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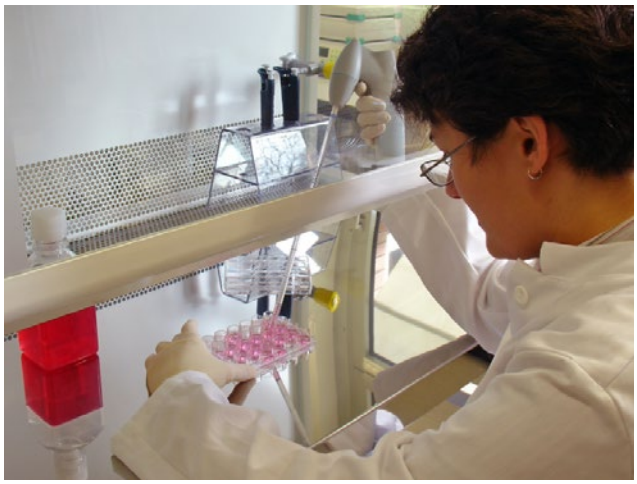
3R is routine today

Basic researchers adopt new ways of protecting laboratory animals

"Awareness of 3R in the scientific community has increased enormously over the last few years. Swiss researchers today make huge efforts in their laboratories to reduce, refine and replace animal experiments." This is the conclusion of Prof. Rolf Zeller, biomedical specialist at the University of Basel, who is intimately familiar with Switzerland's place in research. The kind of day-to-day laboratory routine mentioned by Zeller is illustrated by the example of Annette Oxenius, professor at the Institute of Microbiology, Federal Institute of Technology (ETH) in Zurich. In her research, the biologist is looking at how the human immune system reacts to infections. Here Oxenius not only works with HIV patients, but is also dependent on laboratory animals (mice). "Because complex systems like the nervous system or the immune system are difficult to recreate in the test-tube", explains the Zurich researcher. Animal experiments are "a necessity that arises from the complexity of the research object".



Scientist workung with cell culture

Cell culture

Breeding for many immunologists

At the same time, Oxenius firmly believes that every encouragement must be given to observing the principle of the 3Rs in basic research. She also does her utmost to keep down the number of animals and to design those experiments that are indispensable in a way that is as tolerable as possible for the animals in keeping with the requirements of the law. Where the experimental design permits, she and her researchers work with cell lines (e.g. macrophages). Where mice are used, the groups of animals for each experiment today are usually only half the size they were 15 or 20 years ago. This marked reduction became possible because researchers use genetically identical mice, which facilitates statistical analysis. Measurement methods have also improved, and the importance of the laboratory animal now takes prime place in the awareness of the researcher, as Oxenius emphasizes.

The change in awareness is reflected in the introduction of a centralized maintenance breeding system for genetically modified mouse strains. Until a few years ago, every research group bred its own transgenic mice. Today, researchers go to a central maintenance breeding unit: The Swiss Immunological Mouse Repository at the Institute of Laboratory

Animal Science of the University of Zurich has about 64 mouse strains available any one time. The number of research groups that use this central repository has grown from a handful to more than 30, including groups from Southern Germany and Italy. “As a result, we have to breed far fewer animals”, says Oxenius with regard to the flagship project.

If laboratory animals in academic basic research are used very selectively today, then this has to do not only with a keener awareness, but also with new technical possibilities. Thanks to the use of imaging procedures such as MRI, CT and PET scans, researchers no longer have to euthanize laboratory animals, but can study them alive, for example when the study concerns tumor models in the mouse. Today,

CAT scanner with cat

there is also analytical equipment on the market that is so sensitive that the volume of blood needed for a test can sometimes be reduced to a tenth – the number of laboratory animals needed is falling dramatically. Also of great importance are optimized mathematical simulations with which parts of the experiment can be anticipated and laboratory animals thus spared. Such simulations have to be verified experimentally, because they inevitably have to make certain assumptions that may not conform to the complexities of reality.

Human medical advances for primates

The 3R principles are widely established in academic research today, especially in research using non-human primates, in other words monkeys. “For anyone who works with primates, 3R is a part of day-to-day work, because a monkey is a creature to which, as a researcher, you always feel emotionally close”, says Prof. Stefan Treue. Treue is Director of the German Primate Center (DPZ) in Göttingen. The DPZ is a leading public research institute, which at the same time breeds laboratory animals for academic research not only throughout Germany, but also in Switzerland and other countries. The primate benefits directly from advances in human medicine; it is helping to reduce the stress on laboratory animals. This applies in particular to improvements in surgical techniques. Implants are no longer made of stainless steel, but of coated titanium. The coating promotes the growth of bone. The implants are more durable than predecessor models; and the animals live longer and have fewer infections. Researchers can also be much more accurate in their evaluation of the measurement data obtained thanks to more powerful computers and better mathematical analysis. This development is making a major contribution to the principle of refinement.

At the DPZ, Prof. Hansjörg Scherberger is engaged in research on movements of the fingers and hands, which is only makes sense to study in primates, because they have a highly developed locomotor apparatus. In his research work, the 47-year-old neurobiologist has closely followed the continuous improvements that have been made in the handling of laboratory animals – for example in the recording of eye movements, a standard measure in behavioral studies with monkeys. Previously, a coil was implanted in the animal’s eye for this purpose. When a magnetic field was then created, researchers could use the coil to determine the position and movement of the eye. Today, a camera records the positional changes of the pupil, and a surgical


Common marmoset

Outdoor enclosure for the rhesus monkeys at the German Primate Centre in Göttingen

procedure is no longer necessary. “The new method is precise enough for most experiments”, says Scherberger. in the spirit of 3R the researcher also sees progress in electrophysiological experiments. If ten or even 100 or 200 electrodes are implanted in the monkey brain instead of a single one, far more data can be obtained in a single experiment. The German researcher sees a whole new development in neuroprostheses, as used in the US today for example in patients with paralysis. “In the future, it will likely be possible to avoid animal experiments for certain studies by obtaining the required data directly from humans”, says Scherberger.

Enrichment for the benefit of animals

The public likes to hear about spectacular advances. Yet the improved conditions in the lives of laboratory animals often take place in small steps. This is confirmed by biologist and behavioral researcher Hanno Würbel, who became the first - and so far only - Professor of Animal Welfare in Switzerland at the University of Bern a year ago. “It is often only small differences in housing conditions that make a big difference for animals”, says Würbel. He is referring here to the species-appropriate enrichment of cages, as is standard practice for laboratory animals today thanks to animal welfare law. This improvement provides animals with opportunities to withdraw, and ensures they have material to build a nest, which helps the thermoregulation of the animals. Another unspectacular, but every effective improvement is a more considerate handling of the animals entrusted to the care of the investigator. While it was previously standard practice to grab mice by the root of the tail and put them in place, today they are guided to the experiment through a tunnel or cupped in both hands by the investigator. Anyone who mocks these procedures as a “molly-coddling mentality” underestimates the doubly positive effect, says Hanno Würbel: “Such procedures reduce anxiety and stress in the animal – and avoids a falsification of results.”

Würbel encourages the Swiss National Science Foundation to be proactive in motivating basic research to be more consistent in implementing the 3R philosophy. He points to the example of Great Britain, which has set up a national center of excellence for this purpose. What is important here is not only the continual optimization of the principles of Reduce, Refine and Replace, but also to raise the public profile of these principles. As Dr. Cornelia Exner, Animal Welfare Officer at the University of Marburg, says, there is a problem of perception. “The public is too little aware of the contribution academic research has made towards reducing animal experiments”, says Exner. Academic research, she adds, is constantly providing new methods that serve the interests of the 3Rs. The fact that this progress is not properly perceived by the public is also partly down to the researchers themselves. As she explains, the researchers themselves are not even aware of the extent to which they are working to avoid animal experiments, and accordingly they do not promote their efforts enough to the public.

Transparent information

This is the point where Richard W. Bianco has been applying the lever for some time. Bianco is a professor at the University of Minnesota in Minneapolis/ USA. He has been working in medical research for around 40 years and has repeatedly called for trans-

Lab sheep

parency with regard to animal experiments. Which means that researchers not only put forward good arguments in public to defend the need for animal experiments, but also inform the public how they are reducing the number of laboratory animals used and the stress on these animals. Bianco's own research area is the development of cardiac valves, especially those used in children with heart failure. He uses young sheep for his research. These animals enable him to study the conditions to which heart valves are exposed in the child. The animal model has the advantage here that it allows pediatric growth to be simulated in fast motion, as it were.

"Once we had developed the sheep model, we could reduce the number of animals used", recalls Bianco – not only the actual laboratory animals, but also the animals in the control group. The researcher also touches on an area that is not usually the focus of public scrutiny: education. "We have completely banned the use of live animals from our physiology courses for undergraduate students", says Bianco. Simulations and other techniques are sufficient for illustrating basic principles.

Using the scope

The replacement of animal experiments – this is the most logical implementation of the 3Rs. But especially in academic basic research, this step is usually not yet possible without jeopardizing research. Basic research clearly differs from standardized research focused, for example, on the toxicity of new pharmacological active substances, says Prof. Patrick Matthias, Senior Group Leader at the Friedrich Miescher Institute for Biomedical Research in Basel. "In basic research, every experiment is something new. Here it is much more difficult simply to replace animal experiments." In academic research, therefore, the main emphasis is on the refinement of indispensable experiments within specified limits. Gregor Rainer from the University of Fribourg shows how the scope available within these limits can be used. Rainer works in basic neurobiological research. Amongst other things, he is studying deep brain stimulation, an electrical stimulation of certain regions of the brain, which already works very well in Parkinson's patients; the technique is still in its infancy with Alzheimer's patients. The experiments can only be carried out in vivo in the intact brain.

Lab Rat

Rainer works with healthy animals, namely rats and primates (rhesus monkeys). To reduce the use of primates, he falls back on tree shrews where possible. These are not primates, but they are close relatives. Deep brain stimulation, visual perception and visual memory can be studied very well in tree shrews, because these animals – unlike in rats – are very visual animals. Working with these animals, says Rainer, it has become standard practice to use only positive rewards, e.g. in the form of banana pellets. A further improvement over previous procedures is also that the animals can be given much more freedom to move about by using wireless technology during the measurement of electrical activity. "This is much more pleasant for the animals", says Gregor Rainer.

It would be ideal if we could understand the complicated mechanisms of a body without stressful animal experiment. Unfortunately that is not yet possible today. But the dilemma will remain for a long time to come: basic research without experiments in animals would mean abandoning any medical progress. Mausblick aims to explain why and therefore reports on medical success stories that were only possible thanks to animal experiments.

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