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Processes in cell culture

Introduction

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Many aspects of cell behaviour have been studied in vitro since Harrison[1] first successfully grew cells outside the body. Cell culture has enormous scope and much of the information obtained would still not be available to us without this technique; it also has its limitations. Care must always be taken when we attempt to extrapolate from in vitro findings back to the situation in vivo. Since Harrison first explanted a fragment of tissue onto a solid substratum, covered it with a nutritive medium, kept it in aseptic conditions for some weeks and showed that nerve fibres grew out in such conditions, culture techniques have been improved, extended and used by an increasing number of biologists. It is now one of the most widely used and possibly most successful methods in biological and medical research. By its application a very rich harvest of scientific information has been gathered. It cannot simulate the immense complexity of the conditions in vivo, but cells in culture can aid in understanding how cells function in the body and how they might be manipulated to control certain abnormal states.

Initially the technique acquired an aura of difficulty. Even in the late forties, although Honor Fell at the Strangeways had developed the technique of organ culture, Warren Lewis had filmed cells and Carrel, a surgeon by training, had, by using meticulous care and aspetic conditions, kept cells in culture for several years, many research workers believed that it was impossible to use the technique as a regular research tool. The advent of antibiotics led to an explosion of activity. Much of this activity was directed towards the idea of producing a defined medium in which to grow the cells, and a large body of tireless workers in this field are remembered by the names of media used in many laboratories today, Parker, Waymouth, Eagle, etc. etc., but to this day, except in the case of plants, at least for primary cell culture (cells direct from the embryo) the addition of serum or some similar product of animals is required.

A great increase in activity in what can truly be described as cell culture, rather than tissue or organ culture, has occurred since the fifties as a result of Moscona's work[2]. He demonstrated the efficiency of trypsin in dissociating the tissues of avian embryos and whilst he was concerned more with the reconstitution of embryonic tissues, other workers used his technique to obtain large numbers of cells and eventually to produce the cell lines which are now so reproducible and can be bought in vast quantities. It must always be remembered that there may be an important difference between cells of primary cell lines and established lines, sometimes established lines develop from the primary smoothly and gradually but sometimes there is a marked transformation stage. Transformation can also be brought about by viruses and since Rous' initial description of virus infection, a great body of work, important for its direct application to medicine in the production of vaccines, has also led to a vast increase in our knowledge of the changes involved in malignancy.

One of the really major breakthroughs due to tissue culture has been in the increased awareness of the importance of the ability of animal cells to move and

the factors influencing this ability. Although it was known that movement was important in embryonic development, and of course in wound healing, from very early times, it was difficult, if not impossible, to analyse such movements experimentally. Almost all the activity within the organism was inaccessible to direct observation. The cell locomotion involved is so slow that direct observation gives no immediate impression of anything going on.

In culture, cell locomotion is usually active and using time lapse cinemicrography it became possible to begin to formulate a general picture of the locomotory behaviour of cells and the factors influencing it. Abercrombie and Heaysman[3], as a result of studying many cells in this way, evolved the concept of contact inhibition of locomotion, the stopping of a cell in the direction which has produced a collision with another cell. It has been suggested that this could be a question of differential adhesion or a direct inhibition of the cellular machinery which produces cell locomotion. The problem is still not solved to the satisfaction of all, but has at least led to much work on differential adhesion[4] and on the locomotory mechanism, some of which will be described by J.M. Vasiliev. In the last few years, the latter study has been facilitated by the development of new techniques, particularly the use of fluorescent antibodies to localize specific proteins within the cells.

Anchorage to a suitable substrate is a fundamental condition for growth of normal cells in culture, the type of substrate, the surrounding medium and time all affecting cell locomotory behaviour, as F. Grinnell explains. The substrates used are often not at all like those the cells are associated with in vivo and the cells are usually growing on a 2-dimensional substrate. Many newly isolated tissues grow much better on a physiological substrate, such as plasma or collagen, whilst others appear to grow just as well on glass or plastic.

At the same time as many tissue culturists were working on locomotory behaviour others were more concerned with the possibility of investigating growth in controlled conditions. Mitosis as well as locomotion is important in embryonic development, indeed it is much more obvious and many old embryologists concerned themselves entirely with cell multiplication. Thus 2 distinct groups of workers appeared in the fifties and sixties, some concerned with locomotion, others with mitosis, with some resultant confusion. The phenomenon of contact inhibition of locomotion was for many years equated with contact inhibition of mitosis, or, as it is now designated, 'density dependent inhibition of mitosis'. We still do not know if these 2 phenomena are inter-related, but at least it is now generally accepted that they are not the same thing.

One of the main driving forces in the development of animal cell culture techniques has been the conviction that it could provide the means for coming to grips with the problem of cancer. Even before Harrison[1], Beebe and Ewing[5] had cultured an infectious canine lymphosarcoma in blood from resistant and susceptible animals in the hope of finding some radical difference in effect. A vast amount of information has been accumulated with regard to the differences in structure and behaviour between normal and malignant cells, as M. Mareel will describe, particularly since the increased use of tumour viruses in the early sixties.

Once again, the 2 approaches so typical of tissue culturists are obvious. Much research and discussion of malignancy tends to consider the problem as one of mitotic control or the loss of it. It is, however, universally agreed that invasion is one of the essential features of malignancy and here cell locomotion and its control must be of at least equal importance to mitosis. It has been suggested by Abercrombie[6], in a paper completed just before his recent death, that defective contact inhibition of movement of malignant cells contributes to their invasiveness. As he points out, there is no quantitative measure of invasion in vivo, one has to depend on the histological description, but some idea of malignant invasion can be observed in a simple tissue culture situation.

The application of knowledge gained in tissue culture to events within the organism remains extremely difficult, but although so much effort has been expended, it is probable that the technique is still relatively in its infancy. Tissues and cells have been grown from a relatively restricted range of animal species, though this range is continually being expanded, and only a fraction of cells have shown themselves capable of giving rise to strains which can be maintained for extended periods. Use of substances such as trypsin obviously remove, if only temporarily, factors from the cell surface. Care needs to be taken to avoid extrapolating even from one culture situation to others. The term 'cell adhesiveness', for example, has meaning only in relation to a particular method of assay. We must be very careful when we attempt to extrapolate from the 2-dimensional situation we generally study in tissue culture to the far more complex situation found in the organism, but the relevance of our in vitro studies to an understanding of in vivo cell behaviour is, or should be, of primary concern to the tissue culturist. Tissue culture should be considered as a means to an end, never as an end in itself.

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