The problems in producing a highly infectious preparation derive from two factors:[†] rapid decay of virus and inhibition of viral infectivity by proteoglycans released by the packaging cell line. The proteoglycans have very high molecular weight. If the virus is concentrated by ultrafiltration, the proteoglycan is also concentrated by nearly the same factor. While the high virus concentration would be expected to increase the number of infected cells, the presence of concentrated proteoglycan leads to increased inhibition of infection, so that the number of cells infected does not increase significantly and in some cases even decreases with the concentrated viral preparation.

Another approach to increase viral titer is to produce virus at a reduced temperature (e.g., 28°C versus 37°C). While both the rate of virus production and viral decay decrease with temperature, the rate of decay is more sensitive to temperature. Thus the amount of potentially active virus is severalfold higher when produced at 28°C (e.g., 2×10^5 cfu/ml vs. 6×10^4 cfu/ml; cfu is colony-forming units and measures the number of active viruses in a dilute solution). However, the transduction efficiency did not increase, presumably due to changes in proteoglycan concentration.

These results should inspire the reader to consider bioreactor options beyond batch growth followed by ultrafiltration. Clearly, the reader would want to consider a bioreactor configuration in which the virus is removed as soon as it is produced and then purified and stored at low temperature to reduce decay. Further, a method of concentration of virus needs to be developed that does not also concentrate proteoglycan. Enzymatic digestion of proteoglycans has been suggested, but due to multiplicity of proteoglycan structures such a strategy is difficult to implement. Selective adsorption and desorption of virus is attractive, but the sensitivity of the virus to these processes is a concern. A packaging cell altered in its capability to produce proteoglycan is another possible strategy.

The primary point is that bioprocess technology is intimately connected to production of agents that can be effective in gene therapy. The solution to the biomedical problem requires a solution to the bioprocess problem.

15.4. BIOREACTORS

Mass production of cells for transplantation or the use of bioreactors as artificial hybrid organs are subjects of intense development in medicine. These are issues in which bioprocess engineers may make significant contributions.

15.4.1. Stem Cells and Hematopoiesis

Some animal cells are capable of extensive replication and self-renewal. Others are highly differentiated and perform specific functions; typically differentiated cells cannot replicate (at least, replication is very limited). A *stem cell* is an undifferentiated cell capable of continuous self-renewal that can also produce large numbers of differentiated progeny, depending on extracellular factors. The best studied system is the hematopoietic system. There are eight major types of fully mature blood cells in the human circulatory system. A hematopoietic stem cell gives rise to two types of *progenitor cells*. The progenitor cells

[†] J. M. LeDoux, H. E. Davis, J. R. Morgan, and M. L. Yarmush, *Biotechnol. Bioeng. 63*:654 (1999).