

Gene-Watchers' Feast Served Up in Toronto

International Genetics Congresses are held every 5 years at various locations around the world. The most recent, the XVth, became the largest ever when it attracted some 4000 participants to Toronto, Canada, between 20 and 27 August. The congress proved to be a veritable smorgasbord of recent developments in genetics, with sessions devoted to topics ranging from the most fundamental, such as how genes are controlled, to current efforts aimed at the genetic engineering of plants and animals. A few highlights follow.

Putting Foreign Genes into Domestic Animals

At the genetics congress, researchers reported that it may be possible to use domestic animals to produce large quantities of human clotting factors and other hitherto scarce proteins of medical value. According to results presented by A. John Clark of the Edinburgh Research Station of the Agricultural and Food Research Council, human genes can now be introduced into sheep in such a way that the protein products of the genes are made in the mammary glands and secreted into the animals' milk.

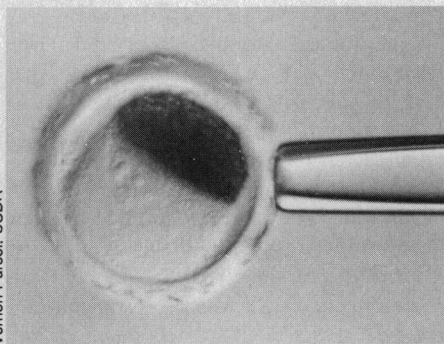
This should permit the harvesting of the proteins from the milk without any inconvenience for the sheep. "The sheep are to all intents and purposes completely normal, despite carrying the human genes," Clark told the congress participants.

The human genes transferred by the Edinburgh group encode factor IX, a protein that is needed for blood clotting, and the enzyme inhibitor called α_1 -antitrypsin. A deficiency of factor IX causes a form of the bleeding disease, hemophilia. α_1 -Antitrypsin inhibits the protein-degrading enzyme elastase. A

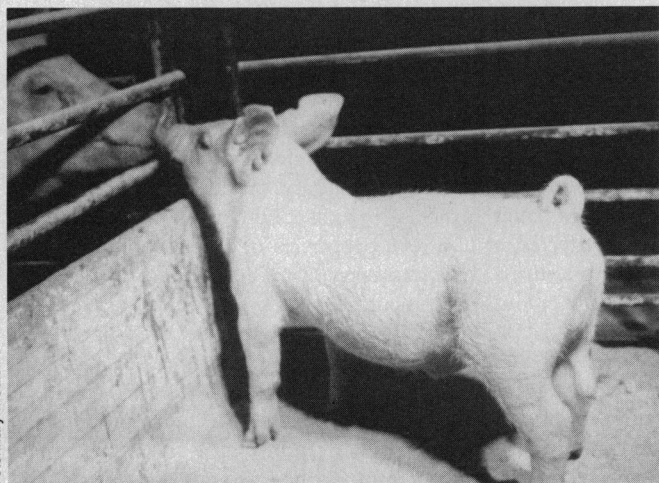
lack of the inhibitor in the lungs contributes to the development of the degenerative lung disease, emphysema.

It might be possible to prevent the lung degeneration by having emphysema patients inhale an aerosol containing α_1 -antitrypsin, but this would require very large amounts of the inhibitor, which are currently not available. Although many human proteins can be made in bacteria, producing them instead in animals might be advantageous. The proteins of higher organisms often contain sugar additions that bacteria are unable to duplicate but which may be required for normal activity of the proteins.

The Edinburgh workers transferred the factor IX and α_1 -antitrypsin genes into



Vernon Pursel, USDA



Transgenic pig and pig embryo. The first transgenic pig born at the USDA laboratory in Beltsville is shown here with his mother. The pig developed from a newly fertilized egg, such as the one shown in the micrograph, that was injected with a foreign growth hormone gene.

sheep by injecting them individually into newly fertilized sheep eggs, which were then implanted into foster mothers to develop. Before the genes were injected into the eggs, however, each was inserted into the gene for β -lactoglobulin, a protein found only in milk. This maneuver was done to endow the factor IX and α_1 -antitrypsin genes with regulatory sequences that would redirect the synthesis of the proteins from the liver, where they are normally made, to the mammary gland for secretion into the milk.

Although some of the sheep that developed from the injected eggs carried the human genes and secreted the proteins into their milk, as expected, the milk concentrations of the factor IX and α_1 -antitrypsin were generally low. Nevertheless, the experiments of Clark and his colleagues demonstrate the feasibility of using the gene transfer strategy to make human proteins in domestic animals. The yields may ultimately be increased by tinkering with the structure of the transferred genes, especially with regard to the regulatory sequences used.

In addition to using gene transfer technology to produce otherwise hard-to-obtain therapeutic proteins in domestic animals, researchers are also attempting to apply the technology to improve the characteristics of the animals themselves. For example, at the genetics congress, Robert Wall of the USDA Agricultural Research Service in Beltsville, Maryland, described efforts to develop leaner, faster growing strains of pigs by introducing human or bovine growth hormone genes into the animals.

So far, these experiments, which are being carried out by USDA researchers in collaboration with Ralph Brinster of the University of Pennsylvania School of Veterinary Medicine in Philadelphia and Richard Palmiter of the Howard Hughes Medical Institute at the University of Washington in Seattle, have produced mixed results. The animals that acquire the new growth hormone genes display the desired characteristics, but they also have a variety of physiological abnormalities.

The growth hormone genes were transferred into the pigs by egg injection. "The production of transgenic pigs is the same as the production of transgenic mice and sheep, albeit the surgery table is bigger," Wall told the genetics congress participants. ("Transgenic" is a term used to designate animals carrying a transferred gene.)

In their early experiments with mice, Brinster and Palmiter had shown that the acquisition of new growth hormone genes makes the animals grow significantly faster and larger than control mice. This did not appear to happen with the transgenic pigs, at least in the early experiments.

The animals weighed less at birth than control pigs and did not appear to gain weight faster or use their feed any more efficiently, when fed a standard pig diet. More recent experiments indicate, however, that transgenic pigs grow faster than controls if they are given a high-protein diet.

The transgenic pigs are considerably leaner than controls. This effect was expected because growth hormone favors the conversion of nutrients to proteins rather than fats. Having leaner pork would be considered a benefit in this health conscious era.

The transgenic pigs display several abnormalities that are not beneficial, however. The females never go into estrous and are sterile, possibly because they are so lean that they do not produce hormones normally. Animals of both sexes are lethargic, have muscle weakness, and are susceptible to developing arthritis and gastric ulcers, which often prove fatal. These effects are probably the result of excessive growth hormone production, Wall says.

Current work is aimed at getting better control of the expression of the transferred growth hormone genes. Until now, they have been linked to a promoter sequence from the metallothionein gene. This promoter responds to zinc ions by turning on the genes to which it is attached, and the researchers had hoped that they could determine when the foreign growth hormone genes would be active by adding zinc ions to the pigs' diet. However, the genes are apparently turned on all the time in the animals, even without the added dietary zinc.

The investigators are now looking for regulatory sequences that will allow them to turn the genes on—and off—as desired. Young pigs normally undergo a critical period of growth between the ages of 3 and 6 months. Limiting expression of transferred growth hormone genes to that time might be advantageous, Wall suggests.

Are Aging and Death Programmed in Our Genes?

The answer to this question is likely to be yes, according to data presented at the genetics congress by Olivia Pereira-Smith of Baylor College of Medicine in Houston. "Our results tend to support the hypothesis that cellular senescence in human cells is the result of programmed internal changes, not the accumulation of damage," she says.

Pereira-Smith, James Smith, who is also at Baylor, and their colleagues came to this conclusion during studies on cells growing in laboratory cultures. It is very difficult to "immortalize" cells so that they divide con-

tinuously in culture. They ordinarily become senescent and die after a limited number of doublings that depends on the species and age of the organism from which the cells were taken. Only under certain circumstances, such as when cells become cancerous, will they divide forever in culture.

Results obtained by the Baylor group suggest that immortalization results from mutations in a small number of genes, which would otherwise act to restrict the cells' proliferative potential. When the researchers fused different types of immortalized cells, they found that some of the hybrids became immortal again, and senesced and died. The two immortal parents of the hybrids apparently had different gene defects so that each could provide the function that the other was lacking when they were fused.

Other hybrids remained immortal, a result indicating that the parents had the same gene defect. According to Pereira-Smith, mutations in as few as four genes could have given rise to the 26 immortalized cell lines used in the fusion studies. "The work indicates," she says, "that a limited number of cellular genes are involved in normal cellular senescence and can be altered to make immortal cells."

The identities of the senescence genes are currently unknown, although the Baylor group has identified a possible candidate. In earlier work, they found that senescent cells contain molecules, including a membrane-associated protein and messenger RNAs, that inhibit DNA synthesis, and should therefore block cell division, when they are injected into young cells that still have the capacity to divide.

The assumption is that the growth-inhibitory messenger RNA directs the synthesis of the membrane-associated protein. The Baylor group has now cloned a gene corresponding to one of the RNAs and find that it encodes the protein fibronectin. Fibronectin is membrane-associated and there are reasons to suspect it might have growth inhibitory effects. Actively dividing cancer cells, for example, generally make little or no fibronectin, whereas senescent cells produce increased amounts. Pereira-Smith and her colleagues are trying to purify enough of the membrane-associated, DNA synthesis-inhibiting protein from senescent cells to determine a partial amino acid sequence and establish whether it is fibronectin.

Finally, there is growing evidence for the existence of genes that suppress cell growth and might therefore help to prevent the out-of-control growth of cancer cells. The properties of the senescence genes are reminiscent of those of the tumor-suppressor genes. It will be interesting to see whether any of the two types of genes are identical.

New Method Found for Making Mutant Mice

Researchers have found a novel way of making developmental mutants of mice. At the genetics congress, Martin Breitman of Mount Sinai Hospital Research Institute in Toronto reported that mutant mice can be produced by introducing a gene into mouse embryos that programs the destruction of specific cell types during development.

Such mutants should be useful for determining how one cell type influences the developmental fates of others. Breitman and his colleagues are applying the new method to study eye development, the role of the lens in particular. "It puts us in a position to make some rather strong conclusions about the role the lens plays in inducing the development of other eye structures," he says.

The gene that the Toronto group uses for making the mutant mice encodes the A chain of diphtheria toxin, an enzyme that kills cells by bringing protein synthesis to a halt. The researchers linked this gene with a regulatory sequence, called a promoter, from one of the genes encoding the crystallin proteins of the eye lens. This promoter directs the expression of its genes exclusively to the lens fiber cells, the mature cells that make up the bulk of the lens body. If the promoter works normally with the A-chain gene, it should limit expression of the gene—and thus the cell-killing effects of the toxin—to the fiber cells.

That is what Breitman and his colleagues found when they introduced the hybrid A-chain gene into mouse embryos by egg injection. Mice that acquired a functional copy of the gene had smaller than normal eye lenses, although the animals appeared healthy otherwise. They were able to breed and transmit the toxin gene to their progeny.

The majority of the mice that were bred to have a double dose of the diphtheria toxin gene ended up with no lenses at all. Lack of a lens did not prevent the development of most of the other eye structures, including the cornea and retina. However, the vitreous body, which produces the clear jelly-like material that fills the eye cavity, did not develop in the absence of the lens.

In theory, the same approach used for knocking out the cells forming the eye lens should be applicable to any cell lineage provided that the appropriate regulatory sequences can be found and linked to the diphtheria toxin gene. In practice, however, difficulties could arise because destruction of some cell lineages is likely to kill the embryo.

■ JEAN L. MARX