Studies with human tissue allow researchers to come as close as possible to the clinical situation, not only in drug research but in safety evaluation. In a far-sighted contribution to the 26th Annual Meeting of the Society of Toxicology, American scientists led by John Frazier of the Johns Hopkins University, argued that "By utilizing *in vitro* methodologies, the toxicological response of human tissues [to chemicals] can be directly assessed under controlled experimental conditions in advance of human exposure. This approach has the distinct advantage that it eliminates the necessity to extrapolate experimental observations from animal models to man. Thus many of the confounding factors that make interspecies extrapolation a difficult task, particularly when there are conflicting data from various species, are eliminated."[7]

There are already enough examples in the scientific literature to show how human tissue tests could save lives and alleviate suffering. It is now known that the potentially fatal cases of aplastic anemia associated with both chloramphenicol and phenylbutazone, which led to severe restrictions in their use, could have been identified with human bone marrow cells *in vitro*.[8] The drugs caused many deaths but the effects had not been predicted by animal experiments. Thalidomide's harmful effects can be investigated in human cell tests[9] although many animal species have proved resistant to the drug.

Animal researchers often complain that *in vitro* tests cannot mimic effects on the whole body. Ironically, the very complexity of a living animal provides more opportunity for species variation and can actually *mask* harmful effects that would be identified in simpler systems. Oraflex and Selacryn are good examples: both drugs were withdrawn after deaths from liver damage, an effect not predicted by the usual animal experiments but subsequently identified in the test tube using cells from the rat's liver.[10] Although in this case *in vitro* tests with animal cells proved correct, their use always carries the risk of misleading results and scientists at Britain's Lister Hospital argue that "any *in vitro* method using human tissue gives a degree of reassurance not provided by [live] animal experiments or by *in vitro* ones based on tissues from other species."[8]

Nevertheless scientists have found a way to make *in vitro* systems more closely resemble a living person. Sometimes chemicals only become hazardous when metabolized by the liver, so researchers include liver cells in their *in vitro* tests to mimic the body's main metabolic processes. Although ingenious, the obsession with animal experiments means that liver cells are usually derived from rats, mice or hamsters rather than people. The problem is well illustrated by the antidepressant mianserin. Doctors have been warned to monitor their patients closely because the drug can cause serious blood disorders. The effects had not been predicted by animal experiments and later research found that only *in vitro* tests with *human* tissues could correctly identify the hazards. Mianserin is thought to produce its harmful effects following metabolism by the liver, where it is transformed into another substance which is toxic to the blood. This can be observed in the test tube by incubating white blood cells with liver tissue from *human* donors but not from mice, rats, rabbits or guinea pigs. According to the researchers, their findings demonstrate that "extrapolation of *in vitro* toxicity data from animals to humans is a poor substitute for use of human tissue."[11]

The use of animals to detect cancer-causing chemicals is so expensive and time consuming that dozens of *in vitro* systems have been developed, which at least makes it possible to assess the thousands of untested chemicals currently in use. Some of these tests use human white blood cells where the effect of chemicals on chromosomes can be monitored *in vitro*. Scientists have demonstrated their value by correctly identifying well known human carcinogens such as arsenic, vinyl chloride, benzene and chromium.[12] Human sperm has also been used to show the carcinogenic action of the estrogenic drug diethylstilbestrol. Most research however, has concentrated on developing *in vitro* tests using bacteria, and cells from other species.

Even so, just one of these tests – the salmonella bacterial assay – is at least as good as the traditional two-year animal experiments in identifying human carcinogens[13] and considerably more accurate in identifying non-carcinogens.[14]

Another area where there is great scope for replacing animals is the production of monoclonal antibodies. Antibodies are proteins which bind specifically to foreign substances and form part of the body's immune response to disease. Scientists hope that one day they will be able to make monoclonal antibodies that are better able to target tumors in the body than conventional, toxic treatments. Traditionally, antibodies have been produced from living animals but they have not been very successful when administered to people and often cause toxic side effects because of allergic reactions to what the human body sees as "foreign" tissues. One answer is to produce the antibodies from human white blood cells *in vitro*.[15] The cells can be derived from tonsils, spleens and lymph nodes obtained surgically or from human blood.

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In many areas of research, it is advantageous for human cells to be cultured to allow their continuous growth. These "tissue cultures" allow researchers to increase the number of cells available for study and they also enable the effect of drugs on cell growth to be monitored, providing a measure of toxicity. To allow cells to grow effectively, they are kept in a nutrient medium usually derived from fetal calf serum. But there may often be advantages in using synthetic growth media where the exact composition of nutrients is known, or indeed human sera. Scientists at the University of British Columbia compared the growth of human breast cancer cells incubated with either human or fetal calf sera. As might be expected, they found that use of human serum most closely resembled the clinical situation not only in growth of the cancer cells but also in their sensitivity to chemotherapeutic drugs.[16]

New FRONTIERS

The power of modern computers allows the simulation of complex biological systems, providing more rapid insights into the nature of disease. This technology has seen the emergence of a growing number of simulations of the immune system. Dr DeLisi and co-workers at the National Cancer Institute have used mathematical modeling techniques to show that the immune system can not only fight cancer growth but stimulate it as well. According to Dr DeLisi, "if our model had been around ten years ago, it could have predicted what it's taken scientists countless man-hours and animals to figure out. This is the value of mathematical modeling – it comes up with things you might otherwise miss."[17]

Computer programs are enabling drug discovery to emerge from the dark ages. Based on the idea that drugs must be the right electronic shape to trigger their effects, scientists are using 3-D computer graphics to design new treatments, thereby avoiding the old hit and miss approach in which thousands of chemicals were subjected to animal tests.

Computer scientists are also able to predict harmful effects. Toxicity is influenced by factors such as the shape of the chemical, the presence of certain reactive groups within the molecule, and the way these reactive fragments are linked together. The computer works out the importance of all these factors by analyzing substances whose toxicity is already known. The information is then used to assess new chemicals purely on the basis of their chemical structure.

Another innovative approach to predicting harmful effects is called COMPACT (computer optimized parametric analyses for chemical toxicity). It is based on the knowledge that interaction of a drug with special chemicals present in the body (P450 enzymes) can create or reduce toxicity. The shape and electronic distribution of the chemical are first determined and if the computer discovers that these "fit" into the P450 enzymes like a lock and key, then toxicity can be expected. The method could have predicted the toxicity of Selacryn, a drug withdrawn following unexpected cases of liver damage.[18]

Together with physical models, videotape recording, *in vitro* experiments and human studies, computer simulations offer great scope for replacing animals in medical education. An example is the "Mac" family of interactive digital computer programs for use in clinical, physiological and pharmacological teaching and research. The student can monitor important physiological variables such as heart rate, blood pressure, arterial oxygen and carbon dioxide pressures, acid-based status, urine output, plasma potassium, hemoglobin and plasma concentration of drugs, and by altering one or more of these variables, can study the effects over a period of time.