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Alternatives to animal experimentation

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Introduction

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Well-planned and properly conducted experiments in animals have played a decisive role in the advancement of knowledge in biological sciences. The recognition of fundamental physiological processes governing the normal function of cells, tissues and organs, the elucidation of mechanisms of diseases, the evaluation of therapeutic agents or the development of modern operative techniques would have been impossible without the use of suitable experimental models in live animals. Despite numerous arguments in favor, however, animal experimentation is increasingly criticized not only by animal protection societies, but also by members of the scientific community. Beside rejection on principally philosophical grounds, the main objections against the current praxis in biomedical research include the alleged lack of correlation between findings in animals and man, the use of unnecessarily high numbers of animals in routine toxicity testing, insufficient measures to prevent suffering of animals, and the availability of 'alternative methods' to replace live animals in certain types of experiments. It is against this background that in 1982 the Swiss Federal Government decided to launch a coordinated research program (NFP 17) under the auspices of the Swiss National Science Foundation to promote the development and to evaluate experimental techniques suitable to reduce and, if possible, replace the use of animals. Out of a total of 37 projects submitted 10 of the most promising research plans were selected by a scientific advisory board for financial support for a maximum period of 3 years. The projects were started in autumn 1984 and concluded by the end of 1987. In the following multi-author review research groups describe and discuss their results, which were also presented at a symposium held at the University Hospital in Berne in April 1988. On that occasion, the use and acceptability of alternative techniques in the preclinical testing of drugs and other chemicals was discussed by an international panel of experts representing drug regulatory agencies of different European countries and the USA.

The search for alternative methods centered around three main themes:

1) Teratogenity testing by in vitro embryo cultures,
2) the use of cell cultures for toxicological evaluation
3) problems of infection and immunity.

In the two studies dealing with teratological testing it could be demonstrated that malformations of the cardiovascular, central nervous or skeletal systems after the administration of known teratogens, such as phenytoin, dexamethasone or cytotoxic agents, are rapidly recognizable in rat embryos cultured in human serum, and in chick embryos maintained under standardized conditions in an 'artificial egg'. The findings in both test systems correlated well with the types of malformations observed in classical teratogenity tests in live animals. Thus, such in vitro embryo cultures have a definite potential for reducing the number of animals required for teratological screening of chemically related new compounds. Only the least damaging agents have to be considered for further development and evaluated by standard in vivo methods.

Adult rat hepatocytes maintained in a medium containing 2% dimethylsulfoxide retained their functional capacity for several days and responded to an enzyme inducing hepatic carcinogen (nafenopin) as in the intact organism. Another test system using co-cultures of hepatocytes and rat liver epithelial cells stabilized the activity of metabolizing enzymes for more than two weeks. Observing the cellular DNA-content by flow-cytometry the effects of various chemicals on cell poliferation could be analyzed. Since hepatic metabolism is a major route for inactivation and elimination of chemical substances, such liver cell cultures may become an important test system for evaluating the biotransformation and poten-

tial toxicity of new drugs. A well-standardized method with aggregating cultures of fetal brain cells was also introduced to screen drugs for neurotoxic effects. Damage to brain cell development and differentiation could be demonstrated by measuring total protein and DNA content together with specific enzyme activities.

Among the projects dealing with problems of infection and immunity a study on the identification of enterotoxine producing bacteria with DNA probes is of particular interest. Gene probes for *E. coli*, *Shigella* and other diarrhea producing bacteria could practically eliminate the need for the hitherto employed ileal loop model in rabbits. In a further study, cultures of human neutrophils and monocytes were shown to be suitable for testing the activity of various chemical and biological substances on the function of these important blood cell lines. Stimulation or inhibition of enzyme activity was used as an indirect measure of phagocyte activity, which plays a decisive role in inflammatory disorders and in defense against infections. The test system allows a rapid screening of a series of compounds and thereby limits the use of animals for this purpose. Other projects included the development of cryo-preservation of helminth parasites (e.g. *Echinococcus* and *Toxocara*), allowing long-term storage of infective larvae without the need of serial passages in experimental animals and the introduction of an ELISA technique to assess the potency of rabies vaccines. Finally, the results of a statistical project on the use of robust regression methods are also reported. The authors describe the use of this type of statistical analysis for the evaluation and comparison of experimental data obtained by the previously mentioned embryo cultures and aggregating brain cell cultures.

The results obtained in the frame of NFP 17 are encouraging. It could be shown that a concentrated effort of scientists at universities and other research institutions may speed up progress in a field of particular interest, such as the development of alternative methods to animal experimentation. At the same time, however, the remaining problems concerning standardization and validation of new techniques also became more apparent. How do the different cell culture methods correlate with each other? Are they reproducible in different laboratories? Will the new techniques predict the risk of toxicity, teratogenicity and carcinogenity reliably enough to replace, or at least reduce, routine tests in live animals for drug development? These questions can only be answered by long-term comparative multicenter evaluations. As it was clearly pointed out by the representatives of drug regulatory agencies during the round table discussion at the symposium in Berne, in no European country nor in the USA would new drugs or other chemicals for human use be presently accepted without a series of established tests in different species of live animals. Thus, by developing new in vitro tests we have only completed an initial step in the right direction but much more work remains to be done to gain widespread international acceptance of alternative techniques. Further methodological studies and large-scale validation experiments should be encouraged and financially supported by governmental, industrial and private funding. Coordinated international efforts and cooperation of all interested circles are necessary to promote the cause of 'alternative techniques'.

Acknowledgment. The successful completion of NFP 17 within a very limited time is the great merit of the research groups presenting their data in this special review. A major contribution to this program was also made by the members of the scientific advisory board (M. Vallotton (president), E. Bonnard, S. Debrot, M. Dolivo, H. Flühler, F. Oesch, A. Steiger, H. Weber and E. Weibel). Their professional competence and cooperative spirit was an invaluable help to all participants. The excellent coordinative work of Dr F. Kästli at the Scientific Secretariat deserves a special mention.

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