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# Contents

# Introduction

## *1.1. Background*

Although a number of books are available giving a great deal of information about various aspects of cell culture (Harris, 1964; Willmer, 1965; Habel and Salzman, 1969; Vago, 1971; Whitaker, 1972; Fogh, 1973; Sato, 1973; Paul, 1975; Kuchler, 1977) this book is designed rather for the biochemist or molecular biologist, whose interest in cell culture extends only as far as this technique provides him with material with which he may perform biochemical experiments.

Before a biochemist will apply himself to using the technique of cell culture he must be assured that it offers him significant advantages which outweigh any disadvantages. Furthermore, he must not imagine that the methods are too laborious for routine use or that some degree of black magic is required before success can be achieved. To some extent such fears are based on the experience of workers in the field up to about 1960. In the preceding thirty years nearly all major cell types had been cultivated for varying periods and much descriptive information obtained but this was only as a result of constant dedicated effort.

Since 1960 many of the obstacles have been removed from the path of the biochemist as a result of three advances. Perhaps the most important is that commercial companies now supply media, sera, cells and culture vessels which enable cells to be cultured occasionally or routinely on a scale varying from a growth surface of less than one square centimeter up to several square metres. This

service is available only as a result of the description of simple media in which the cells grow well and the development of simple methods for isolation of primary cells, selection of clones and storage of cell lines.

The other major fear is one of cost. On a weight for weight basis cultured cells are several orders of magnitude more expensive than, say, rat liver. Thus a rat costs under £2 and yields about 10 g of liver. $10^6$ cells obtained from a commercial supplier cost about £1 and so 10 g of cells ($2 \times 10^{10}$ cells) could cost £20,000 although significant price reductions are obtained when buying in quantity. One can produce the same number of cells in one's own laboratory for less (the cost of medium may be as little as £15), but this hides the cost of overheads. Nevertheless, the use of cultured cells must offer marked advantages before it is worthwhile to embark on large scale production, and there is seldom any justification for using cultured cells as a source of material for an enzyme purification when sources like rat liver or rabbit kidney would do as well. From the other point of view many experiments may be performed with $10^2$–$10^6$ cultured cells at a cost equivalent to the alternatives.

## *1.2. Some advantages*

One major advantage offered by cultured cells which cell biologists make full use of but which is often ignored by biochemists is that the living cells may be watched under the microscope. It is essential that healthy cells are used in an experiment and that they remain alive throughout the experiment. That this is the case may be monitored regularly and moreover quantitative estimates of the proportion of viable cells are readily obtained. It is often impossible to know the state of an animal's kidney until the end of the experiment and then usually only in a qualitative manner.

Cells in culture offer a homogeneous population of cells of virtually identical genetic make-up growing in a constant environment. Moreover, the environment may be changed, within limits, at the whim of the experimenter who may thereby investigate the

effect of pH, temperature, amino acid and vitamin concentration etc. on the growth of the cells. Growth may be measured over a short time period either by measuring an increase in cell number or size, or by following the incorporation of a radioactive tracer into DNA. These are real advantages over a whole animal system, placing cultured cells on a par with microorganisms as an experimental system. Using cultured cells, the growth requirements of human cells were analysed in a few weeks thus confirming decades of work with people of different genetic background living in different environments (Eagle, 1955a, b; see § 2.2).

Moreover, significant results may be obtained with very few cells. An experiment which may require 100 rats or 1000 humans in order to clarify some point may be statistically equally valid if 100 coverslip cultures are used. If each cell is regarded as an independent experiment then one coverslip culture may yield more reliable results than a hospital full of people. This is obviously a major advantage as far as man is concerned but also overcomes the ethical problems which often arise when large numbers of animals are used for experimental purposes. However, in the final analysis, many experiments must be performed on whole animals, but this is no justification for not using cultured cells for the preliminary work.

Because cells in culture are easily available for manipulation by the biochemist, radioactive tracers, drugs or hormones etc. may be applied in a known concentration and for a known time period. The amounts of such compounds required may be an order of magnitude less than with comparable experiments on whole animals. There is no fear that the drug whose effect is to be investigated is being metabolised by the liver, stored in the muscles and excreted by the kidney. It is usually a simple matter to establish that a substance added to a cell culture remains in contact with the cells in unchanged form at a known concentration for a given time. This enables experiments to yield realistic figures for the rates of incorporation or metabolism of compounds. Such experiments are not without hazards in cultured cells (see Chapter 12) but are very difficult to interpret in whole animals. However, when the aim of the experiment

is to find the effect of a drug or cosmetic on an animal, factors which are problems to one biochemist may be the essence of the experiment to another.

## *1 3. Applications*

Cultured cells have given us great insight into the phenomena of cell growth and differentiation and the general characteristics of the growth of cultured cells are discussed in Chapter 2. It should be clear, however, from reading later chapters that, although the detailed nutritional requirements and growth control mechanisms are complex, it is now a simple matter to culture cells in small or large quantities in order to perform biochemical experiments.

### *1.3 1. Differentiation*

The study of differentiation in higher eukaryotes is extremely difficult, but a number of systems are now available which undergo differentiation in vitro and some of these are considered in Chapter 15. The in vitro systems have the advantage that, following a given stimulus, a population of cells will undergo a change which can be easily recognised and quantitatively monitored. The change may be the production of a protein (e.g. haemoglobin by the Friend cells – § 15.1) or more complex alterations in structure and growth pattern such as those occurring during differentiation and fusion of myoblasts (§ 15.5) or differentiation of epidermal keratinocytes to form a system resembling the stratum corneum of skin (§ 15.2).

A large number of cell strains have been derived from the central nervous system including differentiated glial cell lines (Benda et al., 1968; Lightbody et al., 1970; Ponten, 1973) and neuronal cell lines (Augusti-Tocco and Sato, 1969). In addition, Pfeiffer and Wechsler (1972) have isolated clones from tumours of the peripheral nervous system (neoplastic Schwann cells). A book (Sato, 1973) has been written about the special techniques required for the culture of cells of nervous origin. Neuroblastoma cells in culture will extend neurites and, when cultured with differentiating myoblasts, synapse forma-