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Animal Biotechnology [and Discussion]

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Animal biotechnology

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Biotechnology has taken two directions in efforts to speed up animal production above the rates achievable by selective breeding. Recombinant DNA methods have been used to engineer protein gene products for direct administration to livestock, as in recombinant growth hormone to stimulate lactation in dairy cows or yield faster-growing, leaner carcasses in meat animals. Cloned cellulolytic genes have been inserted into ruminal microorganisms with a view to improving ruminant nutrition. The other direction is to use advanced breeding technologies to enhance performance. These include laboratory culture of large numbers of viable embryos for non-surgical transfer to surrogate mothers, development of methods for sexing sperm and embryos, cloning embryos by nuclear transplantation and gene transfer to create livestock with superior performance traits. In all cases material progress will depend upon a deeper understanding of the underlying physiological and developmental control mechanisms and public confidence that due regard is being paid to animal welfare, and to social and environmental implications.

1. Introduction

Since prehistoric times humanity has depended upon domestic animals for food, clothing ornament, sport and companionship. Animals were chosen not at random but for specific genetic dispositions such as docility, fertility, gregariousness and a dominance hierarchy that allowed man to usurp the commanding role (Clutton-Brock 1981). Over thousands of years we have sought to improve valuable traits by selective breeding, an art that became a science with the discoveries of Mendel. In the modern era the rate of livestock improvement had been much increased by advances in reproductive biology including artificial insemination of cattle. However, the annual genetic gain for desirable traits like growth rate, leanness or milk yield in ruminants is generally under 2% (Smith 1984). This response rate to selection can be doubled by using multiple ovulation and embryo transfer (MOET) technology, for this proliferates the progeny from a single mating (Nicholas & Smith 1983). Nevertheless, the quest for even more efficient animal-production systems received its greatest boost from the new insights of molecular and developmental biology.

There have been many recent review articles and popular accounts of animal biotechnology from the standpoint of genetic manipulation (Wagner 1985: Hodges 1986; Fehilly & Willadsen 1986; Simons & Land 1987; Pashen 1987; Land 1988; Wilmut *et al.* 1988; Sreenan 1988). Two broad strategies have emerged. In the first, improved productivity is procured by circumventing the tedious business of selective breeding through direct administration of the gene product, growth hormone, for example, in the form of recombinant bovine somatotropin (BST). In the second accelerated production rates are sought by artificially manipulating the embryonic genome, as in cloning or transgenic techniques.

For present purposes animal biotechnology can be categorized into breeding and non-breeding strategies.

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2. Non-breeding strategies

(a) *BST and milk production*

The strongly galactopoietic action of anterior pituitary growth hormone has been known for many years, and Brumby & Hancock (1955) stressed the increased energetic efficiency of milk production in dairy cows. Food intake does not initially rise commensurately with extra yield, partly because urea excretion is diminished as nitrogen is retained in milk protein. The prohibitive cost of hormone from the natural source precluded commercial exploitation of these basic discoveries until gene cloning and recombinant DNA technology made possible the large scale production of recombinant bovine growth hormone or somatotropin by companies such as Monsanto and American Cyanamid (Bauman *et al.* 1985).

Extensive trials have been done with BST, mainly in cattle but also in sheep and goats. The galactopoietic response to the recombinant product is essentially the same as that to the purified natural hormone. At a recent meeting of the American Dairy Science Association (Anon. 1988) results of cattle trials in a score of research institutions involving thousands of cows were reported. Milk yields increase within days of commencing treatment, reaching 15% to 25% greater or more in a few weeks when food intake begins to rise. As compared with untreated high-yielding controls, no significant adverse effects were found either in the health of the animals or the composition of the milk even with doses of BST several times those needed for commercial purposes. All the findings are consistent with the notion that cows under treatment behave as would be expected of genetically superior dairy cattle (Peel & Bauman 1987). This conclusion is reinforced by the finding that in growth-hormone release tests Friesian calves from a high milk-yield line consistently gave larger responses than calves from a low-yielding line (Løvendahl *et al.* 1989).

The need for frequent BST injection can be obviated by slow-release implants and the effects are fully reversible. The technology therefore not only offers the possibility of leap-frogging twenty years of selective breeding but also the ability to adjust herd production to meet short-term market or quota requirements. There is little doubt that full implementation of the technology would materially lower production costs. However, reactions of the public and farmers have so far been unenthusiastic, owing to worries about the social, economic, welfare and safety implications.

Some of the misgivings might be lessened by further work on the physiological mechanism of BST galactopoietic action. Babraham workers (Prosser 1989) have found in goats that responding animals show raised concentrations of the insulin-like growth factor (IGF-1) in their plasma and milk. Moreover, close arterial injections of IGF-1 into the udder stimulate a 30% rise in milk secretion as compared with the uninjected contralateral gland, indicating a direct action on the secretory cells. Growth hormone itself does not have this direct effect and is thought to act by releasing IGFs from other organs, such as the liver. The increases of IGF-1 in the circulation and in milk following BST treatment do not exceed those occurring under natural conditions.

(b) *Growth and meat production*

The physiology of growth in farm animals is still poorly understood despite the multiplicity of hormones and growth factors known to be implicated. Among these growth hormone has a central role in the young animal, akin to that in lactating adults, of directing metabolism towards protein synthesis (Wray-Cahen *et al.* 1989), in this case in bone and muscle, not milk.

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An equally relevant metabolic effect of the hormone in growing animals is its anti-lipogenic action (Vernon & Flint 1989; Sinnett-Smith & Woolliams 1989), which reduces the percentage of adipose tissue in the carcase.

It is not therefore surprising that many attempts have been made to increase live weight gains and leanness by treatment with recombinant growth hormone (Spencer 1987). Results have been variable. In pigs, growth-rate increases of 10–20% and improvements of feed conversion of 25–30% have been reported (Chung *et al.* 1985; Etherton 1989). On the other hand, high-performance German pig breeds showed no significant weight gains with recombinant porcine growth hormone but even the lean Pietrain breed showed a highly significant further reduction in carcase fat attributable to the growth hormone treatment (Ellendorff *et al.* 1989). In lambs a similar phenomenon can be seen. In favourable circumstances a 36% enhancement of growth rate could occur after two months treatment with ovine growth hormone associated with a 30% reduction of visceral fat. However, with higher levels of feed intake, fat deposition in untreated lambs may obliterate the body-weight difference (Pell 1989).

There are good grounds for supposing that IGF-1 is a mediator of the protein anabolic action of growth hormone but probably not for the anti-lipogenic effect. Moreover, its availability to tissues is much reduced by the presence of powerful IGF-binding proteins. For these reasons recombinant IGF-1, which is now available commercially, would be unlikely to compete with recombinant growth hormone as a means of obtaining more efficient meat production (Etherton 1989).

A variety of immunological manipulations have been tried in attempts to influence growth performance. They include active immunization against somatostatin, a hormone produced in the hypothalamus that inhibits release of growth hormone. This treatment may produce growth increments of 10–20% in cattle, sheep and pigs (Spencer 1987). Certain monoclonal antibodies have been found to enhance the biological activity of injected growth hormone (Holder *et al.* 1985). The mechanism of the latter action remains obscure.

Confidence in the rational handling of all the above procedures will increase when we have a more comprehensive knowledge of the fundamental interplay of the various hormonal and other factors. None of the treatments is likely to reproduce the dynamic pulsatile endocrine changes that are now known to occur naturally (Robinson 1989).

(*c*) *Ruminant nutrition*

Breakdown of cellulose in ingested herbage by cattle, sheep and goats is effected by microorganisms in the complex ecosystem of the rumen. In ruminants receiving normal diets, a significant proportion of dietary fibre remains undigested and an improved conversion of forage into meat, milk and wood could be expected if cellulose and ligno-hemicellulose complexes were more efficiently degraded in the rumen (Armstrong & Gilbert 1985; Hazlewood & Teather 1988). Recombinant DNA techniques and the introduction of genes encoding important degradative enzymes such as cellulase and hemicellulase offer means of modifying the function of the ruminal microflora and improving the efficiency of fibre digestion. A 10% improvement in forage digestibility could have a value to the U.K. dairy industry in excess of £10M.

The technical problems of working with obligate anaerobes and the paucity of fundamental knowledge of their genetics have impeded progress towards that objective. Nevertheless,

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workers at Babraham and elsewhere have obtained encouraging results with cloned genes in ruminal organisms *in vitro*. Cellulase genes have been cloned and expressed in *Escherichia coli* from the highly cellulolytic thermophile *Clostridium thermocellum* (Hazlewood *et al.* 1988) and from the rumen bacteria *Butyrivibrio fibrisolvens*, *Ruminococcus albus* (Romaniec *et al.* 1986), *Ruminococcus flavefaciens* (Barros & Thomson 1987) and *Bacteroides succinogenes* (Teather 1985). The genes and their encoded proteins have been extensively characterized and in some instances, primary sequences determined (Hall *et al.* 1988). In other work at Babraham the *celE* gene of *C. thermocellum*, encoding endoglucanase and hemicellulase activity, was inserted and expressed in *Enterococcus faecalis*, *Bacillus subtilis* (S. P. Mann & J. I. Laurie, unpublished results) and *Streptomyces albus* (Romaniec *et al.* 1987).

Whether genetically manipulated organisms would survive extended periods in the rumen is uncertain. However, Jones (1981) and C. G. Orpin and S. D. Mathieson in unpublished experiments at Babraham have found that naturally occurring variants of known rumen bacteria can become established when introduced into a 'foreign' rumen, even that of a different species. This suggests that inoculation with an engineered organism having the right phenotype would have a good prospect of success. Nevertheless, from a commercial standpoint a more attractive option might be a probiotic approach whereby viable microbes are added to the feed to produce a short-lived effect on ruminal digestion. Alternatively, bacterial silage inoculants could be enhanced by inclusion of genetically engineered organisms. In this regard the *celE* gene of *C. thermocellum* has been successfully inserted and expressed in *Lactobacillus plantarum* (H. J. Gilbert, G. P. Hazlewood & S. P. Mann, unpublished results).

Another area where recombinant DNA techniques may find application is in protein nutrition of ruminants. The introduction into rumen bacteria of a synthetic gene encoding a polypeptide of defined amino acid composition is being actively pursued with industrial support as a means of meeting the protein requirement of highly productive dairy cattle (Teather 1985). More speculative objectives include reduction of methanogenesis and proteolytic activity in the rumen and the introduction of a nitrogen-fixing capability (Armstrong & Gilbert 1985).

Among the earliest domesticated animals, the ruminants are distributed in all continents. Significant benefits would follow if we could learn to manipulate the enzymic function of populations of ruminal microorganisms. Sadly, commercial interests have so far been hesitant to invest in the necessary preparatory research, which is still at the basic and strategic level (Hazlewood & Teather 1988).

3. Breeding strategies

(*a*) *Laboratory production of embryos*

Early developments in artificial insemination, low-temperature preservation of sperm and sire evaluation have enabled fully effective exploitation of the genetic potential of the male. Until recently no comparable methods were available for genetic exploitation of the female. The gap was narrowed by the introduction of systems for superovulating animals with gonadotrophic hormone, mating them, flushing out the fertilized ova and transferring the embryos to the uteri of surrogate mothers by surgical or non-surgical means (Adams 1977; Polge 1978). Embryo transfer in the cattle industry is now a highly organized technology (Sreenan 1988).

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There remains a gross inequality between the number of sperm obtainable from the male and the number of ripe ova obtainable from females during their breeding life. To expand the supply of fertilizable ova, Moor and his colleagues at Babraham have investigated in depth the molecular events associated with maturation in culture of oocytes harvested from livestock ovaries at the slaughterhouse (Staigmiller & Moor 1984; Moor & Gandolfi 1987). From this work reliable culture methods have been developed.

An essential adjunct to culture of oocytes is their fertilization *in vitro* to form zygotes. Though *in vitro* fertilization of human oocytes had been reported as early as 1969 (Edwards *et al.* 1969), viability of the resulting embryos remains disappointingly low principally due to the imperfect completion of oocyte maturation under the laboratory technique employed (Osborn & Moor 1988). Successful *in vitro* fertilization of livestock oocytes was achieved much later (Cheng *et al.* 1986) and culturing conditions for further development of the resulting embryos were quickly devised which by inclusion of oviduct cells in the medium provided essential growth factors for the formation of blastocysts. It then became possible for the first time to take immature oocytes from slaughterhouse ovaries and produce from them entirely *in vitro* blastocysts suitable for implantation into surrogate mothers (Gandolfi & Moor 1987). Previously it had been necessary to insert the early embryos into ligated sheep oviducts in order to grow to the blastocyst stage and then to extract them for transfer to the final host uteri.

The technology of oocyte maturation, *in vitro* fertilization and embryo culture developed in Cambridge has been taken up by at least two commercial enterprises that intend to market low-priced embryos for non-surgical transfer to cattle. There are various potential applications for the technology but the one that has attracted immediate interest is the production of beef embryos for insertion into dairy cows two at a time by a process hardly more complicated than artificial insemination (AI). Subject to proper welfare considerations for calving, high-quality beef animals can be obtained from dairy herds with substantial economic advantage. Current trials being run by Animal Biotechnology Cambridge Ltd and the Milk Marketing Board indicate that annual calf production per hundred cows might rise from about 90 to 130 or more.

Embryo culture under tight laboratory conditions is a prerequisite for many subsequent genetic manipulations for livestock production including sexing, cloning and gene transfer, which will be considered below. A similar consideration applies to the poultry industry and much effort has gone into investigating techniques for culturing chick embryos under laboratory conditions. The embryonic lifespan of the chick is 22 days, all but the first of which are spent encased in albumen and shell. Perry (1988) has now devised a three-stage culture system that permits visual monitoring of all stages of development from the fertilized ovum to hatching. This achievement, the first case of complete *in vitro* production of any homoiothermic animal, should ensure that genetic manipulation of poultry prospers at least as well as that of other farm animals.

(*b*) *Sex selection*

The widespread use of cattle AI since the Second World War has stimulated many attempts to sex spermatozoa in semen to eliminate unwanted bull calves in dairy herds. Methods were based upon supposed physical differences between X- and Y-bearing spermatozoa. No claims of success have so far received unequivocal validation, but it is possible that new techniques for identifying specific X- or Y-products with suitable probes coupled with developments in flow cytometry may eventually succeed. If so, the sexed sperm could be used either for direct

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insemination into cattle or perhaps more effectively for *in vitro* fertilization of oocytes for embryo production.

An alternative strategy that could be more generally useful is embryo sexing if, as seems likely, embryo transfer replaces AI as the principal vehicle for genetic improvement. For this purpose the technique should be non-destructive and applicable to early embryos. Such a method has been described (White *et al.* 1987) with a fluorescent probe for the H-Y antigen. The probe was effective in bovine embryos as early as the eight-cell stage, but not at four cells, and the H-Y antigen could be detected in the cells of the inner cell mass of expanded blastocysts. Of 258 embryos tested 132 (51%) were fluorescent and karyotyping confirmed that 80% of these were XY embryos, whereas 89% of the non-fluorescent embryos had XX karyotypes. A big advantage of the sexing procedure over previous cytolytic techniques is that both male and female embryos can be preserved for transfer or further genetic manipulation. There seems little doubt that sexed cattle embryos of known genetic merit will become commercially available within a few years.

(*c*) *Cloning*

The methods of embryo culture thus far mentioned produce animals each of which has a different genetic constitution. The goal of cloning is to multiply desirable genomes and produce 'identikit' animals.

Pioneering work on cloning livestock was done by Willadsen in Cambridge, building on embryo-transfer techniques. He developed a system for separating embryonic blastomeres by microdissection *in vitro* at the two-, four- or eight-cell stage to form sets of genetically identical individuals from surrogate mothers (Willadsen 1979). Blastomeres at the eight-cell stage rarely developed into viable embryos owing to insufficient cell mass and trophectoderm (placenta) inadequacy, but this could be overcome by inserting cells from other embryos to form the placenta. In this way Willadsen produced genetically identical sheep quintuplets each, produced from one blastomere from an eight-cell Jacob's × Finn embryo and one blastomere from a eight-cell Suffolk embryo. These experiments provided invaluable knowledge about early development and chimerism but the method had little commercial value and Willadsen then perfected a simple technique for producing identical twin lambs and calves by transection of blastocysts across the inner cell mass so that two reconstituted blastocysts could be transferred back into the dam or surrogate mother (Willadsen & Godke 1984). This technique is now offered as a service by Cattle Embryo Transfer companies.

It would be possible to use Willadsen's embryo-splitting technique, say at the four-cell stage, and repeat the process *seriatum* until tens or hundreds of identical embryos had been produced in culture and stored in an embryo bank. The phenotype could be tested by transfer of some embryos to surrogate mothers for assessment of performance. However, most workers have chosen nuclear transfer as the method of forming cloned embryos. Willadsen (1986) was first to clone a farm animal by nuclear transfer. Nuclei were obtained from blastomeres from eight- to 16-cell sheep embryos and electrofused with enucleated oocytes. The nuclear characters (Suffolk breed) were imposed on the recipient egg (Welsh × Cheviot) in each case. Since then, working for the Granada Company of Houston Texas, Willadsen produced three cloned Brangus bulls by transfer of nuclei extracted from a 32-cell male embryo and electrofused into enucleated unfertilized ova. Granada have subsequently produced a group of seven cloned Brangus calves by similar means. Success rates in cloning by nuclear transfer are affected by fine details of the technique employed, including the age and source of the nuclei and recipient

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oocytes. Thus, for example, Prather *et al.* (1987) reported only two successful live births from seven pregnancies resulting from the transfer of 19 embryos into 13 heifers.

In my Institute much attention has been given to electrofusion methodology, improved culture conditions, nuclear cytoplasmic interactions and new sources of viable nuclei by Moor in Cambridge and Wilmut in Edinburgh. In sheep embryos the nuclei retain their totipotency for at least the first seven cleavage cycles and lambs have been born at both centres from embryos constructed by electrofusion into enucleated oocytes of nuclei extracted from inner cell mass cells (Smith & Wilmut 1989). Another potential source of nuclei for farm animals is embryonic stem-cell lines (E. S. cells) like these already established in mice (Evans & Kaufman 1981). As stem cells are similar to inner cell mass cells but can be maintained in culture they would form an ideal bank of identical nuclei for cloning purposes.

It is likely that the principal commercial value of cloned livestock will be to make possible large-scale production of standardized animals of known marketability, e.g. for beef. Though cloned animals would contain no genetic benefits over their precursors the methodology could provide means of upgrading genetically inferior herds, and incorporated into breeding programmes could accelerate the rate of genetic improvement (Nicholas & Smith 1983). The potential of these methods for raising animal protein production in Third World countries is obvious but so far untapped.

(*d*) *Gene transfer*

It was to be expected that following the many biotechnology successes involving gene transfer to *E. coli* there would be attempts to upgrade domestic animals by gene transfer. The field received enormous impetus from the spectacular success of Palmiter *et al.* (1982) in producing giant mice by injecting a human growth hormone gene fused to a metallothionein promoter into the pronucleus of fertilized mouse eggs. Of the resulting mice a third had the human gene incorporated into their genomes but the variable growth response was not correlated with the number of DNA copies incorporated. This pioneering work thus exposed not only the dramatic possibilities of interspecific gene transfer but also some of the problems in the way of effective exploitation.

To improve significantly on conventional breeding the gene insertion must produce at least a 10% increment on the desired trait and to be of permanent value in future progeny must be incorporated into the germline. Most economically useful traits such as fertility, meat, milk and wool production or disease resistance are polygenic. So far very few single genes have been identified that could be of value. If the protein product of a gene is known, e.g. casein (milk) or keratin (wool), it would be possible in principle to deduce the DNA sequences coding for the proteins and fabricate suitable constructs for injection into fertilized oocytes. However, apart from the generally low viability of injected zygotes (Walton *et al.* 1987) and the rarity of successful integration of the gene into the germline there is also the major problem of controlling gene expression.

Attempts to improve growth rates and leanness in pigs and sheep by gene transfer of human or bovine growth hormone have been disappointing even when immunoassayable hormone levels were elevated in plasma (Pursel *et al.* 1987; Rexroad & Pursel 1988). Only about 0.5% of injected embryos resulted in transgenic offspring, though more than half of these expressed the growth hormone. Carcase fat was sometimes reduced but little acceleration of body-weight gain was observed and in some cases arthritic lesions and lameness developed in transgenic pigs. Reduced carcase fat was also observed with chronically high blood levels of growth hormone in

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two transgenic Merino ewes bearing a fusion gene construct of ovine growth hormone with ovine metallothionein promoter, but severe lesions were found in kidneys and liver in addition to joints (Nancarrow *et al.* 1989). Work at Babraham in collaboration with Embryogen Inc., Ohio, U.S.A. and Animal Biotechnology Cambridge Ltd, U.K., has created transgenic pigs bearing a bovine growth hormone gene construct linked to a prolactin promoter sequence (Polge *et al.* 1989). These pigs are healthy and exhibit low blood levels of bovine growth hormone, and none show enhanced growth rates. Of particular interest, however, is the fact that successive peaks of bovine growth hormone reminiscent of physiological patterns of release could be elicited by injections of the thyrotrophin-releasing factor, a known stimulator of prolactin, but not of growth hormone. The porcine growth hormone gene is now becoming available and Vize *et al.* (1988) have reported accelerated growth with no adverse effect on health in one of six transgenic pigs containing this gene with human metallothionein promoter.

A radical development combining tissue specific expression with potential production of medically useful proteins was promoted by Clark and colleagues in Edinburgh. Transgenic sheep were prepared by pronuclear injection into the fertilized eggs of gene constructs either for human α_1-antitrypsin (useful in certain respiratory disorders) or human blood coagulation Factor IX (for the treatment of haemophiliacs). By incorporating regulatory DNA sequences from the sheep β-lactoglobulin gene, Simons *et al.* (1988) obtained transgenic animals that expressed the gene only in the mammary gland. The inserted Factor IX gene entered the germline in three animals which transmitted the gene to their lambs. At maturity two lactating females secreted human Factor IX into the milk. Though quantities were not large the results advance the prospect of harvesting a product for haemophiliacs with no risk of contamination with AIDS virus from a small number of transgenic animals showing no outward signs of their exceptional genome.

Trials on farm animals are very expensive and most exploratory work is still done in mice. Powell & Moor (1987) discuss some of the rules that appear to determine the successful integration and expression of transgenes. Linear DNA is preferable to circular, vector sequences must be removed before integration, 100–200 copies appears optimal but there is a poor correlation between copy number and expression. When all the rules are followed only 1–4 % of injected zygotes develop into young bearing the transgene and of these only 10–50 % will express it.

Clearly much more fundamental research will be necessary before transgenic animals become a practical proposition for commercial production. The molecular embryology department at Babraham has such a programme. An important discovery is that parental source exercises a determining effect on gene expression in the progeny. This process of genomic imprinting (Reik *et al.* 1987), which involves gene methylation, could explain the different performance in the progeny of reciprocal crosses, e.g. more milk from progeny of Friesian female × Ayrshire male than vice versa. A novel approach to identifying chromosome sites that are actively transcribing has been to generate reporter genes with weak enhancers that are only expressed in transgenics when integrated near active genes (Howlett *et al.* 1988). This work may reveal enhancer sequences that confer position-specific expression of integrated transgenes. Further understanding of the developmental processing of the genome comes from the demonstration of overlapping patterns of expression of homeobox genes in mice (Gaunt *et al.* 1986; Gaunt 1988).

The low success rate and high cost of producing transgenic livestock by pronuclear injection

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has stimulated research on alternative gene insertion systems, notably by using retroviruses to convey the DNA into the embryo nucleus, or by inoculating EK cells which are undifferentiated and contribute to the inner cell mass of the developing embryo. Alternatively cultured stem cells can be used to carry the DNA into adult animals where they can colonize the appropriate tissue, e.g. bone marrow and secrete their gene products into the host animal. These methods are potentially capable of increasing the efficiency of gene transfer by ten to a hundredfold but they are not sufficiently developed to constitute a viable commercial proposition. In recognition of the great potential of transgenic biology both as a means of illuminating developmental obscurities and to foster new initiatives in livestock improvement the Agricultural and Food Research Council has recently initiated an extensive programme on transgenic animals involving several Institutes and Universities with supporting finance from the Advisory Board for the Research Councils.

4. Potential hazards to animal biotechnology

The biological revolution initiated by Watson and Crick has led to wide interest in the application of biotechnology to the animal industry and this in turn to considerable popular fascination with its more sensational aspects. It is often assumed that commercial development of available options is inevitable and that new applications will materialize at a rapid rate. There are, however, many countervailing influences, some of which are common to all technology transfer and others special to animal biotechnology (Cantley 1987; Sundquist 1987). They include the following.

1. Shortage of trained scientists. The rapid pace of fundamental biological advances in the last few years has outstripped the supply of high-quality recruits for exploiting the new discoveries.

2. Intellectual property constraints. The negotiation of sustainable contracts between research workers and commercial developers can be lengthy and delicate. Exclusivity and confidentiality terms expected by companies can be detrimental to scientific progress. Pursuit of knowledge and pursuit of profit are not always compatible.

3. Legal issues. Patent law is uncertain as it affects transgenic animals where U.S. rulings are at variance with E.E.C. practice.

4. Economic and social implications. If, for example, milk yields of cattle were much improved by recombinant DNA technology (e.g. transgenic cows or BST) many small producers could be forced out of business and countries with larger dairy units (U.K., Netherlands and Denmark) would benefit while those with small units (Greece, Italy and Germany) would suffer.

5. Animal rights movement. Transfer of a gene to an animal from a human or other animal source has been held to violate 'the right of a species to exist as a separate identifiable creature' (Rifkin).

6. Political considerations. Public attitudes to animal biotechnology are coloured by concern for animal welfare and fears that controls over safety of products may be inadequate (hormones in meat or milk, release of transgenic animals).

7. Industrial hesitancy. Many companies are reluctant to invest in expensive technology development, which may be subject to unpredictable public policy intervention.

8. Scientific concern that gene pools may be disregarded and inadequate attention given to

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planning breeding programmes that will subject the newer artificial breeding technologies to rigorous testing by quantitative genetic methods.

Conclusion

The armoury of technical procedures becoming available to the livestock industry is now formidable. It includes artificial insemination, superovulation, surgical and non-surgical embryo transfer, oocyte reclamation from slaughterhouse ovaries and maturation in culture, *in vitro* fertilization, *in vitro* culture of embryos to blastocyst stage, embryo sexing, cloning by embryo manipulation or nuclear transplantation, gene transfer, low-temperature storage in sperm, oocyte or embryo banks, and treatment of livestock with products manufactured by recombinant DNA methods. Before the more recent developments become recognized technologies much refinement will be necessary and in some cases, such as gene transfer, successful application will be dependent upon fuller comprehension of the underlying molecular processes.

Predictions are hazardous, especially in the light of the constraints mentioned above. However, it would be unduly pessimistic not to expect that within another eight years cattle embryos of known sex and genetic merit will be available to meet the livestock needs of people throughout the world. Whether they will include superior genomes engineered by gene transfer is more problematical. We can be reasonably assured, at all events, that options will be to hand which can take account of economic, social and political problems in the future.

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Discussion

C. R. Lowe (*Institute of Biotechnology, University of Cambridge, U.K.*). I have three questions. Is there a problem of security with these valuable transgenic sheep producing Factor IX? What happens about sterilizing the product from milk? Finally, what has been achieved for the downstream processing of Factor IX in milk?

B. A. Cross. All good questions! But so far little Factor IX is being expressed in the milk so not much progress has been made. Sterility should not be too difficult but on downstream processing only modelling studies have been done with Factor IX added to milk. The transgenic sheep are securely housed but special measures might well be necessary when animals are produced with significantly higher expression levels.

K. James (*Department of Surgery, University of Edinburgh Medical School, U.K.*). Although not directly involved in the work going on in Edinburgh in transgenic animals I do know that some of the points raised by Dr Lowe are being addressed. I am aware, for example, that immunoaffinity techniques have been developed to purify Factor IX from various sources and that the biological activity of Factor IX expressed in work is being examined. Does Dr Cross have any information about the stability of the genetic information in the transgenic mice and sheep?

B. A. Cross. It seems that the human Factor IX gene is stably transmitted over many generations in mice, and so far also in three generations in sheep.

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