Molecular Biology Lies Down with the Lamb

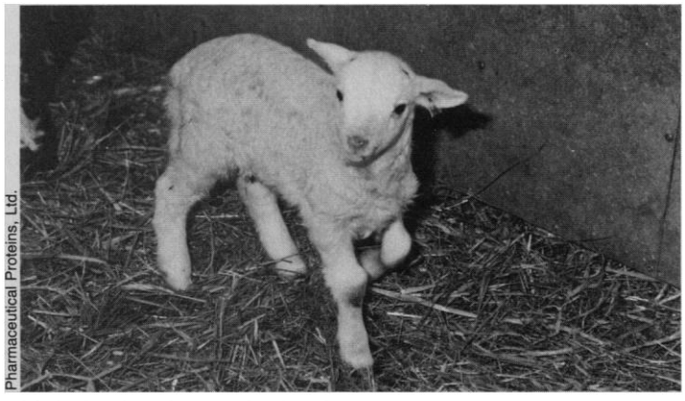
Edinburgh has become the center of an unusual collaboration that produces cutting-edge work on transgenic animals

JOHN ANSELL HAS HIS OWN IDEA about why Edinburgh has become a world center for research on transgenic animals: the city's water. "We could grow things up here that no one else could grow," says Ansell, an immunobiologist who was in on the early days of embryo culture. In those days, biologists tried everything they could to improve their techniques for culturing cells, including embryonic cells, a crucial step in developing transgenic animals. That included distilling the water again and again. Outside Edinburgh, the results improved up to the sixth distillation. "Up here we just used to do it once or twice, and it was fine," said Ansell, assistant director of the new government-sponsored Centre for Animal Genome Research (CAGR).

Whatever the underlying reasons, Edinburgh has indeed become a world leader in the transgenic work. Researchers from the University of Edinburgh and units supported by the U.K. Agricultural and Food Research Council have scored numerous successes over the past few years. Among them: the first sheep to carry human genes and the first demonstration that genetic defects can be corrected in an animal and it correction passed to the offspring. In addition, the transgenic sheep are being exploited as factories for making key human proteins that may be in clinical trials next year. The Edinburgh scientists are also on their way to creating animal models of specific human diseases.

These accomplishments put the workers in Edinburgh on the cutting edge in their field. Of course, they aren't alone there. These days transgenic animals—animals that carry genes from other species—are a hot commodity, both for pure research, in the hands of people like Ralph Brinster of the University of Pennsylvania and Richard Palmiter of the University of Washington, and for applied uses in the hands of groups like the one led by Vernon Pursel at the U.S. Department of Agriculture's Agricultural Research Service in Beltsville, Maryland. But the work at Edinburgh is unique in one respect: it involves a most unusual and fruitful collaboration among scientists in government, university labs, and private companies.

And there were many factors other than the water that conspired to bring the collaboration about, as even Ansell would admit. For one thing, a long research history had resulted in the creation of a core group of scientists. The university's Department of Genetics had accumulated several people with a deep understanding of quantitative genetics. Units of the Agricultural and Food Research Council's (AFRC) predecessor [the Agricultural Research Council (ARC)] had considerable expertise in problems of selection for so-called production traits, such as more rapid growth in farm animals. And the molecular biologists, under the guidance of people such as Ed Southern, director of the Medical Research Council's Mammalian Genome Unit attached to the university's Department of Zoology and inventor of the DNA blotting technique that bears his name, were also a force to be reckoned with.



Pharmaceutical Proteins, Ltd.

Little lamb, who made thee? *Tracy, a sheep who, like her mother, carries the human gene for* α1-antitrypsin.

But this accumulated knowledge might never have come to fruition if it hadn't been for a strategic reassessment that came in 1983. In that year the ARC announced that research on farm animals, of the sort being carried out at its Animal Breeding Research Organization (ABRO) in Edinburgh, was not as productive as it might be. Indeed, the ABRO was threatened with closure. But instead of resigning themselves to their fate or fighting a holding action, Roger Land, director of ABRO, and other Edinburgh scientists convened an informal but high-powered team and asked themselves how research on animal production could be ushered into the age of genetic engineering.

And at those early meetings a critical decision was made. As Nick Hastie, a molecular geneticist and section leader at the Medical Research Council's Human Genetics Unit in Edinburgh, who was there, recalls: "They decided there wasn't anything simple and straightforward you could do that was worthwhile in terms of altering the production characteristics of farm animals. They made the big decision then that instead of doing that they should use them as factories."

This went against the grain of what most scientists were doing with the new molecular biology. Elsewhere, scientists opted to tinker with growth hormone genes in the hope of producing speedier, more efficient converters of feed to meat. But the Edinburgh workers saw that as a dead end. They knew, for example, that chickens selected for very rapid growth actually make less growth hormone than normal chickens. Tinkering with growth hormone genes wouldn't be productive, they decided.

Instead, the informal group decided that before the ABRO went down the drain, its scientific staff should try to make a farm animal that would produce useful amounts of human proteins such as Factor IX. People lacking this protein are hemophiliacs and need regular injections of Factor IX to enable their blood to clot.

But what animal should serve as the factory? Most of the mammalian transgenic work up to that time had been done in mice. But Rick Lathe, a molecular biologist, had a different notion: sheep. The attraction of using another mammal to manufacture human proteins was that it would probably process the proteins properly after making them, something other genetic engineers were having trouble persuading bacteria and yeast cells to do. And if they could attach the human gene to a gene for a protein produced in milk, they could reasonably hope that the product—whatever it was—would be secreted in the milk and have no effect at all on the host. "The sheep's body wouldn't know its mammary glands were producing human protein," explains Lathe.

With hindsight Lathe's recommendation seems obvious, but in 1985 it was a gamble. Others had tried and failed to put foreign

DNA into sheep, and conventional wisdom held that the task was difficult—or impossible. Undaunted, Lathe assembled a team at the ABRO, just before that group went out of business by being absorbed into the AFRC's Institute of Animal Physiology and Genetics Research.

One member of the group was John Clark, a molecular biologist, who isolated, cloned, and then sequenced the gene for β-lactoglobulin (BLG), a small protein found in the milk of ruminants. To the BLG gene Clark stitched one of two genes for useful human proteins: FIX, the gene for human Factor IX, or the gene for α1-antitrypsin (AAT), a protein that helps to keep cells elastic.

Simultaneously, Paul Simons, a molecular geneticist, and Ian Wilmut, a reproductive biologist, set to work on the technology for culturing sheep embryos. The problem was to be able to see—and hence to inject with foreign genes—the sheep embryo's pronucleus, where the DNA is busy dividing after fertilization. In the mouse the pronucleus is clearly visible, but in sheep it is much harder to see. Simons watched through a microscope as Wilmut flooded fertilized eggs with fixative, making the pronucleus stand out. Slowly, Simons began to be able to pick up the pronucleus in living cells that had been centrifuged to move some of the obscuring granules out of the way, and eventually he could detect the pronucleus in almost all embryos with no outside assistance.

Clark handed over the transgenes, Simons injected them into the embryos, and Wilmut put them into prepared ewes. While the lambs came to term, the team did it again in mice. But it was the sheep they were really waiting for. "It was pretty nerve-wracking," Wilmut says, but by May 1986 they had what they wanted.

A total of 511 injected embryos gave rise to 109 animals. Clark analyzed the DNA in blood samples taken from each lamb; six were transgenic. "We didn't believe the first one," Lathe recalled, "it was the worst blot I've ever seen. So we did it again. After that one came out right too, we said it was okay to go out and get drunk."

These were the world's first healthy transgenic sheep. Brinster's lab in Philadelphia had produced one transgenic sheep the year before, but it was stillborn and the inserted gene was scrambled. Now here were six healthy lambs. What is more, when they matured, the transgenic animals transmitted the human genes to their offspring and the ewes secreted either FIX or AAT in their milk.

"It was pretty amazing to score so quickly," Clark admits. "Perhaps it was just luck, but also we did some things differently." Minimizing the delay between injecting the egg with DNA and putting it into the ewe was one. The choice of genes may have been another.

Armed with that success, the Edinburgh scientists began to think of expanding their program. John Bishop, a molecular geneticist at Edinburgh University's Department of Genetics, first conceived a new plan: to set up an interdisciplinary research center (IRC). IRCs are a new trend in British research funding. The idea is to create large centers of excellence, bringing together a critical mass of scientists from different fields. Bishop's suggestion was taken up enthusiastically by the AFRC, which invited proposals for an IRC. Bishop assembled a team that included Ansell, Clark, and Hastie, and they formulated Edinburgh's successful bid. It took just 18 months from Bishop's first idea to breaking ground on the new center.

"A few years ago, the AFRC didn't know what to do with us in Edinburgh. Now we have so many good projects we hardly know where to begin."

—Rick Lathe

One of the reasons for Edinburgh's success in attracting the CAGR to its home turf was an independent breakthrough in the university there—a breakthrough that has pushed the transgenic work ahead at a much more rapid clip. In the Department of Pathology, Martin Hooper, a cell geneticist, developed techniques for genetically altering embryonal stem (ES) cells. These are cells of the very early embryo that still have the potential to develop into any of the final tissues in the fully grown animal. Working with ES cells makes possible far more genetic precision and therefore enables researchers to attempt manipulations that would have been prohibitively time-consuming and wasteful with other transgenic techniques.

In the past, transgenic animals had to be allowed to mature before one could determine whether they had taken up the foreign DNA successfully. By growing engineered stem cells in culture, researchers can establish that the foreign DNA has been successfully integrated *before* recreating a whole animal. The engineered stem cells are then put back into a developing embryo; the resulting animal is a chimera, or mixture, of ordinary cells and transgenic cells. In some cases, transgenic cells form the chimera's reproductive cells, which means its offspring, will be entirely transgenic.

Hooper and his colleagues selected cells that had a mutation in a gene called *HPRT* and turned them into whole mice. Some of their offspring were completely deficient in *HPRT*, a condition that in humans gives rise to Lesch-Nyhan syndrome, a severe—and usually fatal—genetic disease. Working with David Melton, a lecturer in molecular biology at Edinburgh, Hooper went on to correct the *HPRT* deficiency by using gene targeting procedures in ES cells. This was the first demonstration that a precise genetic alteration could be introduced into a mammal's germline—and may be the harbinger of some important advances in treating human genetic disorders.

Manipulating ES cells will be one of the key techniques at the CAGR. Indeed, Hooper, its inventor, will be an assistant director there. "The thing about the ES step," he says, "is that you can select those clones that are doing what you want them to be doing." That will make possible development of models of many human genetic diseases—from common disorders such as cystic fibrosis to rarer conditions such as Lesch-Nyhan syndrome and Tay-Sachs disease. Animal models for these diseases—and many others—have not so far been discovered by simple searching. With ES cells, you can make them to order.

An even more far-reaching line of research, which Ansell describes as "the most speculative of all the projects," is to see whether the differentiation of the stem cells can be controlled. Lindsay Williams, a molecular geneticist who is moving to the new center from the Ludwig Institute in Melbourne, will have a big hand in that work, along with Austin Smith, originally from Hooper's lab, Hooper himself, and Ansell. The payoff could be enormous. If scientists can direct the differentiation of the stem cells they could, for example, produce, more or less, to order bone marrow cells carrying specific genes. And that would have profound implications for human gene therapy.

But it shouldn't be assumed that the work done at the new center will all be far-out cutting-edge stuff. Some of it will involve collaborations with industry aimed at making the new methods into workable forms of technology. Some of those collaborations may well involve Ron James, an organic chemist who heads Pharmaceutical Proteins Ltd., a company set up in Edinburgh to exploit transgenic technology for making therapeutic proteins.

James' company employs 20 scientists. Some are trying to make sheep more efficient producers of FIX, the salvation for many hemophiliacs, and AAT, which might be used to treat emphysema patients. Others are working on means of purifying the proteins from the ewes' milk. "There are already well-established techniques for getting rid of the fats in milk and precipitating out some of the proteins," says James. "We have to go a bit further."

Transgenic animals will no doubt be used for making proteins that cannot be obtained any other way. For an entrepreneur like James, however, their great advantage is economic. Making a gene construct, demonstrating it in mice, and producing the first transgenic sheep might cost a couple of million dollars. Building up a milking flock might cost another million. "That's nothing by pharmaceutical standards," he says.

James calculates that to satisfy the potential market for AAT in an economic fashion he will need sheep that can secrete 2 grams of AAT per liter of milk. Clark and his team have a paper in press reporting the highest ever levels of AAT in milk, 7 grams per liter. The method is not yet practical because the source of the milk is mice, who make only microliters each. But these results do demonstrate that transgenic animals may soon revolutionize the supply of therapeutic compounds. James has his eye on tissue plasminogen activator, for dissolving blood clots, erythropoietin, which controls the synthesis of red blood cells, and many other proteins. Other companies, too, are eyeing transgenic animals for making these products, and it will be a race to see who can make the first sales. But James is confident. "I can't tell you what our first product will be," he says, "but we will have one, and soon, I can tell you that."

The kind of thing James is doing is only the first practical fruit of the work that will be done at the new center, which the AFRC last year pledged to support for 10 years to the tune of some £15 million (about $25 million). Among the others might be cows that produce milk better suited to human digestion, disease-resistant livestock, substances that reverse the transformation of ordinary cells into cancerous ones, and maybe even genetic studies of such things as aging and memory. And all this stemming from a government-supported research unit that was on the verge of dissolution a mere 7 years ago.

"It is extraordinary," says Lathe, now director of the CAGR. "A few years ago, the AFRC didn't know what to do with us in Edinburgh. Now we have so many good projects we hardly know where to begin."

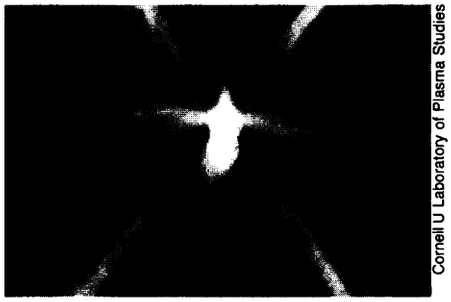
■ JEREMY CHERFAS

X Marks the Spot

A recently developed method of creating powerful x-rays with an x-shaped pair of wires could be x-actly what the semiconductor industry needs. A group of researchers at Cornell University in Ithaca, New York, has shown that a simple x-ray source could lead to a relatively inexpensive way to make advanced computer chips.

Today, the patterns on integrated circuit chips are etched out of semiconductors using optical or ultraviolet lithography. The semiconductor is first covered with a layer of photosensitive material, or photoresist, and this in turn is covered by a stencil-like mask in the shape of the desired electric circuit. When the chip is exposed to visible or ultraviolet light, a chemical change takes place in all of the unprotected areas. Then, depending on the process, either the exposed or the unexposed part of the photoresist—with the semiconductor lying directly underneath it—is etched away by acid, leaving a pattern in the shape of the mask.

But if computer chips are to continue getting smaller, industry experts say, lithography will probably have to be done with x-rays, which have shorter wavelengths than visible or ultraviolet light and thus can create much smaller circuits. The problem is that machines that can produce x-rays powerful and concentrated enough for lithography are both expensive and cumbersome. One option, for instance, is to extract x-rays from a synchrotron—a ring-shaped particle accelerator in which electrons give off x-rays as they are deflected in a strong magnetic field. Several Japanese companies are building compact synchrotrons for x-ray lithography, but these machines cost tens of millions of dollars and, at 10 meters across, they are only relatively "compact."



Cornell U Laboratory of Plasma Studies

X-ray crossing. *The bright spot is producing x-rays of wavelength 7.2 to 7.8 angstroms.*

The Cornell method may make the synchrotron extraneous, however. The team of David Hammer, Daniel Kalantar, Nian-Sheng Qi, and Kailash Mittal at Cornell's Laboratory of Plasma Studies generated high-intensity x-rays, comparable to those from a synthrotron, by sending short, powerful pulses of electric current—in one experiment they used an 80-nanosecond, 450,000-ampere pulse—through crossed wires of aluminum or magnesium. The intense current vaporizes the very fine wires, Kalantar explains, and also ionizes them, leaving the atoms with only one or two electrons. These electrons are in excited energy states, and when they drop down to the ground state they emit x-rays.

Even after the wires are vaporized, the resulting plasma continues to carry the electric current, and magnetic fields created by this current concentrate the plasma and further intensify its x-ray emissions. The magnetic fields also pinch the plasma in toward the center of the "x," creating an intense concentration of plasma at that point, so that much of the x-radiation comes from one small spot, called the x-pinch.

When the Cornell group measured the radiation from the x-pinch, they found that the aluminum wires generated about 23 joules of x-ray radiation with wavelengths from 7.2 to 7.8 angstroms. With six magnesium wires all meeting at one point and a current of 550,000 amperes, they measured 85 joules at 8.4 to 9.2 angstroms. That should be good enough, Hammer says, for x-ray lithography, although the group has not yet tried to expose a photoresist with x-rays generated by their device.

If the x-rays test out well on photoresists, the researchers will build a prototype machine to do x-ray lithography on computer chips. The major tasks here will be building a mechanical device that replaces the wires after they have been vaporized and designing a pulsed power generator to supply electrical pulses with optimum characteristics. It should not be difficult to engineer a machine with these capabilities, says Cornell colleague Steve Glidden, who has joined with Hammer and Kalantar to form a company to build the machine. The researchers will also have to find a way to protect the computer chips from any debris created by the vaporizing wires and to make sure that the x-pinch produces no harmful radiation along with the desired x-rays.

Glidden predicts that they will be able to build an x-ray lithography machine that generates ten pulses of x-rays a second for under $500,000. It would also be much smaller than a compact synchrotron—probably only a few cubic meters, Glidden says. All in all, he says, the prospects are x-cellent.

■ ROBERT POOL