

## SUMMING UP THE INTERNATIONAL WORKSHOP ON THE APPLICATION OF TISSUE CULTURE IN TOXICOLOGY

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### SUMMARY

A summary is given of the present state of the use of tissue cultures in toxicology, as it emerged from the workshop. The need for better education in *in vitro* toxicology and for extensive comparison of results with those from *in vivo* studies is pointed out. Some of the important difficulties in the application of tissue cultures to toxicological problems are highlighted, as are the needs for basic research on tissue cultures and those for better communication between scientists, legislators, money sources and laymen.

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The steadily rising use of ever new chemicals in all aspects of life, the demands from society, governments, legislators and regulators to get a grip on the consequences of the use of these chemicals for all living beings, as well as the justified demands for a reduction in the number of animals that have to be used for toxicological evaluations prompted the organizers of this workshop to invite scientists with interests in this area to gather to an informal meeting on the use of tissue culture in toxicology. The idea was to find out the present state of this rather young art (and when it comes to tissue culture the word "art" so far seems justified), its weak points, its sure points and its future possibilities.

A very obvious fact came forward almost immediately; the lack of proper education in this field. The participants came largely from other, admittedly related disciplines such as cell biology, biochemistry, neurochemistry, physiology, medicine, immunology etc. This led sometimes to unexpectedly stimulating discussions, including misunderstandings that had to be removed or, in other words, several of us probably were in for a few useful hours in school. Everybody present must have realized how necessary it is to create funds and courses for extensive teaching of *in vitro* (and other) toxicology and *in vitro* toxicological techniques.

The contents of the papers and posters presented are obvious from this issue and shall not be repeated here. A number of interesting questions and problems were, however, taken up in spontaneous discussions and more or

less spontaneous "round tables" which always strongly invited (and got) the participation of the entire workshop membership.

One such discussion concerned the need for and possibility to standardize key tissue cultures on an international level in order to make comparable results available for safety evaluation and the setting of safety standards. Efforts in this direction are going on at the level of international organisations but may run into difficulties as national bodies want their own testing. Before solving the problem of international standardisation much needs to be done concerning the selection of culture types and culturing techniques, including the development of reproducible cultures, cultures for studies on chronic toxicity, relevant markers etc. Extensive studies on the effects of large series of model chemicals with known toxic effects in other *in vitro* and *in vivo* systems are important here. Several such studies were described.

It was emphasized that a more vivid discussion between *in vitro* and *in vivo* toxicologists would be of great value. The obvious advantages of the *in vitro* systems (which apart from tissue cultures, single cell systems, isolated organs, tissue slices in such a discussion also should include model systems) are the possibility to study target-directed toxicity and carcinogenesis under defined conditions and to do this on human cell material, once such material is obtainable in a large enough number of laboratories. An *in vivo* toxicologist may argue that results obtained in the absence of blood flow, metabolism, detoxification reactions which may control the formation of reactive metabolites, excretion, hormone and nutrition variations are of limited value or not valid at all. Such assumptions must be analyzed in the light of a great number of results and in the light of time, cost and animal care viewpoints.

Toxic chemicals belong to essentially two groups: either they act directly on their target(s) or they require metabolic activation before they become toxic. The latter motivates the search for coupled or mixed tissue cultures including, e.g. cultured hepatocytes. The difficulties in keeping liver cell material active in a way comparable to intact liver was thoroughly discussed and it was pointed out that lipid peroxidation phenomena might cause trouble and that antioxidants should be tested.

Toxic effects of chemicals may be acute or chronic. Where studies of chronic effects are of interest — and they often are — the viability of tissue cultures becomes a problem, as do possible changes with time in their biochemistry and morphology. This emphasized the need for rigorous control of cultures and was but one example where the need of extensive basic research in this area was very obvious. Most presently available tests do not cover accumulative effects of chemicals.

It is hoped, as results become available from many chemicals and culture types, that it will be possible to relate specific patterns of damage with particular cell disorders. Such a possibility was well illustrated by the advances made during recent years in the study of neurotoxic effects of n-hexane and derivatives on neuronal cell cultures. A classification system based on the cellular target site of toxic substances was suggested. As pointed out

by Spencer, an understanding of the cellular target of, e.g. a neurotoxic substance would allow the prediction of the pattern of the caused neurological disease and the design of tests to detect subclinical defects in the cellular system affected. The study of morphological changes may further facilitate the identification of a particular organelle or cellular target and may help creating ideas on the mechanism of the pathogenesis. Biochemical investigations should form the next step in the analysis of the toxic action. Work with cell cultures in toxicology obviously must involve advanced techniques from a number of disciplines, such as advanced chemical-analytical techniques, electron microscopy including X-ray microanalysis, electrophysiology.

Toxic action in the nervous system, so highly important, presents problems which seem impossible to be solved with tissue cultures. Low dose-long term effects of neurotoxic agents may cause symptoms such as early ageing, behavioural changes, memory and learning changes, aggression, drowsiness, changes in awareness which we will not be able to study on that level for quite some time. Once we have any ideas about the molecular changes being behind these terms, the situation may change.

Among problems for the future the possibility of genetic alterations and manipulations of cells by chymera production, cell hybridisation, the insertion of genes and chromosomes etc. was discussed. The production of special cell cultures by genetic manipulation was seen as an interesting project. Another aspect concerned coupled in vitro-in vivo techniques, such as using the embryo for cell culture and the mother for metabolic activation studies.

In summary, there remains much to be done before we really can say that tissue cultures can be used for safe risk evaluation and before we can use them for more specific studies on the hazards caused by toxic agents. There is very good hope, however that they will be one of the most important tools in toxicology, once we know more about them. To achieve this, money is necessary. In view of the total cost for toxicity testing, funds for the development of reliable, reproducible, long living, biochemically in vivo-like tissue culture systems must be almost negligible.

Money for basic research often comes from other sources than money for goal-directed research. It is essential that scientists who are interested in the field of tissue culturing for whatever purpose including that of developing methods for reliable risk assessment talk to governments, legislators and the producers and consumers of chemicals — in their language — and make it clear to them that before we can provide them with what they want, we have further to study the cultures themselves and their properties and that money is needed for that. There is no point in hiding the need for basic research before we understand enough of the system to apply it for practical purposes; on the contrary this must be clearly explained and understood. By old tradition, scientists have felt that what they are doing cannot be told in words that can be understood by the public. That of course, is utter nonsense, having its roots in lack of training, arrogance and clumsiness.

Thus we have to make policy by interacting with massmedia, other

scientists, government bodies, unions etc. Apart from developing the science itself, money is needed for publications, for talking to each other, for books in the new area of tissue culture toxicology, for the organisation of national and international courses for scientists with previous experience from other areas and for students. The young are clearly interested in the questions at stake and their enthusiasm should be made productive. Today, we cannot yet answer an unlimited yes to the question of using in vitro systems instead of animals for most tests in toxicology. However, present knowledge is probably good enough for crude screening and good enough to allow the prediction of a bright future.

On a bridge for pedestrians over one of the main roads out of Stockholm, outside one of Sweden's major research institutes, someone has written "Don't cry, do research!". I very much agree, but would like to add "and write and explain".