GENETIC ENGINEERING OF LABORATORY AND LIVESTOCK MAMMALS

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Summary
Recent advances in recombinant genetics have made possible the transfer of cloned genes from one organism to the genome of another. Research with mice made transgenic by insertion of rat or human genes has provided direct evidence that transferred genes can be incorporated into the germline and expressed in the recipient. Current technology for gene transfer involves microinjection of the recombinant genes into the male pronucleus of the zygote. Resulting transgenic mice, when mated as adults, produced offspring that contained and expressed the transgenes. These observations serve as indications of the possibilities that exist for genetic engineering in livestock species. Although there are some technical problems to be overcome before livestock embryos can be genetically altered by these means, the genes for producing growth hormone transgenic livestock are currently available, and research groups are working toward this objective. In addition to this work with growth hormone genes, there are many other potential applications for genetic engineering livestock to produce more highly efficient production; however, there is considerable research to be done before the full potential of this technology can be achieved. It will be necessary to identify other genes that have potential for improving the production efficiency of livestock, and it will be necessary to gain a more complete understanding of the developmental and molecular biology of livestock. The potential impact of this technology in farm animal production is enormous, but, in the short term, it will be a costly endeavor.

(Key Words: Genetics, Molecular Biology, Genetic Engineering, Growth, Mammals, Production.)

Introduction
The history of agriculture is largely a history of genetic engineering. From the earliest efforts in agriculture, man has practiced genetic selection, often inadvertently, to produce choice agricultural products. Within the past 100 yr, geneticists have established protocols for scientific selection for desired traits, and a great deal of progress has been made in animal productivity. Nevertheless, animal scientists and livestock producers are interested in further improvements. The various disciplines within animal science are devoted to achieving these improvements, but progress seems slow compared with the desired result.

Within the past 3 yr, the potential for a dramatic rate of change has been created using a molecular approach to genetic engineering of mammals. Cloned genes have been incorporated into the genome of laboratory mice (Gordon et al., 1980; Brinster et al., 1981; Constantini and Lacy, 1981; Gordon and Ruddle, 1981; Wagner et al., 1981a,b; Palmiter et al., 1982; Wagner et al., 1982; Palmiter et al., 1983). These studies demonstrate that foreign genes can be expressed by the transgenic animals, and the technology is now available for use in livestock species. In this paper we review the progress in molecular genetic engineering of mammals and discuss possibilities for its use in livestock species.

Gene Recombination in Laboratory Animals
Recombinant genetic techniques allow the
transfer of specific cloned genes from the genome of one organism to the genetic makeup of another. Utilizing bacterial plasmids, gene recombination was first achieved in bacteria (Cohen et al., 1972) and later in simple eukaryotic species such as yeasts (Beggs, 1978). During the decade following the first successful gene transfers in bacteria, a major effort was mounted to achieve gene recombination in higher eukaryotic organisms including plants and animals. Until 1981, gene recombination in mammals was restricted to the transfer of genes into mammalian cells in tissue culture. The first successful transfer of a cloned gene into the genome of a mammalian cell line was accomplished by Paul Berg, using a recombinant SC40 virus genome containing an insert with the rabbit \( \beta \)-globin genomic clone ligated into the viral genome (Mulligan et al., 1979). Balb 3T3 mouse cells were transformed with the recombinant mammalian virus and transformed cells selected in media that allowed only the growth of the viral transformed cells. Only 1 in \( 10^5 \) cells were transformed and these cells were oncogenically transformed as well as genetically transformed. The resulting line of SV40 recombinant viral-transformed cells contained the rabbit \( \beta \)-globin gene and produced rabbit \( \beta \)-globin proteins (Mulligan et al., 1979). A major improvement in the methodology for the genetic transformation of mammalian cells in culture was developed by Wigler et al. (1979). Using the Herpes virus thymidine kinase (TK) gene, mouse L cells deficient in the TK gene function were transformed by exposure to a CaP0\(_4\) precipitate of the cloned Herpes TK gene. The 1 in \( 10^7 \) to \( 10^9 \) cells that take up and are genetically transformed by the Herpes gene are selected by growth in HAT media (media that interferes with nucleotide biosynthesis in deficient TK\(^-\) mouse L cell line). This TK selection method may be used to genetically transform TK\(^-\) cells with any cloned gene by co-transformation with the Herpes viral TK gene (Wigler et al., 1980). Because on entry into the nucleus of the recipient cell, the cloned DNA fragments are randomly ligated together to form a DNA polymer containing a mixture of the cloned DNA segments linked end-to-end, transformation with Herpes TK gene and any other gene results in the chromosomal integration of both genes linked together yielding TK selection and stable transformation with the desired gene.

It has long been the goal of molecular and developmental biologists to introduce cloned genes into the germline of complete mammals in order to examine the interrelationships between the expression of genes and their regulation during development. Also, the optimum system for the study of the native function of a cloned and well characterized gene would clearly be the whole animal. Gene transfer in whole mammals would also allow the animal scientist to specifically alter the genetic makeup of livestock species with a selectivity unapproachable by traditional genetic selective procedures.

Germline gene recombination in complete animals must be approached by the genetic transformation of early embryonic cells from which a recombinant animal may develop. Because these cells may not be collected in large numbers and may not be propagated over long periods of time, the techniques used to genetically transform mammalian cells in culture cannot be used. In 1981, several laboratories successfully accomplished the germline genetic transformation of laboratory mice by microinjection of cloned DNA into the pronuclei of fertilized mouse eggs at the one-cell stage. The importance of using a single cell was to reduce chances of producing mosaic mice and ensure foreign gene integration into all cells of the developing animal. The relatively large pronuclear regions of the fertilized eggs also provided an easy target for microinjection. In addition to these reasons for pronuclear microinjection, Wagner et al. (1981a) suggested that the early male pronucleus may provide a highly specialized nuclear environment for the incorporation of DNA sequences and for their inclusion into a functional chromosomal region. The period of time during which sperm chromatin loses its protamine regulated structure and acquires a maternally determined histone structure may be a fortuitous time for incorporation of foreign DNA into a structure that is transcriptionally active. The molecular events within developing pronuclei were first investigated in the sea urchin. It has been suggested that sperm chromatin dispersion and male chromosomal gene expression may be manifestations of changes in the nucleoprotein content of the paternally derived chromatin within the developing male pronucleus. Kunkle et al. (1978) have shown that soon after fertilization of the sea urchin egg, the male pronuclear chromatin acquires proteins, probably maternally inherited, of molecu-
lar weights greater than 80,000 daltons and a nuclear protein composition similar to that of the female pronucleus. These researchers postulate that such changes in male pronucleus composition may allow the paternal genome to participate in RNA synthesis (Longo and Kunkle, 1977). Recently Perrealt et al. (1984) have detailed some of the molecular events in mammalian pronuclear development. Also, Laskey et al. (1977a,b, 1978) have isolated a group of enzymes from the frog oocyte that organize cellular histones into the nucleosomal chromatin units and that may function in structuring the sperm chromatin into transcriptionally functional chromosomal units during early male pronuclear development. Both of these observations suggest that extensive "rebuilding" of functional chromosomes occur within the early male pronucleus after removal of protamines from the sperm DNA during sperm decondensation. Therefore, similar early molecular events within the mouse male pronucleus might assist in assuring an appropriate nucleoprotein structure for the microinjected DNA and integration of exogenous DNA sequences placed into the early male pronucleus. Although this rationale for microinjection into the early male pronucleus may be supported by some molecular data in nonmammalian systems, no definitive proof that pronuclear or male pronuclear microinjection is advantageous has, as yet, been presented. Also, unpublished data suggests that gene transfer may, in some cases, be accomplished by late pronuclear or two-cell nuclear injections.

Zygote microinjection is carried out using two micromanipulators controlling two micropipettes. One-cell embryos, maintained in a microdrop of embryo culture media, are held individually at the polar body by suction applied via a microsyringe attached to a fire polished micropipette (25 μm id, 80 μm od). Injection is accomplished using an injection micropipette (.7 to 1.2 μm od) attached to a .5-ml, screw driven, microsyringe to deliver 1 μl of DNA solution into the male pronucleus, oriented approximately 180° from the polar body. Successful injection is monitored by observation of an expansion of the pronuclear volume by approximately 25%. Microinjection is performed under 400X magnification using an inverted microscope. Embryos are held in a specially constructed depression slide above a sterile cover slip. The equipment for microinjection is shown in figure 1. A photomicrograph of the microinjection of a mouse egg is shown in figure 2.

Mice derived from one-cell embryos micro-
injected with specific cloned genes have been scrutinized on the molecular level. Experiments for determining the copy number, integrity, stability of integration and germline transmission of the introduced genes have been performed to investigate the fate of the foreign DNA. Some laboratories have also tried to determine the functional expression of the incorporated DNA.

The first successful incorporation of a cloned foreign gene into mice was reported by Gordon et al. (1980). In this study, a construct containing the SV40 virus origin of replication, pBR322 plasmid sequences and the Herpes virus TK gene were introduced by microinjection. Southern blot hybridization showed fragments of this gene construction rearranged in the chromosomes of several mice. Almost simultaneously, several other laboratories announced the successful incorporation of other specific cloned genes into mice by DNA microinjection into fertilized embryos (Brinster et al., 1981; Constantini and Lacy, 1981; Gordon and Ruddle, 1981; Wagner et al., 1981a,b). From these experiments there are now mouse lines that contain stably integrated SV40 DNA, Herpes Simplex Virus thymidine kinase (HSV-TK) gene, the human leukocyte interferon gene, the rabbit β-globin gene and the human β-globin gene. Gordon and Ruddle (1981) and Wagner et al. (1981b) demonstrated by Southern hybridization studies on undigested DNA from fetuses and live mice derived from injected embryos that the injected genes were stably integrated into mouse chromosomes. Constantini and Lacy (1981) also established chromosomal integration and determined the site of chromosomal integration for injected rabbit β-globin genes by in situ hybridization.

These results indicate that the cloned DNA has recombined in some manner with the host chromosomal DNA and is stably integrated. If this were the case, germline transmission of the DNA would be expected and hence, the establishment of lines of transgenic mice for studies of foreign gene function. Constantini and Lacy (1981) analyzed offspring from four male mice that contained rabbit β-globin genes and

Figure 2. Photomicrograph showing the microinjection of a pronuclear mouse egg. The egg is held by a 60 µm fire polished holding pipette and injected with a beveled 1.5 µm injection pipette.
showed by Southern hybridization that a portion of the group had complementary sequences within their genomic DNA. Wagner et al. (1981a) found the presence of rabbit \( \beta \)-globin protein in the blood of offspring of injected embryos. Transmission of human interferon genes in mice developed from embryos microinjected with this gene was also seen by Gordon and Ruddle (1981). Brinster et al. (1981), using a mouse metallothionein/HSV-TK fusion gene, also demonstrated the presence of the gene in second generation mice and the presence of the HSV-TK protein as well. These results suggest stable integration and Mendelian germline transmission of the foreign added gene in gene transfer in laboratory mice.

The main thrust of this line of research is to establish lines of animals that not only contain stably integrated foreign DNA, but ones that also express the inserted genes. Wagner et al. (1981) have demonstrated expression of injectable rabbit \( \beta \)-globin sequences in their mice.

The stable integration of intact foreign genes into the mouse genome allows analysis of foreign gene expression in these mouse lines. Wagner et al. (1981a) found that this was indeed the case for some of the rabbit \( \beta \)-globin genes present in transgenic mice containing rabbit \( \beta \)-globin genes. Immunodiffusion analysis showed the presence of a protein in the blood of these mice that crossreacted with mouse antirabbit globin antisera. This reaction was not seen in control animals and could be reversed by immunoadsorption of the antisera with rabbit globin. This finding was further corroborated by two-dimensional protein gel electrophoresis of the transgenic mouse hemoglobin demonstrating the presence of a globin species co-migrating with rabbit globin (Wagner et al., 1982). In addition, rabbit globin mRNA was detected in the bone marrow of these mice by Northern hybridization (Wagner et al., 1982).

Constantini and Lacy (1981), using a different construction of the same rabbit \( \beta \)-globin gene, did not observe the rabbit globin protein in their mice, but demonstrated the presence of rabbit \( \beta \)-globin mRNA in muscle tissue.

The difference in results between Constantini and Lacy (1981) and Wagner et al. (1981a) highlights the present lack of understanding regarding the control of gene expression in animal systems. Clearly, the function and expression of the \( \beta \)-globin gene depends upon more than the DNA sequences introduced into these lines of mice. Chromosomal position, flanking DNA sequences or other factors may be of importance in gene function. It has been suggested that control of the expression of developmentally controlled genes may involve at least two determinants; the upstream flanking DNA sequences involved in promotion-regulation and the local DNA environment in the region of the chromosome where the gene resides (Jaenish et al., 1981). Until the role of nonadjacently placed DNA sequences can be properly evaluated, gene transfer with complex, developmentally controlled genes may be a "hit-or-miss" proposition.

Palmiet et al. (1982) have devised an elegant method to avoid the problems of gene expression in complex, developmentally controlled genes. These researchers have used the mouse metallothionein-1 gene promoter-regulatory sequences to act as a promoter of gene expression for almost any structural gene of interest in the mouse. Because the metallothionein-1 gene does not appear to be under the complex control found with other genes coding for proteins with more complex functions, this promoter appears to function well in any chromosomal location or condition. By fusing the metallothionein-1 gene promoter-regulator to the structural genes of interest, high levels of expression of the desired genes may be achieved. Initially, Brinster et al. (1981) used the metallothionein-1 (MT) promoter to express the HSV-TK gene in a MT-TK fusion gene injected into mouse embryos. The resulting mice produce substantial amounts of the TK protein. The universal value of the MT promoter was demonstrated by Palmiet et al. (1982) by fusing the MT promoter to the rat growth hormone (rGH) structural gene at the transcriptional initiation site and introducing their fusion gene into the germline of mice by zygote microinjection. Of the 21 mice that developed from these zygotes, seven carried the fusion gene and six of these grew significantly larger than their littermates. Several of these transgenic mice has extraordinarily high levels of the fusion mRNA in their liver and growth hormone (GH) in their serum. Not only does the approach of Palmiet et al. (1982) have implications for the study of the biology of GH in intact laboratory animals, but it may provide a way to deastically accelerate animal growth.

For GH transgenic animals to be economically useful for accelerated growth or for other functions, the transgenes must be stably integrated, transmitted and expressed in sub-
sequent generations. Otherwise, the cost of producing the transgenic animals by current technology would be orders of magnitude greater than the gain that could possibly be realized. In this regard, Palmiter et al. (1983) demonstrated that GH transgenic mice do transmit the transgenes to their offspring in Mendelian fashion. When transgenic mice, expressing the transgenes and exhibiting accelerated growth, were outbred, approximately one-half of the offspring inherited the transgenes and grew at an accelerated rate (comparable to their transgenic parent) while the other one-half of the offspring did neither. This important contribution, as well as the report of Wagner et al. (1981a) involving offspring of $\beta$-globin transgenic mice, confirm the feasibility of producing lines of transgenic animals with improved production efficiencies.

In order to apply the procedure of gene transfer to livestock species, it is necessary to establish efficient procedures for the recovery of pronuclear livestock embryos, observation of their pronuclei for effective microinjection and to transfer these injected eggs into recipient females with an overall efficiency approaching that now available in laboratory mice. The cloning of livestock genes is well underway and those genes of greatest interest are available or will be available in the near future. The bovine GH (bGH) gene has recently been cloned by Woychik et al. (1982) and efforts to introduce this and other GH genes are underway in our laboratories as well as the laboratories of others.

Problems in Animal Sciences That May Be Addressable Through Recombinant Genetics

Growth Efficiency. Traditionally, the greatest research emphasis in the animal sciences has been, and continues to be, growth efficiency, with particular priority on feed efficiency, rate of gain and body composition. The reason for this is obvious because feed is the major single variable cost of production. Growth efficiency in farm animals has increased substantially over the past 50 yr (Smith et al., 1980) by both improved nutrition and superior genetics of breeding stock. While it is clear that many important traits relating to growth efficiency are heritable, continued improvements by traditional animal breeding methods will depend on the range of genetic variability in current breeds. Nutritional improvements have taken advantage of the improved genetic capabilities of the species and now diets more adequately meet the nutrient requirements of animals at various physiological phases of growth and reproduction. While it is clear that both disciplines will continue to improve growth efficiency, only incremental increases are foreseeable with these conventional approaches.

The question of the likelihood of achieving a dramatic improvement in growth and feed efficiency through recombinant genetics is relevant. Studies dating to those of Evans and Simpson (1931) have demonstrated that long term treatment of animals with growth hormone (GH) or adenohypophysis extracts can substantially improve both growth rate and feed efficiency in animals. Evans and Simpson (1931) found that at 200 d of age, female rats receiving chronic daily injections of an extract from the anterior pituitary gland, rich in GH, reached approximately 475 g while controls were approximately 270 g in weight (table 1). At the same age, male rats weighed 530 and 410 g, respectively. At 400 d of age, GH-treated females weighed about 570 g while controls

<table>
<thead>
<tr>
<th>Sex</th>
<th>Treatment</th>
<th>Days of age</th>
<th>Weighta, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td>Control</td>
<td>70</td>
<td>230</td>
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<tr>
<td>Female</td>
<td>GH</td>
<td>70</td>
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<td>Male</td>
<td>Control</td>
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<td>300</td>
</tr>
<tr>
<td>Male</td>
<td>GH</td>
<td>60</td>
<td>360</td>
</tr>
</tbody>
</table>

aApproximate values derived from figures presented by Evans and Simpson (1931).
weighed 240 g. At the same age, males weighed 850 and 520 g, respectively. These are dramatic increases in growth. Evans and Simpson also noted that the effect of GH on body weight in rats was not particularly evident until after the animals had reached 100 d of age. Thus, this early study provides evidence that the physiological stage on the growth curve of animals is important in determining the degree of response to exogenous GH. Therefore, use of GH in young animals might be expected to yield poor response.

Lee and Schaffer (1934) performed experiments similar to those reported by Evans and Simpson (1931), but they also evaluated treatment effects on body composition of experimental rats (table 2). They found, in pair feeding experiments, that while consuming the same quantity of feed as controls, rats treated with pituitary gland extracts gained more weight than did the controls. At the same time, body composition revealed an increase in water, nitrogen and ash content, but a decrease in fat (ether extract) content. Even with small numbers of animals, it is evident from the data presented that GH exerted little effect on the younger rats (rats started on treatment at 52 d of age and slaughtered at 129 d of age gained a mean net of —2.5 g, while rats started at 171 d of age and slaughtered at 227 d of age gained a mean net of 45 g in response to GH). This is potentially an important point, for it suggests that the response to increased GH may depend on age at treatment. In consideration of the results of both Evans and Simpson (1931) and Lee and Schaffer (1934), it has been clear for half a century that daily treatment with exogenous GH can greatly increase nonfat body growth in experimental animals.

Presumably due to lack of available hormone in sufficient quantity, studies of the effect of exogenous GH on farm species were done only recently. Machlin (1972) reported that exogenous porcine GH (pGH) positively affected growth in pigs; however, high doses equivalent to those used in the rat studies proved toxic to pigs. In general, the results with lower doses of pGH revealed increased muscle growth and decreased fat deposition (table 3). Five experiments with various numbers of pigs were reported, and effects of pGH on rate of gain and feed efficiency were significantly improved in some experiments. This study makes evident two especially important points: 1) pigs are adversely sensitive to very high levels of exogenous GH, and 2) muscle growth, in particular, in pigs can be increased by the hormone. Therefore, moderately increased levels of GH in the pig would appear to be advantageous if the cost of hormone and its administration did not over­ride the benefits to be gained. Until large quantities of inexpensive, pure pGH are available

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control</th>
<th>GH-treated</th>
<th>Difference due to GH, g</th>
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</thead>
<tbody>
<tr>
<td>No.</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Initial wt, g</td>
<td>198</td>
<td>196</td>
<td></td>
</tr>
<tr>
<td>Final wt, g</td>
<td>262</td>
<td>304</td>
<td>42**</td>
</tr>
<tr>
<td>Empty carcass wt, g</td>
<td>250</td>
<td>290</td>
<td>40**</td>
</tr>
<tr>
<td>Empty carcass composition, % of carcass wt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>58</td>
<td>62</td>
<td>36**</td>
</tr>
<tr>
<td>Fat</td>
<td>19</td>
<td>13</td>
<td>—11*</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>3.0</td>
<td>3.2</td>
<td>1.8**</td>
</tr>
<tr>
<td>Ash</td>
<td>4.0</td>
<td>4.1</td>
<td>1.7**</td>
</tr>
</tbody>
</table>

a Values derived from table 3, Lee and Schaffer (1934). Pairs varied in age at initiation of treatment (52 to 224 d of age) and age at end of slaughter (129 to 287 d of age).

b Values derived from table 6, Lee and Schaffer (1934).

*P<.05.

**P<.01.
TABLE 3. EFFECT OF EXOGENOUS PORCINE GROWTH HORMONE (pGH) ON GROWTH AND CARCASS CHARACTERISTICS OF SWINE

<table>
<thead>
<tr>
<th>Item</th>
<th>Exogenous pGH (mg/kg body wt(^{-1}) (\cdot) d(^{-1}))</th>
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<th>.13</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td></td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Initial wt, kg</td>
<td></td>
<td>45.9</td>
<td>46.0</td>
</tr>
<tr>
<td>Slaughter wt, kg</td>
<td></td>
<td>94.3</td>
<td>94.0</td>
</tr>
<tr>
<td>Avg daily gain, kg</td>
<td></td>
<td>.74</td>
<td>.86**</td>
</tr>
<tr>
<td>Feed/gain</td>
<td></td>
<td>3.33</td>
<td>2.89*</td>
</tr>
</tbody>
</table>

Carcass

<table>
<thead>
<tr>
<th>Item</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dressing percentage</td>
<td></td>
<td>71.5</td>
<td>68.9**</td>
</tr>
<tr>
<td>Backfat, cm</td>
<td></td>
<td>3.48</td>
<td>2.79***</td>
</tr>
<tr>
<td>Loin eye, cm(^2)</td>
<td></td>
<td>25.8</td>
<td>31.9***</td>
</tr>
</tbody>
</table>

\(^{a}\)Experiment 2 from Machlin (1972).

\(^*\)P<.05.

\(^{**}\)P<.01.

\(^{***}\)P<.001.

with appropriate delivery systems, treatment of growing swine with exogenous GH will have little practical value.

Growth hormone treatment studies have also been performed in ruminants. Studies by Wheatley et al. (1966) and Reklewska (1974), the former with adult sheep, found no increase in growth of sheep in response to exogenous bovine GH (bGH). Moseley et al. (1982) reported that GH treatment of steers increased nitrogen retention, supporting the data from rats and pigs on increased muscle growth in response to exogenous GH. A recent report by Muir et al. (1983) also found no increase in growth of lambs in response to exogenous ovine GH; however, in this study, feed efficiency was significantly increased (by 7.4%). Wagner and Veenhuizen (1978) reported increases in both growth rate (20% increase) and feed efficiency (13.6% improvement) in lambs started at relatively large size (40 kg). With heifer calves, Brumby (1959) found that exogenous GH increased growth rate, but he did not report feed efficiency data. Thus, in ruminants, as in swine, increased levels of GH could prove advantageous if the costs were not prohibitive.

Currently, the advantages of exogenous GH treatment of livestock can not be realized because of the lack of sufficient, economical GH. However, this situation may change in the near future because a number of companies are planning to produce GH by large scale culture of recombinant microbes. Practical use of exogenous GH is likely to be labor intensive because daily treatment or sustained release implants will probably be required to achieve the desired effect. As an alternative, if the genetic capacity of livestock can be manipulated to produce extra endogenous GH with adequate specific regulation of expression so that particular production traits desired in a specific group of animals would be improved (in this case, growth of meat producing animals—but not necessarily their progenitors), a great advantage would be available to the livestock industry.

**Milk Production Efficiency.** Currently, and in the foreseeable future, there is an excess of milk produced in the United States and dairy producers are faced with minimal profits. While increased milk production per se might not be desirable now, a dramatic improvement in the efficiency (cost) of milk production would be of great benefit to producers and consumers in the face of continued excess milk supply. There is reason for optimism that recombinant DNA (rDNA) technology can be used to dramatically improve the efficiency of milk production.

A number of recent studies have demonstrated that exogenous GH treatment improved milk production efficiency (Brumby and Hancock, 1955; Bullis et al., 1965; Machlin, 1973; Bines et al., 1980; Peel et al., 1981,
1982; Gorewit et al., 1982). With low-producing dairy cattle, these studies demonstrated up to a 50% improvement in milk production with more milk produced per unit of feed consumed. Peel et al. (1981, 1982) used high-producing dairy cows and observed significant (9.5 and 15.2%) increases in milk yield, with slight (nonsignificant) increases in feed efficiency in response to 51.5 IU/d exogenous bGH for short periods (10 to 11 d) early in lactation (10 to 18 wk). In these studies, yield of fat, lactose and protein were increased. Gorewit et al. (1982) using high producing cows in late lactation (~35 wk), found dramatic improvements in milk yield during two limited treatment periods with bGH. Milk yield in this study was improved by 31 to 35%, milk fat yield increased by 32 to 38%, milk protein yield increased by 18 to 22% and milk lactose yield increased by 34 to 37%. The studies of Peel et al. (1981, 1982) and Gorewit et al. (1982) suggest that stage of lactation and, thus, milk production rate of cows at the time of treatment greatly influences the response to exogenous GH.

In view of the potential for use of rDNA technology to insert exogenous genes into the genome of animals, it is within current capability to use this in dairy cattle. The limitation, in addition to the fundamental skill required to microinject pronuclei of bovine eggs, is to make a gene construct with a promoter controlled so that GH production would be regulated with respect to mammary gland function of the cow. Without such precise control, the inserted GH gene, if continually expressed to produce excess blood GH levels throughout adulthood, might cause giantism, without or in addition to, increased milk production. Such a result would probably be undesirable. This circumstance requires that precise control of GH gene be incorporated into the construct and that control be experienced in the producing animal.

**Animal Health.** Currently, biotechnological applications in animal health are focused on development of vaccines with almost immediate target dates for utilization. These developments hold great potential for improved animal health; however, it is conceivable that rDNA technology can have even greater impact by the development of genetic constructs that would produce inherent specific disease resistance. It is intuitively obvious that most diseases are, at least to some degree, genetically predisposed because most diseases affect only a few species. Furthermore, resistance to some diseases is known to be related to particular genetic loci, e.g., Marek’s disease in poultry (Payne, 1973) and many human and mouse diseases (Bach, 1982). Another example of genetically determined disease is internal parasite resistance (e.g., Preston and Allonby, 1978). These diseases should be responsive to recombinant genetic approaches, involving insertion of the appropriate genes. The ability to perform these alterations will require considerable effort in domestic animal molecular biology to identify gene products and development of gene constructs, particularly those involved in nonspecific disease control.

**Reproductive Efficiency.** It is generally recognized that the single greatest limitation to maximizing overall efficiency of animal production is reproductive efficiency (Cartwright et al., 1980). In some of the farm species, the current level of reproductive efficiency is less than 50% of the level that is possible. Little improvement in reproductive efficiency has been made through conventional animal breeding methods because heritability of reproductive traits is low (Smith et al., 1983).

Generally, advances in reproductive efficiency will depend on the identification of rate-limiting gene products. For example, gene products identified by Murray et al. (1978) and Segerson (1981) have the potential to improve embryonic survival in pigs, cattle and sheep and may find utility through rDNA approaches. These gene products are powerful immunosuppressive polypeptides produced by the uterus during the peri-implantation period. They would be natural agents promoting embryo survival through the implantation phase, which is the time during which the largest portion of embryo loss occurs (Ulberg and Rampacek, 1974). As with other potential improvements through rDNA approaches, the benefits can be realized with no increased labor input and would be genetically transferred from generation-to-generation, once success is achieved.

**Use of Recombinant Animals for Physiological Study.** It is conceivable that genetically engineered animals might be useful as models in studies of specific gene product function in a manner analogous to hormone treatment experiments performed currently. The advantages to be gained with recombinant animals are that the inconvenience, stress, and imprecision associated with traditional animal experimentation might be overcome to a significant
degree. In studies with recombinant animal models, laboratory animals might be made transgenic for a particular gene and, with regulated expression, the specific effects of the gene product could be evaluated separately from, or in association with, other related gene products. In their elegant studies on expression of metallothionein/rat and human GH fusion genes in mice, Palmiter et al. (1982, 1983) have presented information on a variety of aspects of GH function and regulation. These studies illustrate the concept that transgenic animals can be used effectively in physiological research. Although the list of currently available gene products from livestock species is short (essentially limited to peptide hormones, milk proteins, some secretory products, some enzymes and histocompatibility antigens), the potential list is huge. Each gene product would offer possibilities for study of physiological effects through production of specific transgenic animals. With the accumulation of this knowledge, the capacity to control and exploit multigene traits may be attained.

The Promise of—and Problems Restricting Progress Towards—Recombinant Livestock

Laboratory experiments performed during the last 3 yr clearly demonstrate that specific cloned genes may be transferred into the germ-line of mammals, and that these genes will function to produce their specific gene products, phenotypically altering the resulting recombinant animal. The animal used for these studies has been the laboratory mouse. Primarily, this is because a great deal is known about the genetic makeup of this animal and embryological procedures of handling and culturing mouse embryos are well established. Although all published experiments, to date, have used the mouse system, there are not substantial restrictions to utilizing other laboratory or livestock species in such experiments.

Although procedures for collection, handling and culture of livestock embryos are not as well established as those for the mouse, livestock embryology has advanced to the level where one-cell pronuclear eggs may be recovered, cultured, micromanipulated and transferred to recipient females with sufficient efficiency to allow gene transfer experiments in these species. Two major obstacles to successful gene transfer in livestock do remain: (1) the low efficiency of the present gene transfer process, and (2) the dense, pigmented cytoplasm in most livestock embryos that makes the pronuclei, making injection difficult, if not impossible (figure 3). It seems likely that these obstacles will be overcome in the near future, opening the "door" to a revolution in livestock genetics.

Little is known of the developmental biology of domestic animals. This is important because the control of gene function varies with the developmental stage of the individual and with physiological status. Controlled expression of genes in laboratory animal development is reasonably well understood, but to date practically no research has been done in this critical area in livestock species. Thus the ability to control gene product production in relation to a particular physiological state, function, or organ system is critical if the desired effect is to be realized.

Another handicap to gene engineering in livestock species is the lack of knowledge of gene products important for functions related to production. It will be necessary to identify, characterize and isolate gene products important to animal production before the genes specifying these gene products can be isolated. This will necessitate reorientation of animal scientists or molecular biologists to carry out this important function. Without such knowledge, only a limited number of genes will be usefully available for gene transfer.

With regard to the limited set of genes available for use in livestock genetic engineering, an often-raised assertion is that, because most important production traits are controlled by multiple genes, genetic engineering will have little impact on most aspects of production. The response to this assertion must be offered at two levels. First, the multigene traits offer not only a greater challenge because the complexity of control of the trait is almost certainly greater than would be true to single gene traits, but the multigene traits probably offer greater opportunity for benefit because at least some of the genes could have additive and(or) cooperative effects, if the entire trait is not controlled by a single gene (a "bottleneck" in a pathway). On the other hand, if the "bottleneck" situation exists, then once the limiting gene is identified the multigene trait would behave as a single gene trait until the next limiting gene function became restrictive. Clearly, this would be more complex and challenging than truly single gene functions, but there...
are benefits to be gained not only in terms of improved animal productivity, but also in the fundamental knowledge of the biology of livestock. The second response to the assertion is that the very first anticipated use of genetic engineering in livestock will be in an area—growth efficiency—that is certainly a multigenic trait. Several of the genes that are involved in growth are known, and it seems certain that more genes that influence growth efficiency ultimately will be identified. Some of the gene products involved in regulating growth efficiency are: GH, GH releasing factor, somatostatin, insulin-like growth factors, enzymes controlling steroid production, enzymes controlling thyroxine production, et cetera. Yet, as reviewed above, GH transgenic animals can be expected to have dramatically improved growth and milk production efficiency. As more knowledge in livestock genetics at the level of the gene, as opposed to the “trait” is obtained, the utility of genetic engineering of the various production functions will be more certain.

Promoters are the control portions of genes that allow structural genes to be expressed at the appropriate time and at an appropriate rate for the function of an organ in a particular physiological status. Thus, promoters not only initiate gene expression, but they also regulate the amount of expression that takes place under a specific condition. Therefore, knowledge of a variety of gene promoters for use with gene transfer in livestock species will be a necessity. Eventually, it may be possible to program animals to perform differently in response to specific triggering mechanisms (such as feed additives, environmental factors, or semiochemicals) if promoters sensitive to the triggering mechanisms were inserted upstream from the structural transgenes. For example, an animal might be designed with GH genes, genes for increasing litter size and genes for resistance to internal parasites. If a sufficient variety of promoters were available and if promoter/structural gene constructs were specifically chosen, it might be possible to cause the female to grow to normal size at the normal rate, yet her offspring would have accelerated growth rates in response to a specific feed additive. At the time of breeding, a pheromone might be used to cause the promoter for uterine gene products responsible for enhancing embryo survival to be expressed. At periodic intervals, triggering agents (for example, photoperiod) might be used to promote parasite resistance. Therefore, with appropriate promoters, a variety of functions might be programmed into the same animal and expressed at the will of the livestock manager. However, it must be emphasized that these possibilities are relatively far

Figure 3. Sheep zygote. Cytoplasm of livestock animal zygotes is dense and pigmented, making observation of pronuclei difficult.
into the future because only a very few promoters, over which currently we have little control, are presently available for this use.

While genetic engineering of animals presents opportunities, the cost and complexity are significant considerations. At present the genetic engineering programs underway involve personnel from several institutions bringing together the required expertise in several fields. Although the assembly of requisite expertise at one location would be a distinct advantage, cost and lack of availability of expert personnel has been a detriment. Several types of expertise focusing on production of transgenic animals is needed. A minimum combination of expertise would include mammalian rDNA technology (for isolation of structural genes and promoters); microbial rDNA technology (for cloning and multiplying genes); nucleic acid hybridization and blot transfer techniques; protein analytical techniques; developmental and physiological biology, retrovirology; embryo collection, culture, and manipulation; animal husbandry and animal performance analytical techniques. Some of these capabilities may be resident in a single person, but efficiency would require more than one person collaborating on some areas. Thus, it could be anticipated that a minimum of four or five scientists concentrating on the group objective would be a necessity. Cost of setting up and operating is substantial also. To start a program from “scratch” currently would require several hundred thousand dollars to equip and several thousand dollars per month to supply each investigator, assuming that some of the equipment would be shared. In addition, technical assistants and graduate students would add to the cost. Another consideration is that gene constructs and some aspects of the technology will be protected by patents.

Clearly, gene transfer and recombinant livestock offer a means to alter the fundamental genetic makeup of livestock to a greater extent, in a few decades, than may have been achieved in the entire past history of the science of livestock genetics. Those of us involved in this research look forward to the challenge and promise of this exciting new technology.

**Literature Cited**


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