Section of Comparative Medicine

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Studies on Cells in Culture

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Continuous-flow Culture of Mammalian Cells

The cultivation of animal cells in thin cell sheets on glass surfaces, known as the monolayer technique, is the simplest and most reliable method for the mass cultivation of animal cells. In contrast, the cultivation of animal cells in suspension, which was developed just after the monolayer method, has proved far less reliable or predictable. This implies that the conditions required for suspension culture are more exacting than those for monolayer culture but which conditions are more exacting remains to be discovered. Despite this drawback the suspension culture technique is of the greatest interest because of its numerous advantages for the study of cell physiology. One of these advantages is that it permits continuous-flow culture; by this means we can hope to learn more about the requirements for growth in suspension culture and consequently to make it more predictable.

In continuous-flow culture the cell may reach equilibrium with its environment or, more accurately, a steady state while it is growing and this greatly facilitates the determination of environmental influences. During the growth phase of batch culture the cell rarely, if ever, reaches equilibrium with its environment. The conditions under which this equilibrium may be reached have been discussed elsewhere (for a review, *see* Gerhardt & Bartlett 1959). In order to maintain a steady state, the cell must be able to react instantaneously to a change in its environment, say the concentration of a nutrient. However, it has been reported (Cohen & Eagle 1961) that in continuous-flow cultures of HeLa cells at low flow rates oscillations occurred in the population density, whereas at higher flow rates steady states were obtained. In my laboratory no oscillations were induced by low flow rates but they did occur in ERK cell cultures when the flow rate was *suddenly* increased by a large amount (from about 0·5 to 1·0 culture volumes per day). In L cell cultures a similar alteration in the flow rate of a culture in a steady state led to an inhibition of growth, the growth rate, instead of increasing as the theory predicts, fell by about 20% and remained at the lower level for at least two days. Finn & Wilson (1954) have shown that oscillations in the population will occur if there is a lag in the response to an environmental change. Oscillations are not generally met with in bacteria or mould cultures. Animal cells may show a lag in response to environmental change as a result of greater complexity of function.

The continuous-flow technique of greatest value is the chemostat type in which the dilution rate is fixed and the population density is allowed to find its own level. The 'turbidostat' technique in which the population density is fixed and the dilution rate is allowed to find its own level is of great interest but this technique has not proved sufficiently reliable in maintaining control over the population density. Steady states have, so far, only been achieved by the chemostat technique. Animal cells have been cultivated by a continuous-flow technique which retains all the cells in the culture vessel (Graff & McCarty 1957) but this method does not allow equilibrium to be reached between the growing cells and their environment.

The culture apparatus used in this laboratory and the results obtained have been described elsewhere (Pirt & Callow 1963). Over periods of two weeks population densities of L or ERK cells in continuous-flow cultures could be maintained within $\pm$ 15% of the mean at a flow rate of 0·3 culture volumes per day. The medium consisted of amino acids, vitamins, salts and serum. Variation of this order was hardly significant. A comparison of the cell production rates in continuous-flow and

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Table 1
Cell production rates

	Continuous-flow		*Monolayer*	
Cell type	*Maximum yield (millions/ml)*	*Production time (days)*	*Maximum yield (millions/ml)*	*Production time (days)*
L	1·5	2·5	0·9	3
ERK	0·6	3·3	0·9	3

monolayer cultures is given in Table 1. The yield and rate of production of the L cell was higher in continuous-flow culture than in monolayer culture, but the reverse was the case with the ERK cell. Apparently the optimum conditions for growth of the ERK cell were not realized in continuous-flow culture.

There was much variation in cell yields. Control of the variation seems to require identification of the growth-promoting factor in serum which is essential for optimum growth of nearly all animal cells. This perhaps is the outstanding problem in the *qualitative* definition of nutrients. The *quantitative* relationships between cell yields and nutrient supply remain almost entirely unknown. Contributions to knowledge in this field may be expected to lead to increases in population density and rate of growth. The development of animal cell culture should soon require the application of the more elaborate continuous-flow culture techniques such as: (1) Multi-stage systems necessary for processes which have successive stages requiring different environmental conditions. (2) Feed-back of some of the cells produced so as to give a higher population density and at the same time maintain a steady state.

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Growth of Baby Hamster Kidney Cells in Suspension

In recent years the widespread successful use of monolayer tissue cultures has considerably furthered our knowledge of cytology and virus/cell interaction. Monolayer techniques are, however, limited in some respects, especially in large-scale applications for biochemical and biophysical manipulation and for the production of virus vaccines. Because of these limitations, interest has arisen in the culture of suitable tissue cells in suspension. Considerable use of suspension culture has already been made in bacteriological research and in brewing, but translation of the principles developed in these fields to cells which *in vivo* normally have spatial connexions with their neighbours has not always been successful. As a consequence of the difficulties involved in vessel design and the lack of fundamental knowledge of the principles of tissue cell suspension cultures, most workers have used cells of tumour origin, which are less exacting in their environmental requirements. This has resulted in the impression that only cells of tumour tissue origin will grow satisfactorily in suspension culture and the technique has fallen into disrepute because of the limitations imposed by this type of cell.

The baby hamster kidney cell strain (BHK 21) originated from normal hamster kidney tissue and the early monolayer transfers of the cloned strain (clone 13) were considered to be diploid (MacPherson & Stoker 1962). Since Capstick *et al.* (1962) first described the growth of the cloned strain in suspension cultures, we have concentrated on determining the precise environmental conditions for optimal growth of the cells in vessels commonly used for bacterial cultivation. This paper records the results of these experiments.

Methods

The cells were grown as batch cultures in vessels of two designs: a small glass flask containing 200 ml of culture fluid and a 5-litre vessel similar to that described by Elsworth *et al.* (1958). The vessels were agitated mechanically and pH was controlled by manual adjustment of the proportions and flow rate of the CO_2/air surface aeration mixture. The medium consisted of Eagle's basal medium modified to contain twice the normal concentration of amino acids and vitamins with the addition of 10% Seitz filtered bovine serum, 10% tryptose phosphate broth and 0·1% methyl cellulose. Antibiotics – penicillin, streptomycin, neomycin and mycostatin – were usually incorporated in the medium. The growth characteristics of the cells were similar in both designs of vessel.