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ANIMAL BIOTECHNOLOGY ON THE HORIZON

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Animal biotechnology, the application of the findings of biological research to animals, has been with us in a very practical way for most of this century for farm animals. The use of artificial insemination was the first animal biotechnology to have a major impact in this century. That impact became tremendous for dairy cows and pigs after the ability to freeze semen was developed. More recently, the biotechnologies of embryo cryopreservation and transfer have resulted in the worldwide rapid dissemination of extremely valuable genetic stocks. In the past few years, commercial ventures have been started based on the biotechnology of cloning animals. Cloning, in this context, means the production of multiple identical animals each derived from a single embryonic cell. These technologies will have impact for many years as plans are made to preserve as many species as possible to protect the genetic resources of the world.

The animal biotechnology that I will focus on today results from the revolution in molecular biology that permits us to permanently or temporarily modify the genetic composition of animals. Permanent modification means inserting genes into animals in such a way that the new genes can be passed on to their offspring. These animals with permanent genetic modification are called "transgenic" (Gordon and Ruddle, 1981). Temporary modification means that only the treated animal sees the new genetic material and perhaps only for a short time. Such animals I will call "somatogenic", indicating that only somatic cells, not germ cells, are modified.

The production of transgenic animals, while inefficient, is straightforward from the biologist's point of view. One-cell embryos are collected from donors shortly after insemination, and DNA solution containing the genes of interest is injected into a nuclear structure in the embryo. Embryos are either immediately transferred to recipients or cultured until they develop to a transferrable stage, e.g., the blastocyst stage in cattle. Only a small percentage, about one-half of 1% of injected eggs, results in the production of a transgenic farm animals (Rexroad, 1992).

The molecular biologist's view of this process is perhaps more complex and is beyond the scope of this talk. However, the power of transgenic technology is based on the molecular biologist's ability to modify and rearrange DNA molecules, thus creating genes with unique properties not found in nature (see Fig. 1 for an example). Another example is the ability to combine the part of one gene that serves as a mammary gland genetic switch with the part of another gene that specifies the production of some desired protein. Such new genes are being used to produce valuable protein pharmaceuticals such as human α-1 antitrypsin (Wright et al., 1991) in the milk of transgenic farm animals.

The production of somatogenic animals is also simple in concept. Two basic approaches are possible and will be only briefly considered because research in this area is limited for farm animals. The first approach is similar to that proposed for gene therapy in humans in which cells are removed from an animal and treated with DNA containing the genes to be expressed. The cells are returned to the animal. The animals will have the ability to produce the gene product as long as the cells persist. This complex procedure is probably not on the horizon for farm animals unless "universal donor" cell lines that can be given to any animal can be produced.

The second approach is elegant in simplicity. The DNA containing the gene of interest is injected by a pneumatic injector into the tissue in which it is desired to have the gene product produced. This "gene gun" is of the type used in the past for mass immunizations in the military. Gene gun therapy may have merit for inducing immunity in animals by introducing the gene for an antigen rather than the antigen. This approach might reduce problems associated with reactions to antigens other than the one of choice that result from the production of vaccines in culture. In addition, this approach might enhance our ability to produce multivalent vaccines for disease organisms that mutate frequently. Another possible use is simply to produce the gene product, such as a valuable pharmaceutical, in the injected tissue. While gene gun somatogenics may be on the distant horizon, Dr. Robert Wall in my laboratory in collaboration with a group from the NIH have demonstrated gene expression resulting from the injection of DNA into the mammary gland of sheep (Furth et al.,1992).

Three goals readily discernable for the use of genetically modified animals in agriculture are improvement of production characteristics, of animal health, and of product quality. I will provide brief examples of current research to demonstrate how genetic modification of animals might lead to achieving goals of each type.

The first transgenic animals reported in the scientific literature were produced at Beltsville (Hammer et al., 1985). These transgenic animals were genetically modified by the incorporation of additional growth hormone genes that produced growth hormone in many tissues. This widespread production caused increased levels of growth hormone in the blood of pigs and sheep. The important result of this research was the demonstration that permanent genetic modification could be made to farm animals. Subsequent important findings are that the growth hormone transgenic pigs grew faster (Fig. 2A), used feed more efficiently (Fig. 2B), and were very lean (Fig. 2C) (Pursel et al., 1989; Solomon and Pursel, 1992). Utilization of these findings awaits better understanding of the control

of the inserted genes to prevent detrimental effects associated with overproduction of growth hormone, such as reduced libido and leg problems (Pursel et al., 1987, 1989). Another genetic modification of pigs has been the insertion of a gene called SKI that very specifically enhanced muscle growth in mice. In swine, SKI causes variable phenotypes that are still under evaluation (Pursel, personal communication). These studies have demonstrated that the fundamental knowledge of growth is not sufficient to permit controlled manipulation. Intensive studies on the genetic regulation of muscle development and on regulation of genes suggest that we may profitably genetically modify animals for improved growth in 10 to 15 years.

Genetic modification of animals for improved health offers a great deal of promise because of the resources directed to understanding mechanisms of infection and host resistance. Experiments in nature in which mice secreting a retroviral-like protein are resistant to viral infection demonstrate one potential method for genetically modifying animals for resistance to viral infection. Chickens made transgenic by retroviral infection of the embryonic egg developed resistance to further infection when an incomplete retrovirus incorporated into their genome (for review, see Crittenden and Salter, 1990).

Retroviral infection of cells is thought to proceed by the attachment of specific viral coat protein to a specific cell protein, a receptor, on the surface of susceptible cells. Attachment may be a weak link in the infection process (see Fig. 3).

At Beltsville in cooperation with Bill Narayan and Janice Clements of Johns Hopkins University, we have genetically modified sheep with retroviral DNA that produces the attaching retroviral coat protein in susceptible cells. Our prediction was that we would flood the cell surface receptor and prevent retroviruses from attaching to the cells and being taken up to start the process of infection. These genetically modified sheep are potentially valuable resources for understanding retroviral infection mechanisms and understanding other processes of concern in the genetic modification of animals. Investigations are underway to determine if these transgenic sheep are likely to be protected against retroviral infection.

The horizon for the genetic modification of animals for increased disease resistance is not clear. Incorporation of transgenes into production cattle would take 15 years if a genetically modified cow with desirable traits were available today. Most of the time would be consumed in breeding the gene into the production population and testing each generation for the persistence of the trait in a new background. The time lines for introduction into swine or sheep would be much shorter because of the shorter generation intervals. Nonetheless, genetically modified animals with enhanced disease resistance as a permanent trait seems to be at least 10 to 15 years in the future. The horizon could be much closer for those genetic modifications schemes that need only to produce somatogenic rather than transgenic animals. Thus, if only antibody production rather than specific cell production of a protein is needed, then animals could be injected by a "gene gun" with DNA coding for an appropriate antigen. This technology could be valuable in the 5 to 10 year range.

The goal for genetic modification of animals that is closest to fruition is alteration of product composition. The driving force for product quality research has been the prediction that proteins with pharmaceutical value could be produced correctly and in large quantities in one of nature's best bioreactors, the mammary gland. An example of this research is the production of the pharmaceutical α-1 antitrypsin in the mammary gland of sheep (Wright et al., 1991). α-1 antitrypsin deficiency results in susceptibility to emphysema. Transgenic sheep produced in a government-industry collaboration in Edinburgh, Scotland, produced α-1 antitrypsin as 2 to 50% of their milk protein. The transgenically produced material was bioactive. The bioactivity of the material indicated that the mammary gland is capable of correctly processing proteins normally produced elsewhere in the body. The value of the technology can be quantified because it was sold for an estimated $25,000,000. Pharmaceutical research will impact agriculture in several ways. Some small population of milk animals will be diverted from food production to pharmaceutical production, perhaps as much as 5% of the U.S. dairy herd in the next 20 to 30 years.

Research on pharmaceutical production in milk will make it possible to conceive of experiments to modify milk in other ways to improve cheese production, reduce fat concentration, and reduce mastitis susceptibility (Wilmut et al., 1990). Other modifications may make cow's milk more like human breast milk. The horizon for food products from genetically modified animals is not clear and will depend on both continued research and the regulatory environment for products from genetically modified animals. The potential for modifying the composition of meat and eggs is under consideration but progress is such that predictions cannot yet be made.

Genetic modification of farm animals is in its infancy. The growth and development of the field is in part sustained by biomedical interest in transgenics. The usefulness of genetically modified animals is dependent on better understanding of the genes that regulate growth and development and on better understanding of how to regulate genes that are inserted into animals. Continued research on genetic modification of animals depends on the scientific community transmitting to the consumer that this research has tremendous potential to safely improve the quality of products consumed and at the same time offers the opportunity to improve animal health and productivity.

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Figure 1. New Genes By Recombination

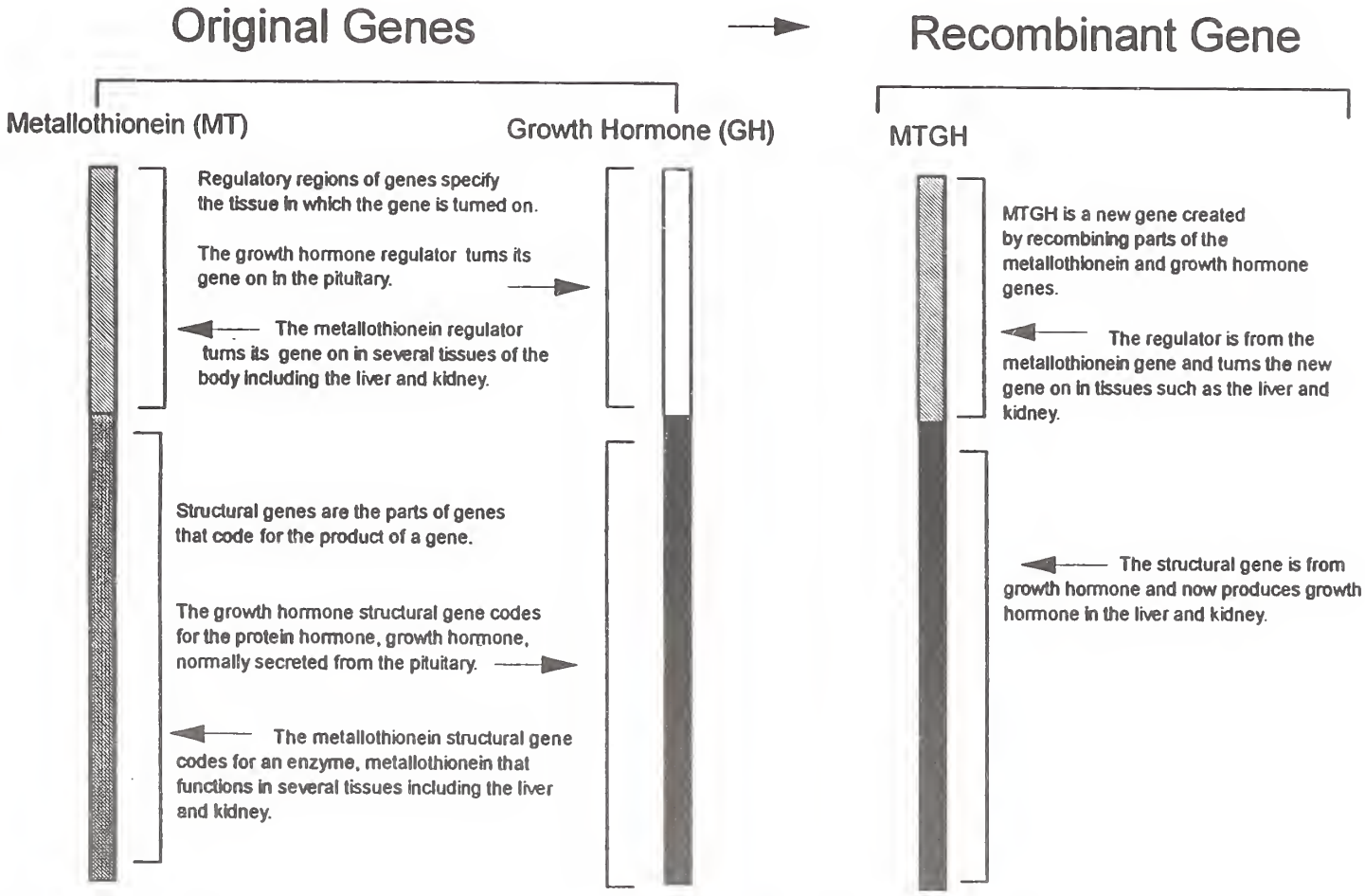

Figure 2. Growth performance Of Transgenic Pigs

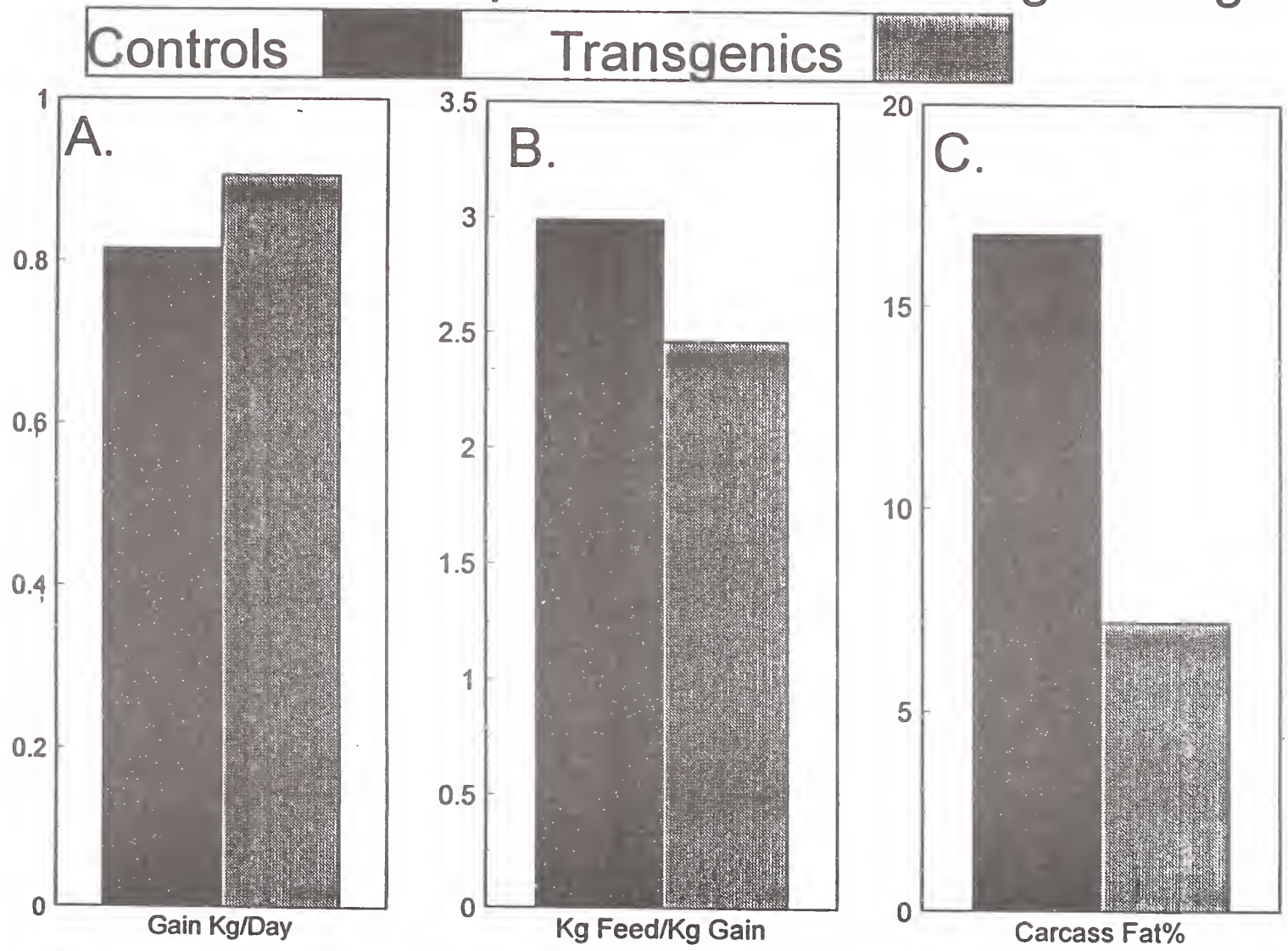

Figure 3. Model For Blocking Retroviral Infection