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REPLACING ANIMAL TESTING: HOW AND WHEN?

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An important discussion in today's society is whether we should make animals suffer for the sake of science and product development. In this article, I present four examples of animal tests that were introduced in the past to protect patients and consumers, and I discuss attempts to replace those animal tests with other methods. When we started using small animals such as mice and rats for testing more than 100 years ago, there were not many alternatives. Today, we have more knowledge and a greater number of options. Scientists can now create tiny functioning organs in the laboratory, and even combine multiple mini-organs, to help us understand how the human body works when it is healthy or sick. This increased understanding will allow scientists to move beyond the use of animals in many cases, which will improve both the accuracy of the scientific tests and the welfare of animals.

Thanks to the endeavors of the Swiss 3RCC, these articles have been translated into the three main Swiss languages of German, French, and Italian.

ANIMAL TESTING IS CONTROVERSIAL

There is much debate over whether we should allow laboratory animals to suffer for the sake of science or the development of products such as cosmetics, drugs, and pesticides: about 50% of Americans and 60% of Europeans oppose animal testing, but individuals hold varied positions in terms of what should be allowed and what should not. In 1959, two scientists named Bill Russel and Rex Burch developed the **3Rs principle** (reduce, replace, refine), which is a sort of compromise. Instead of completely banning animal research or allowing it in all cases, they called on scientists to do as much as possible to replace animal testing. Where replacement is *not* possible, scientists are encouraged to reduce the numbers of animals used and refine their experiments to minimize animal suffering. Russel and Burch said, "Refinement is never enough, and we should always seek further for reduction and, if possible, replacement."

3RS PRINCIPLE

An attempt to replace animals with other forms of testing, reduce the number of animals used in tests, and refine animal tests so that they are more humane.

Back when Russel and Burch came up with the 3Rs principle, there were not many alternatives to animal experiments—but knowledge of the life sciences doubles every 7 years, so we now know over 1,000 times more than we did then! Scientists know much more about growing cells in the laboratory and, using human stem cell technologies and **bioengineering**, we can now recreate the structure and function of some organs in the lab and even combine multiple lab-generated organs to create a "human" system in the laboratory. A detailed understanding of how the body works in health and disease will help researchers create tests that are more accurate than animal testing and that save the lives of laboratory animals.

BIOENGINEERING

The field of engineering that applies the life sciences, physical sciences, mathematics, and engineering to solve problems in biology and medicine.

HISTORY: ANIMAL TESTS TO SOLVE RESEARCH PROBLEMS

Now I will describe four different medical-safety problems of the past that were solved through animal testing. These historic cases have shaped how we ensure the safety of drugs and consumer products, and the examples can help us understand the progress made using new technologies.

Pyrogens

The term **pyrogen** comes from a Greek word meaning something that generates fire. Today we use the word pyrogen to mean something that generates fever. In the early 1900s, scientists started to synthesize disease-curing drugs, including some that had to be injected into the body. Physicians often observed fever in their patients following drug injections, and sometimes even life-threatening reactions. They named the unknown fever-causing substances pyrogens. In 1912, the rabbit pyrogen test was invented: a dose of the drug ten times greater than what would be used in humans was injected into rabbits. If the rabbits did not develop fever, the drug was deemed safe for

PYROGENS

A group of microbial substances that lead to fever and inflammation.

human use. Today we know that these pyrogens come from bacterial contamination during drug production, and even killing the microbes by sterilization does not eliminate them. When the patient's immune system recognizes the bacterial pyrogens, fever results.

Eye Irritation

The eyes are especially sensitive to chemicals. In the US in the early 1930s, a cosmetic used to dye the eyelashes (called Lash Lure) led to more than 3,000 cases of eye irritation, five cases of blindness, and one death. Subsequently, the rabbit eye test was developed to prevent this from happening again. A drop of the chemical is applied directly into the eye of a rabbit and the animal is observed for several days.

REPEAT-DOSE TESTING

Giving a drug to animals multiple times over 28–90 days, to look for unexpected toxic effects on their organs.

Unexpected Toxicities

In 1936, more than 100 children died in the US from a cough syrup (Figure 1). The antibiotic contained in the syrup had been used for years without problems, but a substance called glycol, used to dissolve the antibiotic, was toxic. This started what is called **repeat-dose testing**, usually performed in rats and dogs, in which the drug is given for 28 or even 90 days orally, by inhalation, or on the skin (depending on the use of the drug). Afterward, the animals are killed and their organs are checked for possible effects.

Figure 1

Scandals leading to the introduction of animal testing. Scandals prompted a lot of the animal tests we use today. Two examples are the repeat-dose testing for unexpected toxicities and embryotoxicity in response to health problems caused by Sulfanilamide and Thalidomide.

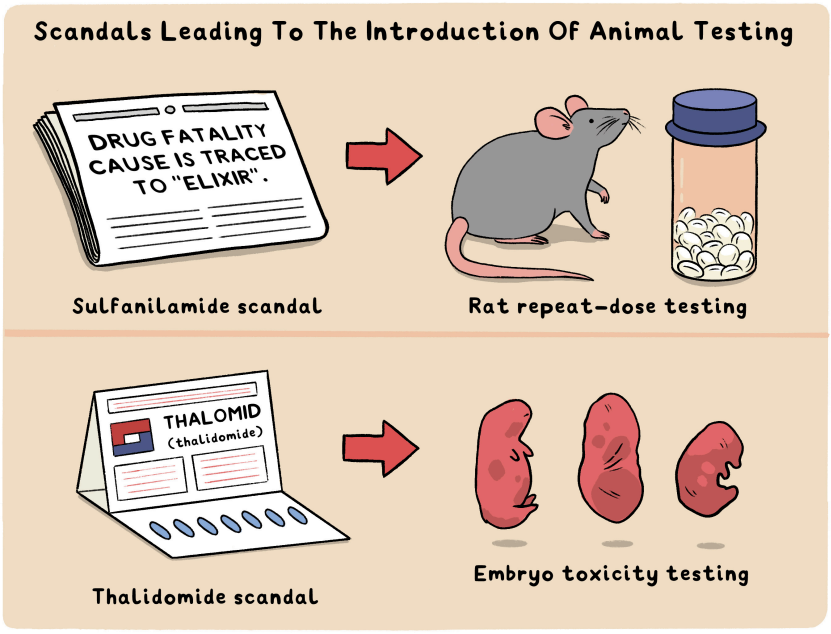

Figure 1

Embryotoxicity Testing

In the late 1950s, a German pharmaceutical company introduced a drug called thalidomide that became very popular for "morning sickness"—the frequent nausea experienced by pregnant women. About 2,000 unborn babies died from the drug and more than 10,000 children were born with malformations of their limbs (Figure

1). In response, broad testing of toxicity against embryos, called **embryotoxicity testing**, was introduced—using 3,200 rats and 2,100 rabbits per drug (Figure 1).

EMBRYOTOXICITY TESTING

Animal testing of drugs on pregnant animals, to see if the drugs are safe or will have dangerous effects on (human) embryos.

In all these cases, the scientific solution was to use animals to make sure drugs and other chemicals were safe for use in humans. But the use of animals to mirror what might happen in people is far from perfect—mice and rats predict each other's response to drugs often not better than 60%, and sometimes specific strains of mice react completely differently from each other in these tests. Sometimes animals react like humans in response to drugs or other chemicals, but sometimes they do not.

REPLACING ANIMAL TESTING: A PYROGEN EXAMPLE

HORSESHOE CRAB PYROGEN TEST

A test using the blood of horseshoe crabs, which coagulates (clots) in response to an important group of pyrogens.

A timeline of pyrogen testing is shown in Figure 2. Scientists first discovered certain bacterial contaminations of drugs that were causing fever reactions back in the 1950s. In the 1960s, it was discovered that the same bacterial substances made the blood of horseshoe crabs clot. This spurred the development of a new test relying on sampling horseshoe crabs' blood, the **Horseshoe crab pyrogen test**, which replaced 90% of rabbit testing starting in the 1980s. Then, in 1995, another laboratory test was developed based on the advancing knowledge of the human immune system—particularly,

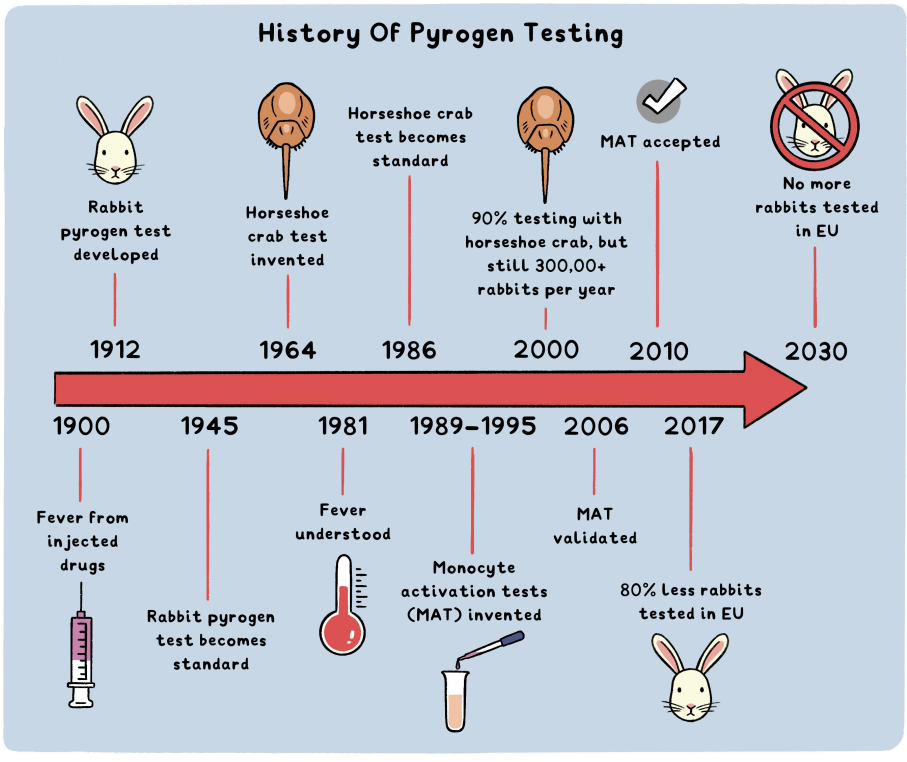

Figure 2

History of pyrogen testing. In the early 1900s, scientists and doctors noticed high fevers and some deaths in their patients after the injection of certain drugs. In 1912, the rabbit pyrogen test was developed to screen injected drugs for these dangerous effects. Since then, various techniques have been developed to decrease or completely end the use of rabbits in these tests. These attempts are ongoing and, by 2030, rabbit testing should be eliminated in the EU.

white blood cells called monocytes, which emit the chemical signals that cause fever. These tests are now called **monocyte activation tests**, and they measure whether substances are contaminated with pyrogens based on the reaction of monocytes. I developed one of these tests and led an international study with other scientists who had developed similar tests, demonstrating that such tests could replace the animal test for pyrogens [1, 2]. Following a thorough review by experts, the monocyte activation test was validated in 2006 and accepted by a number of agencies across the world in the years that followed. However, the actual replacement of the animal test is still ongoing: by 2017, 80% of rabbit pyrogen testing had been replaced in Europe, and by 2030 all rabbit testing in Europe should end. Other parts of the world lag behind. So, it took about 30 years for the horseshoe crab test to replace about 90% of rabbit testing, and another 30 years for monocyte activation tests to replace the remainder. Too slow, but we learn from these forerunners! Once scientists understand what happens in the human body, it will be easier to use non-animal test systems.

MONOCYTE ACTIVATION TEST

A laboratory test that measures whether substances are contaminated with pyrogens based on the reaction of monocytes (cells of the human immune system).

PROGRESS IN OTHER AREAS

Eye irritation testing has seen enormous progress (Figure 3A). Several new tests use simple cell cultures of skin cells, others the eyes of chickens or cows that are killed for our food. Bioengineered human eye structures have also been developed and validated. Unfortunately, no single test can fully replace the rabbit test yet. Some can only identify strongly toxic substances; others can only identify substances that have no effect. Some tests work only with certain types of chemicals. But various combinations of new tests *can* replace animals for most uses.

Embryotoxicity testing is the most demanding animal test, with respect to the numbers of animals needed. Some tests require more than 5,000 rats as well as rabbits and their embryos. Progress to replace animals for embryotoxicity testing is slow, primarily because embryo development is extremely complex and varies between species. Only three out of five chemicals tested in one species give the same results in a different species. Major progress in recent years has included the development of stem cells, which allow scientists to learn more about early human embryo development (Figure 3B). The stem cell tests that have been developed are bringing scientists closer to replacing the animal test.

Unexpected toxicities are still a key problem. How can we prepare for the unexpected? There are hundreds of tissues in the human body, and each could be the target! However, as it becomes more obvious that animals often react differently to toxic substances than humans do, we have no choice but to develop new, human-relevant tests (Figure 3C). Enormous progress has been made with modern

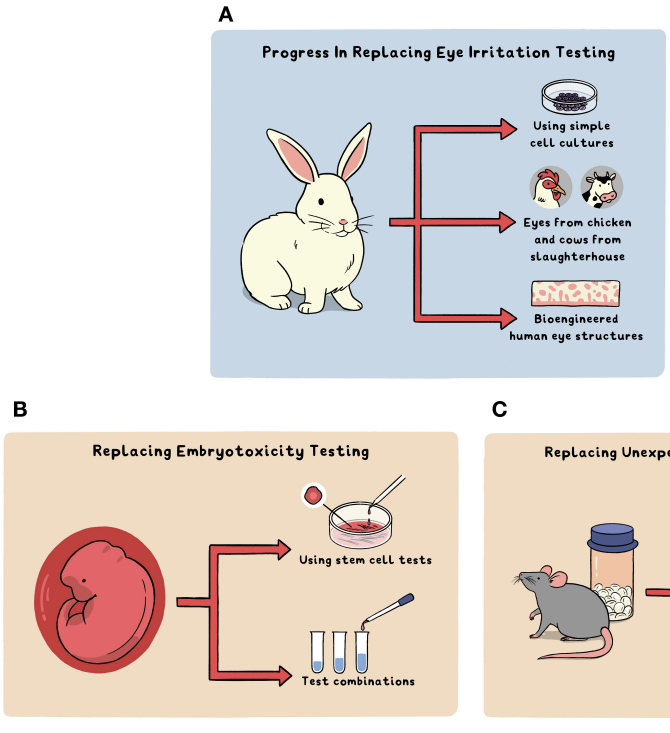

Figure 3

Progress in replacing animal testing. **(A)** Eye irritation testing. **(B)** Embryotoxicity testing. **(C)** Unexpected toxicity testing. Various technological advances allow the replacement of animal tests, including cell culture, slaughterhouse materials, bioengineered organs, stem cell technology, test combinations, and computational methods.

cell culture: bioengineering allows us to recreate the structure and function of bodily organs in the lab. These **organoids** can be combined on chips and connected by tiny fluid-filled channels that act like blood vessels. These human-on-chip models are exciting because they enable scientists to study reactions in human-like systems. At the same time, artificial intelligence (AI), which involves the increasing ability of computers to learn and analyze data, is helping us combine the accumulated knowledge of recent decades. Millions of scientific papers and tons of data from experiments can be combined by AI systems to predict unexpected effects of substances on the human body and thus avoid animal tests. So, gains in computer power are helping scientists to model what is happening in the body and to make sense of large datasets, to predict toxic effects.

ORGANOIDS

Cell cultures that replicate organ architecture and function. They can be 2D or 3D, on "chips," and multiple organoids can be joined together to create human-on-chip models.

THE CHALLENGE AHEAD

These examples illustrate that science is continuously advancing. While this article has focused on the safety of drugs, similar stories could be told for other areas of research. New laboratory and computer-based methods can be used on their own and are even more powerful if combined. These new approaches are often as good or better than traditional animal experiments. The challenge now is figuring out how to change the habit of relying on animal testing for safety assessments of drugs and new consumer products. Recent

advances have made testing processes more relevant to the human body and, most importantly, more humane.

FURTHER READING

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CONFLICT OF INTEREST: The author consults a number of companies on alternative methods.

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Thomas Hartung has spent more than three decades of his career promoting technologies to replace animal testing. From 2002 to 2008 he led the European Center for the Validation of Alternative Methods (to animal experiments) of the European Commission in Italy and since 2009 Centers for Alternatives to Animal Testing in the US and Europe. He is active in many different fields of science: starting with studies of biochemistry, human medicine and mathematics/informatics, he became first doctor (MD Ph.D.) and then professor for both pharmacology and toxicology. He expanded his work to immunology, microbiology and engineering. Today he holds five professorships at Johns Hopkins University and Georgetown University in the US as well as at the University of Konstanz in Germany. He is chief editor of Frontiers in Artificial Intelligence. *THartung@jhu.edu