ALTERNATIVE STRATEGIES

Replacing animals in research and testing

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In the heated debate surrounding animal experiments, it is easy to forget that vivisection is just one method of research: there are others. Most crucial to the advance of medicine are human studies. Vital clues come from monitoring the health of large numbers of people whilst scanning techniques like positron emission tomography and magnetic resonance imaging enable researchers to investigate disease in individual patients. Further information can be derived from autopsy findings and studies with healthy volunteers. Much research can be carried out in the test tube, and countless *in vitro* systems have been devised to assess both the beneficial and harmful effects of drugs and chemicals. Then there are computer simulations of biological systems which aid drug design, medical research and education.

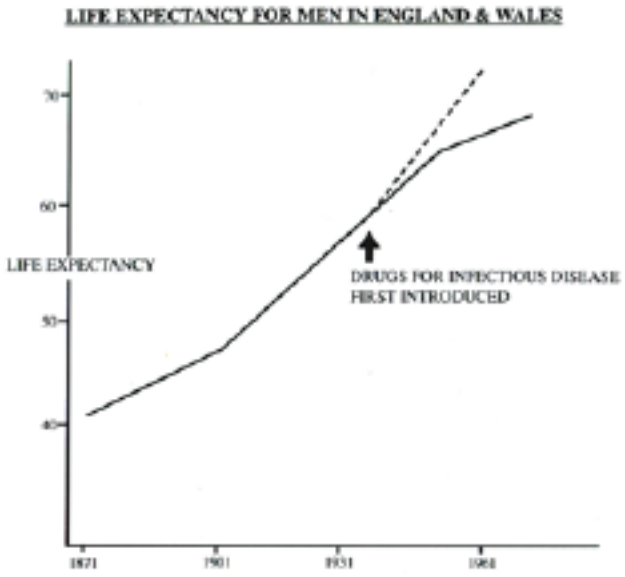
The importance of non-animal methods, and the way in which they are used, can be gleaned from their contribution to the prevention and cure of disease.

Combating the Major Killers

Epidemiology is the technique which allows doctors to find the causes of ill-health so that preventive action can be taken. The method is based on comparisons: epidemiologists obtain clues by comparing disease rates in groups or populations with differing levels of exposure to the factor under investigation.

Early epidemiological studies revealed that people who lived and worked in dirty, overcrowded and unsanitary conditions with little food or clean water, were much more likely to die of infectious disease. Average life expectancy during the 19th century was therefore very low. However, the findings were used by social reformers to bring about much needed improvements in public health and social legislation.[1] As a result, by the time the first drugs to treat infectious disease were introduced in the 1930s, life expectancy in Britain had already increased by 20 years.[2] In Sweden, between 1856 and 1936 the corresponding increase was 24 years! Edward Kass, a President of the Infectious Disease Society of America, described the decline in infectious disorders and their correlation with improving socio-economic conditions as “the most important happening in the history of the health of man”.[3]

Contemporary comparisons reinforce the connection between living standards and health: in Africa, where the average individual income is only $637 per annum, male life expectancy is 51.5. In Asia, yearly income is £1,848 per capita and people can expect to live for 63 years. In Europe and the Americas where individual income is more than $10,000, average life expectancy is nearly 70. Clearly poverty is the world’s deadliest disease.



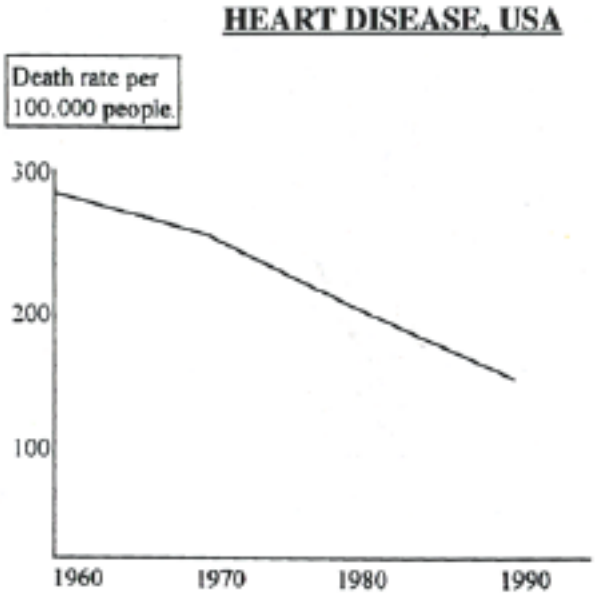
Today, the main killers in Western society are heart disease, cancer and stroke, comprising over 60% of all deaths in the United States. But epidemiology has again shown how most of these can be avoided. Prior to 1948, when the famous Framingham study was initiated, little was known about the causes of heart disease. There were clues however, and during the 1930s epidemiology showed that atherosclerosis is only prevalent in countries with a high consumption of animal fats.[4] Atherosclerosis is the build up of fatty deposits in the arteries eventually leading to heart disease and strokes.

The Framingham project aimed to determine "factors influencing the development of heart disease" and for years monitored the health of the people in the small Massachusetts town who participated in the study. The findings, which indicted smoking, elevated blood pressure and high cholesterol levels, now form the bedrock of preventive health policy. Further epidemiological studies identified lack of exercise as another important risk: London bus drivers were compared with their more active colleagues, bus conductors.

The Framingham results were confirmed in many other countries.[5] A Chinese study, for instance, found that as a result of the largely vegetarian, almost vegan diet in rural China, cholesterol levels are, by Western standards, extremely low, with heart disease rarely recorded as a cause of death. According to Richard Peto, a co-author of the study, "The Chinese experience shows us that most Western coronary heart disease is unnecessary."[6] In fact, as long ago as 1958, Stamler wrote that "significant atherosclerosis is rare in people whose diet over the lifespan is predominantly vegetarian", a conclusion even then based on decades of epidemiological research.[4]

Since the 1960s, when the US had one of the highest death rates for coronary disease in the world, mortality has fallen sharply, in line with changes in diet and lifestyle. Specific medical treatments had only a small impact, at best.[7]

Like heart disease, little was known about the chief causes of cancer before 1950. For decades epidemiology had taken second place to animal experiments which some thought



held the key to success. Mistaken ideas flourished and many believed, for instance, that most (if not all) cancers were caused by viral infection, a view derived from animal experiments where it is easy to transmit the disease in this way.[8]

Fortunately, following the second World War, interest in the epidemiology of cancer was reawakened. The most striking discovery, by Doll and Hill, was that smoking causes lung cancer.[9] Further population studies subsequently linked many other types of cancer to cigarettes so that smoking is now held responsible for 30% of cancer deaths. Put into human terms, this represents 150,000 American lives.

Doll and Hill's success paved the way for more epidemiological discoveries and today we know that 80 - 90% of fatal cancers are potentially preventable. The culprits include diet, smoking, alcohol, radiation, pollution and occupational hazards such as asbestos. Viral infection is associated with about 5% of Western cancer deaths. According to Doll, the knowledge gained through epidemiology "has led ... to nearly all the steps that have been taken to reduce the incidence of cancer in practice".[10]

And as with heart disease, there were early clues that a diet rich in fruit and vegetables could be beneficial: in 1933 epidemiological research by London University indicated that people consuming larger amounts of fruit and vegetables were less likely to develop

cancer. Although this received little attention at the time, later research confirmed the findings: by 1992 128 epidemiological studies had shown the protective effect of fruit and vegetables.[11]

Stroke is the third commonest cause of death in Western society, also resulting in much disability. Every year in the US alone, there are some 500,000 new cases with 150,000 deaths. Yet epidemiological studies have shown that stroke is preventable.[12] The research has identified high blood pressure as the chief cause, but other public health measures identified by epidemiology include avoiding smoking, heavy alcohol consumption and fatty diets.

Epidemiology has also shown that reducing salt intake can effectively combat high blood pressure. Careful comparative studies revealed that some cultures, such as the Alaskan Eskimoes, consume very little salt and do not suffer from high blood pressure. In fact, evidence showing that dietary salt restriction might reduce blood pressure dates back to the ancient Greeks! But the dangers of salt were finally confirmed by researchers at London's St Bartholomew Hospital who analysed 78 previous trials involving 47,000 people. They found that a daily individual reduction of 3 grams of salt would be more effective than drugs in lowering high blood pressure. The findings indicated that individual action, together with reduced levels of salt in processed food, would cut Western stroke by a massive 39% (and heart disease by 30%). This means preventing an estimated 56,000 stroke deaths every year in the US, together with much disability. As the researchers point out, "Few measures in preventative medicine are as simple and economical and yet can achieve so much."[13]

Developing Cures

Human epidemiological studies have the power to save millions of lives, showing that major advances can be achieved without animal experiments. Although prevention is obviously better than cure and deserves the greatest emphasis, not all disease can be avoided. Again, non-animal methods make an important contribution to the cure of disease and are increasingly replacing animals. For instance, analysis of British figures shows that between 1977 and 1995, the use of living animals to develop and test drugs fell by around 60%, reflecting the increased use of more humane techniques. In fact, almost any useful drug effect can be identified in the test tube using cells, tissues and enzymes from the body.[14] The AIDS treatment AZT originated from *in vitro* experiments[15] (as did the drug combinations now being used against HIV), whilst anti-cancer agents can be assessed in the test tube using human cancer cells. Based on the idea that medicines must be the right shape to trigger their effects on the tissues, scientists are employing 3D computer graphics to design new treatments. At present however, *in vitro* methods are usually seen as preliminary tests prior to experiments on animals.

Discovering whether a potential new drug has useful effects is only the first step in development. The next stage is to assess its safety prior to clinical trials with healthy volunteers and patients. Although animals are overwhelmingly used for the purpose, they are not the only way and hundreds of *in vitro* tests have been developed. These include bacteria to test mutagens and carcinogens, yeast to measure phototoxicity, and human tissues to predict skin and eye irritancy. Indeed, tests with *human* tissue have the advantage over animal experiments that results are directly relevant to people. Although the method has not hitherto been widely used there are sufficient examples to show that it could improve safety:

*Human bone marrow cells can identify drugs such as chloramphenicol and phenylbutazone which cause potentially fatal aplastic anaemia,[16] a side effect not predicted by the original animal tests.

*Human liver tissue can detect hepatic toxicity associated with the anti-epileptic drug valproic acid[17] although extensive animal experiments prior to marketing failed to identify the problem.

*Thalidomide's effect on the foetus can be studied with human embryonic tissue[18] although many animal species proved resistant to the drug.

Theoretical techniques to predict toxicity are also emerging. Unilever has developed a computer method called DEREK which can identify potential skin sensitisers on the basis of chemical structure. DEREK compares the new chemical with substances whose sensitisation is already known. Unilever use DEREK as the first stage in identifying suspect chemicals: test on animals usually follow.[19]

Another innovative approach to predicting harmful effects is the computer graphic technique COMPACT (for computer optimised parametric analysis for chemical toxicity). It is based on an understanding of chemicals, known as P450 enzymes, that are naturally present in the body and which are responsible for metabolising drugs and foreign substances. Some of these enzymes interact with drugs to create toxicity whilst others have a detoxifying effect. A drug can only react with a P450 enzyme if it has the correct shape and electronic distribution, that is, if it fits into the enzyme like a key into a lock. This is determined by the computer which then announces whether a new chemical will be potentially toxic or carcinogenic, or safe under ordinary conditions of use.[20]

Ultimately, whatever preliminary tests are carried out, it is human trials that represent the most valid method since only they can reveal how a drug is processed by the human body. This view is reflected by Professor Dennis Parke, former member of the British government's Committee on Safety of Medicines, the body responsible for approving the introduction of new drugs. Parke explains that "... there are indeed more appropriate alternatives to experimental animal studies and for the safety evaluation of new drugs,

these comprise short-term *in vitro* tests with micro-organisms, cells and tissues, followed by sophisticated ... studies in human volunteers and patients".[21]

Laboratory tests are not the only way to discover useful therapies. Clinical investigation of *existing* medicines often leads to important advances as physicians discover new uses for these drugs. One example is the early beta-blocking drug, propranolol, which was first introduced for heart problems but then unexpectedly found to lower blood pressure in patients.[22] As a result, beta-blockers are now a major treatment for high blood pressure. The discovery of phenobarbitone's anti-epileptic effects in 1912 has been described as the most important advance in medical treatment of the disease. During clinical investigation of phenobarbitone's sedative properties, Hauptmann noticed that the drug also reduced epileptic attacks. He then carried out further tests with epileptic patients to confirm his observation.[23] Today, phenobarbitone is still an important treatment for epilepsy.

In cancer research, the first effective anti-tumour agents originated with the discovery that one of the long-term effects of the mustard gases used in World War I was damage to the bone marrow.[24] Doctors noticed that exposed soldiers and workers experienced a dramatic lowering of their white blood cell count and suggested the chemicals as a possible treatment for leukemia and lymphoma - cancers characterised by an over production of white blood cells. The nitrogen mustards are now used in combination with other anti-cancer drugs to treat conditions like Hodgkin's disease.

This approach - the careful analysis of drug and chemical side effects - has enormous scope since all substances have multiple actions, and whilst many are obviously harmful, others can be harnessed for the patient's benefit.

Human clinical studies must necessarily be used if, for some reason, drug researchers are not able to experiment on animals. In the case of Alzheimer's Disease there are no close "animal models" on which to test treatments, but drug development has still proceeded: researchers have been guided by human tissue studies instead. An example is the development of tacrine, the first drug specifically for controlling Alzheimer's Disease. Analysis of tissue from Alzheimer patients revealed a defect in brain chemistry which leads to decreased production of the nerve chemical acetylcholine. Tacrine was suggested as a way of combating this and although it is not a cure, it has been found to improve symptoms.[25]

The effective drug combinations used for the treatment of some cancers, especially childhood leukemia and Hodgkin's disease, were again derived through human clinical studies and *not* because of animal test results. As the medical textbook *Principles of Cancer Treatment* (1982) explains, "While much research has been done testing drug combinations in transplantable rodent tumours, no system with established predictability has emerged."

Human clinical research has played the leading role in developing other forms of medical treatment such as herbalism, osteopathy, homoeopathy, acupuncture and dietary measures. One example is the use of a vegan diet to control high blood pressure in

people worried about the side effects of powerful drugs.[26] Another study showed that a combination of dietary, lifestyle and psychological treatments was actually able to cure advanced heart disease. The researchers found that a low fat vegetarian diet, moderate exercise, and reduced stress led to a "... regression (reversal) of even severe coronary atherosclerosis after one year, without the use of lipid-lowering drugs."[27]

Alternatives - A Question of Attitudes

Although in some countries the use of animals is gradually declining, alternatives still face major problems. Perhaps the greatest is the attitude of scientists who regard animals as the disposable tools of research. Those whose daily work involves the infliction of suffering and death must inevitably become hardened and desensitised, whatever their initial qualms. As one animal researcher recalls, "When I first started, I would feel badly when we killed an animal. In the larger sense. I would think of analogous situations like executioners in a state prison or the Holocaust - being a guard in a Polish concentration camp or something. Of course, every now and then you still think, 'My god, we're killing animals here left and right, left and right. Are we getting anything useful out of it'? but I find it fairly easy to recover from these thoughts now."[28]

During a 3-year study of vivisection laboratories in New York, sociologist Mary Phillips witnessed numerous painful experiments. These included lethal poisoning tests with a toxic substance, injection of cobra venom, and cancers in rats and mice. No pain killing drugs were administered, nor were they usually given following major surgery (although anaesthetics were used *during* operations), yet Phillips reports that "Over and over researchers assured me that in their laboratories animals were never hurt."[29] Could it be that researchers simply did not perceive that any of their animals were suffering?

Because scientists do not feel strongly about the unnecessary loss of life, it is not surprising that humane options are often neglected or left underdeveloped. In 1972 Britain's TB Reference Laboratory reported that a test-tube technique could be used instead of guinea pigs to diagnose tuberculosis. Nevertheless, 14 years later the Medical Microbiology Department at the London Hospital was still *routinely* inoculating guinea pigs for the diagnosis of TB.[30] Later still, in 1991, a correspondent to *The Lancet* criticised the guinea pig test, describing it as costly, hazardous and insensitive so that "the time has come for it to be abandoned once and for all".

The testing of hormones like insulin and somatotropin has traditionally employed animals. In 1995 a report by the European Centre for the Validation of Alternative Methods (ECVAM) noted that in Europe and Japan, animal tests were no longer required and had been deleted from official guidelines. However, ECVAM found that in the United States, the use of animals to test these hormones continued even though it had been stopped in other countries.[31]

Thousands of animals die simply to provide parts of their bodies for *in vitro* experiments when human tissue from volunteers, biopsies, surgical waste and post-mortems would provide more meaningful results. Little is known about the number of animals

sacrificed in this way, but figures for the Netherlands suggest that 14% of experiments fall into this category.[32] At Italy's Mario Negri Institute, where thousands of animals are used for drug and cancer research, the proportion is put at 25%.[33] In Britain this would mean that at least 400,000 animals are killed every year as a source of cells and tissues.[34] Scientists usually cite lack of availability of human tissue as the chief obstacle to its more widespread use, and certainly more could be done to establish and coordinate tissue banks to prevent post mortem material and surgical specimens going to waste. But attitude is also vital, as shown by Pharmagene Laboratories Ltd, the first in Britain to focus exclusively on human tissue for research. According to co-founder Dr Bob Coleman, "It is true that the acquisition of human tissue for research is not easy. However, we have worked hard to establish collaborations with major teaching hospitals and their associated tissue banks. It is also our aim to establish a greater acceptance of research as an acceptable use of donated human tissue. Finally, and most importantly, we make ourselves available 24 hours a day, 365 days a year to receive, process and use that tissue."[35]

Some researchers seem reluctant to think of *in vitro* and other techniques as "alternatives", perhaps because they are seen as a threat to careers based on animal experiments. In an analysis of the arguments used to defend vivisection, Professor Wiebers and his colleagues describe how "Many animal research advocates have recently enlisted another argument. They would abandon the term alternatives, and instead endorse other techniques as adjuncts to animal research. The term alternatives arouses the fear that conceding any replacement for animal research might lead to widespread replacement. This reluctance to look more broadly at the full range of research methods and designs ignores the fact that for much research, better science dictates a shift to cellular, molecular, and mathematical systems."[36]

The Draize rabbit eye test is a classic example of the mindset and lack of imagination that can delay the development of alternatives. Since 1944, this procedure has been employed to test the irritancy of a wide range of chemicals including pesticides and consumer products. Usually no pain relief is given and the experiment can proceed for 7 days during which the cornea, iris and conjunctiva are monitored for signs of opacity, ulceration, haemorrhage, redness, swelling and discharge. It had long been recognised that the rabbit eye is a poor model for the human eye, but toxicologists simply suggested using different species. Only during the 1980s, when animal protection groups focussed attention on the test, did attitudes finally start to change. Since then, dozens of *in vitro* techniques have been devised and some are now routinely used.

One of the most successful is EYTEX which is available in the form of a kit and can take as little as one hour to perform. Irritant chemicals produce turbidity, or cloudiness, in a mixture of plant proteins which mimics the corneal opacity produced in a living person or animal. The turbidity is easily measured using an optical instrument. EYTEX was developed by America's National Toxicology Corporation as "a practical alternative to *in vitvo* (live animal) methods." It can rapidly identify moderate to severe eye irritants.[37] In addition, a human tissue system which models the outer layer of the cornea can distinguish between innocuous, mild and strong eye irritants whilst a combination of results

from two other *in vitro* systems has been accepted by German regulatory authorities for identifying severely irritant chemicals: one of these uses the chorio-allantoic membrane of the hen's egg and the other utilises a cell culture assay.[38]

The Draize campaign encouraged scientists to be more positive towards alternatives with the result that far fewer animals are now used. In Britain during 1980, 13,294 rabbits were subjected to eye irritancy tests, falling to 4,216 a decade later.[39] Another way of focussing the scientific mind is for humane research organisations to fund research into alternatives to specific animal tests. For instance, an early award by Britain's Lord Dowding Fund helped develop quantum pharmacology, a theoretical technique for predicting the useful effects of drugs. Funding had been difficult to obtain as the method was considered speculative, but when the initial studies proved successful, sponsorship was taken up by government and industry. Now quantum pharmacology is an established part of drug design. From 1989 animal welfare organisations contributed to the Swedish-based MEIC programme for assessing *in vitro* toxicity tests. Many laboratories took part in the study which showed that human cell cultures could predict harmful effects in people.[40] And more recently, the Alternative Research and Development Foundation, a department of the American Anti-Vivisection Society, has funded research into a tissue culture method for producing monoclonal antibodies, substances traditionally made using a painful procedure with mice. Monoclonal antibodies are widely used for research and medical diagnosis.

Final Acceptance?

Nevertheless, in toxicity tests, progress in completely replacing animals is frustratingly slow. All too often *in vitro* systems are seen as a preliminary means of assessment, to be carried out prior to final confirmation in animals. In the case of the Draize test, *in vitro* methods are used to identify and eliminate the most severe irritants. Although this can reduce animal suffering, and the number of rabbits used, it means that chemicals not thought to be severely irritating, go on for final assessment in animals. This is reflected in the British statistics: after falling substantially through the 1980s, the number of rabbits remained fairly constant thereafter. In 1995, 4,037 were used, just 179 less than in 1990.[39]

Many non-animal tests have been devised to identify human cancer-causing chemicals, and they too are seen as "pre-screens". Here, they are used to alert toxicologists to hazardous substances which are then subjected to animal experiments. The result is that the use of animals for carcinogenicity testing has only slowly declined.

The perception is that test-tube techniques must be superior to their animal counterparts before finally being accepted. Clearly it is necessary to make sure that any new test predicts human responses with reasonable accuracy but *in vitro* tests are subjected to far greater scrutiny than animal experiments have ever been! The process is known as "validation": a number of chemicals whose effects are already known, are assessed using the new technique. Some scientists have suggested a "pre-validation" stage to ensure that the validation process itself is properly planned. In fact, a scientific

workshop on the subject identified five main stages - test development, pre-validation, validation, independent assessment, and progression towards acceptance by official government bodies.[41] Actually devising a non-animal test seems a minor problem compared to getting it accepted.

The irony is that animal safety tests have never been properly validated[21] and even the briefest study shows only a 5-25% correlation between human and animal test results.[41] It should therefore be common sense that any new *in vitro* test is assessed using human data. Unfortunately most validation studies utilise previous animal research findings. Using vivisection as a basis to assess new methods imbues animal experiments with a validity they do not deserve. Furthermore, any lack of concordance between animal and *in vitro* results will reflect poorly on the alternative technique, yet it could be accurately predicting how people react.

New Generations

The Draize campaign demonstrated what can be achieved with sufficient motivation. This suggests that one of the factors necessary to change scientific attitudes is an informed public which finds the abuse and exploitation of animals unacceptable. After all, it was public opinion which persuaded many companies to adopt more ethical procedures with the result that, in Britain at least, the use of animals to test cosmetics is now illegal.

There are other ways to facilitate change: concerned individuals can influence the funding policy of medical charities, persuade governments to end specific animal tests, and carry Humane Research Donor Cards to improve the supply of human tissue for research. Public opinion is especially important in those areas where the alternative is simply not to embark on the research in the first place. For instance, in medical terms much pharmaceutical research is unproductive since 70% of new drugs offer no improvement over existing products.[42] The proliferation of "me-too" drugs, similar to those already available, does not significantly add to therapeutic options but does represent a comparatively safe financial investment since they require little or no innovation. The development of transgenic animals, for instance, to improve farm animal productivity, is again unwarranted because health studies stress we should be *reducing* our intake of animal products.

The second requirement for changing attitudes within the research community is a new generation of scientists who no longer regard animals as mere laboratory tools. So it is essential that students do not have to begin their careers by exploiting animals. And with the many alternatives available for education and training, including sophisticated models, videos, computer simulations, safe self-experimentation and *in vitro* techniques, it cannot be said that animal experiments are "necessary". Indeed, a survey of training courses by 246 departments of human medicine, veterinary medicine and natural science in Austrian universities, revealed that in only six cases were the use of animals compulsory. Two hundred and forty departments either did not think laboratory animals were essential for their courses or they practised alternative methods.[43]

Recognising the importance of education, animal protection groups are now putting great emphasis on student campaigns. Already the initiatives are paying dividends. In the United States, animal laboratories are no longer required by any civilian medical school for teaching purposes. In some of these medical schools the use of animals is now optional; in others the procedures have been discarded altogether. In Britain, dissection is no longer required by any school examining board, and has actually been banned in Argentina. And a survey of computer-based alternatives in undergraduate teaching in Britain and Europe, found that in 15 out of 20 university departments, students had objected to using animals. The survey acknowledged that "Although there has always been some degree of student objection to using animals, it has never been so apparent as in recent years."[44]

These are all important and encouraging trends, for the students of today are the scientists of the future.

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