

Laboratory animal science in the future: a vision

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What is the vision for the future of laboratory animal science? I believe that the answer to this lies with us who are active in the field. We now enjoy the benefits of those who provided superb leadership in the past, and we must now do the same for those who will follow. When I think of the recent past, I am reminded of the "Nordic Giants" who have made our current field possible:

Jörgen Carstensen
Stian Erichsen
Bengt Gustafsson
Osmo Hänninen
Karl Johan Öbrink
Lars Wass

These giants have left big footsteps for us to fill. Are we up to this task? I think so. I believe that our future will be heavily influenced by two key scientific achievements which are imminent in the next few years:

- Nanotechnology
- Definition of the genome

Nanotechnology is the science and engineering of assembling materials and components, atom by atom, or molecule by molecule, and then integrating them into useful devices. Some refer to this as building from the bottom up; and it is likely to change the way that almost everything is

made. Instead of miniaturizing what we now have, we will construct them from bottom up using the most basic elements. It will require the interfacing of physics, chemistry, and biology. In our field, this will require research using animals to test drug delivery to individual cell types, rapid and more efficient genome sequencing, and "smart patches" that can monitor for specific conditions and, using a closed loop, dispense drugs that will counteract potentially adverse states, e.g., glucose and insulin control in diabetes mellitus. Are we prepared to provide defined animals that will complement this type of research? Or will the benefits of nanotechnology enable us to cancel out retrovirus particles that have become incorporated into the genome or even the presence of subclinical viral infections?

Defining the genome will have a more immediate impact on our future. Completion of the human genome is projected for 2005; a working draft of the mouse sequence is expected by 2003; and the complete mouse genome by 2008. This new era will highlight just how little we know about the phenotypes of genetically-engineered animals. New technologies will be required to identify, interpret, and document the phenotypic expression of physiologic, pathologic, and behavioral

characteristics. In some respects, these are “new” animals.

As we deal with nanotechnology and genomics, I would like to focus on four areas of our future:

- Apply What We Know.
- Nutrition.
- Communication.
- Cost

Apply What We Know

It may seem a bit strange to suggest that our future may depend on the application of what we already know. But ask yourself the questions – do you? Or do you sometimes revert to “common practice”? There are a lot of environmental factors that can affect the interpretation of research data; I am sure that we follow them most of the time, but do we really adhere to them at a level that would be consistent with the standards of nanotechnology and genomics? For example, we know that drinking water is variable in quality; and it can be a source of contamination. Most animal facilities have some provision for water treatment, either chlorination, acidification, or other treatment (*Hall et al, 1980; Hermann, 1982*). How many of us routinely monitor the quality of the water; and do we check to see if it changes from day to day, especially if we are using water bottles?

We are also aware that there are numerous chemicals in relatively low concentrations in the environment of the animal room that can significantly alter hepatic microsomal enzyme activity (*Vesell et al., 1976*). These chemicals include aromatic hydrocarbons in softwood bedding, eucalyptol from aerosol sprays and disinfectants, and chlorinated hydrocarbon insecticides (*Pelkonen et al, 1989; Törrönen et al, 1989*). Even the frequency of bedding change can alter hepatic microsomal enzyme activity (*Vesell, 1973*). Not only must we rigorously control these chemicals that can cause adverse physiological

effects; but we need to ensure the maintenance of those chemicals normally produced by the animals, i.e., pheromones. Have you ever considered that maybe we change the cages too often or, perhaps, keep them “too clean”? New and developing technology should help us to provide definitive answers to these questions in the near future.

Noise is known to have various physiological effects on animals, but the behavioral responses to sound are largely unknown. Recent data suggest that rats react with intense startle and flight response when they hear a sudden sound caused by the tearing of ordinary paper, but they were non-responsive to rat screams (*Voipio, 1997*). This may give new meaning to the sounds made by pulling a paper towel out of a dispenser, ripping a page out of a notebook, or wearing paper disposable protective clothing in an animal room. Transportation, from the breeder to the facility, or even within the facility, can alter an animal’s physiologic behavior. Such movement elevates plasma steroid levels and, in turn, suppresses the immune response (*Landi et al, 1982; Drozdowicz, 1990*). Even though we know this to be true, do we routinely acclimate all newly-arrived animals so their physiology returns to normal before using them in a research project? Do we encourage investigators to come to the animals instead of transporting them to the laboratory?

Even the type of cage may affect an animal’s physiologic response, e.g., a recent study suggests that rats prefer stainless steel cages to those made of plastic (polycarbonate or polypropylene) (*Nevalainen et al, 2000*). We often use plastic cages for our convenience, i.e., the animals can be visualized through the cage rather than using space to open the lid. Furthermore, many of these cages are housed in ventilated racks to further increase the density of housing. Although these steps may help to alleviate space problems, we need to address whether or not there are any adverse

effects on the animals. Does the increase in the number of animals cause any distress or discomfort? Is there something in the cage material that causes some other effect, such as increased light and/or blockage of sound or electromagnetic fields (EMF)? Animal research facilities have been designed largely on the basis of HVAC requirements – which are heavy electrical energy users. Furthermore, EMF levels vary significantly within an animal room, even among the cages on a single rack and certainly among animals on a single research project. Unlike humans, these animals are typically confined to a constant exposure field on a continual basis for the duration of the study. Therefore, an animal in one cage could be exposed to 1500+ milligauss on a continual basis; whereas, another animal just a few cages away – and perhaps on the same project – might not be exposed to more than 5-10 milligauss. The motors on ventilated cage racks may increase these levels. I was intrigued by a recent study by Eskola *et al*, 1999, suggesting that animal enrichment could reduce the number of animals required for statistically-significant data. I am never quite sure whether we are enriching an environment or eliminating stresses. I recently overheard a conversation where one person questioned the need for enrichment and asked what could be better than living in a comfortable environment with everything provided. The response was, “You’ve taken away their job.” In the current economy of downsizing, I think that many people can relate to that type of stress.

Nutrition

It is interesting to note that the focus of the first Bengt Gustafsson symposium in 1983 was on nutrition. In looking through the program for this symposium, I see that there are several papers on nutrition. What have we really learned about nutrition in the last seventeen years, and what can

we expect in the future? The best guides to adequate nutrition of the various animal species are the reports published by the National Research Council (1995). These NRC reports do not describe the exact requirements of any specific animal, because nutrient needs are influenced by genetic and environmental factors. There are known significant differences in the nutrient requirements of stocks, strains, and species. The life cycle stage is probably the most important environmental factor influencing the nutrient requirements of animal species; i.e., changes associated with growth, reproduction, lactation, or maintenance. This is further complicated by diet consumption and nutrient loss after manufacture of the food. It is well known that mammalian species eat the amount of food that satisfies their energy requirements; thus, diets that are high in fat will decrease the total amount of food that is eaten. The hardness of pelleted diets may also influence diet consumption. We typically complain if we find too much ground feed in the bottom of a bag, i.e., we think we are wasting feed. The manufacturer’s response is to make the feed harder – and more difficult – for the animals to eat the food. Nutrient loss is known to occur during the manufacturing process, during transport and storage, especially where the temperature and humidity are high. Sterilization with heat also causes significant loss of nutrients.

As a result of the relatively large number of factors that can influence the dietary nutrient requirements of research animals, manufacturers add nutrient concentrations that are in excess of the estimated requirements. However, there are inconsistencies in the magnitude of these “safety factors.” When feed manufacturers establish these excess amounts, or safety factors, they take into account the stability of the vitamins and the bioavailability of minerals.

In essence, commonly-available diets for research animals are adequate to compensate for diet treatment and storage conditions. The future of nutrition will continue to focus on the amount of

protein, fat, and fiber; but there will be a new emphasis on harmonization. The harmonization of diets will probably have the most influence on biomedical research as we pursue such things as gut mucosal immunity, nutrient absorption as a function of genotype, etc. Pharmaceutical companies have become multinational and need harmonization of diets in order to conduct the same research at multiple sites; investigators need it to build on the research of others. The big problem is that the sources of ingredients vary from country to country. This will probably be overcome by harmonizing the amount of sugar, amino acids, and fatty acids, regardless of nutrient source.

Communication

How we communicate what we do, how we do it, and why we do it will have an impact on our future. In the past five years, the number of animals used in biomedical research has dramatically increased; and most of this increase has been in the production of genetically-engineered animals, especially mice. Genetically-engineered animals will pose a significant challenge because we know so little about their phenotypic expression in terms of physiology, pathology, and behavior. To deal with this challenge effectively, we will have to develop an even greater measure of team effort. We are well aware of the fact the animal technicians have more involvement with the animals than we as biologists, veterinarians, or investigators; and we have to rely the animal technicians to recognize – and to report on – subtle changes in food and water consumption, activity patterns and other changes in behavior. We must train them to know what a normal animal is so they will recognize what is abnormal phenotypic expression. Their powers of observation and attention to detail must be continually reinforced and given appropriate recognition. I have long advocated (*Lang and Harrell, 1972*) that the qualifications of the animal care personnel should be equal to those of technicians working in a

research laboratory, and this will be even more so in the future.

We, as biologists and veterinarians, need to develop appropriate support programs to translate these findings to our colleagues and investigators. For years, we have advocated the need for diagnostic laboratory support, i.e., histopathology, clinical pathology, microbiology, and serology. The future will expand these laboratory services to include those that will enable us to recognize phenotypic expressions on a molecular biology level, e.g., PCR and microarray. After we have made these assessments, we have to be able to effectively communicate this information to the investigator. All too often, we keep to ourselves, almost to the point of thinking that this would be a great career if it weren't for the investigators. Why is this? I think that it may be that we really don't understand what they are doing; and when we do try to discuss things about their animals, we have the feeling that we are not really communicating. No one expects us to be fully conversant about all of the animal-related research projects in our institutions; but we should – at least – have a general knowledge of their research. We can do this by reading their entire research grant applications, reading their publications, and attending in-house seminars. This small investment of our time and visible presence will help us to better interpret their findings and convince the investigators that we are, in fact, academicians who understand and support in the research.

We have not been as successful in communicating with the public about animal research. In a free society we uphold the rights of speech and differing opinions. However, the voices of those who oppose the use of animals are louder and usually heard more often than we are. Since the era of Sputnik, the public has had a love hate relationship with science. They want the benefits of science, but don't want to live near a nuclear power plant, see Mickey Mouse in a cage, or worry about genetically-engineered

animals or plants out of control. Unless you are majoring in science, there is very little science taught in our schools; and, as a result, a large percentage of our society is scientifically illiterate. They are totally unaware of the rules and regulations governing the use of animals. In the United States, and I suspect in each of your countries, the care and use of laboratory animals is among the most strictly regulated activities, ranking close behind air traffic safety and the manufacture of safe drugs. An animal activist can tell a crowd that an animal has no more rights or protection than a chair or a table, and the crowd will believe it. If we don't tell them the truth, who will? We must always remember that animal research is paid for by the public; either by taxation or profits on the sale of drugs. The public, including our elected officials, must be educated about the vital role of research animals and the safeguards that ensure their humane care and use. Only an educated public can provide tomorrow's scientists, and provide the funds that are necessary to promote scientific progress.

Cost

A recurring theme is the investigator's perception of the high cost of maintaining research animals. Investigators can spend approximately 300,000 SEK developing a genetically-engineered mouse and then complain about spending 1 SEK to keep that mouse in your facility. Nevertheless, this perception of the high cost of animal care will prevail – and maybe we should do something about it.

The trend will continue with specialized animals, i.e., genetically-engineered, flora-defined, etc. This will require regional gnotobiotic centers to re-derive animals, either to eliminate disease conditions or to populate the animal's intestinal tract with an assortment of defined flora for specific research projects. The housing requirements for this broad array of research-tailored animals will pose significant challenges.

The biggest challenge is to meet equitably both investigator needs and those of maintaining healthy animals. Investigators need open and ready access to the animals to efficiently conduct their research; disease control, on the other hand, requires restricted access. Furthermore, restricted access – filter covers, ventilated racks, or built-in barrier facilities – significantly increases the cost of animal care. (Lang, 1995 and 2000). One solution to this dilemma is to keep a foundation colony in a germ-free flexible isolator, and keep the production and research colonies in conventional virus-antibody-free rooms. This combination: (1) is the most cost-effective means of housing; (2) provides easy investigator access and manipulation; and (3) permits retrograde reestablishment in the event of inadvertent contamination. The relative costs of these systems are:

Isolator	0.12 USD/mouse/day
Conventional	0.05 USD/mouse/day

Plastic isolators provide a practical means of germ-free housing. They can be sterilized and maintained under positive pressure, filtered air which provides an effective barrier against outside contaminants. These isolators can be made of either rigid or flexible plastic. Flexible plastic isolators are less expensive, permit more flexibility in working movements, and have the advantage of being more easily disposable if they become contaminated with toxic or radioactive substances. Flexible film isolators can be stacked, one on top of the other, to conserve floor space. Those on the upper level are accessible for both investigative and animal care procedures by using an elevated platform. Access to the animals is through plastic tubes with a rubber glove at the end. The standard rubber gloves may be too cumbersome for some technical procedures; in such cases, one can transfer the animals to another isolator equipped with surgical gloves, complete the procedure, and then return the animals

to their original isolators.

Supplies and animals are placed into or removed from the isolator using a transfer cylinder. The cylinder is designed to connect to the isolator using a transfer sleeve.

Conclusions

We have a rich legacy to maintain and further build. Our future will be influenced by new developments such as nanotechnology and genomics, which will lead to “designer” animals. To successfully meet the challenges of the future, we need to pay close attention to, and eliminate or stabilize, environmental variables that can affect the interpretation of research data. This new era of research at the cellular level will push the demand for harmonization of diets, both for accuracy of data and to make it easier to build on research information conducted in multinational laboratories. Genetic engineering on a large scale may further increase public concern about what we do; therefore, it will be imperative that we do all that we can to educate the public and our elected officials. The cost of animal research will continue to be a concern, and we must seek ways of controlling these costs in a manner that is consistent with the research project. You are familiar with the 3Rs by Russell and Burch (1959).

- *Reduction* - Any decrease in the numbers of animals used to obtain information of a given amount and precision.
- *Refinement* – Any decrease in the incidence or severity of procedures applied to animals necessarily used.
- *Replacement* – The substitution of conscious living higher animals by non-sentient material.
- *With the same scientific result.*

I would like to propose a complementary set of 3Rs:

- *Reduction* of environmental variables that can affect the interpretation of research data.

- *Refinement* of diets.
- *Replacement* of common practices with scientifically-based reasons.
- *With improved scientific results.*

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