# Large-Scale Cell Culture in Biotechnology

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**The purpose of this article is to review the current state of large-scale cell culture in terms of its applications, problems, and potential. Because of the commercial and proprietary nature of most large-scale cell culture processes, this review does not contain many detailed scientific results although an attempt is made to address some key issues and findings. Much of this summary deals with processes having an established, commercial track record but some attention is given to more recent innovations with interesting potential applications. Reference is made to plant cell culture but the main emphasis is on mammalian cells.**

## Problems and Limitations in Large-Scale Cell Culture

Contemporary large-scale cell culture has its origins in work done more than 20 years ago by Capstick, Telling, and colleagues (*1, 2*) with suspension-adapted baby hamster kidney (BHK) cells. Since then, the principles and processes established have been refined to form the basis of a multimillion dollar industry in Europe, South America, Africa, Japan, India, and elsewhere. The impetus behind this development lay in the profitable manufacture of vaccines against foot-and-mouth disease (FMD), which affects cattle and swine. Such vaccines are still produced by means of large-scale cell culture and the size of this industry may be appreciated from a recent paper by Radlett *et al.* (*3*). They showed that the volume of cell culture medium used by the Wellcome group of companies alone in 1983 for the production of FMD vaccines was more than 2.1 million liters. In the late 1970's large-scale cell culture processes were adapted by various companies and institutes to produce interferons from continuous cell lines (*4, 5*).

Recently, much effort and investment has been made to exploit large-scale cell culture science and technology. Forcing the pace has been the need to produce monoclonal antibodies and other elaborate glycoproteins in genetically modified mammalian cells. Many complex proteins of pharmacological interest such as viral antigens, mammalian antibodies, and enzymes have precise folding and glycosylation requirements (*6, 7*) that cannot be met in bacteria or yeast. Animal cells, however, often can be persuaded by use of hybridoma or recombinant DNA techniques to produce and secrete useful quantities of these proteins. It is for this reason that in the next decade large-scale culture of animals cells will assume major importance in the manufacture of pharmaceutical products.

The major hazard to large-scale cell culture is microbial contamination. Complexity of equipment, media, support systems, and operational manipulation all lead to risks of contamination. This is a problem with any cell culture, but is most acute at the large scale where losses of production or inoculum vessels can be very costly in materials and time. Animal and plant cells grow relatively slowly (doubling times of about 12 to 100 hours or more) and are easily overwhelmed by most microbial contaminants. Mycoplasma pose a major threat to animal cell culture because they are not easily detected and are highly contagious (*8*). Thus, absolute requirements for successful large-scale cell culture are well-characterized working cell banks [with appropriate supporting master banks (*9*)], in which the frozen cells are known to be free from contaminants. Establishment and testing of suitable cell banks, including screening them for viruses and other adventitious agents (*10*), is a major exercise. Viral contamination is not a great problem with most large-scale systems although certain infections, such as parvovirus (*11*), can have long (and therefore expensive) latent periods before degenerative or lytic effects become apparent. Generally, the source of viral contamination is animal serum or inadequately screened cell banks.

It is common practice in many laboratories to use antibiotics in cell culture medium. Undoubtedly this helps to reduce the incidence of bacterial contamination although it is impossible to estimate the full benefits since the practice can give rise to antibiotic-resistant microbes that pose a threat themselves. Despite contamination problems, high success rates are achieved with appropriate designs for vessels, filtration systems, and pipework and by careful attention to operational detail and staff training. By following such criteria as these, we [as well as other investigators (*3*)] have commonly experienced success rates in excess of 95%. Similar success has been achieved for several years at one installation in South America without any use of antibiotics.

Contrary to expectation, animal cell fragility is not a major problem with many large-scale systems because of the selection of relatively robust cells. (Exceptions to this occur with some shear-sensitive cells such as fragile hybridomas.) Conditions within conventional culture vessels are designed to allow good mixing of the cell suspension; liquids can be added and gases sparged (bubbled) into the culture without causing undue damage to the cells. Appropriate mixing with agitators or airlift mechanisms will maintain homogeneous cultures in which temperature, dissolved oxygen, and *p*H can be controlled easily and from which representative samples of the culture may be obtained.

Cell culture media often contain 50 or more individual components and may be supplemented with serum and peptones. Quality control of raw materials and production of media pose considerable challenges at the large scale. There has been a trend toward the use of more defined media in which serum, or components of it, are minimized (*12, 13*). Cell toxicity, associated with trace metal or organic contaminants in water (*14*), is more likely in serum-free media. Use of the highest purity water, therefore, is desirable for media preparation and clean steam generation. Very pure water is quite corrosive because of its ability to leach elements from metals; therefore, appropriate treatment steps are necessary where it contacts stainless steel vessels and pipes. Cell culture media are usually

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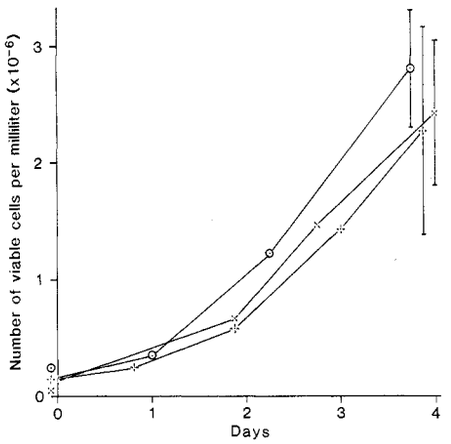


Fig. 1. CHO cell growth in enriched Eagle's medium containing 1% bovine serum at the 10-liter (○), 100-liter (×), and 1000-liter (+) scales. Vertical bars represent 2 SD's around the means, as measured in nine determinations.

sterilized by filtration through depth or membrane filters or both. Sterility can be guaranteed almost routinely with the use of appropriate membrane filters; even mycoplasma will be retained by filter membranes with pore sizes of 0.1 μm. Producers of cell culture–derived material intended for use in humans have to demonstrate control over all aspects of production and downstream processing as a prerequisite for licensing by the Food and Drug Administration (*15*). Thus, for commercial and regulatory reasons, the importance of these criteria cannot be overemphasized.

## Systems and Design Criteria for Large-Scale Cell Culture

The largest cell culture processes, in terms of media volumes and cell numbers involved, utilize homogeneous populations of cells that have been adapted to growth in suspension. In the conventional systems, cells are grown in stainless steel fermentation vessels with height to diameter ratios ranging from 1:1 to about 3:1. The cultures are mixed with agitators that have one or more impellers based on bladed disk (Rushton) or marine propeller patterns. Agitation and mixing conditions vary with different vessel geometries but, generally, low agitation rates are used that give homogeneous cultures and acceptable mixing times.

In most cultures, cell population densities are relatively low and do not exceed about $5 \times 10^6$ cells per milliliter. (In perfused cultures, which will be discussed later, ten- to a hundredfold greater cell numbers are common with attendant problems.) In normal circumstances, oxygen supply and *p*H control is not particularly difficult. It is usual for the dissolved oxygen concentration to be controlled by sparging sterile air into the cultures at less than 0.1 volume per vessel volume per minute. Sterile $CO_2$ gas can be sparged in at similar rates to decrease *p*H, if necessary, and *p*H may be increased by the addition of sterile basic solution (NaOH or $Na_2CO_3$). Automatic control of these parameters is used commonly but not exclusively (*5*). In systems for controlling dissolved oxygen, redox electrodes often are preferred (*16, 17*) because they are less fragile than oxygen electrodes. Where this is the case, redox potentials corresponding to optimal dissolved oxygen concentrations have to be determined experimentally for different cells and media.

Control of the cellular environment with similar agitation conditions in larger vessels has been used successfully as a basis for scaling up cultures of cell lines such as BHK 21, Namalwa, Jurkat, and Chinese hamster ovary (CHO). An example of the latter is shown in Fig. 1 illustrating CHO cell growth in 10-, 100- and 1000-liter vessels in which maximum shear rates were kept constant or decreased with increasing scale. The *p*H was kept at 7.15 ± 0.1, dissolved oxygen at about 20%, and temperature at 37° ± 0.5°C in these vessels.

Foaming in cultures occurs as a result of air being sparged into them and is exacerbated by serum in the medium. This can damage the cells and products and should be controlled with antifoaming additives or appropriate foam-trapping devices. Thus, many large-scale cell culture systems have some analogy to conventional microbial fermentation processes although less power is used per unit volume and batch cultures tend to be run for longer periods of time. The largest reported cell culture processes are operated in Japan at the 20,000-liter scale for tobacco cells (*18*).

Another large-scale suspension-cell culture system is used in airlift fermenters (Fig. 2). This type of fermenter has been used for many years for the cultivation of microbial cells and has more recently been used for the growth of BHK 21 and human lymphoblastoid cells (*19*), as well as CHO and plant cells. Briefly, gas mixtures are introduced into the culture by a sparging device at the base of a central draught tube and rise through the tube to be released at the surface of the culture. The gas causes a reduction in the bulk density of the liquid in the draught tube compared with the density in the outer zone of the vessel, which causes the culture to circulate. Apart from mixing, the gases introduced also supply oxygen to the culture. Hybridoma cells can be grown in both airlift and stirred vessels and there is no evidence that reactor type, per se, has any effect on growth kinetics or antibody production rate. The main advantage of the airlift design is simplicity since it removes the need for motors and agitators associated with stirred reactors. In addition, hydrodynamic and mass-transfer characteristics of the airlift reactor are predictable with increase in scale.

Another important system for large-scale mammalian cell culture is based on microcarrier technology for anchorage-dependent cells. Many useful mammalian cells will not grow in free suspension and require a solid supporting matrix. Diploid cell lines, for example, are grown in culture dishes or small flasks in the laboratory but can be cultured on microcarrier beads in homogeneous suspension. At present this is the most efficient way to provide a large surface area per unit volume of medium for cell growth and is more amenable to larger scales. A. L. van Wezel *et al.* pioneered this work and provided much of the scientific and technical impetus for its subsequent development (*20–22*).

One of the most difficult aspects of scaling up microcarrier cultures is the transfer of cells from one culture to the next. Cells may in some cases form bridges between beads and migrate unaided onto fresh ones, but in most instances proteolytic enzyme treatment of the cells or the microcarrier beads is required (*23*). In large-scale microcarrier cultures it is important to avoid high shear conditions. However, mixing needs to be sufficiently vigorous to prevent aggregation of beads which, if permitted, can cause the beads to

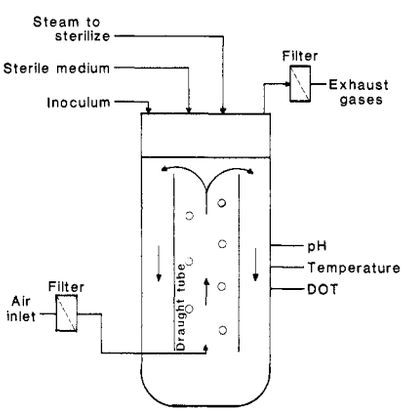


Fig. 2. Principle of an airlift reactor (*52*). DOT, dissolved oxygen tension. [Courtesy of Celltech, Ltd.]

become bound together into large clumps by the cells. It is common for agitation to be achieved with relatively large impellers (a variety of designs are used), rotating at less than 50 rev/min. Dissolved oxygen and *p*H can be controlled adequately when the mixing in unperfused cultures is sufficient to maintain the microcarrier beads in suspension. Problems occur if significant foaming is allowed to form as this traps the beads and results in damage to the cells. Various systems for aeration and mixing of microcarrier cultures have been devised recently (*24*). Because of the complexity of microcarrier-based cell culture, its use will remain restricted to cases where suspension-adapted cells will not suffice either for scientific or for regulatory reasons. An example of the latter occurs in countries where whole virus vaccines are permitted for human use only when derived from anchorage-dependent diploid cell lines. In addition, microcarrier systems are necessary for production of recombinant products that are expressed satisfactorily only in anchorage-dependent cells.

## Optimizing Conditions for Animal Cell Growth at Scale

Cell growth is dependent upon a complex interaction between the cell and its physical, nutritional, and hormonal environment. Imbalances within or between these features can result in suboptimal growth. In batch cultures, cells grow according to the classic pattern of lag, logarithmic, and plateau phases, followed eventually by death unless the cells are subcultivated into fresh medium. Various influences affect these phases but cessation of growth may be caused by exhaustion of nutrients or growth factors, accumulation of toxic components, or in response to external or autocrine hormonal signals (or the lack thereof).

Numerous studies of cell nutrient requirements have been made in the laboratory and have formed the basis for well-known culture media formulations and the understanding of hormonal influences (*12, 25–27*). Published studies of work at the large scale have been fewer for economic and proprietary reasons. One aspect that is overlooked frequently in attempts to quantify nutrient requirements is the variability of cell size in different parts of the cell cycle, growth phases, and culture systems. Average suspension cell diameters (nominally about 11 μm) can change considerably according to conditions; thus, an increase in the average cell diameter from 9.5 μm to 12 μm can mean more than a twofold difference in cell mass. Quantitative estimates of nutrient requirements, therefore, need to be related to cell mass or dry weight rather than cell population density (*16, 28*).

Optimization of medium for large-scale cell cultures is usually preceded by laboratory-scale studies in which many variables can be examined simultaneously. This work may be performed in volumes as small as 100 μl in microtiter plates but usually is refined in larger systems, some of the most versatile being 1-liter fermenters. The transition from static suspension to stirred suspension can be an impediment to scaling up a process, particularly in serum-free medium. An example of differences in hybridoma cell growth in such circumstances are shown in Fig. 3, which illustrates greater cell yields in shallow static suspension than in 1-liter stirred cultures. As a result of this work, further optimization has been done to define conditions in which equivalent cell yields were obtained in stirred suspension culture (*29*).

Continuous cell culture in chemostats may be used to study nutrient requirements and interactions (*30, 31*). As with other microorganisms in chemostats, the method allows one variable to be examined at a time if the cell line is stable. In one such study (*32*), the effect of specific growth rate and a range of nutrient limitations

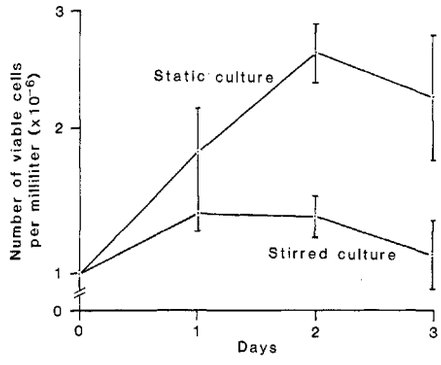


Fig. 3. Growth of mouse hybridoma cells in serum-free medium prior to its optimization for stirred conditions. Vertical bars represent 1 SD around the mean, as measured in three determinations.

on synthesis of an immunoglobulin by a murine hybridoma was examined. It was possible to obtain physiological steady states over prolonged periods and stable antibody production was maintained over many months. In this case, there was no evidence that nonproducing variants were being selected. Under these controlled conditions, antibody synthesis was independent of growth rate and not greatly influenced by the nature of the nutrient limitations. Chemostats can be used to study nutrient dependencies and also the kinetics and stability of protein expression in genetically engineered cell lines that secrete cloned proteins. Chemostat studies are limited to anchorage-independent cells growing in suspension and cannot be applied to microcarrier cultures, in which there is no means of obtaining steady-state conditions at a defined growth rate.

Recent work with various cell lines containing recombinant DNA has shown that they differ considerably in their optimal medium requirements, especially in serum-free media. This occurs between clones from the same cell line that express different recombinant proteins or even mutations of the same molecule. Whether these differences are due to clonal variations or events during the introduction of DNA and subsequent stages is not yet clear. The indications are, however, that recombinant cell lines with large-scale potential will not necessarily all produce optimally in a generic medium.

Crucial to the understanding of conditions in large-scale culture is an appreciation of cell health. Direct microscopic examination, with appropriate fluorescent or other dyes, can give information concerning cell population density, viability, mitotic index, general cell morphology, and amount of cell debris present. For routine cultures this information is usually sufficient. In processes with automatic control of dissolved oxygen, the state of the cells often is indicated by aeration rates although, unlike microbial fermentations, the flow rates of exhaust gases are usually too low to allow meaningful on-line analysis. Other parameters that can be measured relatively easily are glucose and lactate concentrations and lactate dehydrogenase (LDH) activity in the medium. LDH is an intracellular enzyme and can be used to estimate cell damage and lysis. Care and appropriate controls are needed, however, because LDH is less stable in the supernatants of some cell lines than in others (Fig. 4) and is also present in bovine serum.

Animal cell perfusion cultures have become popular in many laboratories. In these cultures, cells are trapped in some contained system to which fresh medium is added while spent medium is removed at the same rate. Frequently, the product is also collected continuously in the spent medium permitting rapid purification starting from the time of secretion. Unlike the traditional chemostat, the cell population densities in these processes usually increase at an uncontrolled rate until they become limited. This latter effect can be circumvented in continuously perfused cultures with biomass feedback, where the extent of cell removal or retention, and thus the population density, can be controlled. In one case at Celltech, a 5-

liter fermenter was operated continuously with biomass feedback and produced 75 mg of murine immunoglobulin M per liter per day. This represented a 5.4-fold increase in output compared with a conventional chemostat. The theory of these processes, which differ from batch cultures in that far greater numbers of cells may be grown per unit of culture volume, has been described (*30*). With some anchorage-dependent cell lines, population densities can become limited by available surface area. This can happen in perfused microcarrier cultures when the surfaces of microcarrier beads become covered by a confluent layer of cells. This self-limited growth, however, depends upon the cell type involved; often such self-regulation is not observed, especially with nondiploid cell lines.

Perfusion systems may be classified into three main groups: (i) microscopically homogeneous systems, such as perfused suspension cell (*33*) or microcarrier cultures; (ii) macroscopically homogeneous systems, where cells are encapsulated inside small beads, gels, or matrices that are mixed in fluidized beds by other means (*34, 35*); and (iii) nonhomogeneous systems, such as cells perfused while trapped statically by filter or ultrafilter membranes (*36*). These systems have been described in detail (*37*). Direct monitoring of the cell cultures is possible with homogeneous systems. This is not true in other processes for which indirect estimates of cell health have to be relied upon, such as LDH activity, oxygen, or nutrient consumption. In the latter cases, the interactions of these parameters have to be ascertained because consumption rates vary with dissolved oxygen concentrations.

With high density cultures, control of *p*H and dissolved oxygen is somewhat more difficult. To control dissolved oxygen, pure oxygen gas usually is required. It may be sparged directly into the cultures or secondary conditioning chambers or, alternatively, introduced via artificial lungs having gas-permeable barriers. Changing the buffering capacity of the medium or inlet *p*H may be necessary to maintain correct *p*H.

Although perfused suspension cell cultures have been run at the 1000-liter scale, most of them involve working volumes of only a few liters. Many advantages are claimed for perfusion processes including low capital costs, higher productivities (due to more efficient utilization of expensive medium components, such as serum), less work between runs, and more rapid product processing. Disadvantages to the processes are their inherent complexity; their need for constant and large supplies of medium; and finally, since advantageous use of the system depends upon long times of operation, their greater vulnerability to contamination and instability of the cells. These points, for and against perfusion, are not new to the pharmaceutical industry insofar as continuous processes are concerned. However, there are few (if any) continuous processes in routine use in this field, where attempts are constantly made to improve profit margins. Despite the arguments cited above, it is too soon to predict the full potential of perfused animal cell culture. Analogies with other microbial processes do not necessarily apply to animal cells, which are maintained at high population densities in vivo (roughly $10^9$ cells per milliliter depending on cell type). Thus perfusion offers the potential for matching the cell to the system in advantageous ways and, in the extreme, the formation of artificial "organs" tailored to specific production needs.

## Present and Future Products Relevant to Large-Scale Cell Culture

*Vaccines.* As mentioned earlier, the products made in greatest volume with large-scale cell culture are FMD vaccines (*3*). Other examples of infectious virus cultures that can be manipulated at the large scale are rabies (*38*), polio (*39*), and bovine leukemia virus

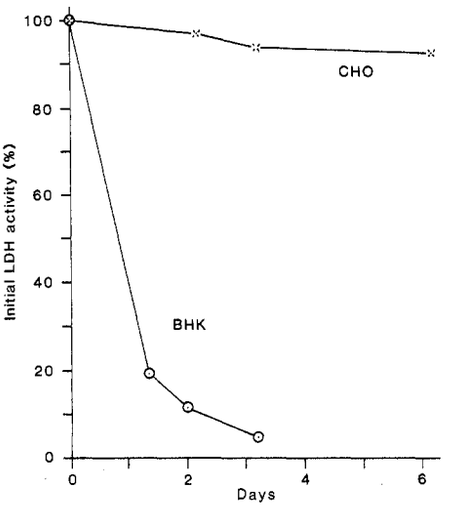


Fig. 4. Variation of lactate dehydrogenase (LDH) activity with time at 37°C in culture supernatants. In this test, initial LDH activities were similar, but the CHO supernatant was derived from a 4-day-old culture and the BHK supernatant was from a 1-day-old culture (*29*).

(*40*); HTLV-I also has been produced at the large scale in this manner (*41*), although in less quantity. A common characteristic of all these processes is the stringent containment and security constraints under which they are conducted. The infectious viruses are confined to isolated equipment designed to prevent leaks or aerosol formation. Infectious virus production is usually done in zones of containment and negative pressure from which effluent gases are filtered exhaustively and in some cases incinerated.

Because it is difficult for their integrity and immunogenicity to be retained during vigorous purification procedures, most whole virus vaccines are relatively impure in comparison with other cell culture–derived products. This has lead to strict regulation governing use of host cell substrates (*15, 42*) because components of the cells (or contaminants) can be incorporated into the vaccines. Since neither the purity of these vaccines nor their impurities could be estimated accurately it was decided that, for human use, vaccines should be derived from normal cell sources—uncontaminated cells with stable diploid karyotype, finite life-spans, and nontumorigenic characteristics. This subject is discussed elsewhere (*42, 43*) but the same constraints need not necessarily apply to the production of more stable and easily purified proteins such as interferons, monoclonal antibodies, and plasminogen activators that have applications in critically ill patients.

Although some whole virus vaccines are produced for human use

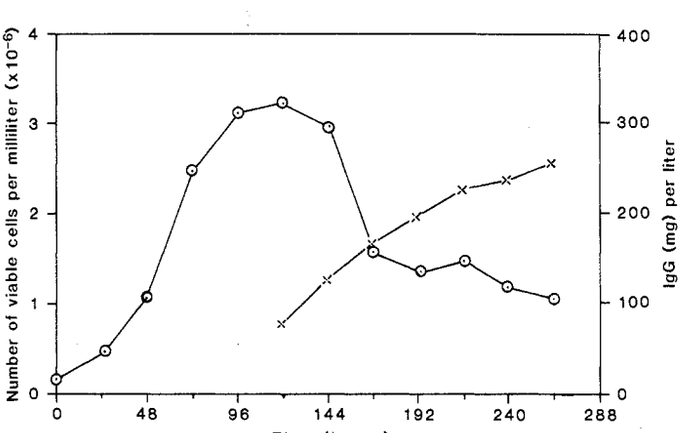


Fig. 5. Growth and antibody synthesis by mouse hybridoma cells in serum-free medium in a 1000-liter fermenter (*53*); ○, cell number; x, antibody concentration. [Courtesy of Celltech, Ltd.]

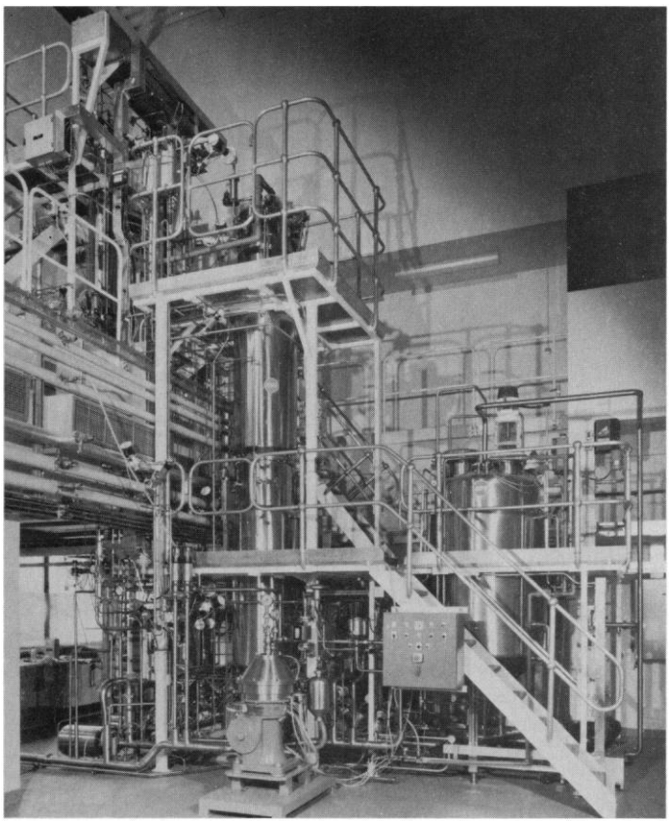

Fig. 6. A 1000-liter fermenter at Celltech Ltd. has the capacity to produce monoclonal antibodies in kilogram quantities per year. [Courtesy of Celltech, Ltd.]

there are still instances where it has not proved possible either to culture the appropriate viruses or make a safe product from them. Recent developments in molecular biology (*6, 44*) have resulted in expression of viral subunit proteins or glycoproteins by genetically modified, animal cell lines that may be grown at the large scale. This has enabled the possibility of purer, safer, and more efficacious vaccines, some of which are in clinical trials now.

Another possibility for future production of viral vaccines centers on the use of vaccinia as a vector into which heterologous viral genes may be inserted and expressed after infection of the desired host cell substrate (*45*). It remains to be seen whether this concept or similar ones will gain acceptance, but large-scale systems could easily be adapted for them.

*Interferons.* Interferons-α and -β have been made in large quantities recently in Europe and Japan. Interferon-α, derived from 8000-liter cultures of Namalwa cells, has been produced by the Wellcome Foundation in response to the demands for clinical trials. These cultures were examples of the largest scale used for growing animal cells and have been described as "Very Large-Scale Cell Cultures" (*17, 46*). Production of interferon-β from human diploid fibroblasts that were grown on microcarriers at scales of greater than 1000 liters was a significant advance in this area (*47*). Problems solved in this effort included microcarrier use, appropriate media development, maintenance of cell karyotype over extended cell life-spans, and superinduction of interferon in these cells to maximize yields.

*Monoclonal antibodies.* The increasing use of monoclonal antibodies for in vitro diagnostics, in vivo imaging, therapy in both humans and animals, and industrial applications such as immunopurification has led to the need for efficient production processes. Many of these applications will not be fully realized unless economic and reliable processes are developed that can meet increasing demand. Ten years ago antibodies were available only in milligram amounts but today there are numerous applications requiring tens to hundreds of grams per year. There are already some systems that require antibodies at the kilogram-per-year level and multikilogram-per-year requirements seem likely to increase rapidly over the next few years.

Two basic approaches have been used for the production of monoclonal antibodies from hybridoma cells—in vivo culture as ascites tumors in mice or rats and cell culture in vitro. The latter method is gaining favor for production because it can be scaled up as a unit operation, which allows significant economies of scale to be realized. For example, a kilogram of antibody that could be produced in a single fermenter would require at least 20,000 mice if made by the ascites route. The cell culture approach has additional qualitative advantages. In particular, the risk of contamination of the monoclonal antibody by extraneous mouse antibodies or adventitious agents of rodent origin is significantly reduced in cell culture. This aspect is especially important when considering the production of material for human in vivo applications.

Since hybridoma cells grow in suspension culture they can, in principle, be grown in both airlift fermenters and in stirred fermenters. In addition, several groups are working with immobilized-cell reactors that involve perfusion type systems (*37*). At Celltech, stainless steel airlift fermenters of 10, 100, and 1000 liters are operated in a cascade system for antibody production. A high level of automation is used in the process, both to increase reliability and to reduce labor costs. A microprocessor-based computer is used to control *p*H, dissolved oxygen, and temperature as well as valve and pump activation sequences (during sterilization of the fermenter, for instance). The computer also provides a system of alarms and emergency action in the case of failure of critical equipment. After fermentation, cells and debris are removed by continuous-flow centrifugation and the culture supernatant containing the antibody product is concentrated by means of a tangential-flow ultrafiltration unit prior to purification.

The methods developed for growing hybridoma cells in airlift fermenters have proved to be generally applicable for numerous cell lines of mouse, rat, and human origin, all producing monoclonal antibodies. Serum-free medium in a 1000-liter fermenter has been used successfully for this purpose (Figs. 5 and 6). In this particular case, 260 g of antibody was harvested over a production period of 260 hours. Production cycles vary from 140 to 400 hours according to the cell line used. Different hybridoma cell lines vary greatly in their productivity and a range of final antibody concentrations from 40 to 500 mg/liter is seen in fermenters. Typically, these yields represent a four- to fivefold increase over those obtained in simple laboratory culture systems such as roller bottles. This increase is the result of systematic optimization of the process, especially with respect to design of the culture medium. The kinetics of antibody synthesis in batch processes are interesting in that the majority of antibody is synthesized during the decline stage of the culture, and the specific rate of synthesis appears to increase during this phase.

*Recombinant products.* Much interest in large-scale cell culture resulted when it was appreciated that many benefits of genetic engineering would not be realized by means of conventional microorganisms, at least in the near future. In contrast, animal cells are able to translate and process large or complex cloned proteins by the precise steps necessary to result in the correct disulfide linkages and glycosylation patterns such as those found in the naturally occurring molecules (*48*). This ability can obviate the need for difficult, if not impossible, renaturing steps after protein purification. In addition, animal cells can secrete desired proteins into the culture medium; it is easier to recover proteins from the medium than from cell homogenates.

Apart from monoclonal antibodies, the protein of most recent interest is tissue-type plasminogen activator (t-PA) (*49*). Clinical trials have shown that recombinant t-PA is safe and effective for dissolving blood clots in patients with myocardial infarction (*50, 51*). The t-PA is expected to have many other applications, such as in treatment of pulmonary embolism and other thrombotic disorders. Projected needs reach levels of many kilograms per year. After considerable work in cell culture at all scales and in protein chemistry for purification and formulation, the science and technology is now in place to meet these demands. In addition, there is ongoing research with the parent molecule and genetically engineered variants.

There are several categories of other recombinant molecules that can be produced in large quantities from animal cell culture. The first contains blood product–related molecules and includes factor VIII, factor IX, protein C, and immunoglobulins. A second category is comprised of hormones and similar molecules, such as erythropoietin, relaxin, kallikrein, and human growth hormone. A third group includes the antigens that are used in viral vaccines for hepatitis B, herpes simplex-I and -II, cytomegalovirus, HIV, and also other complex antigens (such as those found on parasites causing malaria and schistosomiasis). For research purposes, other cell components have been prepared in large amounts including DNA polymerases and kilogram quantities of cell nuclei.

Cells of most immediate large-scale application are those in which the desired genome is integrated in a stable manner. Transient expression, for instance in monkey COS cells, is useful for research but is not well suited to large-scale cultures that must yield products reproducibly. For this reason both the cells and vectors need to be stable and not liable to spontaneous changes.

## Conclusions

In this article, we have attempted to present the current situation and the potential for large-scale cell culture. Recent developments in this field have been very rapid and are the results of successful interaction between engineers, protein chemists, cell biologists, and molecular biologists. The future demand for such molecules as immunoglobulins and plasminogen activators is not yet certain, but these are just two examples of natural proteins that have great potential medical value. In this rapidly evolving science, well-matched skills are needed in diverse areas where pure and applied research have to be transmuted into realistic production protocols.

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