DRUG DEVELOPMENT

SUBSTITUTES FOR HUMAN BLOOD

LONDON—If things pan out the way DNX (Princeton, NJ) hopes, then, as a business at least, transgenic pigs may fly. DNX announced in June that it had succeeded in breeding three pigs which produce human hemoglobin at a level of 10-15 percent of their total hemoglobin. The potential world market for infection-free, safe, efficacious, and universally-compatible blood substitutes is huge. Indeed, estimates vary between $5-10 billion annually if the full range of civilian and military applications are covered.

The blood substitutes market is complicated. At present, most blood comes from human blood donations (or purchases) largely handled through not-for-profit organizations such as the American or Dutch Red Cross or various national health services. Blood centers must, however, type and cross-match donated whole blood, screen it for infection, and store it under refrigeration. These efforts increase pressure for blood substitutes, particularly cell-free hemoglobin. Cell-free hemoglobin must be modified to prevent the formation of nephrotoxic dimers and to compensate for the loss of control over oxygen uptake and release which occurs when the molecule is removed from red blood cells. Working with hemoglobin extracted from donated blood, Baxter Healthcare (Deerfield, IL), the Dutch Red Cross (Amsterdam, The Netherlands), Hemasol (Toronto, Ont.), and Northfield Laboratories (Chicago, IL) are using chemical cross-linking to overcome those problems. Enzon (So. Plainfield, NJ) and Ajinomoto (Tokyo) are developing PEGylated hemoglobin, while Baxter and the U.S. Naval Research Laboratory (Waldorf, MD) are developing hemosomes (hemoglobin-containing liposomes).

The strategy of DNX and other companies developing recombinant hemoglobins—like Delta Biotechnology (Nottingham, U.K.) and Somatogen (Boulder, CO)—is that their products, if produced at the right price, could plug into whichever delivery system works best. The first question that needs to be answered concerns the feasibility of cost-effective production.

DNX hopes to raise expression levels of human hemoglobin to 50-60 percent of the total hemoglobin in each pig. John Logan, DNX's vice president for research, thinks this is realistic, pointing out that 70-80-percent expression levels have already been achieved in mice that are apparently healthy and unperturbed by the high levels of foreign protein. The pigs would be slaughtered and desanguinated at six months. The red blood cells would then be centrifuged out and disrupted to yield a fraction that contained 95 percent hemoglobin. DNX has found that human hemoglobin can be separated from its porcine counterpart through ion exchange on DEAE resin. Each pig yields 10 liters of blood, which in turn might produce the equivalent of 15-20 units of blood. At current blood prices of $150-175 per unit, each transgenic pig could yield $2,250-3,500 worth of hemoglobin. At this production level, a mere 3 million pigs would be sufficient to supply the entire world demand. It should be noted that DNX is collaborating with Pig Improvement Company (PIC, Franklin, KY), the U.S. subsidiary of Dalgety (London, U.K.), the world's largest breeder of pigs.

Other recombinant technologies depend on microbial production methods. Somatogen hopes to begin phase I trials of its *Escherichia coli*-produced recombinant hemoglobin later in the year. Somatogen's product involves co-expression of hemoglobin alpha and beta subunits and incorporation of heme.

—John Hodgson