The Principles of Humane Experimental Technique

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CHAPTER 7

REFINEMENT

... endless forms most beautiful and most wonderful have been, and are being evolved.

Generally Superimposed Procedures

There are many refinement procedures, applicable in many different kinds of experiments, which can be added to or superimposed upon the particular procedure chosen for an experiment. We shall not attempt to discuss all these, but may glance at some of the more important or illustrative.

The progress of experimental biology has often hinged on the development of superimposed procedures of this sort. Endocrinology, for instance, had to wait years upon development of antiseptic techniques--nothing could be discovered while nearly all animals died after operations (Pledge, 1939). General advances in surgery, and improved particular operations of widespread application, continue to further this sort of progress. For instance, until recently bilateral adrenalectomy in the rabbit was performed in two stages, the second of which was technically difficult and occasioned a high mortality from hemorrhages. This meant not only that some important research questions remained unanswered, but also that a great many animals were subjected, without scientific profit, to recovery from the first-stage operation. A new method has been reported for bilateral adrenalectomy in one operation; this is said to be accompanied by few casualties (Zak *et al,* 1957). In the case of one endocrine unit it finally became possible to bypass surgery altogether--by so-called chemical thyroidectomy, using antithyroid drugs (cf., for historical sketch, Charipper and Gordon, 1947). The avidity with which this last technique has been snapped up leaves little doubt that endocrinologists have no love of surgery for its own sake. Advances of all these kinds doubtlessly bear upon reduction as well as refinement. The most generally important of all is that of anasthesia, the supreme refinement procedure. This has occasioned perhaps the greatest single advance in humane technique, and has at the same time been virtually indispensable for the advance of experimental biology (cf. Franklin, 1951).

The general principles and practical problems of anesthesia and analgesia in the laboratory have been discussed briefly (1957d) and comprehensively (1957e) by Croft. She has also (1957a) recently discussed the conditions for veterinary and experimental use of the relaxants or curariform drugs which block neuromuscular transmission (among other effects), and which in general should only be used in conjunction with general anesthesia and (in mammals) facilities for artificial respiration. In view of Croft's thorough treatment of the subject, we shall not dilate on it here; but one potential advance deserves special mention. Local anesthesia and local analgesia for short-term purposes are well understood and widely practiced. 'There is probably more known about local anesthesia and local anesthetics than there is about any other field or any other class of compounds showing physiological activity' (Carney, 1954). But a very recent development has been that of preparations which maintain local anesthesia for long periods, measured in days rather than in minutes. These long-acting local anesthetics (which are known to be effective in birds as well as mammals) were produced to cope with the problem of prolonged local pain in human patients, especially after operations. Harmful long-term effects of some of these preparations, which have prompted caution in their use in human patients, might be of no consequence in animals soon to be sacrificed anyway; but the drugs might spare these animals seriously and certainly interfere with the experiments. Postoperative pain may well give rise to specially serious distress in some instances, which it should by now be possible to specify. The whole subject would repay some research; it has been briefly reviewed by one of us elsewhere (Russell, 1957c).

Closely related to the problem of anesthesia is that of euthanasia. This is perhaps of even greater importance in the laboratory, for it is even more universally applicable, and is a necessary condition for the success of relative replacement. It is also a factor in experimental efficiency, in view of the biochemical and cytological disturbances which may be set up at the moment of a distressing death. Finally, it is a province for humane technique even when no experimentation takes place at all in the ordinary sense--for instance in anatomical, histological, and cytological studies, and in the numerically important case of animals used for class dissection in teaching laboratories. Practical instructions for euthanasia of the common laboratory animals are provided by Croft in her article in the *UFAW Handbook* (1957e), and also in a separate manual by Vinter (1955).

After experimental operations, there are often lesions or pieces of apparatus on the surface of the animal's body, which constitute a continual temptation to investigation and worrying. The animal may thus frustrate the object of the experiment, and hence waste any distress to which it has been exposed; it may also directly hurt itself. Long-acting local analgesics might help here but the sight of the unfamiliar object or area would probably still have the same effect. Hence, for many kinds of postoperative

condition, one important general refinement is the provision of maximally humane devices to restrain the animal's movements. As an illustration, we may mention a restraining collar recently devised for dogs subject to operations, which is described as being well tolerated by animals. "The dog fretted very little and apparently had sufficient latitude of movement to enable reasonable activity and comfort in eating and resting" (Weaver and Bowman, 1956).

The taking of blood from large animals is not very distressing *directly,* but even in such contexts there is always room for refinement. "Most blood sampling" (of experimental farm animals) "involves frequent puncture of the jugular vein which may result in damages to the skin and the vessel" (Bianca, 1955). "It certainly causes a state of excitement ranging from mild tension to intense struggling depending on the temperament of the animal and the skill of the operator." Bianca points out that this excitement is specially unfortunate in hematological work, since it can drastically change the concentration of circulating cells, and hence (as he illustrates with a worked example) give rise to serious error in , e.g., blood volume estimates. Bianca has devised a useful apparatus which can be attached to the animal for long periods. Samplings, after the first one, then cause negligible damage and distress, to the great benefit of their accuracy.

Injection (subcutaneous, intramuscular, intraperitoneal, lymph sac, etc.) is an extremely widespread procedure applied to conscious animals for the administration of substances of all kinds. We tend to dismiss the trauma of injection as a very minor inconvenience, to which we after all often expose ourselves without much trepidation. This attitude is to some extent justified, and refinement here is perhaps not a priority. Nevertheless, especially when *small* animals are injected with needles of the usual size, there are often symptoms of momentary distress which must have their autonomic repercussions. (Every good experimenter uses a control group of animals precisely to control injection trauma--an implicit admission.) Refinement here might, therefore, not be unwelcome, and could certainly operate on a generous scale. Advantage might be taken here (Russell, 1957b) of the possibility of administering all kinds of substances in the form of aerosols, taken into the body by breathing. Where many drugs are concerned, it is already known that parallel effects can be produced by this and the older routes of administration; the aerosol has certain advantages in terms of ease of control by the experimenter (Daeutrebande, 1952).

We may end this far from comprehensive sketch with a reference to those methods of refinement (as well as of reduction) which depend on control of the proximate environment of the animal during the test period. For this purpose, Chance's valuable classification of the factors provides a systematic basis (Chance, 1957c). Enough has been said already of this particular group of refinements, so important for bioassay and pharmacology, but doubtless no less important in other contexts (see Chapter 6).

Suffice it to say that in this sphere, as in others, reduction and refinement go hand in hand.