Scand. J. Lab. Anim. Sci. No. 3 . 1991 . Vol. 18

The basis for standardization of animal experimentation

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Standardization of animal experimentation can be described as defining the properties of the animal (or animal population) and its environment, and the subsequent keeping constant or regulating (controlled varying) of these properties. Aim of standardization is to increase the reproducibility of group mean results from one experiment to another and thereby to improve comparability of results within and between laboratories. Standardization frequently also aims at reduction of the variation in quantitative measurement values of apparently identical animals within a single experiment. Clearly, standardization implies that reporting the results is accompanied by careful description of the potential sources of variation, i.e. the animals and their environment.

It is evident that standardization of animal experiments only involves the potential sources of variation that we know. Variation in measurement values occurs at two levels: between apparently identical experiments (between-experiment variation) and between apparently identical animals within a given experiment (within-experiment variation). In essence, both types of variation are caused by the same sources, i.e. the variable properties of animals and their environment. Prospects and limitations of standardization of the potential sources of variation will be discussed here.

Between-experiment variation

Repetition of a given experiment with an other group of animals will yield different group mean measurement values. This between-experiment variation consists of two noise components: variation in measurement values between individual animals and differences in experimental conditions. The treatment effect (= differences between group means of control and test group) will thus vary between experiments. If there is an interaction between treatment effect and experimental conditions, the treatment effect will differ per experiment. This may lead to false interpretation of results. Moreover, in order to accurately assess the treatment effect, repetition of experiments is required. Decreasing the between-experiment variation of treatment effects reduces the need to perform the same experiments repeatedly and this contributes to a justified use of laboratory animals. From a scientific point of view, experimental results also should be reproducible and thus independent of time and place. A hypothesis for further research must be based on reproducible experimental results.

Within-experiment variation

Quantitative measurement values of apparently identical animals within an experiment will show inter-individual variation. This within-experiment variation is the sum of variation in execution of experimental procedures, analytical variation, intra-individual variation and true inter-individual variation. The true inter-individual variation is caused by the intrinsic contribution of each animal to the measurement value. The intrinsic contribution differs per animal and basically is independent of the type of treatment to which the animal is subjected. Each animal also has a non-intrinsic contribution to its measurement value. This consists of inexplicable and non-standardizable variation within an animal (intra-individual variation), which finds expression in day-

to-day fluctuations of the measurement value.
An increasing inter-individual variation in measurement values (= increasing standard deviation) will increase the number of animals needed per experiment if the statistical power (= chance of detecting a true effect) is to remain constant. Reduction of the use of laboratory animals per experiment is thus possible by decreasing the apparent inter-individual variation of results. This is important on the basis of economical, practical and ethical considerations.

Sources of between- and within-experiment variation
An important source of variation in animal experiments is the animal itself. Differences between animals in one treatment group, such as differences in age, body weight, number of litter mates during the suckling period and further past history, can increase the within- and between-experiment variation in measurement values. Inter-individual differences in genotype, including differences in gender, can also increase the variation in measurement values. Uncontrolled fluctuations in biological (infectious pressure, number of animals per cage etc), physical (light, temperature etc) and chemical (nutrition, bedding etc) environmental factors can increase the between- and within-experiment variation in results.
Basically, the measurement value of an animal is determined by genotype-environment interactions. The response to treatment is also influenced by this interaction. There are various levels at which the environment can interact with the genotype of the animal. The environmental influences from the fetal phase up until sexual maturity are called the primary milieu. The interaction between this milieu and the genotype gives rise to the phenotype. The phenotypical properties are subsequently influenced by the conditions prior to the experiment (= secondary milieu). Thereupon, the laboratory animal is affected by experimental procedures and treatments (= tertiary milieu). Depending on the type of measurement values, genotype and milieu interactions influence the within- and between-experiment variation to a different extent.

Standardization of the animal
Inter-individual differences in genotype can be eliminated by using genetically uniform animals. The individuals of an inbred strain or F_1 hybride (= cross of two inbred strains) are genetically identical and thus will, as a general rule, have a smaller inter-individual variation in measurement values (before and after treatment) than genetically non-uniform animals (random-bred or outbred animals). However, this rule does not hold invariably.
With adequate genetic quality control, it is possible with the use of an inbred strain to have at one's disposal animals with constant genotype from one experiment to another. Certain experiments do not require identically reacting animals, but rather inter-individual variation. Such variation can be standardized by using a population of hybrids (mosaic population). This population is derived by crossings of a number of inbred strains. In this way, constant genetic variation can be created.
The microbiological quality of laboratory animals can influence various types of measurement values. Infectious pressure (latent infections) can increase the inter-individual variation within an experiment and can also differ between experiments. This type of variation can be eliminated by the use of specified-pathogen free (SPF) animals. In certain situations even germ-free animals or gnotobionts can be used. The status of microbiologically standardized animals must be maintained by hygienic measures. It is clear that the microbiological quality of SPF animals is more constant in time than that of conventional animals with unknown or poorly defined status. In this context, microbiological quality control is a prerequisite.

Standardization of the environment

In practice, it is not possible to house individual animals under identical environmental conditions. Within a room in which usually more animals are housed either individually or in groups, there are local differences in environmental conditions. Depending on the ventilation system, the temperature at 1.5 m can be 3 to 4°C higher than at 0.5 m. The location of a cage in a rack will thus influence the temperature in the cage (micro-climate). The micro-climate is also affected by the number of animals per cage, relative humidity and type of bedding. The commonly used fluorescent tubes as lighting are hung on the ceiling which causes a higher light intensity in the cages at the top of the rack. The difference between light intensity between cages at top and bottom of a rack can be four-fold.

Within an experiment, differences in housing conditions between individual animals should be equally distributed between control and test groups. Consequently, the treatment effect will not be biased by differences in housing conditions between control and test groups. Between experiments the local differences in environmental conditions with animal rooms should be constant. Thus, environmental factors must be invariable from one experiment to another.

Standardization of environmental factors is essential to ensure a justified use of laboratory animals. As to the practical realisation of such standardization there are many points to be settled. At which level should environmental factors be standardized? Should this level be constant or rhythmic? On which criteria should the ideal level of environmental factors be based? These criteria can be a combination of optimal welfare of the animals and ergonomical considerations. However, on the basis of these criteria the level of a given environmental factor is not by definition associated with a small within-experiment variation of measurement values. Getting ahead of the availability of sufficient experimental data, the Council of Europe has already formulated recommendations for the housing and care of laboratory animals. These recommendations are based on current knowledge, whether or not scientifically substantiated, and on common practice.

Standardization of experiments and extrapolation of results

In principle, experimental results hold only for the conditions (animals, environmental factors) under which the experiment has been carried out. For validated routine testing using laboratory animals (control of vaccins, toxicity tests), this is not problematical. However, there are many experiments of which the outcome should be generalizable. Standardization of experiments implies a specialization of experimental conditions. Thus standardization is on bad terms with generalization of experimental results. The first question is to what extent the animals used are representative for all comparable animals. Even if we accept that all animals are representative, the results still have a bearing only on a limited population (animals of the same strain, of the same sex, with the same body weight etc) and on limited conditions (the biological, physical and chemical properties of the environment). It is even more complicated when the results obtained with laboratory animals must be extrapolated to another species, in particular man. Clearly, caution is varranted here. The range over which results are extrapolated from experiment to practice or especially from one species to another, probably is very wide in relation to any limitation of generalization of results by standardization. Thus, having to extrapolate the results can be no excuse for dropping standardization.

Efficacy of standardization

The aim of standardization, i.e. reduction of between-experiment variation and often also of within-experiment variation, is only empirically substantiated to a limited extent and only for specific, quantitative measure-

ment values. In certain cases, standardization can have an opposite effect, i.e. an increase in within-experiment variation. Essentially, pursuing standardization is based on theoretical considerations. Nevertheless, it can be stated that standardization must improve the comparability of results from different laboratories, especially because standardization implies careful reporting of experimental conditions.

Ernst Barany död

Ännu en av föreningens Hedersledamöter och de verkligt stora inom försöksdjurssektorn har gått bort.

Idag på eftermiddagen fick jag beskedet att Ernst Barany lämnat oss.

Banbrytande var hans verksamhet inom den svenska och nordiska försöksdjurssektorn. Tillsammans med professor Bengt E. Gustafsson skapade han grundlaget för den verksamhet som bedrivits i dryga 25 år.

Han blev Scand-LAS andra Hedersledamot och den äresbetygelsen var lika välförtjänt som den till Bengt E. Gustafsson. Båda män med en ofantligt stor kunskap, med förmåga att etusiasmera, att skapa något värdefullt och bestående. De fann att försöksdjurssektorn i landet behövde utvecklas och förnyas och satsade helt på den. Vi som kom in i början och fick deras stöd hade en underbar uppgift. Den senaste tiden har jag ofta tänkt tilbaka på "nybyggarandan" och hur fint det var då och hur man saknat de båda stora.

Ernst Baranys stora passion var givetvis hans forskning, men han gav också så mycket till försöksdjurssektorn. Som sekreterare i MFR:s försöksdjursnämnd fick jag ofta orsak att besöka honom och vilka härliga stunder har jag inte upplevt där. Det första internationella försöksdjurssymposiet för svensk del 1967 i Stockholm blev en success och givetvis var Ernst B. og Bengt E. G. bakom.

I Strasbourg för försöksdjurskonventionen gjorde Ernst en fantastisk insats. Otroligt respekterad, avhållen av alla för sin kunskap, vänlighet och humor.

Han kunde så mycket om mycket, det var fantastiskt att få följa honom på Monnetmuseet i Paris, se på fjäriler och skalbaggar också i Paris, glas i Nancy, höra hans utläggning om fjällfloran, vandra med honom, få en bra historia berättad etc.

Livet blev liksom så mycket tommare i eftermiddags, saknaden känns så stor. Men vi skal minnas Ernst Barany med glädje och tacksamhet, försöka att följa hans intentioner i den positiva anda han alltid själv arbetade och ville vi skulle arbeta. En stor, fin människa är borta men han kommer alltid att leva i våra hjärtan med positiva pulsslag.

Lars Wass.

Bidrag till Ernst Baranys Minne kan sättas in på postgirokonto 18 37 97-0. Uppsala Universitet "Fonden för Farmakologisk Forskning".