Cytology and Evolution

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PREFACE

In some ways this is an unusual book. Unlike most text-books on cytology, it is much less concerned with the description of observed data than it is with their interpretation. An early training in zoology, a prolonged study of tissue culture with a temporary excursion into retinal physiology and colour vision, combined with a teaching career in histology and cytology have given the author an approach which is certainly unorthodox and may even be unique.

A description of the main classes of cells which appear in the outgrowths of simple tissue cultures is given in the first few chapters and this forms the starting point for a much wider discussion of certain aspects of cell physiology. A search is made for the origin and significance of these classes both in embryology and in phylogeny. It is then mainly the clues based on the phylogenetic evidence which are followed up, since these seem to be the more suggestive. They lead eventually to an investigation of certain versatile amoebae whose structure and behaviour are directly dependent upon fairly simple and universal factors in their environment. The possible influence of these same environmental factors, which are of such major importance to the amoeba, is then considered as a possible determinant for establishing the basic pattern for a simple colonial metazoan organism, and thus probably for the embryonic forms of the majority of invertebrates and, possibly, of vertebrates also. Emerging from this pattern, a tentative genealogical tree of the families of cells of an organism is then proposed and considered as a key to the manner in which the cells differentiate and to the manner in which they behave in tissue cultures.

The extraordinary importance of the immediate and local environment in the activities and differentiation of any cell is established in the first two parts of the book and the consequences form the basis for the rest. The theme is taken up in an analysis of the machinery which is at work in the various excretory and body-fluid-regulating systems in both invertebrates and vertebrates. By this machinery the tissue fluids in general and the immediate environment of the germ cells in particular are regulated in their own special ways. This study leads to several ideas on endocrinology which are likely to be very different from those current in most texts and which, though at this stage essentially hypothetical, may help in suggesting new methods of approach for further elucidation of this extremely complex field. Similarly, many features in the physiology and cytology of the retina are reinterpreted in the

light of the observations and hypotheses developed in the earlier pages. Homoiostasis is again the dominating idea.

In the final chapter a method for approaching the study of cells is broadly outlined. Emphasis is laid on the necessity for considering both the manner in which patterns of cell-behaviour have evolved phylogenetically and also for an awareness of the way in which cells react to their own very special little environments and of how these are themselves altered by other cells in the vicinity and may be very different from that of the homoiostatically controlled main body-fluid, namely the blood.

In a sense, the book is a series of essays designed to re-orientate current ideas and thoughts. In no sense is it an exhaustive text. The information assembled and discussed is culled from a wide variety of fields, and, since he cannot be an expert in all those fields, the author is frequently conscious of treading either on thin ice or on other people's toes. Nevertheless the ideas which have been developed seem to be of some considerable importance to all those who are interested in the physiology of cells. Embryologists and endocrinologists in particular are likely to find much that is unorthodox, and even the neuro-physiologist may need to question whether the micro-electrode is indeed the genuine Aladdin's lamp.

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Finally, the author wishes to acknowledge the kindness of all those authors and publishers who have allowed the use of illustrations from already published works, and, in particular, of those of his colleagues who have allowed the use of photographs before they were published elsewhere.

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Cambridge, England
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INTRODUCTION

THE conversion of the Victorian carriage into the racing car of today or of the 'Rocket'-type of locomotive into the 'Royal Scot' are object lessons in evolution. Step by step, some features have been modified and adapted; others have been discarded. Some entirely new characters have appeared, while certain basic structures have been maintained relatively unchanged throughout.

A knowledge of this history is not perhaps vital to the automobile or locomotive engineers of today, but it certainly helps the ordinary man to explain and understand the particular pattern which has evolved and it demonstrates the extreme changes which may occur by constant small 'adaptations'. Similarly a study of human embryonic development alone may be sufficient for the practising gynaecologist, but 'what do they know of London, who only London know?' A deeper understanding of the embryology of other animals may lead to better practice.

Step by step man has evolved from more primitive forms which are now for ever lost. So also have other animals. Nevertheless by combining a study of the evolution of a wide variety of animals, including man, with a study of their embryological development, certain basic patterns of the evolutionary process can be discerned; hypothetical, if not actual, 'common ancestors' begin to appear; and evolutionary history becomes more nearly a reality.

The basic units of the animal body are its cells, and, like the organs which they constitute and the animals to which they belong, they, too, have evolved. The evolution of animals is accepted and 'the outline of history' has been written; the evolution of the organs of the animal body is the basis of much zoological training; but the story of the evolution of the constituent cells and tissues is only beginning to be told. Cells are minute, and the methods of biochemistry, refined as many of them now appear to be, are, by contrast, gross. Fossils, which are so valuable in tracing the evolution of bones and organs, cannot help with cells. In recent years, however, the advent of new microscopical methods and the development of better physical and chemical techniques for studying cells in the living condition, and in their interactions with one another, have made the study of comparative cellular physiology a much more practicable and profitable pursuit and with its help some insight can be gained into the evolution of cells and tissues.

The growth of cells *in vitro* by tissue culture methods has thrown into prominence, for the author at least, many important features of cell physiology. For this reason, some account will first be given of cells as they are seen under these conditions. This will be followed by a discussion of some of the problems which are raised when an attempt is made to relate the observations on cell behaviour in tissue culture to cells under embryological conditions and to the possible steps in the evolution of different types of cells. Finally some rather speculative pictures of the cytology of certain peculiarly complex and interrelated physiological systems will be painted on very wide canvases with the use of the rather impure pigments which will have been ground and mixed in the first two sections.

CONTENTS

Chapter I

TISSUE CULTURE AND THE STUDY OF LIVING CELLS

In the animal body each organ is well known to be specially adapted for carrying out certain definite functions, and yet the whole animal is built to a precise plan characteristic of the species. In many ways, therefore, each species of animal must be considered as a somewhat special case and this specialization naturally entails particular problems for the physiologist. On the other hand, it is also well recognized that throughout the whole kingdom of vertebrates, for example, there is a common plan of organization, and that many of the general functions carried out by the different organs may remain much the same in all species; within this common plan, however, there may also be conspicuous differences in detail, from species to species, according to the special adaptations of the animal to its particular environment. These differences, unfortunately, sometimes tend to obscure the main pattern, for often, because of local conditions, human interest or some other essentially trivial cause, there is attached to them a much greater significance than they deserve in relation to the pattern as a whole.

The student of evolutionary cytology is therefore always up against the difficulty of deciding whether any particular structure or function has general significance, or whether it should be regarded as some aberrant specialization, produced by the organism in question to meet the peculiar demands of the environment, this last term being used in its widest sense. It need hardly be emphasized that the various tissues and structures in animals are often extremely complex and intricately organized, so that when the pattern of organization is only slightly modified there may be far-reaching secondary effects, and it is thus a matter of some difficulty to comprehend the nature of the primary modification and to separate it from the secondary results produced. At first sight, the adrenal medulla and a sympathetic ganglion do not appear as very similar structures, but, on further investigation of their embryological and phylogenetic development, the evolution of both from similar cells becomes obvious and the functions of both can be, at least partly, interpreted as variations on a common theme. The same might be said about the inner ear of man and the lateral-line organs of fishes, and about his thyroid gland and the endostyle of *Amphioxus*, and there are many other similar examples.

On the anatomical scale such homologues, among the vertebrates, as pectoral fins, forelegs, wings and arms are all, of course, now universally accepted; but on the cellular level there is still much that is mysterious.

While the classical methods of histology and cytology, with their accent on cell structure and histological organization, can contribute much to the picture of the evolution of many of the various organs of the vertebrate body, they cannot reach beyond a certain point, because functional differences do not always involve parallel and visible structural or morphological differences. It is therefore obvious that the newer techniques which give information about histo- and cytochemistry and, especially, about cell behaviour will, in the future, have an ever-increasing contribution to make.

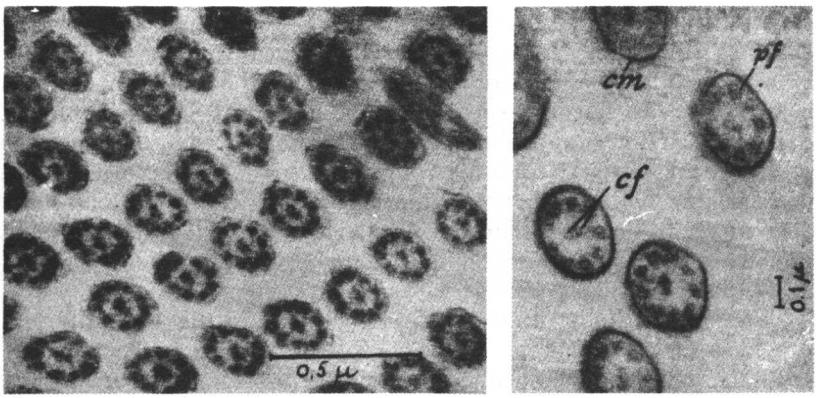
In the chapters which follow, the main accent will therefore be placed on those techniques and their results which allow cells to be investigated during activity, and a search will be made for those features of cell behaviour or their chemical activity which enable cells to be classified together as being similar on the one hand, or differentiated from each other in a fundamental manner on the other. Naturally, there is always the inherent difficulty of deciding what constitutes a fundamental manner and it is in elucidating this point that many of the real problems are raised. A few examples may illustrate the general standpoint which will be adopted.

Ciliated or flagellated cells occur in an extraordinary variety of situations in the bodies of animals belonging to nearly all the phyla. Yet, in all those animals which have been investigated except the Crustacea, the cilia and flagella, as seen by the electron-microscope and other optical techniques, seem to be built on a common plan, with variations of a rather secondary character (Fig. 1.1). They all have a core containing nine peripheral fibres, which are often double, and two central fibres. Is the conclusion to be drawn from this that there is only one possible type of flagellum, or is it that the essential flagellar pattern was developed early in evolution and has continued to be inherited as a useful potentiality by certain groups of cells, even if it is not actually called into play? Surely the latter is the more likely explanation, even if it does raise difficulties concerning the distribution of ciliated cells in different regions or parts of animals and with regard to the factors which cause their appearance or differentiation.

Similarly, muscular contraction and the presence in the cell of the proteins actin and myosin seem to be closely linked; are these proteins the only possible contractile elements? Or is their production again a primitive basic trait which has been handed on from one generation to

another and from one animal to another to be produced, or not, according to circumstances and, when it is used, to be incorporated into special mechanisms adapted to serve particular ends?

The study of any one animal in isolation, whether it be man or amoeba, does not allow the wood to be seen for the trees. The specialization cannot be differentiated from the fundamental and common plan. Consequently it is necessary to try to extract the skeleton-key for the evolution of an organ or structure by studying features common to large numbers of organisms, and in studying the cells of that organ it may often be advantageous to rob them, if possible, of some of their higher flights of specialization so that their more fundamental basic pattern may emerge more clearly.



(*a*) FIG. 1.1 (*b*)

Electron-microscopic photographs of cross-sections of (*a*) Cilia from a mammalian renal tumour and (*b*) cross-sections of cilia from *Paramecium*, showing the typical pattern and arrangement of internal fibres. (Mannweiler and Bernhard, 1957; Sedar and Porter, 1955.)

Fortunately it seems likely, when tissues are grown by certain of the "tissue-culture" techniques, that the constituent cells almost immediately lose some of their morphological and physiological specializations, either temporarily or permanently, and such "simplified" cells may then be able to point to the road along which they have evolved. Admittedly, tissue-culture techniques are not without their specializing actions on cells, for cells have an extraordinary capacity for adapting themselves to their surroundings and, within limits, of adapting their surroundings to themselves. Indeed, evidence is accumulating from the study of "pure strains" of cells that prolonged life *in vitro* leads to many fundamental changes in cell activity, which can be profitably regarded as

adaptations to the particular conditions of cell culture. Nevertheless, judicious comparison between the behaviour of cells in tissue culture, in embryological material, in pathological states, and their behaviour in corresponding or homologous tissues in a wide variety of organisms may often throw the main patterns of cell behaviour into relief. The actual meaning to be applied to the term homologous will have to be considered rather more closely at a later stage.

In bacteriological studies, it has, of course, become increasingly obvious in recent years that bacteria can often become adapted very quickly to life in surroundings which were at first unfavourable to them, even to the extent of producing new enzymes to deal with unfamiliar substrates. Whether this occurs by selection of favourable mutants or by some more directly adaptive mechanism is not always clear but the facts are undisputed, and there is very good evidence that enzymes can actually be produced *de novo* to deal with new substrates. How far similar adaptability is possible among the cells of metazoa is not yet so clear, but it must certainly be kept in the foreground as a possibility, and even a probability, when we are considering how cells could behave when the conditions of their life are altered. For example, there is now evidence that two clones of human cells, both originally derived from the same single cell, have developed quite different properties, one strain being highly malignant, the other not malignant at all (Sanford, Likely and Earle, 1954).

The critical study of cells in tissue-culture can thus, as indicated, provide a first approach to the study of the cells of the higher animals, and this approach is both illuminating and provocative. Some of the various methods used and the results obtainable with them must therefore be first reviewed in outline, for the behaviour of cells in culture depends very greatly on the method of culture employed. Each method imposes different conditions on the cells, and it is necessary to appreciate these effects in translating results from tissue-culture experiments to the behaviour of cells in the animal. Tissue culture, even if it does nothing else, succeeds admirably in emphasizing the plasticity and vital qualities of cells on the one hand, and in pointing towards the existence of a limited number of extremely characteristic and probably fundamental modes of cell behaviour on the other. It is this latter characteristic which is of peculiar interest in the study of evolutionary cytology.

The Method of Tissue Culture

The development of the tissue culture method might have occurred long ago had it not had to wait for two main developments of technique

and of knowledge in other fields. For all tissues from warm-blooded animals a satisfactory aseptic technique had first to be evolved. Tissues isolated in culture have only very limited defence against bacteria and moulds, so that either strict asepsis or the use of antibiotics is necessary and both are nowadays commonly employed. Secondly, the tissues of higher animals only survive and remain active in rather special media, so that much had first to be learned about balanced salt solutions, nutrients, growth stimulants etc., before any real progress could be made in the way of keeping tissues alive outside the body for any length of time. Although there had been earlier beginnings (Loeb, 1897, 1898), Ross Harrison (1907, 1910) is credited with the first successful tissue culture, when he obtained growth, of amphibian nerve fibres, *in vitro,* in a medium of clotted lymph. From these simple beginnings, progress was at first rapid, and numerous techniques, each with its own special application, were quickly developed. To the pioneering work of Carrel and his school at the Rockefeller Institute from about 1912 onwards are due many of the main methods of tissue culture (Carrel, 1912, 1913, 1923). Indeed, this school, together with that of Fischer (1930) in Copenhagen, produced much of the basic description of types of cell behaviour *in vitro.* In England, the division of cells *in vitro* was first described as a continuous process by Strangeways (1922) who, together with Canti (Strangeways and Canti, 1927; Canti, 1928), produced in 1927 a revolutionary, and now classical, cine-film which brought this process, and indeed many other essentials of cell behaviour, vividly before the eyes of the world. Much of the early work was carried out on embryo-chick tissues because these could be readily obtained in a very actively growing state, uncontaminated with bacteria, etc. The medium used for the growth of many of these pioneering cultures was a mixture of fowl blood-plasma and an extract of crushed chick embryo in a physiologically balanced saline solution containing Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, PO_4^{---}, HCO_3^- and glucose. Fowl plasma was used because, unlike that of mammals, it does not clot readily on standing, but produces a firm coagulum in the presence of tissue juices. The "embryo extract" contains both blood-coagulating and growth-promoting factors and can be readily obtained in a sterile condition. The most strict asepsis was preserved throughout the whole technique. This was many years ago and the position has now greatly changed. Since 1950, various synthetic media, in which all the constituents are of known chemical composition, have been successfully developed for certain strains of "normal" cells (Morgan, Morton and Parker, 1950; Evans *et al,* 1953; Healy, Fisher and Parker, 1954; Waymouth, 1955; Morgan, 1958); antibiotics are also extensively used

nowadays to simplify the complicated techniques of asepsis, though there are certain limitations and possible dangers in their use. For example, the sulphonamide drugs, while relatively harmless to cells *in vitro* in comparison with their action on bacteria, do produce a reversible inhibition of cell division when used at concentrations of more than about 1:1,000. (Jacoby, Medawar and Willmer, 1941). Sulphanilamide itself is also known to poison the enzyme carbonic anhydrase.

Tissue culture today thus promises to become much simpler and to have more of its initially numerous variables under control. In consequence, more and more problems seem to be capable of solution by its use.

The primary aim of tissue culture is to maintain cells and tissues alive, active, differentiating, functioning, or growing under conditions in which the various processes can be observed directly or indirectly, measured and analysed in a situation uncomplicated by the presence of the interfering influences of other tissues in the body. Naturally the method can be applied to cytological, histological, embryological, biochemical and pathological problems and appropriate techniques have consequently been devised for particular purposes.

It is not necessary to describe the details of all the numerous techniques available; but some of the more important conditions of culture must be mentioned, so that the results obtained with them may be evaluated. The simplest technique is that known as the hanging-drop. In this, a small fragment of tissue (a few cu. mm. only) is placed in a drop of a physiologically balanced salt solution or nutrient medium, very often mixed with blood plasma, on a coverslip, and the whole is inverted over a hollow-ground slide and ringed with paraffin wax in order to maintain an air-tight seal. This, in essentials, was the original technique used by Harrison for the express purpose of finding out whether nerve processes grew out from nerve cells in the spinal cord of the frog, and by which he produced a major piece of evidence in support of the neurone theory of the central nervous system. By this method he was directly able to observe under the microscope how, after a few hours, the processes of the cells thrust themselves outwards as nerve fibres, by a sort of amoeboid movement, into the coagulated lymph which he used as medium (Fig. 1.2). Incidentally, the essentially amoeboid character of the terminals of the nerve processes which he then described is something which is perhaps insufficiently appreciated by neurologists and neurophysiologists of the present day as a potential property of nerve cells within the living body. Conditions *in vivo* and *in vitro* are, of course, very different, but it is unlikely that complete stability of the processes of nerve cells reigns in the body.

The hanging-drop method, with modifications, is eminently suited to cytological studies of short duration. Unless, however, the medium is renewed at frequent intervals or the tissues are transplanted to fresh medium, a limit of a few days only is set to their activity, by the scarcity of oxygen, high CO_2, lack of food, or the accumulation of toxic metabolites. In the early stages of such cultures, cells of various types may

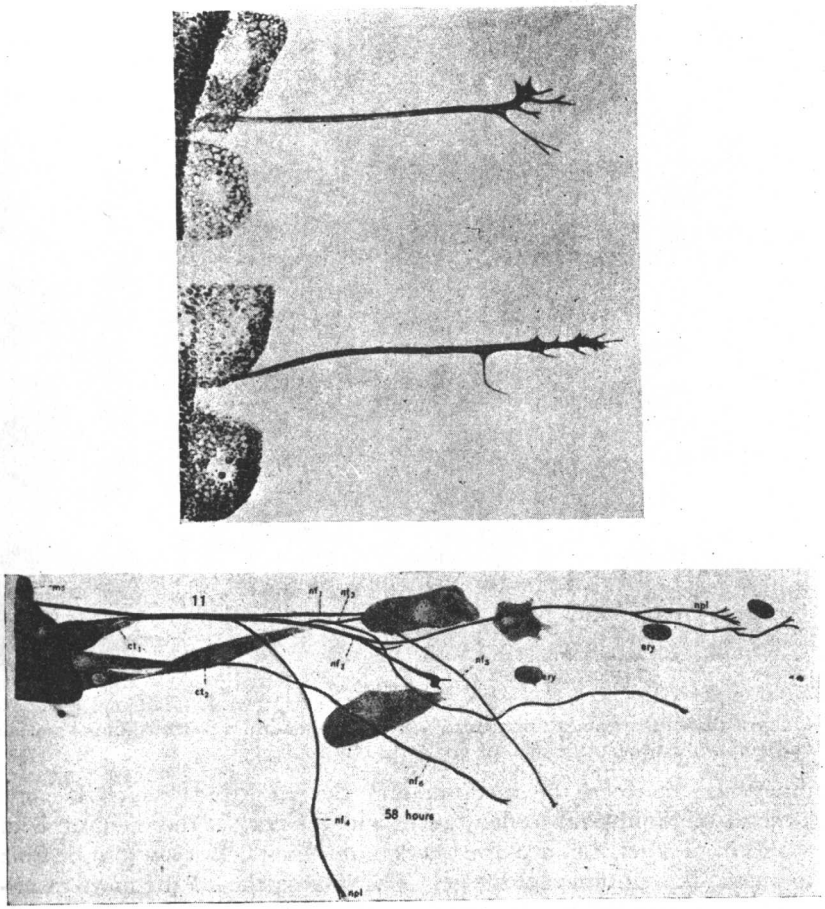


FIG. 1.2

The outgrowth of nerve fibres from the spinal cord of a frog in a medium of clotted lymph.

ms—central mass. nf.—nerve fibre. ct—connective tissue cell
ery—erythrocyte. npl—protoplasmic end of fibre (Harrison, 1907, 1910)

be persuaded by suitable media, temperature, etc., to emerge from the original piece of tissue (Fig. 1.3), especially if this is embryonic in character, or if more adult tissue has been previously exposed to the action of a dilute solution of trypsin (Simms and Stillman, 1937). On the whole, the younger the tissue the more actively do the cells emigrate and, for this reason, embryonic tissues have been most studied. The outgrowing cells mainly creep upon the surface of the glass or on the

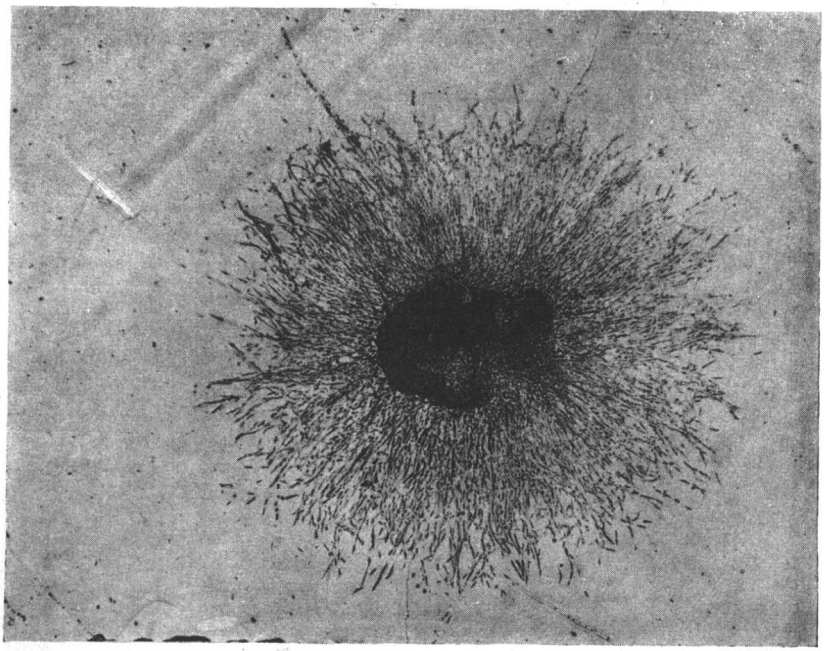


FIG. 1.3
Outgrowth of cells from a fragment of chick perichondrium in a medium of plasma and embryo extract. (Photograph by F. Jacoby).

interphase between the medium and the air. Cells which become loose in the fluid tend to float away and get lost. If the medium is in the form of a gel, e.g. a plasma coagulum, then cells may also be able to invade the substance of the gel. The thinner the gel the more extensively is it invaded by the growing cells. Since, however, some cells liquefy the plasma coagulum, gels which are too thin sometimes break down, and thus there is a limit to the extent to which the blood plasma can be diluted for use as a supporting framework for the cells of each tissue.

In hanging-drop cultures the cells can be directly observed under the highest powers of the microscope, either with direct illumination or with such devices as polarised light, dark-field illumination, phase contrast, or other similar methods. They are also immediately available for experimental study, by microdissection, microinjection and most of the cytochemical techniques. Even more important, perhaps, is the fact that under these conditions the activities of the cells may be photographically recorded on cine-films, or directly on recording paper, either at normal speed, or at some higher speed when the processes to be observed are slow; the photography can also be combined with a stroboscopic device to record any rhythmic movements which may be occurring at too high a frequency for direct observation, as in the beating of cilia.

Although the cells under these conditions are ideally situated for study from the optical point of view, there is one major difficulty. The cells which migrate outwards from the central explant are not often immediately recognizable in appearance as normal constituents of the tissue explanted: in other words, they almost immediately change their shape and pattern as they leave their normal position. Thus, it must always be remembered that the cells which emerge are, in general, not physiologically, and certainly not morphologically, identical with the original cells of the tissue. Not only do the cells themselves change, but also the histological organization of the original tissue ceases to be maintained in the zone of outgrowing cells; it may, however, be so retained in the main mass of tissue, to a greater or less extent depending on the conditions of culture. The change in the outgrowing cells nearly always involves a simplification of their morphology. Moreover, the character of the change in the cells depends on the mechanical and physico-chemical properties of the medium into which they migrate; it depends also on whether the cells find a suitable surface upon which to cling, for when they become detached from the tissue into a fluid medium they immediately round up and remain relatively inert at the bottom of the drop, just as the white cells in the blood are spherical while they are in the circulation, but become more or less amoeboid when they make contact with a surface which they "wet" and to which they can adhere. This inertness of isolated cells may be only relative because it has now been found possible to maintain active growth in cells kept in a constantly agitated fluid medium where the cells are freely suspended (Earle, Schilling, Bryant and Evans, 1954, 1955; Owens, Gey and Gey, 1954).

Cells in culture, to some extent following the lines of least resistance, tend to flatten on to surfaces, to become spindly when enclosed in the