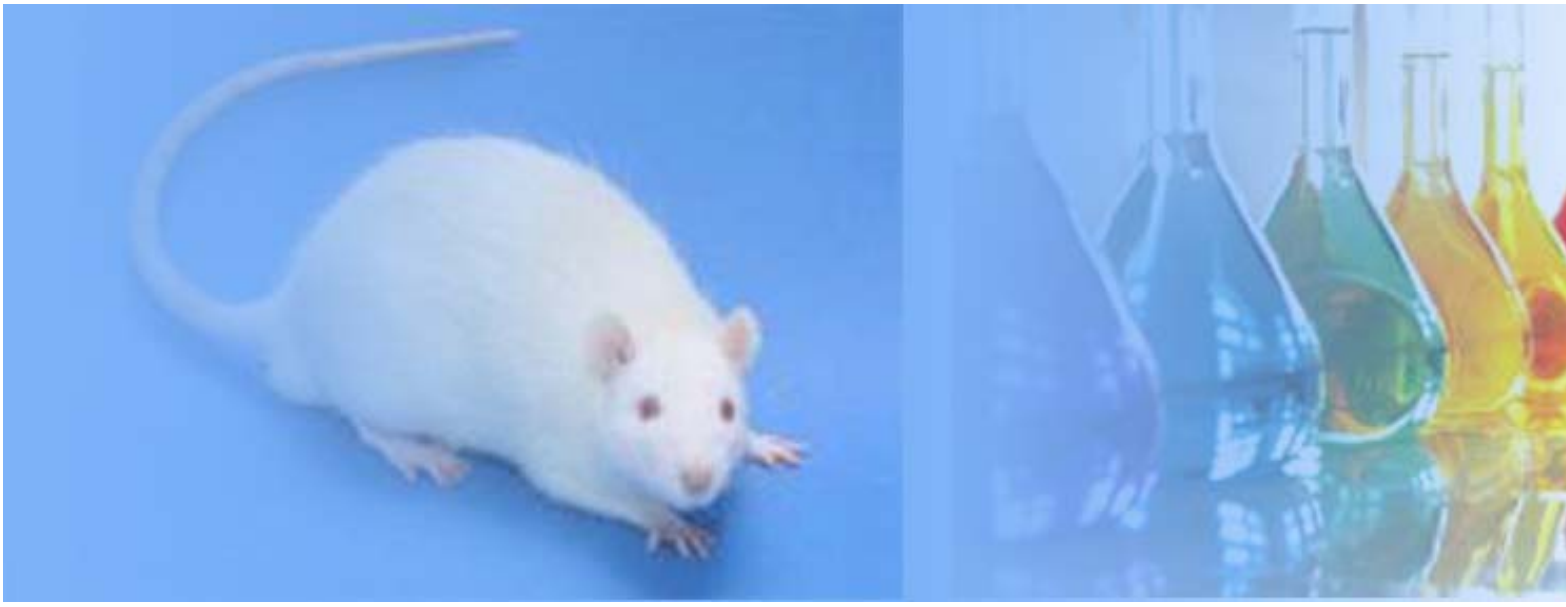
Animal Testing

In

Medical Research

Past, present and future.



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1. From Aristotle to today.

There is no doubt that throughout history, animals has played a central role in medical research. Many of the treatments we have today for serious illnesses have come from animal research. Animal research is defined as the use of non-human animals in experiments[1]. There has been recorded that the use of animals in research has been going on as far back as the Greek writings. Aristotle who lived in 384-322 BC was one of the first to be recorded to use live animals in experiments[2] and Doctor Galen 129-200 AD[3] was known as the “father of vivisection” from his experiments on living pigs. These experiments were not in the name of medical research, they were done to gain knowledge about the animals themselves. In this period man were not set next to animals in comparison of physiology or any other way. Man was creatures above animals. An image of God as it is said in the bible, Genesis 1:26 “Then God said, "Let us make man in our image, in our likeness, and let them rule over the fish of the sea and the birds of the air, over the livestock, over all the earth, and over all the creatures that move along the ground."[4]

The "modern" era of animal research started about 150 years ago when physiology became recognised as a science. In the mid-1800s medical research started to make big progress because they started to do experiments on animals. Among the first discoveries was the functioning of the cardiovascular and nervous systems[5].

In 1859 Charles Darwin came with the theory of evolution[6]. This theory linked humans to other animals and made a link between the groups. This made a path to do research on different animals to learn how human physiology worked. This was encouraged by Darwin, but not with all other people. Darwin also believed animals to have emotions and in the late 1800s the divide between animal lovers and researchers on this point lead to the 1876 British Cruelty to Animals Act to regulate research on animals. But because of the major progresses in medical research by using animals, these regulations fell into the background[7].

An important discovery was proven by Louis Pasteur and Robert Koch late in the 19th century. The two scientists used anthrax to prove that micro-organisms caused maladies to both human and animals. Pasteur developed a vaccine to anthrax, and a few years later he had also made a vaccine to rabies by working with animal testing[8].

Another important medical discovery was the discovery of insulin. Insulin is secreted from the isles of Langerhans in the pancreas. In 1889 a pancreas from a dog was removed to prove its role in digestion. When the pancreas was removed, the researchers discovered flies

swarming around the urine of the dog. They found sugar in the urine which proved the connection between pancreas and diabetes. For the following two decades a lot of research was done on dogs to figure out how to keep the dog alive without its own insulin production. When they had found a way to extract insulin they used cow, pig, horse and fish to produce the insulin needed because the insulin produced by the animals are almost identical to the one produced in humans. Today most of the insulin is grown from human DNA in bacteria, but the process of animal research was important to come to where we are today[9]. Many important medical research the last century are due to animal experiments. Some of them are listed here[10,11]:

1950s

- Kidney transplants
- Replacement heart valves
- Polio vaccine
- Hip replacement surgery

1960s

- Heart bypass operations
- Drugs to treat mental illness

1970s

- Drugs to treat stomach ulcers, asthma and leukemia

1980s

- Drugs to control transplant rejection
- Life-support systems for premature babies

1990s

- Cloning of Dolly

The welfare of animals used in experiment from the scientists became clear when two scientists, William M. S. Russell and Rex L. Burch, in 1959 published “The principles of

humane experimental technique" which included the three Rs that form the basis for the search of alternatives to animals in research.

2. Ethics in animal testing.

How should animals be treated? Is there a general standard for this, or should laboratory animals be treated different from domestic pets? These questions are as relevant today as they were 10 or 20 years ago. There is no correct answer to this, which makes it even more important to discuss.
The use of animals in research is one of many investigative methods used in science today, and has played a crucial role in development of modern medical treatments. Use of animals in research will continue to be necessary as long as researchers seek new improvement and information.

The ethics of animal testing can be viewed from many angles, and the perception to a casual human being is rather negative when introduced to the term animal testing. It very often has a negative comprehension. This can be said to be a misconception in many cases. E.g animals used in cosmetic- and medical testing is not presented in objective ways by animal rights organizations. Biologists, and other people within the science where animal experiments is needed has for the most, formed a firm opinion of the needs and not at least the shades of animal testing.

The general development of animal welfare-opinions has become more and more engaging. Peter Singer wrote Animal Liberation in 1975, which has been a major formative influence on the modern animal rights movement. He wrote that "there are obviously important differences between human and other animals, and these differences must give rise to some differences in the rights that each have." This is meant in a way that justifies the needs and rights every animal have. Needs vary, and there is no reason to necessarily give an animal what you would have given a man.

3. International practice and legislations.

The standards and legislations alter from country to country. In general, the legislations are not very nuanced. Below, is a short list of legislations for different countries.
The lack of nuance and precision in animal legislations, and which animals it includes is quite conspicuous. Also, there is no distinction between animals used in medical research and other areas where animals are used for scientific purposes.

Norway:
Norwegian animal legislation is given by Landbruksdepartementet 20.12.1974. In addition to this there is a legislation for the use of animals in research. This legislation includes living mammals including embryonal- and fetal stages, birds, fish, amphibians and decapods. This means that testing on all invertebrates, except decapods can be done without any approval.[12]

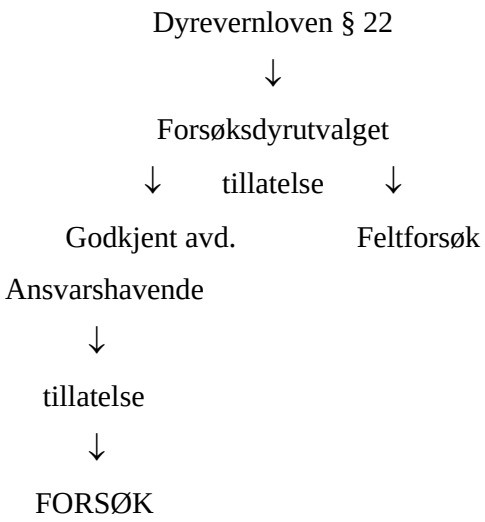

Fig. Handling of the Norwegian animal legislation.

EU.
The aim of the Council of Europe is to expand the unity between its members and co-operate with other States in the protection of live animals used for experimental and other scientific purposes. This convention includes every live vertebrate, (excl. humans) including free-living and/or reproducing larval forms, but excluding other foetal or embryonic forms.
This Convention, in other words, encompass animals used for scientific purposes, not domestic animals or pets. [13]

Invertebrates have been more common in animal research in the recent years. The reason for this is that these animals have a simpler form of nervous system, and no approval is needed for these kinds of experiments.[14] Mostly, the outcome is positive. The three R´s have led to development of new, less harmful methods such as using only blood or cell cultures from an animal instead of donating the whole animal.

4. Present role of laboratory animals.

The number of animals used in laboratory experiments is declining. In the U.K., the Netherlands, Germany and several other countries the total number of animals have fallen with 50% since the 1970`s [7]

In Norway 2005 about 390 animal experiments was conducted by the 65 approved animal research institutions. A total of 1.000.426 research animals was reported, 94% of reported research animals were fish (944.874), 48.968 mammals and 6.235 birds. I addition a large number of animals was used in research related purposes not covered by the definition of research animals. In 2005 this constituted for 1.659.051 fish and 31.086 mammals and birds. Generally there has been a decline in the use of research animals (excluding fish) from 1982 to 2005, however there has been an increase in the use of fish as research animals in the same time period.[15]

The following tables illustrate the number of animals and species used in relation to medical research specifically.

Table 1[15]: The number of research animals used in Norway 2005 in connection with disease and suffering.

		Species		
	All species	Rodents and rabbits	Dogs and cats	Fish
1**	**17278**	16676	0	0
2	**8292**	7989	17	0
3	**1126**	1126	0	0
4	**5427**	5388	0	0
5	**135869**	624	32	135114
Total	**167992**	**31803**	**49**	**135114**

1 Disease in humans: cancer
2 Disease in humans: Heart and vascular diseases
3 Disease in humans: Nervous and mental disorders.
4 Disease in humans: other diseases
5 Disease in animals

Table 2[15]: The number of research animals used in chosen aims for protection of humans, animals and the environment by toxicological- or safety testing in Norway 2005, including testing of products and equipment for use in human- and veterinary medicine.

		Species		
	All species	Rodents and rabbits	Dogs and cats	Fish
1	**0**	0	0	0
2	**0**	0	0	0
3	**0**	0	0	0
4	**0**	0	0	0
5	**2000**	0	0	2000
6	**64713**	2685	0	62024
7	**69234**	13	6	69209
Total	**135947**	**31803**	**6**	**135114**

1 Substances used or intended for use mainly in agriculture
2 Substances used or intended for use mainly in households

3 Substances used or intended for use mainly in cosmetics or toilet requisites
4 Substances used or intended for use in human food items
5 Substances in industrial use or intended for industrial use not covered by category 1-4
6 Potential or real dangers of pollution in the environment not covered by 1-5
7 Safety testing of products or equipment for use in human- or veterinary medicine.

The use of animals in medical research today include:[16]

- Cancer research uses animal experiments to develop methods for treatment and diagnosis.
- An animal model is also used to investigate a long list of diseases in humans and animals. The use of transgenic animals (animals that have had their genes modified by technical intervention has increased exponentially since the 1980`s. And so has the use of animals as donors for body parts (Xeno-transplantation).
- Testing new surgical techniques is often done on animals
- New medicines and vaccinations are often tested on animals before they are used on humans and other animals.
- Animals are used in production of antibodies that are used in diagnosis of diseases in humans and animals and in cancer research.
- Diagnosis of physiological and pathological conditions can be obtained by a diagnostical inoculation. A sample from a patient can be inoculated on an animal to test for a suspected disease.
- Teaching.

5. A critical look at the use of animals in medicine.

In medical research many laboratory animals are model animals, animals that are altered through genetic manipulation, surgery or injections to model human conditions. Human data is often interpreted according to data obtained from non human animals.[18]
In the U.K. Europeans for Medical Progress commissioned a survey amongst general practitioners in 2004. They found that 82% of general practitioners were “concerned that

animal data can be misleading when applied to humans" [17] Indeed there are several examples from the past where animal research has delayed rather than helped medical research. In the case of lung cancer studies had suggested a correlation between smoking and lung cancer as early as in 1963. However almost all animal experiments failed to show that tobacco could cause lung cancer. As a result, health warning were delayed for years.[17,18] In the case of polio research, animal models led to a misunderstanding of the mechanism of infection. Monkey experiments led the researchers to believe that the polio virus was transmitted through the respiratory organs. The polio virus in humans is actually transmitted through the digestive route, this misunderstanding caused failed preventing measures and delayed the development of a vaccine.[17,18]

Taking into account the crucial genetic, molecular, immunologic and cellular differences between humans and other animals, unwarranted focus on animal models may prevent progress in many important medical research areas. Animal models are extensively used in research on cancer, AIDS, psychology/drug abuse and genetic diseases. However the contribution of animal models to these disciplines can be disputed.[17]

6. The three R`s [19]

The three R`s explains how we can diminish and remove inhumanity, and define the search for alternatives to animal experiments.

Russel and Burch defined the three R`s the following way:

1. Replacement as the "substitution for conscious living higher animals of insentient material" Tissue culture, computer modelling and the use of invertebrate species are classical examples.
2. Reduction as using the minimum number of animal necessary "to obtain information of given amount and precision". It was stressed that the important thing is to use the right amount of animals, not too few or to many.
3. Refinement as any decrease in the nature, severity or incidence of inhumane procedures to those animals that still have to be use.

Multiple testing [20]

Animal experiments are still being repeated, for good reasons or not. It's generally accepted that important findings should be successfully reproduced by independent scientists/institutions before it can be accepted as a scientific fact. However in toxicology and safety testing one might argue that more repeating of animal testing than strictly necessary is conducted. There is little sharing of information among manufacturers and regulators, and different test requirements between nations and regions can cause obstacles for mutual recognition of data. This may cause supplementary testing to satisfy different regulators. Sharing of data and equal test requirements between nations can reduce repeated animal testing.

7. The future of animals in medical research. [21]

Since 1959 when Russell and Burch published the principle of the three Rs, the welfare of animals has come into more focus. In the last 30 years we have seen a decline in the use of animals in research, and in countries like Great Britain and Norway the number of research animals has been halved.
With the new and improving technology the future of research animals looks better, and many countries grant big sums of money to find alternative research methods. In 1992 the European Centre for the Validation of Alternative Methods was set up by the EU and they contribute about 80 million NOK each year to find alternative methods to animal research. There are also considerable amounts of money donated by humanitarian organisations each year.

7.1 What are the alternatives?

In the past, the toxicity of a new substance was measured by an "LD50" (lethal dose 50%) test witch required up to 200 rats, dogs or other animals to be force-fed different amounts of the substance, to determine the dose that would kill exactly half that group of animals. A recent change in protocol has put a ban on the LD50 test. In addition, the Organisation for Economic Co-operation and Development says that if a substance kills the first three animals it is tested on, further trails are unnecessary.

Statistics has come to be an important tool when testing vaccines. A vaccine is only considered effective if at least 80% of the vaccinated animals survive after being exposed to a particular disease. However, the disease must also kill 80% of the control group not protected by the vaccine. At the National Institute of Public Health and the Environment in the Netherlands, Coenraad Hendriksen has developed at method to test diphtheria and tetanus vaccines that only requires measuring the level of antibodies in an animal. This method does not only reduce the suffering of the animals, but it halves the number of animals used.
It is also possible to use statistical techniques and patient data to understand how a disease spreads, without using animals.

The use of fewer animals has also become an issue, and at the German centre of animal testing alternatives (ZEBET) they have surveyed decades of industry data on pesticides. They concluded that if mice and rats prove sensitive to a chemical, it does not have to undergo further tests on dogs. Horst Spielman at ZEBET anticipates that this technique will decrease the use of dogs by 70%. There are also efforts by researchers to use lab animals that are less likely to suffer the sensations of pain, and some researchers in Canada are trying to use bacteria in tests instead of rats.

The use of cell cultures has also turned out to be both effective and accurate when producing vaccines. Hormones and vaccines manufactured in cell cultures are purer than those made within the animals. This reduces the need for animal test to check for the safety of the vaccines. This technique has given dramatic changes in the use of monkeys in polio vaccine production in the Netherlands. What used to be 5000 monkeys annually has been reduced to the cell cultures of only 10 monkeys, and these ten monkeys produce enough polio vaccine to supply the whole country.[21] The usefulness of cell-cultures will increase as our knowledge about body cells improve.[22]

In traditional tests for skin corrosivity, it was measured how far a corrosive substance ate into a rabbits shaved neck, but now a method using replacements like reconstructed human skin or a synthetic material called “Corrositex” has been approved. This is likely to reduce the number of rabbits used substantially.[21]

With new technology comes better and more reliable alternative methods to animal testing, and imaging is one of these. Imaging is a broad concept to get pictures from within a living

body without surgical intervention or putting tools into the animals' body. The technique includes low-tech techniques like classical x-ray and ultrasound, while more advanced techniques are magnetic resonance imaging (MRI) and gamma camera. AstraZenica in Sweden is one of the medical companies that use imaging, and Professor Peter Thorèn states that even the less sophisticated methods are often used and have high value to the researchers. It is possible to study the disease progress in the animal, like arteriosclerosis, in a totally different way. Each animal also becomes its own control, since the disease develops in the same animal over a longer period of time and the animal is monitored before, during and after infection. Another major advantage is that imaging creates great amounts of information. Without imaging this is only possible with use of large amounts of research animals. With the old method the researchers kill the animals at different times and study their organs, e.g. the aorta. But this old method only allows the researcher to use the organ one single time and to get statistical relevant results one has to kill a great number of animals.
Even tough imaging is effective and decreases the use of lab animals, it demands quite expensive equipment and a MRI camera for mice is actually three times as expensive as for humans! [23]

7.2 Knowledge about the animals.

At the Swedish Agricultural University in Uppsala, Professor Kerstin Olsson has been pleading that scientists must obtain maximum knowledge about the animals that they use in their experiments, for the sake of both animals and science. Knowledge about the physiology and behaviour of the animal can be vital to the experiments scientific value. For instance, the sensation of pain is individual, and researching human pain it can be better to use humans, because it can be difficult to directly transfer the pain sensation of an animal to a human. If you want to study digestion in a human it is not wise to use cows since they are ruminants, but rather use pigs witch are omnivore as well.
The interest for different aspects of medical science has varied over time. An increase in interest for cell- and molecular biology resulted in a decrease in interest for animal models. Now that the medical science has obtained more knowledge about the details about the human organism, the interest for animal models, as an intermediate, has once again grown. These fluctuations have resulted in a failing knowledge about animals, because more scientists are specialists and not generalists. As a result of these change in scientific trends with more

specialists, there is a need for close co-operation between grouping of scientists like molecular biologists and veterinarians. [24]

A national platform

In 1999, the European Consensus Platform on 3R-Alternatives (ECOPA) was formed. [25] A National Platform is, according to ECOPA a competence centre or forum with representatives from the four stakeholders interested in animal research: [26]

- Government
- Academia
- Animal welfare organisations
- Industry

ECOPA includes several countries like Great Britain, Germany, Sweden and Czech Republic. Through this common European platform it will be granted money to enhance the development of alternative methods to animal research. [25] A Norwegian platform is under construction, and it is the School of Veterinary Science which has got the task to establish such a platform. The main job at the Norwegian Platform is to collect national and international knowledge about research animals and try to fill the blanks where the knowledge is insufficient. One of the goals of the Norwegian platform is to get approved by the ECOPA, because Norway is still just an associate platform. [27]

8. Conclusions

Many medical breakthroughs have been a result of animal research, and animals have been used in experiments since Aristotle. Through time, the welfare of the animals has come into focus, and several legislations have been made to prevent cruelty and unnecessary acts.
The introduction of the tree Rs has been one of the most importantevents for animal welfare, and many animal acts and legislations are based on this principle. Even though legislations are constantly refined, there are still many loopholes. New technology has led to alternative and more humane methods like use of cell cultures and imaging. Due to alternative methods, the

number of animals used in medical research has been greatly reduced. However, there is still much work to do in the field of animal research.

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