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ABSTRACTS FROM THE SCIENTIFIC PROGRAMME

Genetical aspects of laboratory animal breeding

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A reproducibility of the results from animal experiments is only possible if the gene pool of a population can be kept extensively constant over a long period. Therefore the maintenance of the genotype spectrum is an universal problem of laboratory animal breeding.

Breeding inbred strains requires the establishment of a genetic profile in qualitative traits per strain and genetic monitoring over generations for verifying the integrity of the strains.

Outbred populations are used in large numbers in biomedical research as models for human populations. In contrast to inbred strains the maximum genetic variance of characters within an outbred population is an important quality criterion. Neither random matings, nor rotation systems are suitable for preserving the genotype spectrum of outbred populations.

Consequently - using Han: NMRI mice as a model for genetic monitoring in outbred populations - we developed a method for keeping the genotype spectrum widely constant. The following steps had to be taken:

1. Recording of various character groups within the breeding population: Reproduction characters, skeletal measurements, body weights, total body analyses, hematological data and biochemical polymorphisms.
2. Definition of the characters in arithmetic means, variances, specific deviations (skewness and kurtosis) and heritability coefficients. From the total of the arithmetic means and variances from 109 important characters we obtained the population norm for Han: NMRI. These standards were not short term observations, but they have been derived from data gathered from approximately 6500 males and females each.
3. For breeding, animals are selected in consideration to their individual performances or their parent's characters in correspondence to the population norm. The best fit of the new breeding unit to the given population norm is determined by means of a computer (PDP 11/34A), using a quadratic optimization technique. Quadratic optimization is a calculation in which the quadratic deviations of the averages and variance differences between population norm and new breeding unit are simultaneously taken into consideration and reduced step by step to a minimum.

Nutritional aspects of laboratory animal breeding

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The nutrition of laboratory animals must be physiological as well as defined and reproduceable. Unlike the nutrition of agricultural domestic animals economical points of view are here of minor importance. Varying nutrition leads to differences in the biological response of the animals, which often remain unrecognized. With respect of the breeding of laboratory animals two main questions are asked and discussed by means of examples.

1. What nutrient requirements are necessary?
2. How can a defined, reproduceable covering of nutrient requirements be achieved?

The familiar data on the requirement of single nutrients for a special species vary. They depend on the respective test conditions and contain the biological variability. Very precise supply data cannot be reproduced very well. Pre- and postnatal malnutrition influences the development of young animals. Under-as well as overnutrition should be avoided. Practical examples on this subject are presented. To improve the necessary reproduceability of the supply of the nutrient requirement in laboratory animal breeding, food mixes should contain as little as possible crude material, but such of high quality. Presently, a list recommended crude material is prepared by the committee of nutrition of the society for laboratory animal science.

The named aspects are explained by the example of rat breeding and two food recipes, easy to reproduce are presented. A three-step feeding plan is pointed out to reduce the usual protein luxury consumption.

Large scale production of laboratory animals, quality - economy

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Since funds available for biological research are limited, the animals needed must be produced with equal respect to quality and economy.

This favours large scale production units capable of supplying a wide variety of animals (species or strains) to the user's specification, and in the most economical way.
In order to meet this demand careful planning of facilities, working routines, training of personnel and quality monitoring systems are necessary.
With the rapid development in biotechnology it is also necessary – in the interest of reducing the number of animals required for each experiment – to explore the feasibility of employing new methods in the production of laboratory animals.

Health aspects in large scale production of laboratory animals

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The evolution in laboratory animal science began only 40 years ago.
There was a growing awareness of the obvious problems connected with the use of laboratory animals. The quality of the research performed and the data and the results obtained may with our eyes today often seem unsatisfactory. Many valuable scientific discoveries were, however, obtained by clever researchers at that time, despite obvious technical problems and difficulties.
At the same time most of the scientists did not bother about what we today believe is the necessary background information to be considered before we can perform studies of quality with animal models e.g. genetic backgrounds, health status, composition of diet and other environmental parameters.
In this short lecture I will try to describe how I feel the patogen control should be applied within science using laboratory animals.

Excessive but legal use of laboratory animals

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What do the politicians say?
»The purposes for which experiments should be permitted should be clearly defined and limited; there is a need to reduce the number of animals used and the level of suffering, to eliminate experiments for trivial purposes and to avoid unnecessary and duplicate testing«.
Where are the problems?
1. Pyrogentest in rabbits.
Problem: Large variation in response of the individual animal.
Excuse: Traditionally required by authorities for 50 years.
Proposal: General use of Limulus Amoebocyte Lysate (LAL) test in vitro. It is a more sensitive, quantitative, validated method with a much better reproducibility than the in vivo test.
2. Bio-assays.
Problems: The test require unnecessary use of large numbers of animals.
Excuse: Many years ago the only methods available to quantify extracts etc. were bio-assays.
Proposals: Use of Q-HPLC and/or cell culture methods instead of e.g. mice or rabbits for potency of insulin. Use chemical methods for analytical quantification of human growth hormone instead of bio-assays with hypophysectomized rats.
3. Acute toxicity & LD50.
Problem: Performed 7 times (approx. 80 rats each time) for 7 firms on the same chemical at one contract laboratory.
Excuse: Authorities demand.
Proposal: Registration of all acquired data within EEC/OECD. Compulsary check of whether data are already available before start of animal testing. Payment of fee for reuse of data.
4. LD50.
Problem: LD50 used unnecessarily for classification of chemicals.
Excuse: Authorities demand.

LD50 mg/kg b.w.	warning/ labelling
<25	very toxic
25-200	toxic
200-2000	harmful

Proposal: Estimiate of mean lethal dose is sufficient for the purpose.
5. Abnormal toxicity.
Problem: Irrelevant testing of human proteinaceous preparations in mice and quinea pigs.
Excuse: Authorities demand.

Proposal: Replacement by physicochemical analyses which coinside with results from tests in mice and quinea pigs after introduction of GMP.

6. Toxicity testing of peptides in dogs and monkeys.

Problem: Non-rodent species are used for longer term studies with immunogenic proteinaceous products.

Excuse: The tests are on the authorities check list.

Proposal: Maximum duration of such tests should be limited to 2 weeks. Dogs and monkeys are likely to produce antibody response with foreign proteins, resulting in non-relevant pathological reactions.

7. Rabbit convulsion test for toxicity of insulin.

Problem: Induction of very unpleasant convulsions alleviated by glucose injection as an indication of absence of toxic impurities.

Excuse: Authorities demand. Introduced before GMP & GLP.

Proposal: Immediate deletion.

What a correction means in animal lives saved:

Laboratory animals used annually in Denmark 480.000

Medical industry uses hereof	320.000
Estimated number of saved lives	100.000

A survey on laboratory animal activities in Finland

By *Heli Haataja,* National Laboratory Animal Center, University of Kuopio, P.O.B. 6, 70211 Kuopio, Finland

Laboratory animal activities in Finland have not been studied since 1973. Consequently it seemed proper to survey the field. Furthermore the new statute including the idea of purpose breeding of all laboratory animals prompted the ministry of education to go on with the survey.

The methods used in this survey were a questionnaire sent to 141 departments or institutes, personal interviews and visits. 103 departments or institutes having activities on this field answered to the questionnaire. The majority of them belongs to universities and the rest is composed of hospitals, national or private institutes and pharmaceutical companies.

There are 75 breeding or experimental units for laboratory animals, which is the same as in 1973. About 300 persons are working in this field. About 220 000 laboratory animals are used annually in Finland. The number of laboratory animals bred annually is about 250 000. The approximated need of laboratory animals in 1990 is 250 000, too. Compared to the year 1973 the total number of laboratory animals used in 1985 has increased. However, the use of rabbits, dogs and cats has decreased during the same period. A few problems seemed to be essential according to this survey. Although the number of animals bred in Finland seems to be large enough, proper strains and health monitored animals are not always readily available. Sometimes there is even lack of common strains of mice and rats. Diseases are also regarded as a problem in particular with rabbits, rats and mice. The conditions in breeding or experimental units have improved during the last 13 years, but environmental factors still cause problems in many units. Lack of space is common.

Laboratory animal technicians and technologists are better educated today than 13 years ago. Also the scientists have better possibilities to get education on laboratory animal science. However, most of the education rely on contemporary courses. No special programme for the education of technologists exists in Finland. Economic problems are often mentioned to prevent both scientists and technicians from participation in supplementary education.

The laboratory animal activities in Finland have expanded since 1973, and certain trends for centralizing these activities can be observed especially in universities. The education in this field has increased but is not systematic enough. A health monitoring programme should be established at a national level.

A quality assurance program in a rabbit breeding colony

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In order to increase the genetic and microbiological animal quality a commercial rabbit breeder established a closed colony in a purpose-designed, new building. The rabbit house comprises of a 250 m^2 common stock room with flat deck caging system and automatic systems for watering, manure removal and control of positive pressure ventilation, temperature and humidity. All stock is fed on an unsterilized pelleted diet, hay and tap water.

With initial financial support from the Board of Universities and Colleges (UHÄ) a breeding nucleus of totally 45 rabbits of the NZW, NZW-FX1 and Dutch strains were imported from a LABA accredited barrier breeder (Froxfield SPF Rabbits, Hampshire, England) in January 1985. The laboratory animal unit, Karolinska Institutet designed a quality assurance (QA) program for the initial breeding stock and the production for replacement and research.

On arrival and thereafter every three months the QA program included: Clinical examination, breeding and performance record examination, on site inspection of environmental factors, blood sampling for serology (Encephalitozoon coniculi and Toxoplasma gondii), hematology and biochemistry (first three samplings), fecal samples (pooled from each age group/row of hutches) for endoparasites and nasal swabs for bacteriology. At each sampling occasion 10-12 »monitoring cages« covering the distribution of hutches in use, were selected for blood and swab samples. In addition all animals that have died or are observed ill were sent in for post mortem examination. The laboratory examinations were performed at the National Veterinary Institute (SVA), Uppsala, Sweden.

One year after start the herd comprised totally of about 1000 animals (on an average 50-100 does with litters and 400 weanlings). At that time the QA program was extended to health monitoring (performed at the SVA) including post mortem examination of 5-6 animals, two times a year.

On arrival and during the following three months no infection was detected and the stock appeared clinically healthy. Clinically silent light infestation/infection with intestinal coccidia (Eimeria ssp), respiratory Bordetella bronchiseptica and pin worm (Passalurus ambiguus) was detected after 4, 8 and 9 months respectively. These organisms seem to be more or less persistent in the colony, but have not up to now manifestated in clinical disease.

Three colony medications have been performed after the production started in 1985: against coccidiosis (sulfonamides). Bordetella bronchiseptica (oxytetracycline) and pin worm (febantel). All treatments resulted in eradication or reduction in the number of isolates when re-sampled after treatment.

Practically, the breeding program as well as the stock produced, have worked out satisfactory. Results of the QA program illustrates the difficulties of keeping a closed but conventionally reared herd free from infections.

Microbiological surveillance of breeding colonies

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Microbiological surveillance of a breeding colony of laboratory animals is an essential aid to the management of that colony. The many investigations undertaken enable those responsible for the colony to make decisions as to the quality of their animals and to enable them to improve their husbandry methods.

There are many different areas of a production colony that can be monitored. These include the animals, the food, the bedding, the environment and the staff. The most important are the animals themselves. They must be examined for bacteria, fungi, mycoplasmas, parasites and viruses.

In the paper details will be given of the methods used to monitor laboratory animal colonies of mice, rats, rabbits, guinea pigs and dogs. The tests included will cover all aspects of the examination. the skin and hair is checked for ectoparasites and dermatophytes. The upper respiratory tract is checked for respiratory pathogenic bacteria. This site is also checked for mycoplasmas in rats and mice. The lungs are checked in a similar manner. The liver and other major organs are checked for bacteria and the gut for *Salmonella* and *Shigella* species and for endoparasites.

Results over a 2 year period show that consistent data is obtained from breeding colonies. Respiratory pathogens are isolated frequently but mycoplasmas are not found. Zoonotic organisms have only been isolated in two cases. Viral infections occur sporadically and are limited to respiratory viruses. Sendai and Pneumonia virus of mice.

Health monitoring of laboratory rabbit breeding colonies in Sweden. Major findings

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From 9 breeders, groups of 12 rabbits were received for health monitoring at The National Veterinary In-

stitute, Uppsala, Sweden. Each rabbit was necropsied and samples obtained for histo-pathology, bacteriology, virology, and parasitology.
Inflammatory lesions were most common in lungs and intestines.
The bacteriological investigations demonstrated growth of *Bordetella bronchispetica,* often associated with lung lesions, in most colonies. Bacteriological samples from the intestines yielded a mixed flora frequently composed by *Bacillus sp., Bacterioides sp.* and *E. coli.* Two colonies exhibited coronavirusinfection.
Enteritis was frequently associated with infections of *Eimeria sp.*. Liver coccidiosis, *Eimeria stediae,* was present in three colonies.
Trichomonas sp. was found in two colonies. Among the nematodes *Passalurus ambiguus* was the commonest and found in seven colonies.
Among the ectoparasites *Cheyletiella parasitivorax* was very common and found in all but two colonies. *Listrophorus gibbus* and *Psoroptes cuniculi* were also found.

Application of embryo transfer and cryopreservation to laboratory animal production

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In the laboratories of Bomholtgaard a program has been initiated, exploring the application of embryo transfer, cryopreservation and embryo manipulation in large scale laboratory animal production.
The preliminary phase has been concerned with optimization of superovulation, synchronization of donor and recipient, finding the most suitable recipient strains and stocks, adaptation of techniques for embryo transfer and quickfreezing of embryos to laboratory animal production. Moreover, shipment of superovulated, mated females as well as pseudopregnant females over longer distances, has been examined.
Results will be presented concerning 1) optimal doses of gonadotrophins and the age of the donor female with respect to maximum number of fertilized eggs, 2) synchronization by pheromonal stimulation (Whitten effect), 3) analysis of the influence of group size of females prior to mating, 4) evaluation of mouse strains as recipients in oviduct embryo transfer.
The present results indicate that inbred strains are less satisfactory as fostermothers than outbred strains and hybrids (Bom/NMRI, Bom/SENCAR, B6D2F1/Bom).
The experiments with cryopreservation have been concerned with a quick-freezing technique using direct plunging of the embryos into vapor nitrogen (-180°C). The results indicate that this method is applicable, yielding 60-70% morulae developing into blastocyst from frozen-thawed embryos, although the technique still needs refining.

Transgenic animals as a research model: Economical and ethical aspects

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DNA-clones produced by recombinant DNA techniques can be integrated into the pre-nuclei of fertilized egg cells. Foreign DNA is expressed in most cases in the next generation of different animal species. Transgenic animals are very useful when studying gene expression. The benefits of gene manipulation like this depend on the fact if the foreign gene is expressed in germ line.
Transgenic domestic animals are beneficial in an economical way: Many diseases could be cured with gene therapy. Fertility will rise. The weight of bigger animals rises and so meat production increases. Clinically important proteins are produced for commercial aims in future.
However, common laboratory animals could be cheaper producers - working with big domestic animals is expensive, because lots of animals are needed. It is difficult to show the effects of stable, regulatory and hereditary changes. That is why it is important to do far-reaching selections as what concerns the aims of gene manipulation with domestic animals.

Many ethical questions arise. Is it possible to develop only one character without impressing the whole? Is it ethically right to use growth hormone produced in bacteria to promote growth of cattle? Is gene therapy always healthy? For more public discussion on gene manipulation with animals we have to influence the society to get more knowledge in general biology.

Monitoring of water quality by transfer of cultivated rainbow trout

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Aquatic environment receives finally much of the industrial, agri - and silvicultural as well as municipal wastes, although waste water treatment is obligatory. The chemical analyzes of released waters are valuable, but the number of chemicals in each mixture exceeds the rational possibilities for their follow up. Biological monitoring is the only way to get integrative information and learn the responses in the living organisms subjected to the dissolved waste compounds. Fish have a number of advantages in such monitoring. It is possible to get high numbers of progeny from each pair of fish. Fish have a highly developed detoxification system, which is adaptive to environmental pollution. One can also cage them for long periods of time.

We have studied the effects of transportation as well as caging of rainbow trout in waters having different degrees of loading in Lake Saimaa area as well as in laboratory conditions.

Rainbow trout resists transportation in vessels carried in cars with minimal losses and minimal changes in their biotransformation ability. If the rainbow trout is anesthetized with the aid of Tricaine, considerable lowering of the monooxygenase activity occurs.

In polluted waters rainbow trout is rather resistant, although less resistant than the local white fish. The activity of 7-ethoxyresorufin o-deethylase appears to be the mose sensitive indicator of the presence of waste waters of pulp industry, since it is considerably more readily induced than e.g. 7-ethoxycoumarin o-deethylase or benzo(a)pyrene hydroxylase.

Rainbow trouts are readily available. This fish is also easy to handle. One can furthermore collect the bile of the fish for chemical analysis of the compounds studied. The tissues as well as their biotransformation enzymes are also resistant to storage, if proper care is taken. Since the enzymes respond to the chemical loaders like those in pulp industry and oil used in engines, it is quite suitable species for the monitoring of aquatic environment.