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Standardisation of laboratory animals for biomedical research in Poland

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Increasing demands for biomedical research require laboratory animals of good quality. Simultaneously, the scientists aim at limiting the number of sacrificed laboratory animals. Some international organisations like ICLAS, FELASA, SOLAS deal with the promotion of proper breeding and care of laboratory animals. In 1997' The Animal Protection Act' was adopted by Polish Parliament. The standardisation of laboratory animals for biomedical research in Poland is the main task of the Commission on Biology of Laboratory Animals P.A.Sci. and Polish National Committee for Collaboration with ICLAS. In 2000, the Department of Genetics and Laboratory Animal Breeding, M. Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw was designed as National Reference Centre for SPF Animal Breeding.

The department consists of five integral parts: (1) bank of SPF (Specified Pathogen Free) inbred mouse and rat strains, (2) experimental animals laboratory, (3) health monitoring laboratory, (4) genetic laboratory, (5) anatomo-pathological laboratory.

Trends in development of laboratory animal science in Poland and Europe are overlapping. The standardisation of laboratory animals according to international recommendation seems to be sufficient in leading centres.

Key words: laboratory animals; SPF mice research; Poland

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Introduction

Permanently increasing demands GLP (Good Laboratory Practice) laboratory rules as well as Polish law regulations require laboratory animals of good quality for biomedical research.[1] Simultaneously, the scientists aim at limiting the number of sacrificed laboratory animals. The use of animals in biomedical research provokes considerable conflicts and discussion. The international co-operation and collaboration is necessary to solve the problem. Some international organisations deal with the promotion of proper breeding and care for laboratory animals.

The following international organisations are the best known within Europe and worldwide:

1. The International Council on Laboratory Animal Science, ICLAS
2. Federation of European Laboratory Animal Science Associations, FELASA
3. Gesellschaft für Versuchstierkunde, GV-SOLAS
4. Institute for Laboratory Animals Resources, ILAR
5. Fund for Replacement of Animals in Medical Experiments (FRAME) founded as a scientific charity.

In Europe, two intergovernmental organisations have adopted regulations for protection of laboratory animals: The Council of Europe, CE and The European Community, EC.

Although the aims of these organisations are not the subject of this presentation, it should be mentioned that their statutory activities are based on the concept of "Three R-s", namely: **R**eduction, **R**efinement and **R**eplacement.

The standardisation of laboratory animals for biomedical research in Poland is the main task of the Commission on Biology of Laboratory Animals P.A.Sci. and Polish National Committee for Collaboration with ICLAS.

The breeding of high quality experimental animals is not possible without progress in the field of laboratory animal science and appropriate political decisions.

Background of laws and policies that impact protection of animals in Poland (including laboratory animals)

In 1928, the regulation referring to the problem of protection of animals was issued by the President of the Republic of Poland. (The first comparable act has been in force in England since 1924.)

In 1957, the regulation issued by the Ministry of Justice and the Ministry of Interior described the general requirements to receive a licence to experimentation on animals and possible penalties.

In 1959, the Ministry of High Education issued a regulation concerning the problem of using animals for scientific purposes.

Today's legislation

In 1986, in Strasbourg, 11 members of Council of Europe signed 'The Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes'.

In September 1997, the Polish Parliament adopted the 'Animal Protection Act' to harmonise Polish legislation with European Community. General requirements for animal care and use were based on the above mentioned Convention.

In September 1999, the State Committee for Scientific Research appointed National Ethics Committee on Animal Experimentation. Its members are representatives of biological, medical, veterinary sciences and humanities. Simultaneously, 17 Local Ethics Committees were approved.

In November 1999, the Government issued an act that listed 85 scientific insti-

tutes, medical and veterinary faculties, universities as well as pharmaceutical firms that are allowed to perform experiments on animals.

Progress of laboratory animal sciences and alternatives

Polish Commission on Biology of Laboratory Animals P.A.Sci.

Since 1962, the Polish Commission on Biology of Laboratory Animals has been acting under the auspices of Polish Academy of Sciences. The organisation is an excellent place of contact between researchers and people dealing with the breeding of laboratory animals.

In the period 1964-1993, the Polish journal "Laboratory Animals" was published under the supervision of the Commission on Biology of Laboratory Animals. In the last issue, the Convention signed in Strasbourg was published in Polish translation.

The journals 'Comparative Medicine' formerly 'Laboratory Animal Science', 'Laboratory Animals' as well as the Japanese journal 'Experimental Laboratory Animals' are also available in Poland.

Membership in ICLAS

The long-lasting collaboration of P.A.Sci with ICLAS has resulted in the participation of Polish National Representative in the Governing Board of ICLAS. There are three laboratory animal breeding centres in Poland fulfilling the criteria to function as an ICLAS Reference or Monitoring Centre: 'The ICLAS Reference and Monitoring Centre System is a regional, international and inter-institutional collaborative network in line with ICLAS policy'.[2,3]

In 1978, the Reference Centre for Histocompatibility Testing in Mice was designated at the Institute of Oncology in Warsaw. Ten years later (1988), ICLAS Regional Monitoring Centres for Microbiology and Genetics were designated at the Institute of Oncology in Warsaw and at the Institute of Immunology & Experimental Therapy in Wrocław (Poland).

In 2000, the Department of Genetics and Laboratory Animal Breeding in the M. Skłodowska-Curie Memorial Cancer Center and the Institute of Oncology in Warsaw was designated as National Reference Centre for SPF Animal Breeding.

Development of alternatives in Poland

The end of 1980s was the period when Polish scientists were stimulated to develop alternatives mainly in pharmacology and toxicology testing. It has resulted in Poland's participation in EU Fifth Framework Programme in 1999 and numerous joint publications. INVITOX ON LINE service is available from Medical University of Warsaw server. The 13th ESTIV INVITOX (European Society for Toxicology in Vitro) Workshop will be held in Poland in 2004.[4]

Presentation of the National (Polish) Reference Centre for SPF Animal Breeding

The Department of Genetics and Lab. Animal Breeding consists of five integral parts:

1. bank of SPF (Specified Pathogen Free) inbred mouse and rat strains,
2. experimental animal division,
3. health monitoring laboratory (microbiology),
4. genetic laboratory,
5. anatomo-pathological laboratory,

– bank of SPF inbred mouse and rat strains

SPF animals are an essential tool in the investigations in oncology, immunology, and transplantology. SPF mice became indispensable models in oncology because their

longer life span allows to develop numerous, spontaneous tumours with rare different histological patterns. The incidence of chemically or irradiation induced neoplastic tumours is significantly higher when SPF animals are used.

Three inbred strains of rats and 18 of mice (7 inbred and 11 congenic resistant strains) are maintained in the barrier system in SPF condition.[5] They are bacteriologically, virologically and parasitologically controlled. Breeding division consists of 12 breeding rooms, each equipped with lock-chamber. The doors of lock-chambers are blockaded. Room temperature around 22°C, higher pressure and 12 h light/dark are regulated automatically and controlled for each room separately. There are 15 exchanges of air per hour. The air is filtrated by three filters, the last one is the absolute filter. Pelleted food (LABOFEED H), cages with bedding material and bottles with water are sterilised in double-door autoclaves and transferred through the barrier into the clean area. The entrance to the breeding part is allowed only to the employed, specially trained personnel after taking a shower and donning sterile medical suits.[6]

Pelleted food LABOFEED H is produced under the supervision of the Institute of Animal Physiology and Nutrition P.A.Sci.

Animal breeding is carried out according to the single line system and breeding protocols are kept in Excel computer program. The animals are registered at the Committee on Standardised Nomenclature for Inbred Strains of Mice and at the National Academy of Science, NW Washington DC 20418. The symbol is W.

Figure 1. A trained and properly dressed personnel takes care for animals.

– experimental animal division

Conditions in the division for experimental animals are the same as in the SPF breeding part, however animals are maintained in the "semi barrier" system. Experimental animals at this division are kept at the least MD (Minimal Disease) standard. The researchers are allowed to enter the animal and operating rooms after conforming the hygienic rules.

– health monitoring

The health monitoring includes parasitological, bacteriological and virusological control according to the recommended international rules.[7,8,9]

Bacterial colonies cultured from nasopharynx, eye, caecum, colon and vagina are identified on standardized diagnostic media. Bacteriological identification is performed using BioMerieux API kits (French production). Mycoplasma sp. and virusological infections are verified by ELISA – (Enzyme-linked immunosorbent assay) using Perimmune MAT kits (American production). As a rule, three mature animals are sacrificed for examination three times per month.

The list of microorganisms (viruses, bacteria and mycoplasma, ecto- and endoparasites) tested has been published.[10]

– genetic control

In 1981-1998, genetic monitoring including morphological, biochemical and immunological markers was being performed. Five mor-

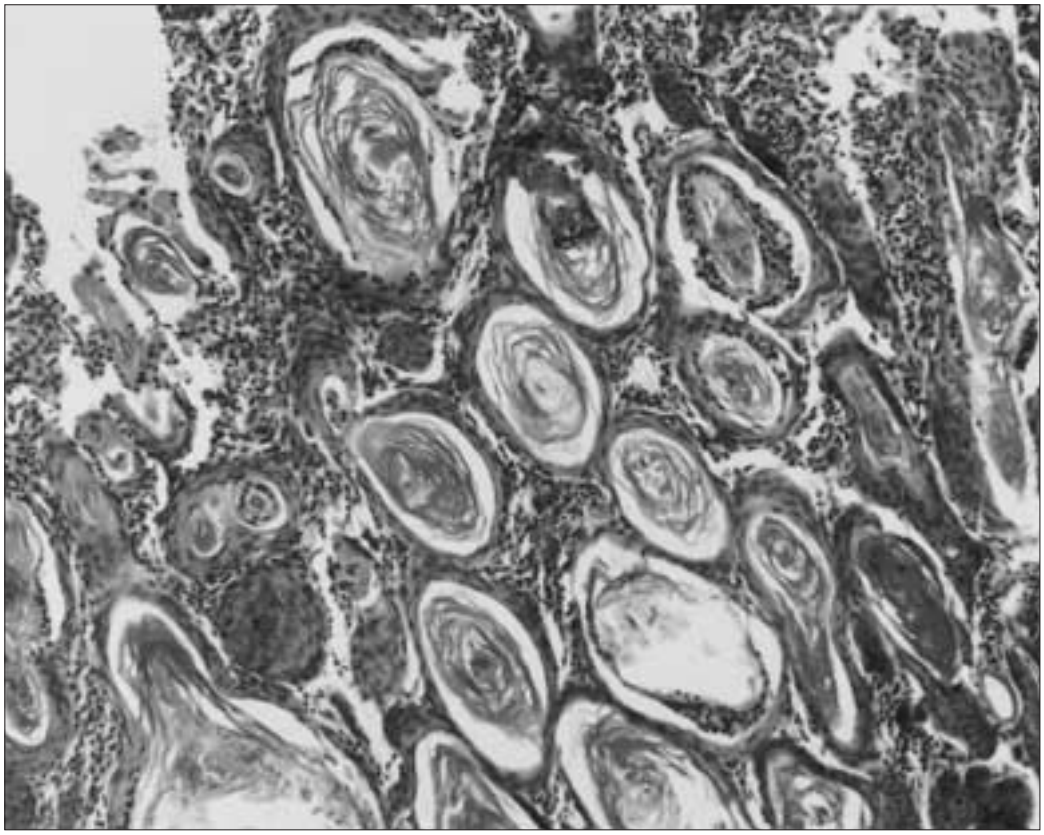

Figure 2. Irradiation-induced mammary gland tumour – *adenoacanthoma*.

phological and 9 biochemical markers were used for genetic monitoring in rats, whereas 4 morphological, 15 biochemical and 9 immunological markers in mice.[10,11,13] Genetic profiles were constructed for all maintained animals. Since 1999 genetic control of mice from seven inbred strains and one congenic resistant line by random amplification of polymorphic DNA (RAPD) method has been performed.[14] Ten arbitrary primers were tested. Moreover, genetic profiles (chromosome No 1 – 8) were constructed for these strains using microsatellite markers.

– anatomo-pathological and histopathological control

Emphasis is being placed on the incidence of spontaneous and radiation-induced tumours.[15,16] The data obtained from 7 inbred strains (AKR/W, BALB/cW, BN/aW, CBAT6/W, C3H/W, C57BL/10PhW, DBA/2W) are collected. The observed tumours are histologically classified. H&E staining as well as histochemical and immunohistochemical methods are routinely used. The incidence of mammary gland, lung, liver and haematopoietic system tumours was analysed.

Today's trends and problems

The mouse strains with defined genetic background are used in fundamental researches. The trends in the development of laboratory animal science in Poland and Europe are overlapping. In oncology, the inbred strains, recombinant inbred strains and recombinant congenic strains are the most preferred.

The high cost of mice and rats supplied from the breeding reference centres to other institutes reduces the number of animals per project, but parallely discourages the investigators to new experiments.

Besides, in practice it is very difficult to prove researchers' ignorance of law and laboratory animal science and punish them especially when an experiment is failed.

Conclusion

Standardisation of laboratory animals according to international recommendations seems to be sufficient in leading centres. However, the general state of laboratory animals breeding in Poland still needs improvement and additional funds.

The law regulation is respected by the institutes conducting fundamental researches; however, it has rather resulted from the awareness of scientists than effectiveness of law regulations.

In future, high priority for development of modern genetics, immunology, oncology and others may cause seeing laboratory animals more as research tools than as companions in scientific investigation.

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