Scand. J. Lab. Anim. Sci. No. 2 . 1990 . Vol. 17

What is laboratory animal science?

by *K. J. Öbrink,*
Department of Physiology and Medical Biophysics,
Uppsala University, Uppsala Biomedical Center, Uppsala.

"Even the learned is ignorant of many things, but he who does not recognize his own ignorance is nothing".

Animal experiments have been used in biomedical research for centuries. Those interested in the historical background to today's use of laboratory animals and the debate around it in society are recommended to read "Of Mice, Models and Men" by *Andrew Rowan* (1984).

Those of us who have been active in biomedical research for the last 3–5 decades may have experienced a change in the use of animals which is interesting in several respects. I write "may" because unfortunately there is a very large group of scientists that have not at all or only just vagualy been aware of what has happend.

When I myself began as a physiologist about 1940 I came to use cats for studies on the physiology of the stomach. These animals were obtained from neighbouring farms. The cats were anesthetized and the stomachs exposed and opened. In nearly all cases we had then to remove a spoonful of worms from the stomach lumen before the experiment could start. This was not at all remarkable. What was remarkable, however, was that I (we) did not object. As physiologists we were of course familiar with homeostatic mechanisms (see below) and knew that the animals could be influenced by practically everything (even endoparasites). But we were brought up in a school where such things were not considered (or perhaps more correctly – could not be considered). Consequently we were taught that the precision of a biological experiment could never be better than about ± 15 %. However, when we used just a few dogs for a great number of experiments under standardized conditions it was possible to eliminate inter-individual and day to day variations to a very great extent. By further standardization of the experimental conditions it should be possible to reduce even further these "biological variations".

The pioneers in this work are to be found among the users of small rodents (especially mice and rats). Because of their short reproduction cycles successful breeding is possible, and as a result of professional husbandry increasingly healthier animals can be obtained. There is one name that is worthy of recall in this connection and that is the English physician Dr William Lane-Petter (*O'Donoghue,* 1988). His knowledge and awareness contributed among other things to the formation of the "Laboratory Animal Centre" in London, of which he became the first managing director. It is fair to say that this was the nucleus from which a great deal of "Laboratory Animal Science"(LAS) developed.

What is new and what makes LAS a scientific discipline of its own? This question has not been fully answered. Some attempts may be mentioned. *Hau et al.* (1989) divided the discipline into two parts, Basic and Applied LAS. The first concerns the animals themselves whereas the second deals with the animal as a scientific model. *Max Lang* in an unpublished lecture (1989) describing training programs for specialists in lab. animal research, gives an excellent introduction to the subject and in fact a list of contents when he states: "Few biomedical investigators . . . have had any formal training concerning the animals used in their research. Many errors have been made in the interpretation of research data, because the investigator did not know – or did not consider –

the effect of husbandry procedures, nutrition, environmental factors, or common diseases (many of which are non-clinical); or because he/she was not familar with the normal anatomic, behavioral, genetic, or physiologic characteristics of a particular species of animal."
It might be of value to formulate a definition as precise as possible because this would facilitate the recognition of LAS as a separate discipline among biomedical scientists.

Definition:
Laboratory Animal Science is a science auxillary to biological and medical research but still a well demarcated discipline both in research and teaching. It consists of four main parts, namely:

1. *Genetics and characteristics of lab.animals.*
2. *Effects of homeostatic mechanisms on animal experiments.*
3. *Lab.animal husbandry and basic animal-experiment methodology.*
4. *Legislation, decrees and applied lab.animal ethics.*

All these parts demand knowledge and this in turn makes education and research necessary.
I shall try to describe the four parts individually:

1. *GENETICS AND CHARACTERISTICS OF LAB.ANIMALS*

The genotype of a laboratory animal plays the same role in an experiment as specification of an analytical instrument and at the same time one is trying to find out how this "instrument" functions. In the same way as an exchange of components in a physical instrument may alter its function, exhanges of hereditary factors may change the biological reactions of the animal. Identical experiments in different genotypic animals may thus give different results. The biomedical researcher must therefore try to understand the genetic background of his "instrument" as far as possible.
LAS has played a major role in this field. Inbred strains with different genetic characteristics have been created. An inbred strains is usually obtained by consecutive brother-sister mating for at least 20 generations, by which isogenicity is almost obtained (Inbreeding coefficient = 0.98). There are at present available about 250 inbred strains of mice and about 130 strains of rats. Among them there are furthermore important mutants (about 110 and 50 respectively). Together with congenic strains and outbred stocks there are at present about 1200 different kinds of mice and about 440 kinds of rats registered.
Outbred stocks can be obtained only by rather complicated breeding. A stock starts with a certain set of genomes, the composition of which should not change with time. Obviously new animals must not be added to this group and free mating would probably result in a certain degree of selection eventually leading to inbreeding. Instead it is necessary to plan for truly randomized mating so as to maintain a constant genomic pattern.
For a modern lab.animal department it is important to be responsible for professional breeding and also to make sure that the investigators get the proper animals. That means that not only shall an animal department deliver all strains that are required but also guarantee by genetic monitoring that the researcher really is working with the special kind of animal he/she believes is used.
The monitoring systems mainly used have been grafting techniques as immunological markers, anatomical measurements as morphological markers and physicochemical tests as biochemical markers. The first is usually done by "skin grafting" (*Festing* 1979). An example of the second type is determination of reference points on the mandible (*Festing* 1973) and the third type is usually electrophoresis of the blood plasma (*Groen* 1977). The latest progress in DNA-

research has made possible comparison of strains through identification of DNA-sequences. The so-called PCR technique (polymerase chain reaction) may be a useful instrument for our lab.animal departments in the future.

Hitherto biochemical scientists have been rather ignorant of the importance of the genotype of their animals but a new tendency is quite evident. An analysis of the papers published in scientific journals shows a steady increase in the use of inbred strains (*Festing* 1979).

The majority of investigators use Sprague-Dawley or Wistar rats or NMRI-mice. None of those are genetically defined. At one time they did come from a certain source but subsequently only the name has been retained, not the original set of genomes. (It is therefore advisable to report not only for example "Wistar rats", but also the name of the breeder, in scientific publications. This will at least give some indication of the source).

Now and then it may be difficult to retain a valuable strain when nobody else is presently using it. Techniques have been developed, however, for deepfreezing embryos (8 cells) and storing them until needed. The same technique is also used to preserve strains that otherwise would change through genetic drift.

In order to facilitate the finding of a required strain or stock anywhere in the world an "International Index" is published by ICLAS (International Council of Laboratory Animal Sciences) (1980) and up to now edited by Dr Michael Festing, London. This will probably be available as a database in the near future.

The characteristics of lab.animals include anatomical, physiological, biochemical and immunological characteristics, that may be of particular use in solving different biomedical problems. Among examples of this are studies on the nerve function in the squid axon because of its giant size, tubular function of the kidneys in birds with their peculiar portal blood flow from the leg to the proximal tubulus in the kidney cortex, and many many other useful characteristics. Physiological, biochemical and immunological data from different kinds of species are often of great help in biomedical research.

2. *EFFECTS OF HOMEOSTATIC MECHANISMS ON ANIMAL EXPERIMENTS*

The animal body has a marvelous ability to keep most of the physiological variables constant. Body temperature, blood pressure and salt concentrations are a few examples. Interference by physical or chemical factors or by microbial invaders are all opposed by feed back mechanisms tending to conserve all physiological variables within normal limits. These feed back systems utilise metabolic, nervous and hormonal mechanisms as well as immunological reactions to achieve this goal. It is therefore obvious that two identical animals that are subjected to different interfering influences (stressors) have different activities in their feed back loops, i. e. their metabolic, nervous, hormonal or immunological activities are engaged to different degrees. Consequently if identical experiments were performed on identical animals but in different room climates, or after different types of feeding or with different microbial burdens it would not be unexpected if differences were reported.

Factors that may influence the results are:

a) Different stages in life cycles, i.e. age, sexual cycles, pregnancy, lactation etc.
b) Physical factors like ambient temperature, relative humidity, light intensity, photoperiods, noise etc.
c) Chemical factors like nutrition, quality of drinking water, type of bedding etc.
d) Microbiological factors like bacterial or viral infections, endo- or ectoparasites etc.
e) Sociological factors i.e. the number of animals in the same cage or pen, relation to animal caretakers etc.

The above mentioned factors must be kept

under control as far as possible. Thus the animal department should deliver animals of the correct age or weight, maybe on a specific day of the sexual cycle or at an exact age of the fetus. Daily control of room temperature, humidity, light cycles etc. must be recorded. Food quality and composition, purity of drinking water and proper bedding must be guaranteed. The highest possible hygiene and the establishment of microbial barriers are essential as are observations of important sociological factors. In this latter case one can expect changes in the secretion of several hormones depending on hierarchic status among the animals. Thus the experimental results may differ markedly depending on the number of animals in each cage or pen. Similar influences may depend on the relationship to caretakers.

Health control is an important part of service to biomedical science. It is essential to diagnose and cure diseases of different origin. They may be due to metabolic, nutritional or toxic disturbances or to microbial infections. This large area of health control, health improvement and therapy is usually comprehended as Laboratory Animal Medicine (LAM). LAS and LAM are not always kept apart and schools for LAM are certainly teaching LAS in a broad sense, but for many practical reasons I feel it desirable to keep the definitions clear.

To summarize: most environmental factors – physical, chemical, microbiological and physiological – will influence the animals and thus the experimental results. LAS should be engaged in exploring this area in detail.

3. *LAB.ANIMAL, HUSBANDRY AND BASIC ANIMAL-EXPERIMENT METHODOLOGY*

Biomedical research involves numerous techniques of different kinds – biochemical ones like separation of biological molecules, physical like measurements of electrical potential differences, physio-chemical like ion mobilities etc, etc.

Two thirds of the biomedical experiments involve the use of laboratory animals and this introduces special techniques to the work. We may mention simple things like proper handling of the animals in order not to cause unnecessary stress; injections, force feeding and blood sampling. All this can be stressful in the hands of untrained people but may cause no discomfort with properly trained personnel. Other basic animal-experiment methods are blood pressure recordings and articficial respiration. Much more complicated are the anesthetic techniques. Anesthesia in veterinary practice is surgical anesthesia, which may differ markedly from anaesthesia in research, because here consideration must be taken of possible interferences with the experiment. This is in fact one of the most difficult problems. Immunization, minor surgery,insemination and euthanasia are examples of other techniques that often need special practice.

In most animal based research special techniques developed specifically for that very project are used, e.g. micropuncture of kidney nephrons, cannulation of brain ventricles, intracardial electrode implantation etc. I suggest that such special techniques should not be included in the definition of "basic animal-experiment methodology".

To keep effective microbial barriers the use of cubicles, filter-top cages and isolators may be necessary and a modern biomedical laboratory should not lack the possibility to provide such services. This needs training and research.

One of the most important problems is to be sure that the way we treat the animals is satisfactory. Do the animals feel well? As we cannot ask them we have to observe their own messages, which mainly are through their appearance and behavior. An uncomfortable animal will always behave differently from a happy one. Therefore ethology and the ability to understand what the different behavioral patterns mean is extremely important. Especially all personnel in the

animal department must aquire a skill in this area.

Unnecessary animal experiments should never be performed and there is a trend to use alternatives whenever possible. As a general rule one may state, however, that alternatives to animal experiments in basic research do not exist. Only when the animals are used for production of biological substances like vaccines, or for testing for toxicity, cancerogenicity, teratogenicity or for assessing the presence and concentrations of biologically active substances, are alternatives possible (*Öbrink,* to be published). In these latter cases other techniques like cell cultures or even chemical analyses may replace the animal. However, an international definition of alternative methods does not only include "Replacement" of animals for something else but also "Refinement" of the experimental techniques and "Reduction" of the number of animals needed. All three taken together are denoted as the three "R". And in all experiments – also in basic research – both Refinement and Reduction are of extreme importance.

Thus for both ethical and economical reasons animal experiments must be conducted by well trained and educated people and the animals cared for in laboratory animal departments of high moral and scientific standard. We are probably using more animals than necessary today because of our ignorance about nutrition, environmental influences etc., but it must be considered inexcusable to ignore those factors that are already known to us. Then the animals are rendered more harm than necessary and also some of them will be used in vain. It is progress in LAS that will provide us with more knowledge in these areas.

4. *LEGISLATION, DECREES AND APPLIED LAB.ANIMAL ETHICS*

As long as animals have been used for experiments there has been a deep concern about it. This has led to guidelines and restrictions of different kinds. The first law to be adopted was the "Cruelty to Animals Act" in Great Britain in 1876. This law was remarkable as it was in force for more than 100 years.

Now most developed countries have adopted their own laws for animal protection. They are generally rather similar but differ in their details. Also the control systems to guarantee the observance of the laws differ. The laws are completed by by-laws and decrees of different degrees of precision.

It is, however, fair to say that laws and decrees are always too remote to give full protection to the animals (*Öbrink,* 1983). In the end it is the attitude of the people that handle the animals that is the most important thing. And promotion and development of such attitudes is one of LAS' important duties. In many countries ethical committees have been set up to stimulate ethical discussions and improve ethical consciousness.

CONCLUSION

Laboratory Animal Science (LAS) is an indispensable speciality for all biomedical research. It comprises important factors for the correct conductance of animal experiments. Knowledge and ethical consciousness are the fundamental corner-stones on which LAS should be based. A clear definition of LAS is furthermore an imperative prerequisite for a proper construction of teaching curricula for all categories of people handling lab.animals. Unfortunately many scientists misjudge their own state of knowledge in LAS. It is certainly not sufficient to have a pet dog at home, but even – and maybe for obvious reasons – persons with qualifications as physician or veterinary surgeon do often not realize their ignorance of LAS. This is the reason for the initial device in this paper.

Sammanfattning

Försöksdjursvetenskap är en hjälpvetenskap till den biomedicinska forskningen men likväl en egen separat disciplin. Den kan sägas omfatta fyra delområden, nämligen

1. Försöksdjursgenetik och försöksdjurens anato-

miska, fysiologiska och biokemiska karakteristika.
2. Homeostasmekanismernas inverkan på de experimentella resultaten.
3. Försöksdjursteknik och grundläggande djurexperimentella metoder.
4. Lagar, förordningar och tillämpad djurförsöksetik.

Det är viktigt att denna avgränsning görs mot andra biologiska vetenskaper så att forskning och undervisning får en ändamålsenlig utformning. Annars föreligger en risk för att personer med utbildning inom medicin, veterinärmedicin eller zoologiska discipliner tror sig automatiskt ha ett kunnande inom ämnet försöksdjursvetenskap.

Yhteenveto / K. Pelkonen

Koe-eläintiede (KET) on välttämätön aputiede kaikelle biolääketieteelle. KET:n tulee perustua oikeaan tietoon ja eettiseen tietoisuuteen. KET pitää myös voida määritellä selkeästi koeeläimiä kästtelevien eri työntekijäryhmien opetusohjelmien laatimista varten. Valitettavasti monet tutkijat arvioivat väärin omien KET:llisten tietojensa tason. Selvää on, ettei lemmikkikoira kotona takaa tietojen riittävyyttä, mutta – sinänsä ymmärrettävistä syistä – henkilöt joilla on esim. lääkärin tai eläinlääkärin tutkinto, eivät tiedosta omien KET:n tietämyksensä vajavaisuutta.

References:

Festing, M. F. W.: A multivariate analysis of sublime divergens in the shape of the mandible in C57BL/Gr mice. *Genetic research* (Cambridge) 1973, *21,* 121–132.

Festing, M. F. W.: Inbred strains in biomedical research. The Macmillan Press Ltd., London, 1979.

Groen, A.: Identification and genetic monitoring of mouse inbred strains using biochemical polymorphism. *Lab. Animals,* 1977, *11,* 209–214.

Hau, J., L. L. I. Andersen, B. Rye Nielsen and *O. O. M. Poulsen:* Laboratory animal models. *Scand. J. Lab. Animal Science,* 1989, *16,* Suppl. 1, 7–9.

International index of laboratory animals. Ed. M. F. W. Festing, 5. ed. Laboratory Animals Ltd. Newbury, England, 1987.

O'Donoghue, P.: Obituary, William Lane-Petter. *Scand. J. Lab. Animal Science,* 1988, *15,* 42–43.

Öbrink, K. J.: Are regulations to protect experimental animals adequate? In "Research ethics" ed. K. Berg, K. E. Tranöj. Alan R. Liss, Inc., New York, 1983, pp 159–166.

Rowan, Andrew N.: Of Mice, Models, and Men. A critical evaluation of animal research. State University of New York Press, Albany, 1984.