Non-animal methods of research – *who* is responsible for holding back progress?

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Is it possible to *replace* every type of animal experiment with a non-animal method? This article will try to show that this is really an unfair question. Let us rather ask: is it possible to *eliminate* every type of animal experiment? In trying to answer this, we need to examine different categories of animal research separately.

[For the purpose of this article, the term 'animal experiments' applies to animal research conducted primarily for *human medicine*. Animal studies are also performed for *veterinary medicine*, but there is an important methodological distinction between the two. Using dogs to study dog diseases makes scientific sense. Using dogs (or other animals) to study human diseases does not. However, even with the intention of finding treatments for dogs, we should not experiment on healthy animals and deliberately make them sick. We should, instead, study dogs who are already sick, and try to help them with therapies that have shown promise in the laboratory.

The fundamental principle should be to make as much use as possible of all the relevant research methods available, to the point where, having exhausted every avenue, there is no other option but to try the experimental drug or therapy on a living animal who is already sick. This ethical principle should apply no less to humans as it does to animals].

We can divide animal experiments broadly into 'basic' and 'applied research.'

'Basic research' refers to animal studies that simply yield data. There is no obligation on the part of the researcher to provide a result that may be useful to human or veterinary medicine. In fact, it is not even necessary to provide a clear result. Often, one study is used to justify the next. In many cases, unanswered (usually unforeseen) questions arising from one study produce the rationale for a later study. A clear example of this can be seen on:
http://www.animalaiduk.com/h/n/CAMPAIGNS/experiments/ALL/719//

While current law makes it difficult to avoid animal testing during the safety evaluation phase of new pharmaceutical products, scientists conducting **basic research** have no legal requirement to use animals. It is left to the individual scientist to judge what is worth studying, and whether or not to use animals (1).

All research proposals involving animals must first obtain approval from an ethical review committee or panel. This panel consists of representatives from the institution where the research is being conducted. The panel normally includes an 'outside' person, whose task it is to represent the public interest. Despite the presence of such a person, the panel is invariably weighted in favour of allowing animal experiments to proceed and very few research proposals are turned down. However, there are certainly more objective ways of assessing animal research than the current peer review system.

One such method is `**citation analysis**' - the process whereby the impact or `quality' of a piece of science is assessed by counting the number of times other scientists mention it in their work. This is particularly suited to animal experimentation in **basic research**, since the justification for such animal studies invariably points to human benefit.

A recently published study on applications of biomedical research involving animal models makes a compelling case for using citation analysis in the peer review process (2). The authors of this study investigated the frequency of citations of the animal research in clinical (human) publications. The outcome was unambiguous - against the use of animals.

Another useful tool is the '**systematic review'**. A systematic review is an in-depth assessment of numerous scientific studies into how a particular medical condition responds to a treatment. A recent report funded by the UK National Health Service commissioned three collaborating research teams to compare in detail the clinical (i.e. human) outcome of six medical treatments with the results obtained from experiments on animals. The areas of research chosen included head injury, blood coagulation and stroke. The research teams found that most of the animal research examined was poorly conducted and produced conflicting results (3).

While the application of citation analysis and systematic review would add objective criteria to the peer review process, this of itself would have only a limited impact on the overall use of animals in basic research. The most important element, or missing ingredient, is to be found in the mindset of the individual research scientist. It is called **compassion.** It is that quality that allows a person to perceive an experimental subject, not as a tool, but as a sentient being. Scientists wanting to conduct basic research can choose between two options: asking a question that can only be answered by means of an animal experiment; or asking a different question, which does not require the use of animals and therefore does not cause deliberate animal suffering.

What happens when an animal researcher suddenly undergoes a 'perceptual awakening'? Here is a shining example*:*

"In 1986 I was awarded an American Heart Association Clinician-Scientist Award, which is a 5 year grant to support original research. It was well understood that almost all of these grants went to basic science investigators, and at that time I still believed that animal research was a fundamental precursor for human investigations. My research involved evaluating the results of cardiac nuclear imaging methods in dogs, and required tying off their coronary arteries to investigate radiotracer distribution patterns under different circumstances.'

'I did this research for several months, until I realized that it was pointless. Dog coronary anatomy wasn't the same as humans, and there were several other differences which made our experiments only interesting but not applicable to humans. More importantly, when I actually had to do these things to dogs, I was forced to see them as the helpless, dependent, trusting creatures they are … I was unable to face my own dogs at home, without shame and regret. I finally had to admit that my research was a sham, and that all I was accomplishing was self-promotion."
"… I designed a new research protocol involving imaging studies in humans …"

JJ Pippin M.D., F.A.C.C. (for full CV, please refer to Appendix 2 of website above).
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Let us now turn our attention to the other major category of animal experiments **– applied research.**

One of the most important forms of applied research is drug safety testing by the pharmaceutical industry, most of which is in fact *toxicity testing*. These tests involve force-feeding animals by gavage (a long tube pushed right down to the stomach) or injection, or both. In other 'procedures', the animals are forced to inhale vapour by sealing them in an air-tight chamber.

The pharmaceutical industry has traditionally defended its use of animals by citing the legal requirements of regulatory authorities. The regulatory authorities in turn have defended their position by stating that they require drug manufacturers to submit 'good data' to show that their products are safe and that they work. How they achieve this, is up to the drug companies, say the regulators.

Taking the UK as an example, animal testing is influenced by both national and EU legislation. Although the Home Office officially claims that all new drugs are required by law to undergo animal experiments, on closer examination, this would appear not to be the case. For example, the UK Medicines Act 1968 and other UK regulations *do not specifically require animal tests.*

The only piece of legislation that specifically refers to animal testing (Annex I of Directive 2001/83/EC) states that toxicity tests 'shall be carried out on two species of mammals one of which must be a non-rodent'. However, this seemingly solid statement is eclipsed by article 7.2 of Directive 86/609/EEC, which states that an animal experiment must not be carried out if a non-animal method could be used to provide the information in question.

It must therefore be concluded that there are essentially no legal obstacles to the replacement of animals in toxicity and drug safety testing.

So, if there are no legal obstacles to the wider use of non-animal methods, why does the pharmaceutical industry continue to rely on animal tests? Their main excuse is that only a few non-animal methods exist and even fewer have been scientifically validated. The definition of v*alidation* is "the process by which the reliability and relevance of a procedure are established for a particular purpose" (4).

The process of inter-laboratory validation of a single non-animal method can take up to ten years to complete. Once a test method has been validated, it must then obtain regulatory approval – which can take up to five years to achieve. At present in the EU there are fewer than 30 so-called validated 'alternative' methods. However, some of these 'alternatives' still require animal use, for example, the mouse local lymph node assay, which is used as a skin sensitisation test (5).

While insisting that any non-animal method must undergo validation before it can even be considered for regulatory approval, the pharmaceutical industry and the regulatory authorities have conveniently overlooked the fact that ***animal experiments have never been validated*** (6,7).

Not all non-animal methods require validation. For example, the use of cell culture is an accepted form of research in basic studies that does not require validation, since this use of cells has been well established over the years and has a sound scientific basis. The issue of validation of a test method becomes relevant when considering products or processes that may impact on health or on the environment.

The following case studies are examples of completely non-animal methods that have been developed for use in research and testing (applied research). The push for non-animal methods of testing has largely been the result of public pressure. Although the pharmaceutical industry possesses the infrastructure and resources to develop and implement non-animal methods, its track record indicates that it lacks the motivation and initiative to do so. A recent EU consumer survey clearly illustrates this point: two-thirds of the pharmaceutical industry's budget is spent on drug promotion (marketing and advertising), while one third is spent on actual research (8).

Pyrogenicity testing

Any pharmaceutical product intended for parenteral administration (e.g. intravenous, intramuscular use) must be free of pyrogens. A pyrogen is any substance capable of producing a fever response, e.g. bacterial endotoxins.

The rabbit pyrogen test was developed in 1940. It involves injecting a minimum of three rabbits with a test substance, and then monitoring their body temperatures for several hours. The rabbits would normally then be

killed. Countless rabbits have been used in this way since 1940. However, despite its long history of use as the gold standard, ‘this test has never been formally validated to establish its reliability or relevance to humans and there are a number of well-documented drawbacks to this test, including marked species and strain differences in sensitivity’ (9).

An alternative to the rabbit pyrogen test was developed at the Johns Hopkins School of Hygiene and Public Health, beginning in 1956 (10). It was based on the observation that bacteria caused intravascular clotting in the horseshoe crab, *Limulus.* Blood from the crab was obtained by heart puncture, after which the crab was released back into the sea. Mortality was reportedly low. The crabs’ blood cells (amebocytes) are used in the so-called Limulus Amebocyte Lysate (LAL) assay as a means of detecting bacterial endotoxins. The first large-scale facility for producing LAL reagent for industrial purposes was established in 1971 in the US, but it was not before 1977 that the US Food and Drug Administration (FDA) formally recognised it as a test method to replace the rabbit pyrogen test. While the LAL test is far more sensitive at detecting endotoxins than the rabbit test, the LAL does not detect non-endotoxin pyrogens. Thus, some rabbit testing still took place.

A major step towards a completely non-animal method occurred in the 1980s, in the form of an in vitro test called the Monocyte Activation Test (MAT). This test detects pro-inflammatory and pyrogenic contaminants not always detected in the rabbit pyrogen test or the LAL test. The MAT uses human mononuclear cells (e.g. monocytes) obtained from human volunteers or from blood banks. In some countries, such as the UK, blood banks routinely discard the white cell fraction of donated blood to eliminate the risk of transmission of prions (vCJD). Some researchers have suggested that this discarded component could potentially supply the white blood cells needed for the MAT.

Once validation studies are completed and the test adopted by the regulatory authorities, reports say that it will save the lives of 200,000 rabbits in the EU alone every year (11). *It should be noted that the MAT was applied 18 years ago (12), but apparently ignored by industry.*

Monoclonal antibodies

Monoclonal antibodies (Mabs) are useful as tools for a wide range of research purposes, e.g. for identifying specific protein molecules. They have traditionally been produced by the mouse ascites method. This involves injecting a specific antigen into a mouse. Once the mouse's immune system has developed antibodies against the antigen, the mouse is killed in order to harvest these antibodies (B cells), from the spleen. These cells are then fused with myeloma cells (malignant B cells). The result is a hybridoma, which is then injected into the peritoneal cavity of mice. The hybridoma multiplies rapidly inside the mouse peritoneum, to the point where, after 10-20 days, the abdomen becomes severely distended. The mouse ascites method has been widely criticized on welfare grounds for many years.

It is useful to examine the history of Mab production. In 1975 Kohler and Milstein (13) published a paper describing a new method for producing Mabs, for which they eventually received the 1984 Nobel Prize. It should be noted that ***the original research was principally an in vitro technique***, but other researchers seized upon the idea that monoclonal antibodies could be produced by injecting the hybridoma cells into the abdominal cavities of rodents using the ascites method. As a result, tens of millions of animals have suffered and died despite the availability of non-animal methods.

A variety of non-animal systems designed for the purpose of harvesting Mabs in vitro have existed for many years. They include the static culture (in flasks), ceramic or hollow fiber, matrix cartridges and roller bottle/dialysis cartridges. These 'bioreactor systems' were designed specifically for the large-scale production of Mabs (14).

A more recent development is the Phage Display technique. This in vitro technology uses recombinant DNA techniques to generate large panels of monoclonal antibodies (15). Selected monoclonals can then be produced in bacterial expression systems. Humanized monoclonal antibodies can also be produced in this manner (e.g. Abbott Laboratories "Humira" for the treatment of rheumatoid arthritis).

The above examples describing the development of non-animal methods illustrate two important principles. The first is that technological solutions not requiring animals can be found even for seemingly insurmountable obstacles, where animals have been relied upon for many years. The second is that industry has the capacity to develop these technologies, but lacks the motivation and initiative to do so. Public pressure will be required for the

foreseeable future to persuade industry to move away from its reliance on animals as 'living test-tubes'. However, a much more fundamental change of attitude needs to occur for real progress to happen – the realization by industry and by the regulatory authorities that animal experimentation represents bad science. Let us replace it with 21st century science that is species specific, ethical and compassionate.

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