The defined animal

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LABORATORY ANIMAL SCIENCE

Good science is characterized by precision and care coupled with a critical analysis of methods and results. The type and precision of the measuring devices, the origin and quality of chemicals and the experimental protocol must be carefully selected and planned in detail. Variables that may interfere with the results must as far as possible be controlled or at least standardized. In physics and chemistry this means for instance working at accurately controlled temperatures, pH and ionic strengths, with carefully controlled sensitive balances and other analytical instruments. The accuracy of the results need not necessarily be given in statistical terms but rather as implied by the number of decimals.

In biology, however, our present state of knowledge is very far from complete and therefore lots of variables that may influence upon experimental results are either not known or difficult to control. The precision of experiments, therefore, and the accuracy of results will vary in accordance with the complexity of the experiment. In a subcellular system the experimental conditions will probably be much easier to control than in a whole animal experiment. The results will be scattered and the precision must be expressed in statistical terms. For instance the systolic blood pressure of a normal rabbit is 110 ± 20 mg Hg (range) (*Kozma et al.* 1974).

In order to increase the precision and to make it possible for other researchers to repeat animal experiments it is necessary to standardize the experimental conditions to as high degree as possible and to report carefully and in detail the experimental conditions in all scientific publications (*Beynen* 1991).

If no precautions are taken in animal experiments the scatter of the results may probably be ± 15 % or more. To compensate for this and for the sake of safety, a large number of experiments (= large number of animals) must be used but even so the interpretation of the results may sometimes be difficult and possibly erroneous. In order to increase the precision and decrease the number of experiments needed it is necessary to study which variables are interfering and to learn how to eliminate their uncontrolled effects (*Hurni* 1969, *Heine* 1985). This task has in the last 20 to 30 years created a new scientific discipline, "Laboratory Animal Science" (LAS), an auxiliary science serving other biomedical disciplines. LAS may be divided into basic and applied types (*Hau et al.* 1989, *Hau* 1991). For the present topic of "defined animals" the basic LAS is of interest and it may be divided into the following four parts (*Öbrink* 1990):

1. Laboratory animal genetics and comparative biology,
2. Effects of homeostatic compensatory mechanisms on experimental results (including microbiological influences),
3. Laboratory animal technology and fundamental experimental methodology and
4. Legislation and laboratory animal ethics.

Each of these parts must be observed thoroughly when performing animal experiments if the danger of the deleterious influence of irrelevant variables is to be avoided or minimized.

Laboratory animal genetics and comparative biology

It is obvious that the use of an instrument, the properties of which are unknown or uncalibrated, necessarily involves risks of uncertainty. This is also no less true for laboratory animals and much work has been devoted to the clarification of their genetic backgrounds (*Lang* 1983). In order to make it possible to perform series of experiments on genetically defined animals purposeful breeding of animals has been practised. As a consequence a large number of inbred strains and other genetic specialities have been developed (*Festing* 1979). For obvious reasons the breeding of such animals has been most successful with small rodents because of their short generation turnover. No less than about 1 200 different types of mice (inbred, mutants, coisogenic, congenic and outbred) are available and also about 440 types of rats (*Festing* 1987). Genetically specified animals are used as models for a variaty of biomedical problems like diabetes, obesity, hypertension, tumour development, immunosuppression etc., etc. Due to the large number of genetically defined but diverse types of animals efficient genetic monitoring is a necessary service to all serious biomedical laboratories (*Festing* 1990, *Hedrich* 1990). Most experiments on rodents are, however, performed on Sprague-Dawley and Wistar rats and NMRI mice, which are nothing but the names of numerous outbred stocks, that are poorly – if at all – genetically characterized.

Even the most careful genetic standardization will, however, not lead to completely uniform animals because biological random variability still persists for, as yet, unknown reasons, maybe from unavoidable selection or from ooplasmic differences (*Gärtner* 1990).

Comparative biology may be a useful tool for solving some biological problems. Different species have sometimes different biological characteristics in for example, anatomy, biochemistry and immunology, that can be of value, but such differences may also provide pitfalls for the ignorant researcher (*Falkmer & Waller* 1984).

Effects of homeostatic compensatory mechanisms on experimental results

The most common cause for low precision and scattering of results and even misinterpretations are the interferences by homeostatic reactions of the animal body, that occur to almost every external and/or internal factor (*Öbrink* 1990). The following factors should be mentioned:

a/ Different metabolic conditions during the life cycles, i. e. age, sexual periodicity, pregnancy, lactation etc.

b/ Physical factors like ambient temperature, humidity, light intensity, photoperiods, sound, pressure etc. These factors have been extensively studied (*Hurni* 1969, *Lang* 1983).

c/ Chemical factors like composition of diet (*Beynen* 1987), including deficiencies or excesses, quality of drinking water, origin and type of bedding, unintentially added impurities like pesticides, detergents etc.

d/ "Animal sociology", which involves the interactions of animal-with-animal, animal-with-cage, pen etc. and animal-with-man. These interactions necessitate observations of animal behaviour (ethology). The homeostatic reactions are mostly of hormonal nature and may interfere with the experimental results in fundamental ways if not recognized and controlled. In this area should be mentioned the assessment of pain and stress, which is of importance for evaluating experimental data (*LASA Working Party* 1990, *Jeneskog* 1991).

e/ Microbiological agents, which are potentially disturbing factors in animal experiments. These agents include parasites, bacteria, mycoplasmas and viruses. A normal animal carries a large microbiological flora living in symbiosis with the host. Deviations from this normal flora, especially the introduction of pathogens, will change the immunological status of the animal and as a consequence even its physical status. This may cause alterations in the animal's reactions and responses to experimental procedures. It must be understood, however, that also latent infections (often of viral nature) may have a significant influence on biological experiments (*Bhatt et al.* 1986, *Rehbinder & Feinstein* 1989, *Rehbinder & Feinstein* 1990, *ILAR* 1991).

Infections may furthermore lead to cell destruction (necrosis) and eventually scar formation or other induced tissue lesions. The homeostatic mechanisms have in such cases not been able to restore the body to its original state but animals with new characteristics have in fact developed. This may lead to serious complications for the evaluation of the experiments. Furthermore, if the deviations from the original state are different in different individuals, scattered experimental results may be expected. Obviously even past infections may leave post-infectious changes that may interfere with the experiments. Consequently it is imperative to define the experimental animal as to its microbiological status – past and present – and to possible anatomical abnormalities. This is accomplished by *"Health monitoring"*, which is the topic of the series of subsequent papers following in the present issue of this journal. "Health monitoring" includes both microbiological screening and patho-anatomical checking of macroscopic and microscopic alterations, that may be the result of microbiological, physical, chemical or nutritional influences.

Laboratory animal technology and fundamental experimental methodology

Handling techniques, routines for changes of bedding and cage cleaning, the hour of the day for blood sampling (circadian influences) etc., etc. are of importance for the experimental results (*Falkmer & Waller* 1984). Consequently, in order to obtain "Defined Animals" it is important that all experimental work is standardized. This is accomplished by the routine of working out detailed "Standard Operating Procedures" (SOPs), which is a practical way for every laboratory to reassure a consequent and standardized handling of every moment in an animal experiment including the analytical procedure. Drawing up SOPs is one way of adhering to the so called "Good Laboratory Practice" (GLP) (*Food and Drug Administration* 1978, *OECD* 1992). GLP furthermore demands detailed descriptions and control of the experimental protocol, which helps to eliminate many otherwise uncontrolled details that would upset the experimental accuracy. This is a prerequisite for the possibility to repeat and reproduce an experiment. GLP will hopefully "infiltrate" all biological laboratories and contribute to a proper definition also of the laboratory animal.

Legislation and laboratory animal ethics

This important part of LAS will not be particularily discussed further in this context, but if all proper measures are taken in husbandry and animal experimentation most ethical requirements will be satisfactorily fulfilled.

THE DEFINED ANIMAL

From what has already been said a laboratory animal is not satisfactorily defined unless several variables are clearly described. A complete definition must include the following statements and checks, namely: the origin and general status of the animal confirmed by genetic monitoring and health monitoring, a description and control of the physical environment, a description and control of the food and feeding system, a description of the standard operating procedures used in the husbandry. The appropriate details should of course be used as a check list already at the start of a scientific project and not when the experiments have been completed and it is time for publication. A proper care including daily observations of the behaviour of each individual animal should exclude deviations from this defined state.

THE PRESENT STATUS AND THE FUTURE

Although Laboratory Animal Science has expanded during the last 20–30 years its impact on biomedical research in general has not yet been adequate. It is true that the use of inbred strains has increased and that sophisticated laboratory animal facilities have been built, it is true that healthier animals are available now compared with before and that education in different fields of LAS are offered to the researchers. The general state is, however, as yet far from satisfactory. This can for instance be judged from perusing "Materials and methods" in scientific papers.

This section will often contain a detailed description of chemicals used (pro analysi, manufacturer, batch number), analytical instruments (model, precision), analytical procedureres step by step etc., etc. but when it comes to the animal model the description will not infrequently be very poor. It may sometimes simply be stated: "10 white rabbits were used"! Only in a minority of papers will the animal models be properly described. This is of course unsatisfactory and must eventually be changed. If GLP were to be accepted as a routine in every biological laboratory it would result in a detailed and adequate description of all known variables relevant to the experiment. This does not mean that every experiment will be performed with the highest possible quality of animal or in the most exclusive laboratory. It only means that all the conditions and procedures used will be reported in detail, thereby making it possible to evaluate the experiment and the results and to repeat the experiment.

There are many reasons for the present status of poor animal definition. Obviously failure may depend on not yet understood mechanisms, and it is the aim of LAS to study such mechanisms and thereby improve the models. Those variations that are due to known variables, however, should not be overlooked by good scientists. But unfortunately there are still too many that are neglectful.

The majority of researchers, however, are failing in defining their animal models probably due to ignorance. Education is the obvious solution for this deficiency. However, voluntary courses have been given for research students in many countries for rather many years by now, and considering the lack of effect on model description we must conclude that these courses have not had a sufficient impact. Probably they have been concentrated too much on technical issues and not enough on LAS, but a further reason might be the conservative attitude found in many research departments.

Defined animals and scientific journals

An absolute demand from the editors of scientific journals for a proper and correct description of the animal models would be an effective way to increase the use of proper animal definition. If the editors would reject every paper that was insufficient in this respect, a change would certainly occur. But such a step is not without problems.

"Gesellschaft für Versuchstierkunde – Society for Laboratory Animal Science" (GV-SOLAS) in Germany and central Europe has worked out a scheme for describing the animal model in scientific papers. The journal "Laboratory Animals" (London), has adopted the scheme. A full description is found in every issue of this journal under "Notes to authors". So far "Laboratory Animals" is the only journal that has adopted the system, but some other journals, also specializing in laboratory animal science, do demand a proper description of the animal model. The reason why all the other biomedical journals do not require a better definition, is probably that a full description will require space that most journals cannot accept. Other ways must be sought.

Some procedure is needed that could squeeze sufficient information about the animal model into a limited space. One way would be a coded system in a table form, another a kind of a bar-code system, and still another the establishment of an international data bank for animal models. In any case, this would necessitate international cooperation.

The "Federation of Laboratory Animal Science Associations" (FELASA) has initiated a work on "Health monitoring", which is now almost complete (*FELASA Working group on animal health*). A scheme will be presented with recommendations on the minimal requirements for a sufficient health definition. In the future it will probably be possible to refer to this document and in few words describe a rather detailed health status.

Hopefully, similar documents in genetics, nutrition, husbandry etc. would make it possible to codify the definition of laboratory animals.

If the FELASA initiative were to spread to other LAS organisations around the world there would eventually be a possibility to put international documents relating to animal definition under the aegis of the International Council of Laboratory Animal Science (ICLAS).

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