Cells in culture in toxicity testing: a review[1]

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The possibility of using cell or tissue cultures as suitable material for testing agents for toxicity has often been suggested. Some of the technical problems attendant on such a procedure have been overcome but many difficulties remain. The background to the use of cultures and the current difficulties in their use in toxicity testing is discussed below.

Cultures

Media

One of the major difficulties encountered when animal cells were first cultured in the laboratory was bacterial contamination. This was overcome by adoption of rigorously sterile procedures which limited to a great extent the availability of the technique. This problem was resolved following the introduction of antibiotics and as a consequence tissue culture has become a standard procedure in many laboratories. Although plant cells can now be cultured in completely synthetic media, animal cells still require the medium to be supplemented with hormones and other factors which are usually supplied in the form of a natural product such as serum, although recent reports suggest that completely defined media may be developed for all eukaryotic cells.

Establishment of cell lines

If tissue of the desired type is cut into slices or small blocks and the tissue fragments incubated in a balanced salt solution containing one of a number of enzymes to break down the intracellular matrix (e.g. trypsin, collagenase, hyaluronidase) a suspension of cells is released which can be cultured *in vitro*. Relatively high yields of disaggregated hepatic cells have been obtained by perfusion of the liver with a medium containing mixtures of collagenase and hyaluronidase (Iype 1971, Seglen 1972, 1973, Williams & Gunn 1974). Cells released by these procedures are filtered through nylon mesh, concentrated by low speed centrifugation, resuspended in medium and placed in a tissue culture vessel. Generally, some of the cells become attached to the surface and this forms a primary culture of the tissue which often contains a number of cell types. Some of these cells quickly die, others persist but do not divide. Yet others may begin to proliferate and become a primary cell line. It is possible to subcultivate or 'passage' these cells many times; the culture is referred to as a secondary culture or an 'established' cell line. Some cells can be passaged indefinitely (tertiary cultures).

This whole progression may be smooth with successive selection of the cell strain showing the best adaptation to the culture conditions. Occasionally a sudden alteration ('transformation') takes place in a culture, often revealed by sudden changes in growth in some areas of the monolayer of cells.

Cell characteristics

A primary cell line possesses many characteristics of the original cells, such as similar chromosomal numbers and the specialized biochemical properties of the parent tissue, e.g. in the case of liver cells the ability to secrete albumin. In contrast, established cell lines invariably have different chromosome numbers and lose a number of specialized biochemical properties of the parent tissues. This latter fact imposes severe limitation on the usefulness of established

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cultures in the design of tests of toxicity. Nevertheless, established cell lines have been successfully employed in a number of fields of medical research. In particular, many aspects of modern virology have been developed using animal cells in culture. Most viruses will grow in suitable host cells in culture and this has permitted investigations into the biochemical events underlying the process of viral replication. The replication of certain viruses in particular cell lines can be useful in diagnosis, and the viability of virus-infected cells in culture can in certain instances give a measure of infection (i.e. titre). The ability of viruses to replicate in cell cultures has led to the adoption of this system for the production of vaccines.

Tests on cultured cells

Antiviral agents

The virus-infected cell culture offers a system for screening potential antiviral agents and permits the rapid elimination of any compound lacking selective action. Naturally, the detection of a compound exhibiting selective antiviral activity in the tissue culture system is only the first step and many of the subsequent studies would require the use of infected animals.

Agents affecting nucleic acid and protein synthesis

The growth of established cell lines in culture is accompanied by rapid nucleic acid and protein synthesis. The addition to the medium of appropriate radioactive precursors which are incorporated into proteins and nucleic acids permits accurate analysis of the turnover and processing of these molecules. Because high specific radioactivities can be achieved, the cell culture system has provided a great deal of information on macromolecular synthesis and has been successfully used to elucidate the mechanism of action of drugs which interfere with RNA and protein turnover. At this molecular level the tissue culture system has in many instances advantages over using whole animals.

Growth factors and 'transforming' agents

Cell cultures have been extensively employed in investigations into factors influencing growth. Cells may be readily transformed in the laboratory by oncogenic viruses and chemical carcinogens and as such provide a useful adjunct to whole-animal studies.

Screening tests on tumour cells

Attempts have been made to develop *in vitro* cancer chemotherapy tests using cultures of tumour cells (Riley *et al.* 1973). Knowledge of the sensitivity of a specific human tumour cell population to a range of possible antitumour agents could assist in deciding on the treatment required. Salmon *et al.* (1978) have reported a retrospective survey of cultures from 9 patients with myeloma and 9 with ovarian cancer in which no case that was resistant *in vitro* was found to be sensitive *in vivo*. The majority of cases where the cultures were sensitive *in vitro* showed a good response to the drug *in vivo*. There is little doubt that this approach is worthy of further investigation and is only limited by the ability to grow the tumour cells in culture.

Toxicity testing

It is obvious that tissue culture has provided a system which has aided in the progress of many of the fields in which it has been utilized. Could it make equal contributions in the area of toxicity testing? Toxicity testing is at present a time-consuming and expensive procedure which is, nevertheless, essential for the protection of man. It is an area where a false positive could be accepted but never a false negative.

Carcinogenicity screening with microrganisms

With the proposal that at least 80% of cancer has an environmental cause and with more than 40 000 man-made chemicals in commercial use, whole-animal testing cannot adequately provide a first screen for carcinogenicity testing of this magnitude. Thus, it is essential that faster and alternative techniques should be developed if a reasonable protection of man

against the environment is to be achieved. The test developed by Ames (1971) offers an interesting short-term test for carcinogenicity subject to the proviso that mutagenicity is equivalent to carcinogenicity and that, since most carcinogens have to be metabolized to reveal their carcinogenic action, a preparation of rat liver provides an activation similar to that which would occur in human liver. (Of course, this latter requirement is not more limiting than if whole-animal testing were used.) The Ames test employs a *Salmonella typhimurium* mutant which does not require histidine in the medium. If the mutant is exposed to a mutagenic substance it reverts to the original wild strain. The test system is combined with a liver preparation to activate the carcinogen and the test has been further developed with the use of organisms more sensitive to the mutagenic action of chemicals. Bridges (1976) reported results from an ICI study which gave a 90% accurate prediction of carcinogenicity on 120 chemicals tested. The validity of the test has more recently been criticized by Ashby & Styles (1978) and defended by Ames & Hooper (1978). Alternative tests depend on the postulated electrophilic nature of the active forms of the carcinogen and their ability to react with DNA. Williams & Rabin (1971) developed a test involving membrane – polysome association and in the ICI comparative study the predictability of the test was 72%.

Carcinogenicity screening with cell cultures

Since cells in culture are capable of transformation by chemicals they could also be used for rapid screening of carcinogens. One of the major difficulties is to determine satisfactory morphological and biochemical criteria that characterize cell transformation in culture. Pitot (1976) has listed the 'accepted' characteristics of culture cells transformed in culture. The ultimate test of malignant transformation is that following the introduction of the transformed cells into the appropriate host there is growth of a lethal neoplasm. If this criterion were to be adopted in screening there would be little advantage over administering the suspected chemicals directly to animals. For initial testing a more limited criterion of transformation must be accepted, such as altered growth properties of the cells in culture or their increased ability to grow in 1% agar. The growth change is most easily monitored in fibroblast cultures and the predictive accuracy of this system is similar to that of the Ames test. Since with fibroblasts one is employing a mammalian cell, it might at first sight appear more acceptable although most human tumours are carcinomas derived from epithelial cells. Although epithelial cells can be transformed in culture, transformation in this instance is not necessarily accompanied by the marked alterations in growth characteristic seen with fibroblasts and is therefore more difficult to monitor.

An alternative to growth changes in cells as a measure of detecting chemicals capable of interacting and damaging DNA is to examine incorporation of radioactive thymidine into DNA. Carcinogens damage the DNA and this is then followed by repair synthesis, i.e. 'unscheduled' DNA synthesis. Han & Stich (1975) have used this approach with cultured human skin fibroblasts with good predictability and now have incorporated a metabolic activation system making possible the detection of procarcinogens. Painter (1977) employed a similar approach with HeLa cells. Williams (1976) has proposed that liver epithelial cultures with their ability to activate carcinogens could be useful in screening for chemical carcinogens. However, activation appears to be labile and high activity is only found in primary culture lines; in addition, the growth criterion with liver cells is not easy to monitor, so it would appear at present that fibroblasts remain the cells most suitable for screening carcinogenicity. It is clear that cells in culture occupy an important position in the group of short-term tests for initial chemical carcinogen screening, particularly as it is possible to use human cells and thereby overcome at least one limitation of animal testing.

Toxic agents

Progress in the development of cell culture techniques for acute and chronic toxicity testing has been considerably slower than in carcinogenicity screening. The main reason for this lies in the wide range of primary insults that toxic agents evoke culminating in cell death, coupled with specificity of certain agents for a given cell type. Established cell lines have often lost the

specialized properties of the progenitor cells and as a consequence might not respond to the toxic action as *in vivo*. Thus, accumulation of triglyceride in tissues, a common response to injury which is the consequence of disturbances in lipoprotein secretion, would not occur in liver cells in culture which are deficient in the appropriate secretory mechanism. A similar problem would arise if a drug inhibited bile secretion, since its toxic action would then be the result of the accumulation of bile salts or bilirubin. This would not be detected in the tissue culture system. Another difficulty in determining whether a drug is cytotoxic lies in the sensitivity of the *in vitro* system. *In vivo*, besides metabolism of a drug, there are many other factors which determine its circulatory levels such as binding to serum proteins, renal excretion etc., and one cannot predict the final concentration that a cell will meet. *In vitro*, if the concentrations of most drugs under investigation are raised there is a level at which most, if not all, will become cytotoxic.

To some extent some of the disadvantages outlined above could be overcome by the use of a range of primary cultures. Continual availability of these systems is difficult but not insuperable, although a range of cultures which would cover all cell types is unlikely to be achieved. Nevertheless, with small groups of such cultures it would be possible to offer a reasonable prediction of whether a chemical under test is very toxic. Low toxicity would be more difficult to evaluate since high concentrations might evoke nonspecific effects which would not bear any relationship to the *in vivo* situation. This system might also give rise to false negatives.

Foodstuffs

In the area of human and animal foodstuffs the culture system has little to offer as the foods would have to be extracted and some purification of the fractions undertaken before testing. Thus, for example, without animal screening the aflatoxins would never have been isolated.

Conclusion

At present it would not seem that for general toxicity screening cells in culture can offer the potential seen in the testing for carcinogenesis. Nevertheless, challenge of a small range of cultures with appropriate dilutions of a new chemical may prove a valuable rapid indicator of major toxicity.

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