Contemporary Themes

*Alternatives to Animal Experiments in Medical Research

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Summary

In laboratory work which involves the large-scale routine assay of biologically-active substances the sponsors of "alternative" (in vitro) methods and the scientists have a common aim: a reduction in the use of living animals. In vitro methods are usually more accurate, easier to perform, and cheaper. Animals are no longer required for the production of certain antiviral vaccines.

Those engaged in medical research where new knowledge is being sought also need no incentive to develop in vitro methods because they make refined analysis possible. Nevertheless, such in vitro methods are usually not alternatives in the sense that they substitute for animals; they complement experiments on animals. A legal requirement "that no experiment on a living animal may be performed if the purpose of the experiment can be achieved by alternative means not involving an experiment on a living animal" would be unenforceable.

The Cruelty to Animals Act (1876) regulates and restricts experiments which may cause pain to any living vertebrate and no experiment permitted by the Act may be performed without a licence from the Secretary of State. Misgivings about the use of living animals for medical research recently led to a proposal that the following words be added to the 1876 Act: "... it shall be a condition of every such licence that no experiment on a living animal shall be performed ... if the purpose of the experiment can be achieved by alternative means not involving an experiment on a living animal." This amendment to the Act was talked out at the report stage in the House of Commons¹ though the support it received will no doubt encourage attempts to introduce similar restrictive legislation in the future. Two other developments have been the establishment of a Fund for the Replacement of Animals in Medical Experiments (F.R.A.M.E.), which publishes abstracts of papers dealing with work in vitro to draw the attention of scientists to existing alternative methods; and the suggestion that an institute be set up to study and develop alternative methods.

We must be clear what the argument is about. The proponents of "alternative methods" do not deny that animal work is necessary; they do not challenge the claims that animal experiments have contributed to the prolongation of human life and to the alleviation of human suffering. They

simply argue that some and possibly a great deal of animal work is unnecessary and that the number of animals used in medical research could be significantly reduced either because alternative methods already exist or, if they do not exist, because it should not be difficult to develop them. Alternative methods mean, for example, the use of chemical methods for the assay of compounds having important biological activity and the use of organ, tissue, and cell culture in research.

In this article I should like to examine the notion that it is simple to identify particular alternatives and, therefore, in principle, simple to enforce them legally. I should also like to comment on the idea that the adoption of in vitro methods automatically does away with the use of living animals and consequently reduces the number of animals employed in medical research. Finally, I will make a proposal about the structure of the proposed Institute for the Study of Alternative Methods. The sectional headings are taken from the Hansard account of the second reading of the Cruelty to Animals Act 1876 (Amendment) Bill (11 May 1973).

"People will not search for alternatives as long as they have animals to use"²

It has been suggested that scientists are reluctant to employ or to develop methods which would replace the use of animals in medical research. This is a misconception. In fact, there are very strong incentives to replace animals by in vitro methods, especially in problems involving large-scale assays and in the manufacture of certain medical products. In these fields in vitro techniques are usually more rapid, more accurate, considerably cheaper, and their introduction has already resulted in a substantial reduction in the use of animals. Thus in this country the use of cultures of established lines of human cells has largely replaced freshly-prepared animal tissue in the manufacture of polio and rubella vaccines, though testing still requires the use of animals. Another development has been the substitution of radioimmunoassay for the older methods of bioassay for hormones. Immunoassays for more than a dozen peptide hormones have been devised and their great sensitivity, cheapness, and ease with which they can be automated not only ensure their routine use in clinical pathology but also allow them to be employed on a far greater scale than was ever possible with bioassays. Again, the technique does not do away entirely with animals since the antisera employed in the assays can only be raised in living animals. Further progress is also being made in the use of methods for the assay of antitoxins in vitro. It needs to be emphasized that cheapness, sensitivity, and reproducibility are not the only factors which will ensure the adoption of such methods. National and international statutory bodies have to be convinced that in vitro methods provide accurate and safe alternatives for the preparation and assay of medical products.

The large body of research which is concerned with physio-

¹Stephen Paget Memorial Lecture to Research Defence Society, 16 October 1973.

logical mechanisms and with the cause of disease also needs no legislative encouragement to employ *in vitro* methods. It is a natural progression from studies on animals to attempt to simplify the complexities of biological material by isolating their components *in vitro*. This may result in dangerous oversimplifications, but analytically such techniques can be very powerful. An example from my own field is the recent advance in our understanding of the cellular mechanism of antibody formation by carrying out the whole process from induction by antigen to antibody synthesis in glass or plastic containers.^{3,4} The way in which T-lymphocytes collaborate with B-lymphocytes in the induction of antibody synthesis will probably be elucidated by *in vitro* techniques and the stimulus for their development comes from immunologists themselves and needs no help from Parliament.

"If alternatives exist we must use them. If they do not exist we must find them"⁵

The argument in the preceding paragraph appears to suggest that there will be a time when the use of animals for medical research will be largely restricted to providing the ingredients for *in vitro* methods. Even in connexion with the assay of drugs there are many obvious reasons why this is not so. For example, radioimmunoassays, though very sensitive and accurate, may not discriminate between biologically active and inactive species of a particular hormone; it may be a waste of time to devise a chemical method for assaying a drug if it only becomes biologically active after modification *in vivo*; the rates of absorption of different preparations of a drug and consequently the varying concentrations achieved in the body fluids require studies *in vivo*.

For the layman there are less obvious reasons why animals cannot be replaced by *in vitro* methods in many areas of medical research which are concerned with the discovery of new knowledge about physiological processes and the causes and cure of disease. For the scientist *in vitro* methods are not alternatives to experiments on living animals in the sense that they are complete substitutes. Rather they provide a powerful way of analysing in isolation one facet of the situation *in vivo*, and the relevance of the findings must always be checked by returning to the whole animal. Experiments *in vivo* and *in vitro* in medical research are usually complementary; they do not present themselves as alternatives. For this reason the arbiter will find himself in an impossible position when asked to decide what is and what is not an acceptable alternative. Indeed, the complexity of the technical arguments which the Secretary of State, the Home Office Inspectorate, or the Courts would be called upon to consider in particular cases would make legislation of the kind proposed in the amendment to the Act completely unworkable. I would like to illustrate these difficulties with three examples from work with which I have been associated.

CANCER IMMUNOTHERAPY

There has been a revival of interest in the possibility of immunizing patients against cancer. This revival has been stimulated partly by the discovery that even highly-malignant tumours sometimes elicit an immune response in the human host which can be detected *in vitro* but which is, unfortunately, ineffective *in vivo*^{6,7}; and partly because tumour immunologists now have available several methods which may enable them to increase the immunogenicity of weak tumour antigens by first modifying the tumour cells *in vitro*.⁸ In Oxford we are attempting to immunize mice against highly malignant tumours by tumour cells which have been first hybridized with a cell partner carrying very strong antigens.⁹ For example, a mouse melanoma has been hybridized with

fibroblasts from a different strain of mouse so that the cell used for immunization carries both the weak tumour antigen and the strong foreign transplantation antigen on the same cell surface. This is a technique of immunization first employed by Watkins and Chen¹⁰ and we are simply trying to confirm and extend their findings. Having immunized our mice the problem is to discover whether the immunity we have raised is sufficient to protect against challenge with a tumour inoculum which will normally kill the mice.

Such assays employ many animals, many of which are killed by the tumour. A glance at the literature on tumour immunology will show that many workers are analysing the way in which lymphocytes, macrophages, and antibodies either alone or in combination are able to kill tumour cells *in vitro*.¹¹⁻¹⁴ Each of the four tumours we have used in our experiments can be stored in liquid nitrogen more or less indefinitely and can be grown up *in vitro* when required. In principle, very many tests could be performed *in vitro* employing the body fluids and cells from relatively few immunized mice.

Why then am I not employing this alternative method? The answer is that different workers have shown that different combinations of cells and antibodies can kill tumour cells *in vitro* but it is not known which, if any, of these combinations is relevant *in vivo*. In other words, no one would claim that *in vitro* tests provide an accurate forecast of whether a mouse has been effectively immunized.

A further point is that there is a great deal of difference between a naked layer of tumour cells in a plastic dish and a tumour *in vivo* with its fibrous stroma and its blood supply. The agents of destruction have ready access to the tumour cell in culture but they may penetrate poorly *in vivo*. Why then do I not follow the exhortation that "if they (alternatives) do not exist, we must find them." The answer is simple. Our present trial will be over by the end of the year but I have no idea whether I could ever devise an *in vitro* test which mirrors exactly the response of the whole animal; I only know I would expend thousands of mice in the attempt. The present *in vitro* methods are not "alternatives" at all. They are important in that they are providing information about possible mechanisms in tumour immunity and they complement studies on the response of living animals.

ANTI-LYMPHOCYTE SERUM

The Wellcome Research Laboratories are producing anti-lymphocyte serum (A.L.S.) for use as an adjunct to conventional immunosuppression in renal transplantation and a clinical trial is being planned. The production of a large batch of A.L.S. posed two problems which are common to the development of any new therapeutic substance for human use—namely, the development of reliable tests to establish that the product is both potent and non-toxic. A new therapeutic substance for human use must satisfy the requirements of the Safety of Medicines Committee and it is inconceivable that the material would have been passed had it not undergone extensive toxicity-testing in animals. There are similar requirements for toxicity-testing for a whole range of products to which human beings may be exposed—cosmetic, industrial, nutritional, as well as therapeutic, and it is to be hoped that the situation would never arise where one government department demanded animal tests while another insisted on alternative methods.

The second problem in the production of A.L.S. was that of assaying potency. Since the aim of treatment with A.L.S. is to prolong the survival of kidney homografts the arbiter of potency is the ability to prolong the survival of skin and kidney homografts in primates. These assays are expensive, tedious, and unpleasant. Unfortunately, despite a considerable international effort extending over several years no satisfactory *in vitro* assay for A.L.S. has been discovered.¹⁵

Several tests have been devised based on the effect of A.L.S. on lymphocytes in vitro but, as with the anti-tumour assays mentioned previously, none of them provides an accurate forecast of the efficacy of ALS in vivo. It is not for want of trying that no in vitro assay exists; it has just proved intractably difficult and no amounts of exhortation or legislation would have altered the outcome.

ANTIBODY FORMATION

The first volume of the abstracts of *Alternatives to Laboratory Animals*, published by F.R.A.M.E., contains a section on immunology among which a quarter of the references (five of the 19) concern techniques for studying the formation of antibody in vitro.¹⁶ The selection is interesting because it illustrates the confusion about what constitutes an "alternative." Thus, anyone wishing to repeat the work described in each of these five papers would have to hold a Home Office licence since each requires the production of special reagents in living animals. The method which has excited the most recent interest is that given in abstract 1 1 G18.¹⁷ Though not stated in the abstract the method described in this paper requires the preparation of spleen cells from mice which have been actively immunized together with spleen cells taken from other mice six days after the administration of 800 rads of whole body radiation followed by an intravenous injection of thymus cells and antigen. As has already been mentioned, this technique promises to add considerably to our understanding of the mechanism of antibody formation but though it is an in vitro technique it is hardly an alternative; indeed, it is a great consumer of experimental animals and by its very popularity may actually increase rather than decrease their use.

I mention the five abstracts concerned with the production of antibody because my colleagues and I have been interested over several years in the cellular basis of antibody formation but we have only employed an in vitro method on one occasion.¹⁸ This is because in vitro methods for the production of antibody do not show a significant switch from 19S to 7S production, they result in the production of antibody of predominantly low affinity, and they do not generate immunological memory. This would be the reply if I were asked why the bulk of our work has been performed on living animals. I make the point in this way to illustrate that arguments

whether an alternative is acceptable would be *exclusively* technical; common sense and legal training could not decide the issue.

"A research institute should be set up to look into this matter"¹⁹

My proposal for the Institute for the Study of Alternative Methods is that to be effective it must have an enormous animal house. In vitro methods not only require a continual expenditure of animals to provide the ingredients for the techniques but to validate them as substitutes for in vivo studies they must be continually checked at all stages of development against the responses of the animals they are attempting to replace. The complexity of biological mechanisms and the intractable difficulty of many of the outstanding problems in medical research make it not altogether cynical to suggest that there are not enough animals available in the research laboratories of the world today to decide whether they can be replaced by alternative methods.

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General Practice Observed

Home or Hospital Care after Myocardial Infarction: Is this the Right Question?

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Summary

This article attempts to lay down guide-lines for the general

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practitioner faced with a patient with a myocardial infarction in his home. A too rigid distinction between home or hospital care should not be attempted. Rather the general practitioner should look at home and hospital care and decide which is more appropriate in a particular case. In particular, he must distinguish clearly between cases he sees very soon after an attack and those he sees some hours later. Two hours is suggested as a useful dividing time between the two groups of patients.