tissue--the technique of tissue culture--is largely a product of the twentieth century. Mammalian tissue cultures (including those obtained from humans at biopsies or operations) have become, since the World War II, one of the most important replacing techniques, and indeed one of the most important developments in biology. We may bracket with tissue culture the use of the hen's egg, its embryo, and membranes.

Tissue culture forms a bridge to the next major division--absolute replacement, in which vertebrate animals are not required at all. For, apart from cultures derived from nonhuman vertebrates and from man himself, there are also invertebrate tissue cultures and, finally, tissue cultures of the higher plants--some of which are eminently suitable for the fundamental study of "both normal and abnormal (cancerous) growth" (Steward et al, 1956). In later sections, we shall not draw our distinctions too fine, and shall discuss all types of tissue culture and even relatively acute isolated preparations under the general heading of work with tissues in vitro. The bulk of this work certainly rests on vertebrate (especially bird and mammal) tissues.

Turning to absolute replacement, we may distinguish four main subdivisions: the use (outside the vertebrate body) of metazoan endoparasites, higher plants, microorganisms (protozoa, bacteria, molds, etcs.), and nonliving physical and chemical systems.

First, there is the study of metazoan endoparasites (nematodes, cestodes, and trematodes) in vitro, as opposed to their study in the living vertebrate host. The preparation of media, in which these parasites can be kept alive outside the body, has offered a formidable challenge, which is gradually being met (Smyth, 1947; Bueding, 1949; Dawes, 1954). This type of replacement is relevant not only to study of the general physiology and biochemistry of the parasites, but to that of the action of chemotherapeutic drugs. The bioassay of these drugs is still performed mainly on living hosts; replacement at this level would be a very real gain.

Second, we may consider the use of higher plants. Perhaps because of the time factor, this mode of replacement has been explored disappointingly little, and less than it

deserves. We may instance the assay of digitalis and related heart-poisons. Reference to Table 13 shows that at least 500 frogs were used for this purpose in 1952, and there is reason to suppose that frogs have continued in use since that year (cf. Anon., Nature, 1954, where a chemical method is discredited, and frogs are still regarded as affording the best method for potency tests of tincture of digitalis). The frog method is a disagreeable one which involves leaving the animals to die overnight (Brit. Pharm., 1948). Non-recovery tests under anesthesia on cats and guinea pigs do not seem to be used exclusively. It might, therefore, be worth reexamining an old observation--the assay of digitalis and other substances by the growth of plant seedlings (Macht and Kranz, 1927). The use of plants might indeed be seriously considered in many other assay and toxicity contexts (cf. also Macht, 1956). Methods based upon them might be relatively slow, and time schedules would have to be fitted carefully to the overall routine of a large drug house (though indeed this also applies to many existing animal assays). But the use of plant seedlings might entail a substantial gain in terms of cost.

If this replacement has not been adequately explored, the same can happily not be said of the next--the use of microorganisms. These have been very extensively used in the context of nutrition, an application which probably by no means exhausts their potential usefulness.

Last of all, there remains the huge field of replacement by nonliving physical and chemical systems--assays of drugs by physicochemical methods, and other uses too numerous to cite. Beyond occasional reference, we shall not attempt to do even summary justice to this vast subject, which deserves at least a monograph to itself even from our present restricted point of view. But we may close this brief survey with a glance at a topical and intriguing example--the use of machines as models for living organisms.

Lord Kelvin is reputed to have said: "If ye canna mak' a model, ye dinna understan' it." This maxim has been followed to a considerable extent by students of the vertebrate nervous system, and a variety of models of great usefulness have been made for various purposes, both mechanical (e.g. Von Holst, 1950) and especially electronic (e.g. Ashby, 1952; Walter, 1953; Taylor, 1956; G. Russell, 1957). In 1955, one of us (W.M.S.R.) arranged a meeting at which Ross Ashby demonstrated an exceedingly simple apparatus to Tinbergen and his ethological students and associates at Oxford. Ashby was able to produce from this machine, more or less on request, analogues of many of the behavioral phenomena which have forced ethologists to develop important concepts. Nobody has ever pretended that these very simple models are of more than negligible fidelity for the system as a whole for whose study they are designed. Until we can build smaller than with transistors or even solid circuits, it will be impossible (if indeed it is useful) to set up a model which approaches the vertebrate brain even in sheer number of elementary unit connections. However, meanwhile

these relatively primitive gadgets will answer many preliminary questions that might otherwise be put to albino rats by electrified grids. Why this is so, is a question we shall deal with in the next section. In brief, the use of such machines is of three kinds. First, they serve to expose logical flaws at early stages of theory construction. "We may need the animals themselves, as it were, on the night; but the machines will do well at rehearsals" (Russell, 1955). Second, such machines often behave unexpectedly, and thus suggest new lines or links of thought. Third, they can be of great value in teaching and class work as substitutes for intact animals or animal preparations. Grey Walter actually uses machines for teaching students or neurology and neurophysiology. He has shown that they have several important advantages over the originals, and has given a clear exposition of the conditions under which they can profitably be used, with examples drawn from demonstrations of nervous activity at several levels (Walter, 1957).

But, in raising the matter of electronic models, we have started an electric hare which can be pursued very much further, into more general regions of interest for our present purpose. This pursuit we shall undertake, or at least signpost, in the next section.