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Toxicology and Drug Development

In toxicology the term drug extends to agricultural chemicals, cosmetics, food additives and even foodstuffs. Moreover, the classification of toxicity tests as acute, subacute or chronic has been extended to include possible teratological or mutagenic effects. Comparative metabolic studies, including man, are already obligatory in America, and long-term animal studies will facilitate assessment of the carcinogenic potential of prospective drugs.

Variation between species does not simplify the selection of a suitable species for chronic studies, despite the common use of rats and dogs. At Huntingdon Research Centre short-term studies and pharmacological screens may involve rodents and sometimes cats, rabbits or dogs. Specific problems in cardiovascular and behavioural pharmacology sometimes require dogs or squirrel monkeys. A variety of species should be used in short-term work: the hen, for example, is valuable in safeguarding man, domestic animals and wildlife from the potential hazards of organophosphorus pesticides. Simple acute toxicity tests have limited value. The view of Paget & Barnes (1964) that the main object of these tests is not to establish a precise LD_{50} but to illuminate some of the drugs' toxic activity is now widely accepted.

Governments justifiably place most reliance on long-term studies, the principal objective of which, as of all toxicity studies, is to determine predictive values for man. We predict great advances in *in vitro* techniques and short-term studies in intact animals, including man. We also believe that, however sophisticated these techniques, governments will still require long-term animal studies.

Dose levels and duration of chronic studies necessary to elicit toxic effects not detectable by a simpler programme are debatable. Increasing, or prolonged dosage may induce a quicker response or merely produce death from acute poisoning. Frequency of dosing and route of administration must also be considered. Dimethline, a respiratory stimulant, was ten times less lethal in the diet than by intubation, and was more toxic in fasted than non-fasted animals. By giving divided doses the normal acutely lethal dose was tolerated for several weeks.

Noel (1962) and Woodard (1965) emphasized the choice of animal as one reacting like man. Since there is no such animal, a compromise is necessary, determined by experience of the existence of known responses. Routinely, the animals should be freely available, able to live and breed comfortably, safe to handle, and remain healthy on diets acceptable to man. Fitzhugh (1959) explained the inevitability of using more than one species for chronic studies, and recommended the albino rat, and dog or monkey. Apart from a greater emphasis on metabolic studies, and an appreciation of the value of monkeys, the present Food and Drug Administration attitude appears to be in agreement with this.

The Committee of the European Society for the Study of Drug Toxicity, presenting a case for shorter chronic studies, emphasized the value of specific pathogen free (SPF) animals and avoidance of inbred strains. Different types of the same species are therefore frequently employed in pharmacological studies.

A wealth of data is available for pure-bred beagle dogs and rabbits. The latter are frequently employed in teratology and fertility studies and for local irritation. Pigs, though less commonly used, have advantages in resembling man in size, diet, anatomy of the alimentary and cardiovascular systems, and renal physiology. Spontaneous changes occur in the aorta and arterial intimæ resembling human atherosclerosis. The skin differs and, of course, large quantities of test drug may be necessary. Most monkeys used in toxicology are obtained wild. The baboon, *Papio anubis* and *P. cynocephalus*, or their hybrids, are more tractable for prolonged studies than *Macaca mulatta*, which is used for metabolic work. We have used the cynomolgus *Macaca irus* for inhalation studies, while the rhesus monkey has been reported to have advantages over the rabbit for predicting ocular effects in man.

Regarding mutagenicity tests, which have been proposed for inclusion in routine safety evaluation procedures, bacteria and drosophila have been employed to detect low frequency mutations in several generations of short life span. There are difficulties in extrapolating such data to man, and the US Food and Drug Administration Advisory Committee considers 'data from tests in mammals would have to be the basis for conclusions regarding mutagenic hazards.'

The currently favoured mammalian mutagenicity test systems are: (1) studies of chromosomes from treated rats, mice or tissue culture examined for evidence of breakages, inversion gaps and translocations; (2) the host-mediated bacteriological assay, using the mouse or rat

peritoneal cavity, to culture the test mixture; and (3) the dominant lethal test, in which male mice or rats are treated with the drug and mated with a series of (usually untreated) females. The latter are killed in mid-pregnancy and counts of implantation and resorption sites, and of viable fœtuses are made.

The predictive value of such tests has yet to be established, as have their advantages over the current well-conducted reproductive and teratological tests used in evaluating safety.

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Vaccine Manufacture, Control and Research

The Biological Division of the Wellcome Research Laboratories is the largest single manufacturer of vaccines, antisera and diagnostic reagents in this country. It is also concerned with research and development aimed at understanding more about the basic mechanisms of immunity and devising more effective means of producing clinical immunity or tolerance, so that existing products may be improved and new ones prepared.

During the year 1969–70 we used very large numbers of animals which cost us about £200,000; in addition there is the cost of their maintenance, housing and feeding whilst in our laboratories. The economic incentive to reduce the number of animals used is therefore strong, yet the numbers used increase inexorably.

The total numbers that we use, including animals that are used to provide meat for media production or normal serum, amount to over 300,000. In addition, we use 750,000 fertile eggs. Usage has been divided into several classes and it is found that the large majority of the animals used are employed in tests to establish potency and safety of products.

The quality of animals is of vital importance, especially for the provision of substrate for virus vaccine production or testing. The solution – partial only at present – to this problem is the substitution of tissue cultures for animals. Cells can be established in continuous culture and stored at low temperatures whilst aliquots are tested exhaustively for freedom from microbial contamination and general safety. The use of such preserved cells has been in large scale use for veterinary virus vaccines like those for foot and mouth and Newcastle diseases. Last year a living attenuated rubella vaccine grown in a human diploid cell strain W138 was licensed in this country. This has reduced the dependence on variable animal hosts and introduced to the substrate for virus vaccine the same seed concept that has been found necessary to control the quality of attenuated microbes. In the early days of vaccinia or BCG vaccines serial passage of the strain occurred, with resulting variations in quality. It gradually became possible, as good techniques of preservation were developed, to produce stable master seeds of vaccine shown to be effective and safe in man and use these for vaccine production. Now a similar seed system can be employed for the cell substrate used for the production of virus vaccines.

Animals have to be used for many purposes, particularly for safety and potency testing. Any reduction in their numbers for these purposes has to come from using larger batches of vaccine, or the improvement of their quality so that more uniform and regular results are obtained. Similar improvements in quality are being sought in other applications so that, for example, higher antibody levels are achieved from animals used for antibody production. By comparison, the usage of animals for research programmes is relatively small, but none the less vital to continued progress in the prevention of disease of man and animals.

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Medicines, Animals and the Law [*Summary*]

Medicines mean medicinal products as defined in Section 130 of the Medicines Act, 1968. In the context of this paper, only a limited area of such a wide group will be referred to. Almost any animals can be and have been involved but the most commonly used are mice, rats, guinea-pigs