Trends in laboratory animal science and welfare

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INTRODUCTION

Approaching year 2000 it seems appropriate to take a forward look at laboratory animal science and welfare.

There is an old saying that it is difficult to make predictions *particularly* about the future – and I will restrict the forecast to the immediate future and not attempt to look far beyond this decade. The development within the new biology is rapid and since the results are often unpredictable it is extremely difficult – if not impossible – to take these into account when speculating what the future holds in store.

There is no doubt however, to my mind, that a substantial part of biomedical research will continue to rely on the use of whole animals.

If one wants to look into the future it is perhaps a good idea to look at the present situation first and find out what the status is right now.

We should perhaps first ask the question:

What is laboratory animal science? and

What is laboratory animal welfare?

Well if you ask a number of different laboratory animal scientists you would probably get as many different answers or definitions, and if you ask other scientists some may still question that it is a scientific disciplin at all.

Many laboratory animal scientists find themselves occupied more with quality control and development of methods and techniques than with pure science, and I suppose that the answer to the question whether laboratory animal science is a science or technology is – yes – simply because there is a large element of technology and developmental work involved in the proper use of animals in biomedical research, e.g. in combination with providing other researchers with assistance in the operating theatre.

And talking about the proper use of animals in research I think we have approached what laboratory animal science is about: – namely how to use animals in research – and how to do it in the most efficient and human manner.

Laboratory animal science is a heterogenous discipline which is difficult to border and define.

When I teach laboratory animal science I find it convenient to subdivide the disciplin into: Basic and Applied laboratory animal science.

Basic laboratory animal science encompasses e.g.:

Laboratory animal biology incl. anatomy, physiology and ethology.

Husbandry and breeding of the different strains and species.

Handling and sampling of biological tissues and liquids.

Diagnosis and treatment of diseases incl pathology.

Anaesthesia, pain relief and euthanasia.

Experimental surgery.

Monitoring – Environment
– Health Status
– Genetic Profile
of the animals.

In other words – basic laboratory animal science is centrered on what has been called the animal as a sophisticated and complicated biological instrument. In order to get to know this instrument and to calibrate it, it has been essential to *define* the animal as much as possible and to minimize interindividual biological variation – the variation between animals – in the reaction to various "treatments".

This goal has been pursued and approached along different parellel avenues simultaneously:

One of these, and this is perhaps the best

known, is the development – and increasing use – of genetically defined animals.

Since the beginning of this century where the breeding of inbred congenic strains began with the early American cancer researchers and their studies of histocompatibility in mice a great number of inbred strains of small rodents have been developed, characterized and maintained in laboratories all over the world.

The individual members of these strains often react as identical twins to a given treatment and this is indeed the whole idea because it leads to significant experimental results using fewer animals then would otherwise have been necessary if arbitrary individuals had been used.

In some cases it may be advantageous to use an outbred stock, and systems have been developed to avoid in-breeding and maintain the genetic material in outbred colonies as constant as possible from one generation to the next – minimising genetic drift with time.

Laboratory animal scientists thus advocate for the use of genetically defined animals, either as individuals or as populations.

Another way to standardise and define the animals has been the development of animals with uniform and known health status.

Many animals are now maintained in a gnotobiotic condition with known intestinal flora in isolators in the animal houses. Most animals used for experiments are small rodents and most of these are nowadays bred behind barriers from caesarian derived gnotobiotic animals and bought as SPF animals free of a longer or shorter list of potentially pathogenic microorganisms.

Among biomedical scientists it is now generally recognized that environmental changes may affect the homeostasis of the animals and thereby also the experimental results.

A lot of resources have been – and are being – allocated to provide the laboratory animals with a defined environment – an extremely stable and well-controlled physical environment.

That knowledge of, and control over, the ambient environment is so important is per-

Table 1. LD50 in mice for amthetamine and caffeine at different room temperatures (*Muller* 1969).

Room temperature (°C)	LD50 (mg/kg) Amphetamine	Caffeine
15	70	260
22	8.5	270
30	2.5	190

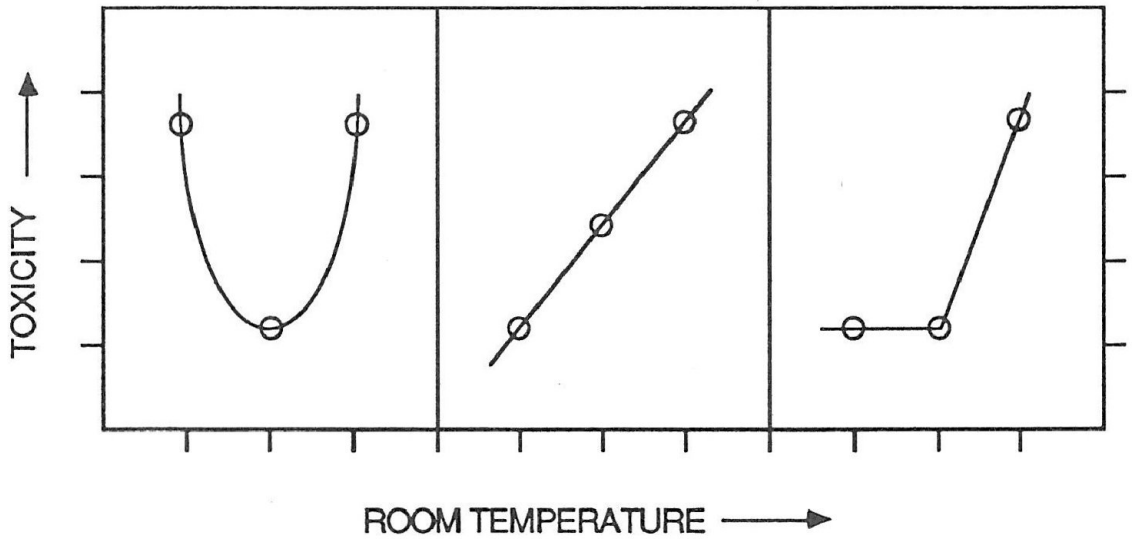

Figure 1. Schematic correlation between ambient temperature and the toxicity of different drugs. (*Fuhrman & Fuhrman* 1961).

haps best illustrated with a single example showing how the toxicity of a substance changes with changes in ambient temperature.

Table 1 shows the dramatic increase in toxicity of amphetamine and of caffeine with increasing temperature (*Muller & Vernikos-Danellis* 1969).

And to make it more complex there are different modes of temperature dependent changes in toxicity for different substances.

The most common correlation between ambient temperature and the toxicity of a drug is schematically shown in the curve to the left on figure 1 (*Fuhrman & Fuhrman* 1961). At a certain temperature which is characteristic of each compound and animal, the toxicity is lowest. This temperature lies between 30 c and 34 c for mice and rats which is somewhere in the thermoneutral zone of these animals.

The importance of being able to maintain a very stable environment has fortunately reached understanding in ministeries and governmental offices, and it has resulted in a plethora of national and international regulations and guidelines ensuring that poor animal facilities in attics and cellars will soon be a thing of the past.

Another important aspect is the biotic environment and the impact of biologic environmental factors on the animals and their metabolic functions.

Because of the laboratory animal species' remarkable ability to adapt to environmental changes by biochemical adaption there is often no visible effect on the animals of an important change in their environment which makes it all the more deceiving.

The importance of the social environment of the individual animals has received little attention.

Table 2 show results from a study by *Gidley Baird et al.* (1986) demonstrating that the number of eggs ovulated by the female mouse is positively correlated with the number of days she has spent with the male prior to mating. Variations like these are of course important when allocating mated female mice to different treatment groups and using litter size as the investigated parameter.

The establishment of new central laboratory facilities at universities and pharmaceutical companies staffed with well trained and educated personnel is an extremely positive development, and there is no doubt that the importance of education will continue to be stressed in the animal welfare debate.

In this context it is a pleasure to note that the EEC will soon have finished rather detailed guidelines on education and training of all categories of personnel in contact with laboratory animals.

The EEC directive regarding the protection of animals used for experimental and other scientific purposes, with which we all have to comply, requires all personnel using or taking care of laboratory animals, including staff with a supervisory function, to have had appropriate education and training.

Table 2. Number of ova recorded in females (group size 30) which mated on days 1, 2, 3 or 4 after housing with intact or vasectomized males.

Day of mating after placing with males ♂	Day 1	Day 2	Day 3	Day 4
No. of ova $\bar{x}$ ± S.E. mated with intact	13.2 ± 0.42	14.3 ± 0.44	15.4 ± 0.35	14.9 ± 0.49
No. of ova $\bar{x}$ ± S.E. mated with vasectomized ♂	13.4 ± 0.38	14.7 ± 0.39	15.6 ± 0.33	14.2 ± 0.45

In the introduction the most recent draft on The Guidelines on mandatory education it is stated that "The most essential element for the translation of controls outlined in any legislation on animal experimentation into daily practice in the laboratory is the provision of well trained and conscientious staff. I think we can all agree with this and the draft operates with three broad groupings of personnel:

- Researchers
- Animal technicians (AT) (incl. senior AT's)
- Animal carers

and

- Laboratory veterinarians as described in Article 19.2. d of the EEC directive, which says:

"a veterinarian or other competent person should be charged with advisory duties in relation to the well-being of the animals".

In the draft on education the curriculum for each group of personnel is specified:

I will not go into detail with the different categories but with regard to the laboratory veterinarians mention that they besides holding a registrable or equivalent qualification, will require additional education and training similar to the other categories of personnel. It will be necessary for the veterinarian, the EEC-draft goes on, to extend his or her knowledge to laboratory procedures and to species with which the veterinarian is normally not familiar. On top of that there are areas where the veterinarian requires much more specialised training than researchers in areas such as microbiology of the relevant species, quality control, health monitoring, pathology and disease prevention and treatment.

What are the other trends then in basic laboratory animal science apart from better animal facilities and heigher level of education? Well there is no doubt that laboratory animal veterinarians will continue to use substantial resources to improve and control the standard or quality of the animals.

The general tendency in the western world is clearly towards the use of fewer animals, but animals of a continued increasing quality.

The producers of laboratory animals will have to document that their animals are free of an ever increasing list of potentially pathogenic microorganisms, and the users will continue to use more and more documented healthy animals.

In the production of laboratory animals more and more animals are being produced under gnotobiotic conditions using animals derived by caesarian section or by embryo transfer into pseudopregnant females kept in isolators followed by association with a known non-pathogenic flora.

Cryopreservation techniques are currently being simplified and deep freezing by the rapid technique – vitrification – where the embryos are plunged directly into liquid nitrogen is an inexpensive method whereby an unlimited number of important stocks and strains may be stored in embryo banks for many many years using little space and at low cost (*Dagnæs-Hansen & Hau* 1988).

One of the major advantages of this method is that it is possible to maintain interesting mutant strains and at the same time avoid genetic drift altogether.

The need to control the genetic authenticity of the animals and the check whether genetic contamination has occurred will certainly still be there in the future too.

The methods currently used for this are:

skin transplantation using tail skin grafting,

biochemical methods using isoenzyme patterns,

immunochemical methods using polyvalent sera,

skeletal morphology using mandible shape, and test matings with coloured animals.

These methods are all indirect and not optimal and several of them have to be used in combination in order to ensure that a major proportion of the genome is monitored.

New techniques render it possible to examine the genome directly in stead of analysing gene products and it is fortunately just a question of time before most if not all of

these methods become obsolete because of the use of DNA fingerprinting technique (DNA mini satellite analysis).
A number of scientsts are working on the application of the technique to distinguish between strains and stocks of the same species, but international recognition of DNA-fingerprinting in the genetic control will require standardization on procedures including the use of similar probes and restriction enzymes at the individual monitoring centres.
But basic laboratory animal science also deals with alternatives to the use of animals, welfare of the laboratory animals and in recent years with enrichment of the environment of the animals. Most people date the concept of alternatives back to the 1959 book by Russell and Burch who published several principles for human experimental technique. Their central issue was that the researchers should follow the so-called three R's:
Replacement, Reduction and Refinement.
When performing animal experiments the researcher should seek, wherever possible, to replace the use of live animals with nonsentient material, to reduce the number of animals used, and to refine techniques so as to reduce animal pain and suffering.
Perhaps the best example of replacement is the development of immunochemical assays replacing bio-assays for measuring e.g. the biological activity of hormones. This development has resulted in hundreds of thousands of animals not being produced and subjected to this use.
The use of laboratory animals in the pharmaceutical industry for development of new products will gradually decline. As a consequence of the accumulated knowledge of biological processes within the cell during the past couple of decades the private research is changing from using whole animals to using organs, cells and subcellular structures.
It is somewhat different in the testing of toxicity of substances which accounts for 20 % of the experiments in Great Britains. The methods employed are usually prescribed by the authorities and before a new product can be approved it has to have been thoroughly tested in order to protect the comsumers.
It is this use of animals which has attracted the most severe critisism by the antivivisectionists; in particular the LD50 test for acute toxicity and the Draize test (*Draize et al.* 1944) for irritative effects on the eyes.
Laboratory animal scientists have for many years fought to get more humane methods of toxicity testing recognised by the authorities. It has an extremely slow process to get national authorities to accept alternative methods and change accordingly rules and international agreements to recognise each others approval procedures.
However, international collaboration has recently resulted in an agreement to recognise an alternative to the LD50 test. The test is called a "fixed dose procedure" and was developed in this country by The British Toxicology Society. The EEC and OECD subsidised an international test of the alternative procedure, which in contrast to other alternatives does not have death of the animal as a necessary endpoint (*Heuvel et al.* 1990). The EEC and OECD now recommend the new method as a replacement for the LD50 test.
An international analysis of four in vitro methods and two methods using isolated rabbit eyes from killed rabbits and membranes from fertilised hen's eggs has recently been scrutinised and published by the EEC: "Collaborative study on the evaluation of alternative methods to the "Draize" eye test". The report concludes that the preliminary results are encouraging and that a more thorough analysis of the most promising methods shall consist of more substances to be testet and a larger number of laboratories participating in the analysis.
I think that we all hope ultimately for the complete replacement of animals by non-animal testing methods.

Now with respect to reduction and refinement much has been accomplished with the use of defined animals in carefully planned experiments designed according to statistical principles, which ensure that the correct number of animals are used.

As mentioned before reduction and refinement are concepts closely associated with the welfare of the animals.

It is difficult to define, assess and discuss animal welfare without indulging too much in anthropomorphism, and it is perhaps easier first to agree on some measures of poor welfare for the animals which include:

Reduced life expectancy
impaired growth
impaired fecundity
body damage
increased disease incidence
increased susceptibility to disease
immunosuppression
increased adrenal activity – physiological attempts to adjust behavioural anomalies – such as stereotypies
self-narcotisation – with endogenous production of endorphins.

All of these parameters are signs of poor welfare – and welfare has been defined by Professor Broom at the Cambridge vet. school in the following manner:

"The welfare of an individual is its state as regards its attempts to cope with its environment." (*Broom* 1988).

An indirect or negative definition of welfare has been given by Professor Webster at the Bristol University:

"Welfare as perceived by the animal can be categorised most simply by "five freedoms", freedom from thirst, hunger/malnutrition, chronic discomfort, injury and disease, fear and stress and freedom to express most natural (socially acceptable) patterns of behaviour." As professor Webster states "This provides a comprehensive checklist for evaluation of any form of animal husbandry and avoids the trap of evaluating welfare on grounds only of behaviour – a foult of welfarists – or of production – a fault among farmers".

The last part of this definition, fear stress and freedom to express behaviour brings us directly to another trend in basic laboratory animal science; and that is environmental enrichment. We have been so focussed on providing the animals with optimal clean microclimatic conditions in completely static unchanging environments that the biotic environment and the behavioural needs of the animals are frequently neglected, which apart from the welfare aspects of it may be stressing for the animals and therefore contribute with biological variation to the animal experiments.

One typical example of maltreatment of animals because of neglect of the behaviour of animals is the housing of several male mice in the same cage. All of us who frequently come in different animal houses have seen these cages where the fighting among male mice results in blood all over the cage.

Another example is the housing of rabbits in single cages with no bedding on the floor. Rabbits are extremely social animal and capable of displaying a complex behaviour when given the opportunity and housed in family groups.

A large number of rabbits are used for production of polyclonal antisera, and fortunately guidelines ensuring that the animals are subjected to as lenient methods as possible are being made in more and more countries. Hopefully this will contribute to put an end to many of the unnessary aggressive immunisation methods using repeated injections of Freund's adjuvant which may cause disseminated granuloma formation in the lungs, kidneys and other tissues ulcerations and abcess formation and ultimately kidney failure (Figure 2 and 3).

With respect to the production of monoclonal antibodies in ascites fluid in rodents, guidelines are also appearing limiting the number of tappings of peritoneal ascites fluid to one or two and putting restrictions

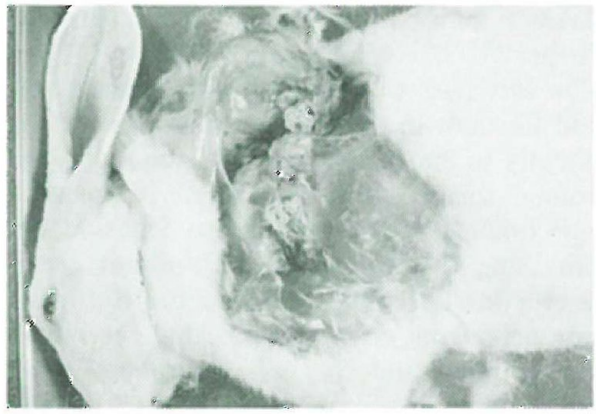

Figure 2. Abscess formation subcutaneously in a rabbit immunised with the antigen emulsified in Freund's adjuvant.

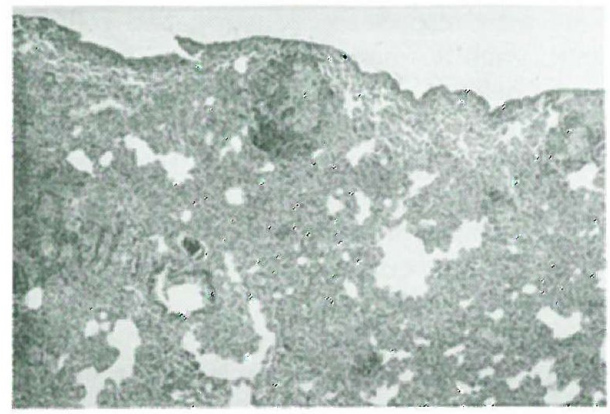

Figure 3. Haematoma formation in the lung tissue in the rabbit shown in Figure 2.

on the volume employed of the primer pristane (0.2 or 0.25 ml).

How can animal welfare be assessed? Well perhaps the only possible way is to assess when welfare is decreased.

With regard to short-lastig negative impact on welfare such as immediate stress or even pain several interesting initiatives have been taken. *Barclay et al.* (1988) have published what they have termed a *Disturbance index* which is an objective method for assessing severity of procedures on rodents. The report describes a method of scientifically – electronically assessing the degree of disturbance to a rodent's exploratory behaviour or activity in a sort of open field caused by subjecting it to an experimental procedure e.g. injection of different substances using different sizes of needles and volumes. Behaviour is thus a tool and the extent of behavioural change is assumed to relate directly to the severity of pain, distress or discomfort which the animal is experiencing.

The indices vary from one species to another and measurements or recordings are performed in different time intervals for the different species.

Another interesting approach to assess the severity of scientific procedures has been made by *James Wallace and co-workers* (1990). The 1986 Act requires that the adverse effects of scientific procedures are categorized and described as being of either mild, moderate or substantial severity. The terms indicate increasing severity but they are of course of a subjective nature and closely associated with the Verbal Rating Scale (VRS) used to evaluate clinical pain in humans. Dr Wallace and co-workers operate with what they have termed an Index of Severity (SI) and they must be congratulated on their thorough work. The problem with this index is its lack of objectivity. The severity of the individual elements of different procedures is evaluated and graduated according to a scale with a maximum of 5. The scores are thereafter added to indicate the severity of the procedure. In spite of the lack of linearity the fact that each procedure has been broken down into small elements which have all been assessed makes the method rather good in my opinion.

Dawkins (1990) has introduced interesting economic terms to the study of animal welfare. She states that in order to study animal welfare impirically we need an objective basis for deciding when an animal is suffering. Suffering includes many unpleasant sensations such as pain, fear, stress and boredom. Captive animals are often deprived of possibilities to perform a certain behaviour for which they are highly motivated. The "price" an animal is ready to pay to be able to perform such a specific behaviour shows

how fundamental or important that behaviour is to the animal. Withholding conditions or commodities for which an animal shows "inelastic demand" i.e. for which it continues to work despite increasing costs – is very likely to cause suffering and decrease the animal's welfare.

To summarise the trends in basic laboratory animal science I predict that laboratory animal scientists will continue to work intensely with health monitoring and genetic monitoring and that they will introduce molecular biology to their tool box.

Biomedical research seems to develop towards biological engineering and there will probably be an increase in the expectations with regard to animal quality and uniformity from those commissioning or funding the research which will nourish the development towards more and more defined animals.

However, I am convinced that welfare, welfare quality control and welfare monitoring will be just as natural and relevant issues for future laboratory animal scientists as health monitoring and genetic monitoring is to day.

There is a need for studies of the normal behaviour of the different laboratory animal species in order to be able to diagnose when the animals' welfare is reduced. There simply is alack of knowledge which should be rectified.

We all know what a happy dog looks like – or an aggressive swan but how about a happy rat or an aggressive guinea pig? It is a tremendous challenge to introduce scientific principles and objective methods of measurement of animal welfare or perhaps various degrees of lack of welfare to basic laboratory animal science in particular' but also to science in general.

Basic laboratory animal science may be considered a necessary platform for applied laboratory animal science because the use of animals in research requires a profound knowledge of technical. Ethical and legal factors concerning their care and utilisation.

Similarly applied laboratory animal science may be considered as a link between basic laboratory animal science and a number of biological disciplines.

This is because applied laboratory animal science deals with laboratory animal models.

How to develop the model, how to evaluate it and how to apply it in biomedical research.

This means that applied laboratory animal science is closely related to comparative disciplines, e.g. comparative physiology and comparative medicine which is defined as "The study of the nature, cause and cure of abnormal structure and function in people, animals and plants for the eventual application to, and benefit of, all living things" (*Bustad et al.* 1976), and in some countries including the US laboratory animal science is often found at the departmet of comparative medicine.

The term "laboratory animal model" has been defined by many people. The definition I like best is the one described by a US national research committee on animal models for research on aging 1981:

"An animal model is a living organism in which normative biology or behavior can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in human or other species of animal."

This is a very broad definition and as you can see it includes also normative biology and behaviour which is quite logical, but differs from the very early understanding of animal models, when the term was often restricted to animal models of human disease.

It is, however, a well known fact that most of our basic knowledge of human biochemistry, physiology, endocrinology and pharmacology has been derived from initial studies in subhuman animal models (*Coffey & Isaacs* 1980), which makes it logical to include animal models for the study of normal biological functions in the definition.

Applied laboratory animal science is thus to

a large extent to create animal models and to make these models available to other scientists, and to ensure that the developed strains do not become extinct.

Research involving laboratory animal models often can be divided into 3 stages:

Development

Evaluation

Application.

Most laboratory animal models are still used to study the cause, nature and cure of human diseases and it is possible to divide these animal models in four groups:

Experimental (induced) models, spontaneous models, negative models and orphan models. Of these four groups the two first categories are important whereas the latter two are of more academic interest only.

Experimental models are often referred to as induced models, and in these systems, the scientist attempts to induce a disease or pathological condition experimentally, e.g. surgically or by administration of biologically active substances. By manipulating the environmental, dietary, endocrine, immunological and infectious state of the animals, animal models for a multitude of human diseases and malfunctions have been and will no doubt continue to be created.

Spontaneous laboratory animal models of human disease occur naturally, and hundreds of strains and stocks of laboratory animals with spontaneous diseases have been characterized and used successfully in the study of the different diseases. A prerequisit for the optimal use of these animal models is knowledge of the cause and pathogenesis of the disease not only in the animal but also in the human. In general the spontaneous model posesses similarities with the human disease without mimicking it completely. The spontaneous models are genetic variants, and a major problem is that financial difficulties have resulted in the loss of many well defined strains and stocks of different species representing usable models of human disease. The cryopreservation techniques and equipment is unfortunately not present everywhere and not yet applicable to all species as a routine and concern from scientists that valuable models continue to be lost prompted the US National Research Council to form a Committee on the preservation of laboratory animal resources (*Barnes* 1986).

Perhaps a similar initiative should be taken in Europe?

Negative models are those which for some reason are resistant or insusceptible to a condition or treatment which usually affects other species or other strains of the same species. Studying differences can often be as valuable as studying similarities.

Orphan models are those in which a disease is first recognised in the animal species, after which a human counterpart may eventually turn up later. Examples of this category are Visna virus in sheep and feline leukemia virus.

Many important human diseases such as diabetes are studied using many different animal models including both induced and spontaneous types.

However, often an animal model can be used to answer only a limited number of sientific questions, and as stated by *Snider and co-workers* (1986) the usefulness of a laboratory animal model should be judged on how well it answers the specific questions it is being asked, rather than how well it mimics the human disease.

What are the trends in applied laboratory animal science?

I don't think there can be any doubt that *transgenic* animals will play a very important role in the future.

Transgenic animals are animals into which DNA from another species has been introduced into the genome, and it is a technique which has been with us for the past decade.

There are several ways of introducing the foreign DNA into the fertilised egg or early embryo. The microinjection directly into the pronucleus of the fertilised ovum is now used routinely on mice in many laboratories. Another method is using retrovirus as a

carrier, but the newest and very promising method is using the embryonic stem cell method. Embryonic stem cells are immortal cells which can be continuously grown in culture. These cells can be transfected with isolated genes after which it is possible to select those cells which have incorporated the foreign DNA into their genome and in which recombination has taken place so that the original gene has been swopped with the new foreign gene.

These cells are then introduced by micro-injection into the blastocyst and it is thus possible to produce animals of a specific genotype.

These animals can thus be designed as models for human diseases by performing specific site mutagenesis and replacing the endogenous gene with the mutated gene.

This opens up for the induction of highly specific disease models and the results from the application of these models will be most interesting.

There are many other potential applications but the immediate value of the transgenic technique is as a tool in basic research on gene regulation and to test engineered genes for expression.

The new technique has resulted in the interest in new laboratory animal species – fish. The main advantage of using fish is of course that the fertilised eggs do not have to be returned to the mother – she might eat them –. One disadvantage associated with the use of fish for this purpose is that it requires the use of fish genes with which oly a limited number of scientists are working.

In conclusion, I think that embryo-manipulation and transgenic animals – in particular the so-called designer animals will play a central part in the future of laboratory animal science. The result of introducing foreign genes into a species where they don't belong, but are never the less expressed, makes it even more imperative to monitore the welfare of these animals. It is easy to imagine that some of the transgenic strains being developed will be characterised by inherent poor welfare.

It can thus be considered a future requirement that the transgenic animal strains must be analysed thoroughly to elucidate the welfare state of the individual strains, and precautions must of course be made in order to ensure that we do not produce new strains of animals with to severe inherent welfare problems.

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