PA produced to alginate bead volume was equal in both bioreactors. Production of hybrid t-PA in alginate beads was lost after 2-3 weeks in more than 60% of bioreactor runs because the beads eventually fell apart.

On the basis of equal surface area for cell growth, the best system for producing hybrid t-PA was the Charles River bioreactor. We expect that the results seen with the Charles River 5200 bioreactor would be linearly scalable to the model 5300 bioreactor which provides up to 400,000 cm^2 surface area for cell growth. Work with other cell lines, not presented here, has shown scale-up to be linear from the 5200 to the 5300 model bioreactor. If the C-127 cell line behaves similarly, one gram of hybrid t-PA could be produced every day in the model 5300 unit. When t-PA concentration was considered, the Bellco bioreactor was the better reactor, attaining 32.5 mg of hybrid t-PA per liter of medium. One disadvantage of the Bellco system, however, was the extensive (non-productive) growth period—three weeks compared to one week with the Charles River system. Optimization of these bioreactors is still under study. In conclusion, the Charles River Opticell and the Bellco bioreactor with a stainless steel matrix were the most productive systems studied.

The Other View

YES, BUT .....

"It [the Wyeth Ayerst study] is rather an unusual case—to test out a set of different bioreactors. Usually you would test things with what is already in house and then that system would develop its own momentum." That statement, from a cell culture expert at a major pharmaceutical company, highlights the infrequency with which direct comparisons of bioreactor performance are performed. The McKillip *et al.* study, therefore, represents a rarely seen level of refinement.

There are obvious problems in putting together such a study. By no means the least of these is cost both of the systems themselves and the personnel to run them. "You're limited in your resources." says Ed McKillip at Wyeth Ayerst: "There were some systems we didn't try either because they weren't scalable or because they didn't look as good on paper. But there was at least one system which we did consider but rejected because the manufacturers wanted too much."

Some of the systems chosen were clearly more difficult to work with than others.

Glenn Ambra of Unisyn Fibertec (San Diego, CA) which bought the CD Biomedical business after the Wyeth Ayerst study was performed believes that C-127 was always going to be difficult to work with in cellulose acetate hollow fibers.

Despite its murine lineage, C-127 has been described as "a dog." Apparently, under certain growth conditions it produces an extracellular glycoprotein matrix and grows in a nodular form. In hollow fibers, it would stand a high chance of being diffusion-limited, and this was, in fact, observed by McKillip *et al.* Experiences with C-127 at Celltech (Slough, U.K.) were not positive either. Growing the cell line experimentally on microcarriers in suspension culture, the company did not find it cost-effective or operable, preferring to use CHO cells, insect cells, recombinant myelomas or other suspension-culturable expression systems. The Wyeth Ayerst researchers were able to grow C-127 in cellulose acetate hollow fibers but they also concluded that product yields were unsatisfactory.

The changing face of the mammalian cell culture market creates another variable in comparative studies. For instance, Charles River Biotechnology, while still supporting the system, has been looking to divest itself of the Opticell business during the past few months. If that was to mean that users were starved of the technical back-up CRB supplies, experimental studies on a novel system (as Opticell then was) would be less easy to countenance. Even where there is support there is still a large inertia barrier to overcome for new technology, contends Rudi Bliem of Bristol-Myers (Syracuse, NY): "Verax (Hanover, NH) fairly aggressively pushed its [collagen bead] technology as a turnkey package. But only a few companies picked it up even though they [Verax] were willing to invest their expertise in the venture." What then are the prospects for a renaissance in microencapsulation technology *a la* Damon Biotech (Needham Heights, MA) or the Static Maintenance Reactor from Invitron (Clayton, MO) in the absence of the companies that developed the method? Is the bioreactor market suffering a hernia? The user still has many choices, but is his (or her) equipment sufficiently well-supported? Shyness of novel techniques may account for the trend towards stirred tank reactors (STRs) with marine impellers for bulk cell culture: traditional pharmaceutical houses trust STRs.

The technology, nevertheless, marches on inexorably, cocking a snook at decisions made in a frozen moment. The CD Biomedical business has, according to Glenn Ambra, been overhauled since its acquisition by Unisyn Fibertec. For instance, Unisyn has recently developed activated carrier matrices (ACM) membranes specifically for the cultivation of anchorage-dependent cells which grow poorly on the cellulose membranes. Ed McKillip and colleagues are currently working with that technology.

A final additional factor that would be considered in any comparison of cell culture systems is the quality of the downstream side. "If I was now faced with selecting a culture system," says Rudi Bliem, "I would be looking at what the cell does in the broth, at what it does at harvest, and at its health status during the process." Cells and cell debris mean DNA. "Therefore," he explains, "the more DNA in the harvest, the better. And that implies that what is needed is either less cells, less cell damage or more cell retention."

John Hodgson

Experimental Protocol

Cell selection by dilution and soft agar cloning. Three different foci of BPV transformed C-127 cells producing hybrid B t-PA were grown to confluency in T-flasks. The cells were trypsinized and diluted to one cell/0.2 ml medium for single cell suspensions. The cell suspensions were used at 0.2 ml $well^{-1}$ to seed 96 well microtiter plates. Wells containing only one cell, as determined by light microscopy, were utilized. Each well was re-fed every 3 to 4 days with 0.1 ml of an equal proportion of DMEM with 10% FBS and sterile filtered conditioned medium. Conditioned medium was obtained from C-127 cells grown for five days in DMEM with 10% FBS.

A modified soft agar cloning method14 was used with concentrations of $5x10^3$, $5x10^4$, and $5x10^5$ cells per 60 mm^2 dish. Cell colonies were observed 1 to 2 weeks after inoculation. Hybrid t-PA activity was detected by overlaying the soft-agar clones with casein agar. Clearing zones appeared in the casein agar around the t-PA-producing colonies within two hours. The casein agar overlay consisted of 1 ml DMEM with 5% FBS and 40 µg plasminogen, 1 ml 20% nonfat milk and 2 ml of 4% Seakem agarose solution (3 ml $dish^{-1}$).

Medium selection. Cells were grown to approximately 80% confluency in a variety of basal tissue culture media supplemented with 10% FBS, 4 to 6 mM glutamine and Gibco PSN antibiotic mixture (growth medium). Cells were then either passed or placed on serum -free medium. The medium was changed every 3 to 4 days. Only the cells on media that supported hybrid t-PA production were continued.

Bioreactor selection. The hollow fiber system was the first to be studied, and