ANIMAL MODELS IN PARASITOLOGY

Edited by
Dawn G. Owen

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A Symposium held at the Royal Zoological Society, Regents Park, London, in March 1981

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DAWN G. OWEN

MRC Laboratory Animals Centre, Carshalton, Surrey, United Kingdom

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Symposium Contributors

J. P. Ackers, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT

D. J. Bradley, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT

W. Bray, Imperial College Field Station, Ascot, Berks. SL5 7DE

F. E. Cox, Department of Zoology, King's College, Strand, London WC2R 2LS

J. D. Dargie, Animal Production and Health Section, Joint FAO/IAEA Division of Isotope and Radiation Applications of Atomic Energy for Food and Agriculture Development, Wagramstrasse 5, P.O. Box 100, A-1400 Vienna, Austria

D. A. Denham, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT

M. J. Doenhoff, London School of Hygiene and Tropical Medicine Field Station, Winches Farm, St Albans, Herts.

D. Dunne, London School of Hygiene and Tropical Medicine Field Station, Winches Farm, St Albans, Herts.

M. F. W. Festing, MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF

P. C. C. Garnham, Imperial College Field Station, Ascot, Berks. SL5 7DE

R. Harrison, London School of Hygiene and Tropical Medicine Field Station, Winches Farm, St Albans, Herts.

O. Hassounah, London School of Hygiene and Tropical Medicine Field Station, Winches Farm, St Albans, Herts.

D. C. Jenkins, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

Michele Jungery, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, Headington, Oxford OC3 9DU

N. McHardy, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS
Diane J. McLaren, National Institute for Medical Research, The Ridgeway, London NW7 1AA
H. Murare, London School of Hygiene and Tropical Medicine Field Station, Winches Farm, St Albans, Herts.
Bridget M. Ogilvie, The Wellcome Trust, 1 Park Square West, London NW1 4LJ
M. Elaine Rose, Houghton Poultry Research Station, Houghton, Cambs. PE17 2DA
A. Sabah, London School of Hygiene and Tropical Medicine Field Station, Winches Farm, St Albans, Herts.
S. R. Smithers, National Institute for Medical Research, The Ridgeway, London NW7 1AA
R. J. Terry, School of Biological Sciences, Brunel University, Uxbridge, Middlesex UB8 3PH
D. Wakelin, Department of Zoology, University of Nottingham, University Park, Nottingham NG7 2RD

Introduction

The symposium reported here was convened in order to examine the model systems in use for the study of those helminth and protozoan parasites which cause diseases of great medical or veterinary importance.

The most frequently used laboratory animal hosts are the rat (*Rattus norvegicus*) and mouse (*Mus musculus*). Beginning at about the turn of the century, many inbred lines have been developed and spontaneous mutations maintained. This has led to the great diversity of rodent stocks currently available. The increasing use of gnotobiotic techniques since 1950 has subsequently allowed the mass production of microbiologically defined animals, and these two factors together have resulted in a highly sophisticated product being available for the research worker.

The first session of this symposium was concerned with the genetics of inbred strains, whilst the third dealt specifically with the relevant features of two particular mutant stocks – the nude mouse and the nude rat; interest in these centres largely around the many useful features related to their athymic condition.

In the second session some less common laboratory hosts (such as primates) and rodents other than rats and mice were discussed and in the final session the application of techniques *in vitro* to immunological and chemotherapeutic studies was considered.

The host, or culture flask, is of course only half of the system, and the choice of model parasite is also of prime importance. In many instances the species of parasite causing disease in man is not amenable to growth in a laboratory host (e.g. *Wuchereria bancrofti* or *Onchocerca volvulus*), or will only grow in a rare, threatened, or impossibly expensive host (such as the human malaria parasites in *Aotus trivirgatus,* the douroucouli monkey). Many parasites have been successfully persuaded to grow in artificial media, and some, such as the asexual forms of *Plasmodium falciparum,* will flourish in a simple culture (Trager and Jensen, 1978). If the organism is also difficult or impossible to culture then a completely artificial system is all that is available. Thus for the filariases, *Litomosoides carinii* in cotton rats (*Sigmodon hispidus*) became the favoured model, despite the taxonomic separation of both parasite and host from their principals in the disease. The establishment, then, of *Brugia malayi* in the peritoneal cavity of the Mongolian jird (*Meriones unguiculatus*) (Ash and Riley, 1970) is momentous for the future of chemotherapy and immunology of human filariasis.

Maintenance *in vitro* of parasites is very desirable for basic biochemistry; for mass production of antigens leading, one hopes, to practicable vaccines; and for many other biological purposes free from the complex immune responses of the animal host. However, the papers collected here make it clear

that many animal model systems flourish and will remain, for the foreseeable future at least, the main basis for the collection of data on the parasitology of man and animals. It is hoped that this volume conveys to the reader some of the thought-provoking atmosphere of the symposium and the vigour of the debate.

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Trager, W. and Jensen, J. S. (1978). *Nature (Lond.)*, **273**, 621.

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The Importance of Genetic Susceptibility

CHAIRMAN: J. G. M. Shire

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1
Genetic Manipulation of the Host as a Method in Parasitology

M. F. W. Festing
(MRC Laboratory Animals Centre, Woodmansterne Road, Carshalton, Surrey SM5 4EF, UK)

INTRODUCTION

A wide range of genetically defined laboratory animals are now being used by parasitologists as a means of investigating host-parasite relationships. These defined animals include inbred, congenic and recombinant inbred strains, F_1 hybrids and a wide range of different mutations. Selection for resistance and susceptibility to the parasite or for immune function is another promising method both of developing better hosts and of studying the host-parasite relationship. The aim of this chapter is to outline the characteristics of the main types of genetically defined laboratory animals and to show the ways in which they can be used most effectively in research. Special attention is given to the use of immunodeficient mutants, as many such mutants are now available, although some of them have not yet been used by parasitologists.

INBRED STRAINS

Grüneberg (1952) stated that 'The introduction of inbred strains into biology is probably comparable in importance with that of the analytical balance into chemistry'. Most research workers will by now be aware that an inbred strain is one which has been derived by 20 or more generations of brother X sister mating. Such strains have many properties which can be of immense value in research. These properties are discussed in detail by Festing (1979), but some key properties may be summarised very briefly as follows.

Isogenicity

All members of an inbred strain should be isogenic, or genetically identical at more than 99 per cent of the loci which were segregating in the original base population. Individuals of such a strain will be histocompatible. As all individuals are genetically identical, genetic typing of a single individual (say at the major histocompatibility complex) is sufficient to type the whole strain. As any pair of individuals taken from the colony will have a complete set of the genes present in the colony, a daughter colony founded by a single breeding pair will be genetically identical with the parental colony.

Homozygosity

All individuals of an inbred strain should be homozygous at more than 99 per cent of all genetic loci. Thus, they will normally breed true and will not carry any hidden recessive genes, apart from the very small number remaining as 'residual heterozygosity' and as a result of new mutations (Bailey, 1977).

Phenotypic Uniformity

There should be virtually no genetic variation within an inbred strain, and this usually leads to a reduction in the observed phenotypic variation. This increased uniformity, which is particularly apparent with respect to immune responses, may have important practical implications. For example, Wakelin (1975) studied the number of *Trichuris muris* worms recovered after an experimental infection of CFLP outbred and NIH inbred mice. The mean number (± S.D.) of worms recovered was 98.3 ± 42.3 in the CFLP and 78.8 ± 14.9 in the NIH. The variation was therefore much larger in the CFLP. Suppose the aim were to study the effect of some treatment on the worm burden in mice, and the research worker set a goal of an 80 per cent chance of detecting a treatment effect as large as 10 per cent of the mean, as given in Wakelin's paper, with a 5 per cent significance level. Using tables given by Cohen (1969), it can be calculated that only 92 inbred compared with 512 outbred mice would be needed. In other words, the use of the more uniform inbred mice can substantially reduce the size of an animal experiment. Indeed, it would be quite impractical to carry out such a large experiment with the outbred mice, and the research worker would normally have to be satisfied with a more modest goal.

Individuality

Differences between inbred strains may be found for virtually every characteristic that has been studied so far. These differences may be of practical importance in research. For example, Howard *et al.* (1980) noted tremendous strain differences in response to *Leishmania tropica* infection. In BALB/c mice a progressive infection could be established with as few as 20 parasites, whereas C57BL/6 mice were resistant to as many as 2×10^7 promastigotes, and the infection, when established in this strain, was not progressive. It has been suggested by several authors that this variation in response to *Leishmania* parasites among inbred strains of mice may parallel the clinical variation observed in humans.

Long-term Stability

As there is no genetic variation present in an inbred strain, selection and inbreeding will have no effect on the characteristics of the strain. This is in strong contrast with outbred stocks, which will constantly be changing

genetically as a result of these two factors (Falconer, 1960). This means that background information on inbred strain characteristics should remain valid for a very long period. The only way in which an inbred strain will change will be as a result of sampling variation acting on some residual heterozygosity (a strain never becomes completely inbred) and the accumulation of new mutations. These lead to sub-line divergence, which is of some practical significance, although it is a much slower process than is likely to be found in outbred stocks.

Identifiability

Inbred strains may be identified by the genetic profile of their known genetic markers, such as immunological and biochemical variants. Thus, given a white mouse, it is possible to use some powerful tests of the hypothesis that it is a BALB/c mouse. In contrast, there is no test which can be applied to the hypothesis that the mouse is a 'Swiss' mouse, as such mice have no known genetic profile. Similarly, there is no way of distinguishing between Wistar and Sprague-Dawley rats (Yamada *et al.* 1979), and there is evidence that in many cases colonies of these two types of rats have become mixed. There may be no real difference between these which are repeatable on a world-wide basis.

Sensitivity

There is some evidence that on average inbred strains are more sensitive to environmental influences than are outbred stocks. This means that more attention should be given to maintaining the correct diet, husbandry and environmental conditions for inbred strains than for outbred stocks. On the other hand, such strains may well be more sensitive to treatment effects, and to that extent they will be better experimental animals.

International Distribution

The more common inbred strains of mice and rats are internationally distributed, so work can be repeated in the USA, the UK and Australia using animals which are virtually genetically identical. This is not possible with outbred stocks such as Swiss mice and Wistar rats, simply because such colonies are likely to be genetically different even though they have the same name.

Background Information

As a result of the genetic stability of inbred strains and their wide international distribution, information on strain characteristics accumulates rapidly. The names of the more common inbred strains are also MeSH subject headings for the MEDLAR literature search system. Thus, it is possible to carry out literature searches of all papers which have used a given strain of mice or rats

and (for example) a named parasite.

Inbred strains are, therefore, substantially better research animals than are outbred stocks, and the use of outbred stocks should be phased out except where the use of an outbred stock can be specifically justified. For example, in some cases no inbred strain is available; this is particularly true for large laboratory animals and exotic species. In other cases the work is such that the type of animal is unlikely to have any influence on the experimental results. Some studies may be of natural host populations, which would not be inbred. Other studies may demand a strain of mice or rats with a very high reproductive potential, which may rule out the use of an inbred strain. Finally, outbred stocks may be justified when the cost of the animal is a very substantial fraction of the total cost of the experiment. In such cases it may be cheaper to use more outbred animals rather than fewer, but more expensive inbred ones.

DERIVATIVES OF INBRED STRAINS

Congenic Strains

Congenic strains have played an important part in immunological research ever since Snell started developing his 'congenic resistant' strains after World War II. The method that he used to develop his original 'congenic resistant' strains is shown in figure 1.1. Snell (1964) used strain-specific tumours and backcrossed

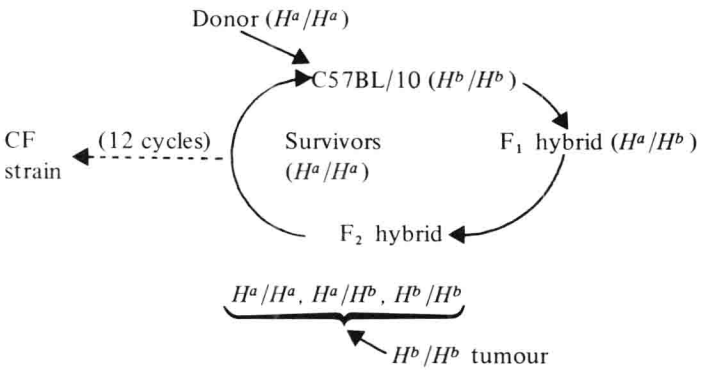

Figure 1.1 Development of 'congenic resistant' strains of mice as described by Snell (1964). The original strains were developed by crossing a 'donor' strain to C57BL/10ScSn mice, followed by the production of an F_2 hybrid. These were then challenged with a tumour specific to strain C57BL/10ScSn, and the survivors (i.e. those that were 'resistant') were again mated to C57BL/10ScSn. A total of 7-12 cycles are needed to develop a good congenic strain. Skin grafting or serological methods are now usually used in place of the strain-specific tumour.

genes conferring resistance to the tumour to a standard inbred strain as shown. The resulting strains, which differed from the standard inbred strain (usually

C57BL/10ScSn) only for the 'resistance factor', were therefore known originally as 'congenic resistant' strains. It turned out that all of these original strains differed from C57BL/10 at the *H-2* major histocompatibility locus. Later, congenic strains were developed using skin grafting or serological analysis rather than tumour resistance, and the term 'resistant' has now been dropped.

Several hundred congenic strains which differ from an inbred partner at a designated locus are now available. The development of congenic strains by backcrossing will result in a pair of lines which differ only at the designated locus, plus a short segment of chromosome associated with the backcrossed locus, which may carry some contaminating genes (Klein, 1975). In most cases such contamination can be ignored, although it should never be forgotten. Indeed, Johnson (1981) has recently shown that there is a very good chance that many congenic pairs of strains differ at more than one histocompatibility locus.

Congenic strains are most widely used in studies of the effects of the major histocompatibility complex (MHC) on a characteristic of interest. The influence of the MHC on response to parasites has been reviewed by Vadas (1980). An example from the work of Blackwell *et al.* (1980) is shown in table 1.1. It can

Table 1.1 Influence of *H-2* on *Leishmania donovani* infection (from Blackwell *et al.*, 1980)

Strain	Haplotype	Long-term response†
B10.G	*g*	'Non-cure'
B10.S	*s*	'Cure'
B10.R III (71 NS)	*r*	'Cure'
C57BL/10	*b*	'Cure'
B10.D2	*d*	'Non-cure'
BALB.B	*b*	'Cure'
BALB/c	*d*	'Non-cure'

† The first five strains all have a 'C57BL/10' genetic background, but differ at the *H-2* complex (plus some unknown contaminating genes presumed to have little importance). It will be noted that spontaneous recovery ('cure') depends on the *H-2* haplotype. The last two strains have a BALB/c genetic background, and, again, the progress of the disease depended on the *H-2* haplotype.

be seen that the congenic strains differed markedly in their response to *Leishmania donovani,* giving presumptive evidence that the response depended on the *H-2* locus. This was confirmed by crossing C57BL/10 ($H\text{-}2^b$) with B10.D2 ($H\text{-}2^d$) mice and looking at the *H-2* type and the parasite response in the subsequent segregating generations. A pair of congenic strains with the BALB/c genetic background was used to investigate whether the response to the parasite was associated with a particular *H-2* haplotype rather than being an interaction between the *H-2* type and the genetic background, as was found for life-span by Smith and Walford (1977).

The term 'congenic strain' is used to refer to any pair of strains which differ from one another at a single locus and which have been produced as a result of backcrossing to a standard inbred strain. Each strain is, of course, a fully inbred strain in its own right. Many mutants are maintained on inbred genetic backgrounds as a result of such backcrossing. However, if a new mutation should occur in an inbred strain, and if this is then maintained as a separate line, it would be designated as a strain 'coisogenic' with the inbred partner. A pair of coisogenic strains will differ only at the mutant locus and will not normally have any of the contaminating genes introduced by backcrossing.

Segregating Inbred Strains

Many mutants can not be maintained by matings of homozygous animals, as one or both sexes may be infertile. An inbred strain carrying such a mutant, which is maintained by matings involving animals heterozygous for the mutation, i.e. with forced genetic segregation, is known as a segregating inbred strain. Green (1981) restricts the use of this term to strains which have been inbred with forced segregating and he does not include strains developed as a result of backcrossing a mutant to an existing inbred strain.

Many mutants are strongly influenced by their 'genetic background' – i.e. the genetic constitution of the strain in which they are maintained. Therefore, if

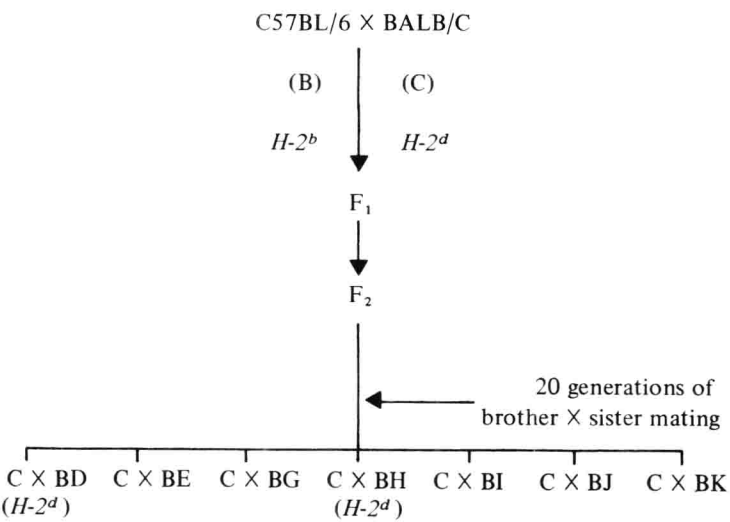

Figure 1.2 Development of a set of recombinant inbred strains (in this case CXB strains as developed by Bailey, 1971). Two standard inbred strains are mated, and brother X sister mating for 20 or more generations from the F_2 inbred results in a new set of strains, each of which is an inbred strain in its own right. In this case seven new inbred strains were developed, and two (CXBD and CXBH) had *H-2* haplotypes like that of the BALB/c parent, and the rest had the *H-2* haplotype of C57BL/6By.