Chapter 3

Emerging Animal Technologies

Photo credit: Grant Heilman, Inc.

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The U.S. livestock industry is immense, and the costs of running it are correspondingly large. Feed and health care costs for the Nation's nearly 100 million head of cattle (beef and dairy), 55 million pigs, 10 million sheep, and 600 million chickens and turkeys amount to billions of dollars annually. Disease and reproductive losses also significantly erode industry profits.

Like any industry, livestock producers strive to reduce costs and losses, and to maximize profits. Feed constitutes almost 70 percent of the cost of producing pigs for pork. Improvements in feed efficiency (i.e., a lower quantity of feed consumed per unit of weight gained) and faster weight gain could potentially lower production costs in this and other sectors of the livestock industry. Animal diseases cost the livestock industry billions of dollars each year. For example, anaplasmosis in cows costs an estimated $300 million a year in losses and disease control. The bacterium *Staphylococcus aureus*, which causes 55 percent of mastitis, costs U.S. dairy producers some $250 million annually. New vaccines and diagnostic kits can help decrease disease in livestock. Other economic losses in the livestock industry result from low conception rates and embryo mortality. Such losses can be minimized by a greater understanding of reproduction as well as by emerging technologies for improving reproductive success.

Biotechnology has the potential to improve feed efficiency, reduce losses from disease, and increase reproductive success in all sectors of the livestock industry, in part by furthering our understanding of animal physiology, and in part through the development and commercialization of new techniques and products.

The term biotechnology refers to a wide array of techniques that use "living organisms (or parts of organisms) to make or modify products, to improve plants or animals, or to develop microorganisms for specific uses' (45). Under this broad definition, biotechnology includes many long-practiced technologies, such as animal breeding and cheese, wine, and beer making. Generally, however, the term biotechnology is used in reference to such new technologies as recombinant DNA techniques (also called genetic engineering), cell culture, and monoclinal antibody (hybridoma) methods. The application of these new methods to the livestock industry has already generated a number of products for improving production, animal health, and food processing, and will continue to do so.

Biotechnology is specifically used to produce products that will promote growth and increase feed efficiency and carcass leanness in growing animals, and significantly increase milk production in lactating animals. New reproductive technologies are providing means to rapidly upgrade herd quality. Transgenic animals are being produced to grow faster, have greater disease resistance, and to produce high-value pharmaceutical products. New vaccines and diagnostic kits are being developed to improve livestock health. Biotechnology is also being used to process meat and dairy products and to detect food contaminants that might be present in those products. This chapter presents some new livestock biotechnologies currently under development. [1]

COMPOUNDS THAT PROMOTE GROWTH, ENHANCE FEED EFFICIENCY, AND REDUCE CARCASS FAT

Compounds currently used in the livestock sector to promote growth and increase feed efficiency, such as anabolic steroids and antimicrobial compounds, will continue to be used. However, new products are also being developed, including protein hormones called somatotropins and catecholamine compounds called beta-adrenergic agents. These compounds increase growth rates in young animals, improve the efficiency with which food is converted to muscle, and significantly reduce carcass fat so that meat products are leaner. Somatotropins also increase milk production in lactating dairy cows. Currently, recombinantly-derived bovine and porcine somatotropins are undergoing Food and Drug Administration (FDA) review for use in lactating dairy cows and pigs, respectively, and one beta-adrenergic agent is undergoing testing for approval in pigs.

[1] Because of the large quantity of research on these technologies, this chapter will mainly cite OTA **commissioned background** papers and other review articles.

Somatotropin

A hormone is a chemical that is produced by one organ or cell and transported to another to cause a biological effect (i. e., it is a chemical messenger between different cells and organs of the body). Hormones can be steroids, proteins, peptides, or modified amino or fatty acids. About 70 percent of the hormones in blood are protein hormones. Somatotropin is a protein hormone produced by the pituitary gland, a small gland located at the base of the brain. All vertebrates (i.e., animals that have backbones) produce somatotropin. In addition, evidence exists that some nonvertebrate animals, such as shellfish (i.e., oysters, clams, etc.), also produce somatotropin.

All major livestock species produce somatotropins unique to each species. Naturally produced bovine somatotropin (bST) **contains 190 or 191 amino acids, and** each polypeptide can contain either the amino acid valine or leucine at position 126, which gives rise to 4 variants of bST. Pigs produce porcine somatotropin (pST) consisting of 191 amino acids. The amino acid sequence of pST, however, differs from bST at 18 positions. In contrast, bST and ovine (sheep) somatotropin (oST) differ by only one amino acid position (3, 16, 40).

Differences in the amino acid sequence of proteins lead to species specificity. The amino acid sequence determines the unique three-dimensional shape characteristic of a specific protein. Only proteins of the appropriate shape bind to a receptor, and thus elicit a biological response. Proteins from one species that differ by many amino acids from the equivalent protein in another species generally do not elicit a biological response in the other species. Conversely, bST and oST that differ only by one amino acid are active in either sheep or cattle. However, human somatotropin differs from pST by 59 amino acids and from bST by 68 amino acids (a 35 percent difference). Bovine, porcine, and ovine somatotropin are not biologically active in humans (20, 23, 49).

Mechanism of Action

Somatotropins affect growth rate, feed efficiency, milk yield, and the proportion of fat and protein in the carcass. These effects occur in response to the coordination of numerous metabolic pathways by somatotropin. These metabolic effects are both direct and indirect. The direct effects include nutrient partitioning among tissues, most specifically liver and adipose (fat) tissue (table 3-1). indirect effects include those mediated by insulin-like growth factor-1 (IGF-I), whose secretion is stimulated by somatotropin.

Somatotropin affects glucose metabolism. Glucose is a carbohydrate used as an energy source by many tissues, or as a raw material for the synthesis of other molecules (as in the production of milk lactose). Administration of somatotropin increases blood glucose levels by stimulating glucose production by the liver, and may possibly reduce glucose use for energy by other body tissues.[2] Thus, additional glucose is available for uses such as increased growth or milk production while normal body functions are still maintained. The changes in glucose use by body tissues and glucose production by the liver appear to be caused by somatotropin altering the response of these tissues to acute signals, such as to insulin and other hormones that affect glucose metabolism (3. 16).

Somatotropin also adjusts lipid (fat) metabolism. In growing pigs, for example, somatotropin redirects nutrients (primarily glucose) away from fat synthesis to providing energy for lean tissue accretion. The adjustments in tissue lipid metabolism depends on the nutritional status of the animal. If an animal's energy (food) intake is greater than its requirements, somatotropin allows for the reallocation of nutrients to support increased lean tissue accretion (growth) or milk production (lactation) instead of storing excess nutrients as body fat. If the animal's nutrient intake is equal to or less than its requirements, somatotropin directs adipose tissue to mobilize deposits of body fat so that these energy reserves can be used to support the increased lean tissue accretion (growth) or milk production (lactation). The former situation is more likely to be the case for young growing animals and the latter situation would be typical of lactating cows in early lactation. Like glucose metabolism, adjustments in lipid metabolism result from changes in the way adipose tissue responds to acute signals, such as to insulin and other hormones (3, 16, 40).

In addition to the direct metabolic effects that somatotropin coordinates, it stimulates the release of other compounds with metabolic effects, most notably insulin-like growth factor I (IGF-I). IGF-I probably mediates the effects of somatotropin on animals such that the cellular rate of milk synthesis is increased and the rate at which mammary cells die is decreased, thus causing higher daily milk yields for a longer period of time during the

2 Evidence in lactating dairy cows suggests that glucose use by tissues other than the mammary gland is decreased when somatatropin is administered. It is still not clear whether glucose use by skeletal muscle is decreased in growing pigs (3, 16).

Table 3-l—Effect of bST on Specific Tissues and Physiological Processes in Lactating Cows[a]

Tissue		Process affected during first few days and weeks of supplement
Mammary	↑	secretory activity and maintenance of mammary glands
	↑	blood flow and nutrient uptake
	↑	synthesis of milk with normal composition
Liver	↑	production of glucose
	o	response to acute signals (e.g., insulin) that allow for greater glucose production
Adipose	↑	mobilization of fat stores to meet needs for increased milk production if nutrient intake is inadequate
	↓	use of nutrients for fat storage so that they can be used for increased milk production if nutrient intake is adequate
	o	response to acute signals (e.g., insulin and other hormones that affect lipid metabolism) that allows for synthesis and breakdown of body fat reserves to be coordinated with changes in use and availability of nutrients
Muscle	↓	uptake of glucose
Pancreas	0	insulin and glucagon secretion reponse to changing glucose levels
Kidney[b]	↑	production of 1,25 vitamin D_3
Intestine[b]	↑	absorption of Ca, P and other minerals required for milk
	↑	ability of 1,25 vitamin D_3 to stimulate calcium binding protein
	↑	calcium binding protein
Whole body	↓	use of glucose by some organs so more can be used for milk synthesis
	↑	use of fat stores for energy if nutrient supply is Inadequate
	↓	use of nutrients to make body fat if nutrient supply is adequate
	ø	insulin and glucagon clearance rates
	ø	energy expenditure for maintenance
	↑	energy expenditure consistent with Increase In milk yield (i.e., heat per unit of milk not changed)
	↑	cardiac output consistent with increases in milk yield
	↑	productive effidency (milk per unit of energy intake)

[a]Changes (↑=increased, ↓=decreased, ø=no change, o=change) that occur in initial period of bST supplement when metabolic adjustments occur to match the increased use of nutrients for milk. With longer term treatment voluntary intake Inceases to match nutrient requirements. demonstrated in nonlactating animals and consistent with observed performance in lactating cows.

SOURCE: D.E. Bauman, 'Bovine Somatotropin: Review of an Emerging Animal Technology," commissioned background paper for the Office of Technology Assessment, Washington, DC, 1991.

lactation cycle ***(3).*** In growing animals, IGF-I stimulates cell proliferation in a variety of tissues (bone, muscle, connective, and adipose tissue) and increases protein synthesis in muscle (16, 40).

Poultry Somatotropin

Research using somatotropin to enhance growth and carcass composition in poultry (i. e., chickens, turkeys, and ducks raised for meat and egg production) is limited. Earliest research involved chickens that had their pituitary glands removed. Administration of chicken somatotropin (cST) was shown partially to restore growth. Chicken somatotropin also has been shown to increase circulating levels of IGF-I (40).

Administration of cST to broiler chickens[3] (i .e., chickens marketed at 6 to 7 weeks) has not been shown to influence growth, feed efficiency, or carcass composition. In young (post-hatched) chicks, the binding of somatotropin to its receptors in the liver is very low, whereas in adult chickens high somatotropin binding has been observed. There appear to be low somatotropin receptor numbers and/or receptor affinity for somatotropin during the early stages of chicken growth, potentially up to the time when broiler chickens are marketed. This might provide an explanation as to why cST has little or no effect in young broiler chickens. The basis for this low binding is not known, but some evidence exists that somatotropin itself regulates the number of somatotropin receptors (40).

While most studies have reported no enhanced growth in young chickens given cST, one study using daily injections of intermediate doses of native cST did elicit improved growth in 4-week-old broiler chickens. This raises the possibility that diet, frequency of cST administration, molecular form of cST, or dose may be necessary conditions to achieve a growth response in broiler chickens. Thus, it cannot be ruled out that optimal conditions have not been employed in most studies. Based on the evidence to date, however, cST administration appears not to be an effective means of promoting growth or productive efficiency in growing broiler chickens (40).

Administration of cST to roaster chickens (i.e., chickens more than 8 weeks old) has been shown to stimulate growth and feed efficiency while reducing carcass fat. The effects of cST on breast meat weight varied depending on the method of cST administration. For example, the weight of the breast meat was reduced when cST was administered in a pulsatile (rhythmic dripping) fashion, but increased when administration was by continuous infusion or daily injection. The extent of growth and of fat tissue accumulation also varied with method of administration and age of the chicken. These results suggest that cST can be used to improve roaster-age

[3]Chicken somatotropin derived from chicken pituitary glands and from recombinant DNA procedures were tried

chickens, but that the mode of administration and dose, and potentially diet, need to be optimized to achieve consistent results (40).

Turkeys that have had their pituitary glands removed have been treated with bST and cST; neither influenced growth. Administration of chicken or turkey somatotropin to intact turkeys has not been adequately explored.

Some evidence exists that bST or pST injections into the egg increase the growth and feed efficiency of male chickens after hatching, and reduce abdominal fat in both male and female chickens.

In summary, it has not been definitively demonstrated that somatotropin can be used to improve growth, feed efficiency, or carcass composition of poultry. More research is needed to determine if this is in fact the case, to optimize conditions needed to achieve growth, and to improve the mode of administration. There is a general lack of research on poultry biology and much basic research is needed to understand growth mechanisms in poultry. There is also a need to characterize fully the structure and control of the receptor(s) for chicken somatotropin, to identify the specific amino acid sequence of somatotropin that binds to the receptors, to understand the signal system used for somatotropin to elicit its biological response, and to identify hormones that may counteract the effects of somatotropin in poultry. Given the state of the art, it is unlikely that cST will be available for poultry production before the later part of the 1990s (40).

Porcine Somatotropin

Pigs administered porcine somatotropin (pST) for a period of 30 to 77 days have been shown to increase average daily weight gains by approximately 10 to 20 percent; improve feed efficiency by 15 to 35 percent; decrease adipose (fat) tissue mass and lipid formation rates by as much as 50 to 80 percent; and concurrently increase protein deposition by as much as 50 percent, without adversely affecting qualities such as taste and texture of meat. Prolonged release formulations and daily injections produced similar growth rates and feed efficiencies. In addition, similar growth rate increases were observed in both barrows (castrated male pigs) and growing gilts (immature female pigs) (16).

Daily administration of pST to gilts weighing between 110 and 220 pounds did not affect the age at which puberty occurred, the proportion of gilts reaching puberty prior to 240 days, or the pregnancy rate. One study did indicate that with pST administration, ovarian function was impaired in prepubertal gilts, and that the onset of puberty was delayed. Withdrawal of pST restored normal reproductive function (16).

The minimally effective dose of pST needed to increase growth performance is approximately 20 micrograms of pST per kilogram of body weight per day. In the commercial setting, pigs will likely be treated with pST for about 60 days during the growing-finishing period (16).

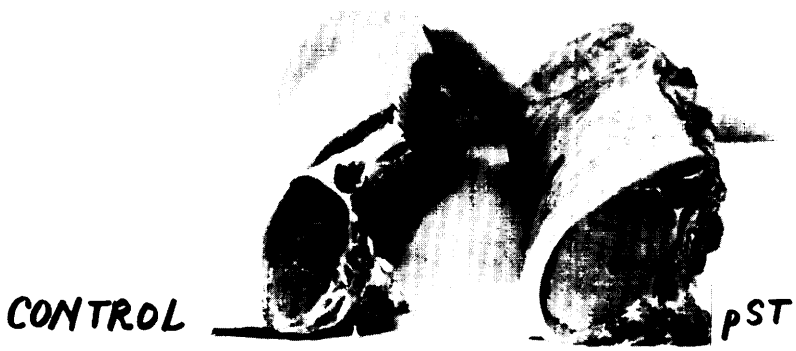


Photo credit: Terry Etherton, Pennsylvania State University.

Comparison of pork loins that show the effect of pigs treated with porcine somatotropin (PST). The loin-eye area of the loin treated with PST is 8 square inches; the control is 4.5 square inches.

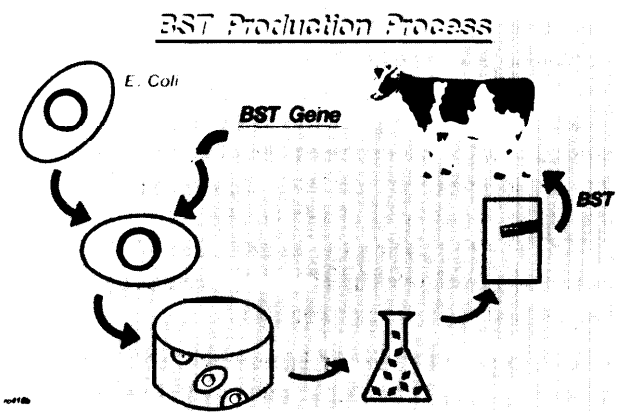
For effective use of pST, prolonged release formulations lasting at least 30 days need to be developed. Optimal nutrient requirements need to be determined. Initial data indicate that the diet should contain about 1.2 percent lysine ***(6).*** Current corn-soybean meal formulations containing about 16 percent crude protein may need to be supplemented with additional lysine, and perhaps other amino acids. Total feed intake will likely increase by 10 to 15 percent with pST administration. The nutritional requirements of pST-treated pigs is currently being studied by the National Research Council.

One **study** found that porcine somatotropin increased milk production between days 12 and 29 of lactation and the nursing piglets have a greater weight gain which matched the increased milk yield (16). However, this increase in milk yield and piglet weight gain has not been consistently observed (2, 8, 9, 10, 11, 42, 43, 44). Also, in some cases, adverse health effects were noted in pST treated sows (10, 42). Porcine somatotropin is currently being reviewed by FDA for commercial use. (For additional information on pST and its effects on carcass grades, see ch. 14.)

Bovine Somatotropin

Bovine somatotropin (bST) is currently undergoing FDA review for use in lactating dairy cows to increase milk production (figure 3-1). While individual milk yields depend on the management ability of the producer, on average, gains of about 12 percent can be expected with bST administration. However, response varies with the stage of lactation. Administration of bST early in lactation (i.e., immediately following parturition and prior to peak milk yield) evokes a small or negligible response (3). Administration after peak milk yields evokes a high response due to an immediate increase in milk yield, and a reduction in the normal decline in yields that occurs as lactation progresses. Maximum milk response is achieved with a daily bST dose of about 30 to 40 mg/day. BST does not alter the gross composition of the milk. The fat, glucose, protein, mineral, and vitamin composition of the milk all fall within the range of values normally observed in milk from cows not given bST (3).

Figure 3-l—Bovine Somatotropin (bST) Production Process



SOURCE: Elanco, a division of Eli Lily

The relative ratio of nutrient requirements of cows administered bST do not change, but the cow will eat more feed to accommodate the increased milk production. The magnitude of the increase in feed intake depends on how much milk production increases and on the energy density of the diet.

BST decreases pregnancy rates (proportion of cows becoming pregnant) and increases days open (days from parturition to conception). Conception rates (services per conception) are not altered. The effects observed are similar to those occurring in high milk producing cows that do not receive bST (3). The implications of using, bST in dairy production are discussed more thoroughly in OTA's 1991 publication U.S. *Dairy industry at a Crossroad: Biotechnology and Public Choices (47).*

A small number of studies using somatotropin to increase growth in growing cattle has been conducted, but research in this area is increasing. Results to date are highly variable due to the fact that studies differ significantly with respect to source and type of somatotropin used; dose and potency of somatotropin; route and frequency of administration; number, sex, type, and age of animals; duration of treatment; level and type of nutrition; and methodology used to determine characteristics measured. Thus, comparisons are tenuous, but on average, administration of somatotropin to growing cattle increases average daily weight gain by" 12 percent, improves feed conversion efficiency by 9 percent, increases carcass lean content by 5 percent, and decreases carcass fat content by 15 percent (15). Additional long-term studies are needed. Optimal dose, nutritional needs, duration, and withdrawal period before slaughter need to be determined.

Ovine Somatotropin

A small number of studies has examined ovine somatotropin (oST) or bST for use as a growth promotant in sheep. Because oST and bST are similar on amino acid sequence they both are effective. Like the studies with growing cattle, investigations with sheep vary sig-

nificantly in design and methodology. These studies suggest that on average, administration of somatotropin to sheep will increase the average daily weight gain by 18 percent, improve the feed conversion efficiency by 14 percent, increase the carcass lean content by 10 percent, and decrease carcass fat content by 15 percent.

Ruminants present some special challenges with regard to supply of amino acids to support high rates of protein accretion. Recent studies with growing cattle and lambs demonstrate that nutritional constraints imposed by rumen fermentation may limit amino acid supply and ultimately the biological response to somatotropin (4, 2 1). Long-term studies are needed, and optimal conditions of somatotropin administration and nutrient requirements must be determined (15).

Fish Somatotropin

Recombinant trout somatotropin injected into yearling rainbow trout increased growth rates by 100 percent as compared to control fish. Body length increased, and the chemical composition of the muscle tissues was indistinguishable from that of the controls (34). However, injection into individual fish is inefficient and different modes of administration are needed. Other studies have tried dipping and incubating test fish in an appropriately balanced salt solution containing fish somatotropin. Results have been encouraging; within 5 weeks, body weight had increased by 1.6 times over that of controls (34).

Evidence exists that invertebrates also produce somatotropins. Somatotropin from abalone has been isolated and shown to enhance growth in juvenile abalone. Recombinant trout somatotropin has been shown to increase the size of oysters (34).

Somatotropin also can be used to increase growth in finfish and shellfish. Research is needed to determine the most effective and practical means of administration. Large-scale production and purification of recombinant fish somatotropin is paramount. Optimum dose, nutrient requirements, and other related conditions must be established for each target species. Most studies to date have been short-term studies. Long-term studies to understand the effects of somatotropin on fish must be conducted. Given the work that is still needed, it is unlikely that somatotropin will be used commercially in the fish industry before the second half of the 1990s.

Somatotropin Related Technologies

Recognition of the role that somatotropin plays in growth and milk production has led researchers to search for means to increase endogenous levels of somatotropin in livestock as an alternative to administration of exogenous somatotropin.

The production and secretion of somatotropin by the pituitary gland is controlled by another protein hormone called growth hormone releasing factor (GRF). Early studies in pigs involved daily injections of 30 micrograms of GRF. Neither growth rate nor feed efficiency was significantly improved. There was a significant improvement in carcass composition (less fat), although the improvement was not as great as with exogenous administration of porcine somatotropin. Using synthetic analogs of GRF that are resistant to degradation by protease enzymes elicits a greater reaction; daily weight gain and feed efficiency increased, and carcass composition changed in a manner similar to that which occurs with exogenous administration of porcine somatotropin (16). There is some evidence that GRF does elicit some effects that are different than those of somatotropin. For example, a small improvement in the digestibility of dietary dry matter has been observed in GRF-treated cattle and this has not been routinely observed with bST-treatment (3, 16). GRF itself can be produced in bacteria, but some of the synthetic analogs cannot, and alternative methods will be required to produce sufficient quantities for commercial use. It is not expected that GRF will be commercially available before the later half of the 1990s.

An alternative way to increase endogenous somatotropin levels is to block compounds that prevent the secretion of somatotropin. Release of somatotropin from the pituitary gland is blocked by a compound called somatostatin. Deactivating somatostatin will increase the levels of somatotropin in the animal. Somatostatin is deactivated by stimulating the animal to produce antibodies to this compound. The process involves coupling somatostatin with another compound that stimulates the immune system in animals. Administration of this coupled compound to an animal causes the animal to produce antibodies that bind to somatostatin and deactivate it, thereby preventing it from inhibiting the release of somatotropin from the pituitary. When used in pigs, this process doubled the concentration of porcine somatotropin and increased growth rates slightly, but it is likely that higher somatotropin levels will be needed to increase growth in pigs significantly. In cattle, use of this method increased growth rates by 10 to 17 percent and improved feed efficiency by 13 percent (16).

A third possible way of increasing the effectiveness of somatotropin is to couple somatotropin with a monoclinal antibody specific for somatotropin. In dwarf mice

that have deficient pituitary glands, a somatotropin-monoclonal antibody complex increased weight gains 400 to 600 percent more than administration of somatotropin alone (16). In lactating sheep, a somatotropin-monoclonal antibody complex increased milk production more than somatotropin alone (16). The mode of action is not known with certainty. It is speculated that the complex is selectively recognized by different target tissues and receptors in preference to somatotropin alone. It is possible that the monoclinal antibody inhibits the receptor from internalizing the somatotropin, which allows the somatotropin to be active for a longer period of time. The use of monoclinal antibodies from species other than the animal being treated, however, may cause an immune response by the animal.

Beta-Agonists

Beta-agonists (also called beta-adrenergic agonists) are compounds similar to adrenaline. They are generally of two types, the beta- 1 agonists that stimulate cardiovascular functions and the beta-2 agonists that regulate smooth muscle function. Beta-agonists are currently used in humans to control bronchial asthma and to relax premature uterine contractions.

Beta-agonists can also act as repartitioning agents. They redirect nutrients away from the formation of adipose tissue (fat deposits) and towards muscle growth (48). Almost all cells have beta-adrenergic receptors. interaction of beta-agonists with the cell membrane receptors initiates intercellular responses that affect fat and protein metabolism and accretion.

Beta-agonists are not currently approved for use as livestock growth promotants in the United States. At least three companies have tested beta-agonists to promote growth and enhance carcass leanness in meat-producing animals. Beta-agonists tested include clenbuterol and cimaterol in lambs, beef, swine, and broilers (American Cyanamid); salbutamol in swine (Glaxo Animal Health, United Kingdom) and ractopamine hydrochloride in finishing swine, beef and turkeys (Eli Lily and Co.). Results of early studies with clenbuterol, cimaterol, and salbutamol were variable and available evidence suggests that none of these compounds are under development as growth promotants for livestock application (48).

Eli Lilly and Company is developing ractopamine hydrochloride to enhance carcass leanness and promote growth in meat-producing animals. In finishing swine (i.e., pigs weighing 100 to 250 pounds), ractopamine is administered as a feed additive, at doses of 5 to 20 parts per million (ppm), usually for a period of 42 to 49 days. Ractopamine is registered under the trade name Paylean, and is currently undergoing FDA review (48).

Trials involving finishing pigs were conducted in the United States, Canada, and several other countries worldwide. Ractopamine increases the rate of daily weight gain (maximum of 8.9 percent), decreases feed consumption (average of 3.9 percent), and improves feed conversion (up to 12.3 percent over untreated controls). [5] Additionally, two measures of carcass leanness—loin eye leanness and the 10th rib fat thickness—improved by a 14.9 percent increase and 13.6 percent decrease, respectively. Total lean content of the carcass increased from 50.9 percent to 56.9 percent as determined by total carcass dissection. Swine with superior genetics for leanness show a greater response to ractopamine than those with low lean-gain potential. Visual and taste panel evaluations of meat palatability characteristics from the ractopamine-treated pigs appear to be unchanged (48).

While total feed consumption decreases slightly, use of ractopamine requires crude protein levels greater than current National Research Council recommendations for finishing swine. Rations containing 16 to 20 percent crude protein or lysine equivalent appear to optimize the growth performance response to ractopamine. However, carcass leanness effects are seen at lower crude protein levels. Addition of fat to the diet, a common practice in swine, did not affect carcass leanness, daily weight gain, or feed conversion responses to ractopamine (48).

Some reports have indicated that beta-agonists cause hoof lesions in swine. No such effects were observed in another study with ractopamine given in amounts up to 25 times the highest intended level of use (550 ppm). Similarly, at three times the intended use level (60 ppm) during the finishing phase, there were no observed effects on the subsequent percent of gilts in heat, the percent

[4] Clenbuterol is currently marketed in Europe, Mexico, Canada, South America, and Asia as a veterinary prescription drug to treat bronchial and smooth muscle disorders in animals (primarily race horses and sheep). It has not been approved for use in the United States. Salbutamol is marketed as an anti-asthmatic in humans (17, 48).

[5] Twelve trials involving 1278 barrows and gilts were fed rations of 16 percent crude protein and administered ractopamine as a feed additive in quantities up to 20 parts per million.

farrowing rate, the number of live or dead newborn pigs, the 21-day pig weaning weight, or gilt weights at the end of the nursing period (48).

Antimicrobial Agents

Biotechnology is being used to produce new compounds that can enhance livestock production, but traditional means will continue to be used for the same purpose. One such traditional method is the addition of antimicrobial agents to livestock feed. Antimicrobial agents are compounds that, when administered in low concentrations, suppress or inhibit the growth of microorganisms. Antimicrobial agents include antibiotics (naturally occurring substances produced by yeasts, molds, and other microorganisms) and chemotherapeutic (substances that are chemically synthesized). Copper also has antibacterial properties when present in relatively high concentrations.

Antimicrobial have been widely used as feed additives for swine, poultry, beef cattle, and dairy calves since the early 1950s and numerous trials have been conducted during that time to document the efficacy of antibiotic use. Approximately half of the 4.65 million kilograms of antibiotics and chemotherapeutic sold in the United States in 1988 were for nonmedicinal use (12). In the early 1980s, it was estimated that approximately 75 percent of pig feeds, 80 percent of poultry feeds, 60 percent of feedlot cattle feeds, and 75 percent of dairy calf feeds contained antimicrobial agents (12). An estimated 90 percent of all feedlot cattle are administered antibiotics (12). Today, approximately 88 percent of the antibiotics used in livestock are given at subtherapeutic levels to promote growth, improve feed utilization, reduce mortality, reduce liver abscesses, and improve reproductive efficiency. Currently, 14 antibiotics and 6 chemotherapeutic have been cleared by the FDA for use as livestock feed additives (table 3-2).

The exact mechanism by which antimicrobial stimulate growth is not known with certainty. Three mechanisms have been proposed: a metabolic effect, a nutritional effect, and a disease control effect. Various antimicrobial have been shown to affect water and nitrogen excretion, to inhibit oxidation reactions that require magnesium ions, and to increase protein synthesis in muscle cells. However, none of these metabolic effects is significant enough to account for the observed increases in growth (12).

The nutritional effect is based on the premise that certain intestinal microbes synthesize vitamins and amino acids essential to animals, while others compete with the host animal for these nutrients. Shifts in the intestinal population of bacteria associated with the use of antibiotics could result in greater availability of nutrients for the host animal. Some antibiotics have been shown to stimulate yeast growth and bacteria that produce vitamins while reducing population levels of lactobacilli, bacteria that require amino acids in the same proportions as pigs and chicks.

Increased intestinal wall thickness and total gut mass, thought to be caused by bacterial invasion or toxins, are reduced by antibiotics. This decreased mass possibly leads to greater nutrient absorption and increases diversion of energy and nutrients away from heat production by the gut to body growth.

Evidence exists to support the hypothesis that the dietary protein requirements of animals administered antibiotics are lower than those of control animals. The most striking evidence in support of the nutritional effect is seen with the ionophore class of antibiotics, which causes an increase in propionic acid and a decrease in acetic acid in the rumen. Biosynthetic pathways using propionic acid are energetically more efficient than those using acetic acid, which could account for the marked reduction in feed requirements per unit of gain for animals administered the ionophores.

The most widely accepted theory as to how antimicrobials promote growth is the disease-control effect.

Table 3-2—Antimicrobial Agents Approved as Growth Promotants for Swine, Poultry, and Cattle in the United States[a]

Antibiotics	Chemotherapeutics
Bacitracin zinc (S,P,C)	Arsanilic acid (S,P)
Bacitracin methylene disalicylate (S,P)[b]	Carbadox (S)
Roxarsone (S,P)	Sodium arsanilate (S,P)
Bambermycins (S,P)	Sulfamethazine (S,C)
Chlortetracycline (S,P,C)	Sulfathiazole (S)
Erythomycin (P)	Lincomycin (S,P)
Lasalocid (C)[c]	
Monensin (C)[c]	
Oxytetracyline (S,P,C)	
Penicillin (S,P)	
Streptomycin (S,P)	
Tiamulin (S)	
Tylosin (S)[b]	
Virginiamycin (S,P)	

[a]The letters in parenthesis refer to the species for which the drug is approved; S = swine, P = poultry, and C =cattle.
[b]Bacitracin methylene disalicylate and tylosin are also approved in cattle to reduce liver abscesses.
[c]Lasalocid and Monensin are approved for use in poultry to control coccidiosis.

SOURCE: Office of Technology Assessment, 1992.

Antibiotics control subclinical disease, thereby allowing animals to more closely approach their genetic growth potential. The fact that antibiotics stimulate growth more in young animals than older animals provides some support for this theory because young animals have lower immunological competency and are more susceptible to disease. Also, the degree of the growth response is strongly influenced by the cleanliness of the living environment and the disease load of the animals involved.

Most of the research concerning antimicrobial is conducted at the pharmaceutical firms that develop these products. Research at universities evaluates the efficacy of already approved antimicrobial agents under different housing, management, and feeding programs. Some clinical studies of compounds in development are also conducted at universities.

Current research is focusing on the development of new antimicrobial, new techniques for screening and evaluating the safety of antimicrobial, detection of residues in meat, and the possible spread of antimicrobial resistance. Genetic engineering techniques can be used to alter the production of antibiotics by bacteria and to develop nucleic acid probes for use in safety evaluation.

Other research is focusing on ways to improve the efficiency of nutrient utilization and microbial fermentation in the gastrointestinal (GI) tract. Techniques that modify membrane function in bacteria can increase the transport of ions and substrates into bacterial cells, which could enhance digestion in ruminants. Alternatively, the use of live antagonistic microorganisms in feed can be used to maintain the optimal microflora.

More efficient methods of delivering antimicrobial, including intraruminal delivery devices, boluses, and rotation of two or more agents, are being developed. The compatibility and synergism of antimicrobial combinations and the effect of the diet are also being explored (12).

Antimicrobial Use in Poultry

Antimicrobial use in chickens up to 4 weeks old increases growth rate and feed efficiency by approximately 7 and 4 percent, respectively. Older chickens also show improvement, although not as high. Young turkeys have shown improved growth rates and feed efficiency of approximately 13 and 7 percent, respectively. When antimicrobial are used in laying hens, egg production improved by up to 4 percent, the feed required per dozen eggs was reduced up to 5 percent, and matchability improved about 3 percent. Similar results were obtained in turkeys. Antimicrobial use also appears to reduce mortality (12).

Antimicrobial Use in Swine

In pigs, antimicrobial have been shown to increase growth rates, reduce feed requirements per unit of weight gain, and reduce mortality and morbidity. Smaller (younger) pigs respond more to antibiotics than heavier pigs. Antibiotics have been found to improve growth rate of pigs weighing between 7 and 25 kg by 16 percent and to reduce the amount of feed required per unit of gain by 7 percent. In slightly heavier pigs (from 7 to 49 kg), the improvements in weight gain and feed efficiency were 11 and 5 percent, respectively. Over the entire growing-finishing period, antibiotics improved weight gain by 4 percent and feed efficiency by 2 percent. Improvements in growth rates, feed efficiency, and mortality rates from antibiotic use are greater under farm conditions than in highly controlled test conditions at universities and research stations. In addition, the effectiveness of antibiotics has not diminished over 40 years of use (12).

Copper gives growth rate and feed-efficiency utilization rates similar to those of antimicrobial, and in young pigs a combination of copper and antimicrobial appears to have an additive effect.

Antimicrobial are not usually continuously administered to breeding animals, but during certain critical stages of the reproductive cycle, such as at the time of breeding, administration of antimicrobial can improve conception rates (by about 7 percent) and increase litter size (by about a half a pig). Use of antimicrobial at farrowing reduces the incidence of uterine infections. Data also indicate a slight improvement in the survival and weight gain of nursing pigs that have been given antimicrobial in prefarrowing and lactation diets. Evidence also exists that the withdrawal of antibiotics from animals that have been administered antibiotics for a long time is associated with a reduction in reproductive performance (12).

In the last 5 years, two new antibiotics were cleared for use in swine. Three more antibiotics are currently under development (12).

Antimicrobial Use in cattle

In beef, growth rates have increased up to 5 percent, and feed efficiency gain has increased up to 7 percent with antimicrobial use. Antimicrobial are also commonly used to reduce, by nearly half, the incidence of liver abscesses. Animals with abscessed livers gained

weight more slowly than those without abscessed livers—about 1/3 pound per day less. Antimicrobial can be used to improve weight gain in dairy calves. but no general beneficial response has been noted in lactating cows (12).

Anabolic Steroids

Steroids are a class of lipid compounds composed of four interconnected rings of carbon atoms linked with various functional groups. Some steroids act as vitamins while others act as hormones. The anabolic steroids used to promote growth are estrogens and progesterone (female sex hormones) and androgens (male sex hormones). Steroids have been demonstrated to promote growth, increase feed efficiency, increase lean meat production, and reduce carcass fat. These hormones have been demonstrated to have growth-promoting properties in beef, sheep, swine, poultry, and fish. Such effects are greatest in ruminants.

Anabolic steroids were first approved for use in livestock in 1954. Currently they are approved for use as growth promotants in the United States only for beef and sheep. It is estimated that 10 percent of heifers and 60 percent of steers are treated with anabolic steroids as calves; 70 percent of stocker cattle; and 90 percent of feedlot cattle are administered anabolic steroids (35). Anabolic steroids reduce the cost of producing beef by an estimated $17 per head, and a complete ban on anabolic steroids in the United States would result in an estimated net-return loss of $2.4 to $4.1 billion in beef and sheep products (35).

Anabolic steroids are used in the United States either singly or in combination, with the most common method of administration being a prolonged release implant inserted at the base of the ear (see table 3-3). A combination estradiol-trenbolone acetate implant is currently under FDA review.

The mechanisms by which steroids act in livestock are still not known with certainty, despite the fact that these compounds have been used for nearly 40 years. It has generally been postulated that estrogens stimulate the production and release of somatotropin from the pituitary gland, and that the increased sornatotropin, in concert with insulin, increases the uptake of amino acids and the synthesis of muscle protein (35).

New studies indicate, however, that estrogens and somatotropins are additive, and act independently, and therefore it is unlikely that the action of estrogens occurs via elevated levels of endogenous somatotropin. This evidence has led to the proposal of alternative hypotheses. One such proposal postulates that because there are estrogen receptors in bovine skeletal muscle, estrogens could directly bind to these receptors and stimulate protein synthesis (35).

Alternatively, estrogens may stimulate the somatotropin receptor sites in the liver; greater binding and receptor capacity has been observed following estradiol administration. However, estrogens do not elicit an anabolic response in rats despite the fact that they stimulate somatotropin release and there are estrogen receptors present in rat skeletal muscles. This evidence suggests that the mode of action of estrogens may in fact be different than any of those hypothesized (35).

Table 3-3—Anabolic Steroids Commercially Available in the United States

Anabolic steroid	Commercial name	Method of use
Estrogens		
Beta-estradiol	Compudose	Implant
Zeranol[a]	Ralgro	Implant
Androgens		
Trenbolone acetate		Implant
Progesterone		
Melengesterol acetate		Feed additive
Combination		
Beta-estradiol/testosterone	Synovex-H	Implant
	Heifer-oid	Implant
Beta-estradiol/progesterone	Synovex-S	Implant
	Synovex-C	Implant
	Steer-oid	Implant

[a]Zeranol is technically not an estrogen (it's produced by a fungus) but has estrogenic properties.

SOURCE: Office of Technology Assessment, 1992

Most androgens have not consistently shown anabolic activity in ruminants, although trenbolone acetate (TBA) used alone, and especially when combined with estrogens. gives good response. TBA significantly elevates plasma estradiol levels, which may explain at least part of its activity. Androgens are thought to work by blocking muscle receptors for another class of hormones, the corticoid hormones. This decreases muscle protein degradation and turnover, rather than increasing protein synthesis (35).

The pharmaceutical industry conducts most anabolic steroid research. Universities conduct some research concerning the mechanism of action of steroids and work in conjunction with the pharmaceutical industry to conduct clinical trials. Current research is focusing on using combinations of steroids and on methods to improve timed-release implants so that they release lower levels immediately following implantation and continue to release for a longer period thereafter. Researchers are also exploring the possibility of administering androgens to pregnant ewes and cows in the hope of increasing growth potential in the offspring (a process known as imprinting). Imprinting has been shown to improve growth. feed efficiency, and carcass leanness in female offspring, but leads to no observed changes in castrated male offspring (35).

A clearer understanding of the mechanism of action of anabolic steroids is needed. Research is also needed to determine the optimum dose of steroids required to maximize anabolic response. Current dosage rates are *14* to 36 mg for estrogens, 200 mg for progesterone, 200 mg for testosterone, and 140 to 200 mg for trenbolone acetate, administered by implants lasting for 90 to 120 days. These doses are probably lower than those that would yield maximum growth; however, to change dosage would require FDA approval (35). Determining optimal dosage for maximum anabolic effects might also help determine the mode of action of these steroids and whether steroids are additive in effect with other hormones.

Further research is needed to determine the nutrient requirements for maximum response and to determine the effects of steroids on meat marbling. Anabolic steroids do not appear to affect the texture, flavor, juiciness. or cooking loss of meat, but some controversy remains concerning the effect of steroids on carcass quality, marbling, and carcass grade, particularly with respect to TBA/estradiol combination (35).

REPRODUCTION TECHNOLOGIES

The field of animal reproduction is undergoing a scientific revolution. For example, it is currently possible to induce genetically superior cows to shed large numbers of eggs (superovulation). It is also possible to fertilize these eggs in vitro with the sperm of genetically superior bulls. Each resulting embryo can then be sexed and split to produce multiple copies of the original embryo, frozen for later use, or transferred to recipient ''surrogate' cows whose reproductive cycle has been synchronized to accept the developing embryo. In the near future, it may be possible to sex the sperm rather than the embryo and to create greater numbers of copies of each embryo than is currently possible. Embryos produced by new reproductive methods are currently being marketed. Techniques now being developed will make it easier to insert new genes into the embryos to produce transgenic[6] animals. Although as yet no transgenic farm animals are commercially available, these new technologies are being used to improve the quality of livestock herds more rapidly than could be achieved with traditional breeding. Currently, however, many of these technologies are still relatively inefficient.

Estrous Cycle Regulation

Research has shed new light on the basic mechanisms controlling egg growth and maturation, and corpus luteum[7] function. This new knowledge is aiding the development of precise methods to regulate the estrous cycle, induce superovulation, and reduce the heavy losses due to early embryo deaths that occur in all domestic animals.

Perhaps the most important development in ovarian physiology in recent years is the discovery of the ovarian hormone inhibin, which decreases the ovulation rate. [8] Some breeds of animals with exceptionally high ovula-

'Animals whose hereditary DNA has been augmented by the addition of DNA from a source other than parental germplasm, using recombinant DNA techniques (46). Transgenic animals can be created that possess traits of economic importance including improved disease resistance, growth, lactation, or reproduction.

[7]The corpus luteum is a temporary endocrine organ that is produced at the site of ovulation during each estrous cycle. It produces hormones needed to maintain pregnancy.

[8]Inhibin decreases ovulation rates by suppressing the secretion of follicle stimulating hormone (FSH), a hormone produced by the pituitary gland.

Photo credit: U.S. Department of Agriculture, Agricultural Research Service.

Animal physiologist prepares an embryo for microscopic examination before implanting it into an animal.

tion rates, such as the Booroola strain of Merino sheep in Australia, are known to have low levels of circulating inhibin. Cattle immunized against inhibin have lower circulating levels in their blood and show increased ovulation rates, The genes controlling inhibin production have been cloned, and the potential exists for producing transgenic animals in which these genes are repressed or deleted (18).

Progress has also been made in understanding the control mechanisms that regulate corpus luteum function and its production of progesterone, a hormone that regulates the length of the estrous cycle and helps maintain pregnancy. Regulation of the estrous cycle is needed to ready surrogate mothers to receive embryos, and also to initiate superovulation. Estrous cycle regulation is reasonably well understood and developed in cattle and sheep. Conception rates in treated cows are similar to those obtained with animals bred at naturally occurring estrus. The estrous cycle of pigs appears to be more complex than that of ruminants and the process of controlling the cycle is not as efficient. Currently, superovulation treatments for cattle use highly purified hormones produced by recombinant DNA technology. About 10 viable eggs are produced, on average, per treatment (compared to the 1 egg a cow normally produces per ovulation) (18). As new knowledge of the factors controlling egg development and corpus luteum function is applied, the number of viable embryos produced by each superovulation treatment is expected to increase.

Once eggs are collected, they are matured and fertilized in vitro. In vitro fertilization occurs only when a capacitated sperm (i. e., a sperm specially prepared to penetrate the egg cell membrane) encounters an egg that is in an optimal maturation state. Great progress has been made in understanding the factors involved in egg maturation and sperm capacitating in livestock. As a result, in vitro fertilization rates as high as 70 to 80 percent are produced in cattle, swine, sheep, and goats, and offspring are successfully produced. Conception rates with superovulated and artificially inseminated eggs in cattle are the same as those obtained by artificial insemination of control animals bred at naturally occurring estrus. Embryos produced with these techniques are currently being marketed. It is estimated that about 100,000 calves are born annually in the United States as the result of embryo transfer techniques. Many more embryos are being exported (41).

Early detection of pregnancy can enhance a livestock producer's ability to identify and rebreed animals that have not become pregnant. Traditionally, pregnancy has been detected by rectal palpation. This procedure can be conducted at 40 days post breeding, but at this early date the possibility exists of damage to the fetus. In practice, rectal palpation is usually carried out at 60 days or later in cattle. An alternative method is to measure progesterone concentration in milk. Concentration can be measured at 20 days after breeding. However, the process is expensive and results in about 15-percent false positives. A new method under development involves using a radioimmunoassay procedure to detect protein B, a gly - coprotein produced by cells of the ruminant placenta (18).

High embryo mortality is a major cause of reproductive loss in all livestock. Embryos of all species must signal their mothers in some way to prevent regression of the corpus luteum, so that the progesterone secretion needed to maintain pregnancy can continue. Early pregnancy recognition signaling systems are complex and apparently differ from species to species. In ruminants, compounds similar to alpha interferon may be early signals of pregnancy. Administration of interferon early in pregnancy is being tested as a possible means of reducing

[9] Sperm capacitation involves the uptake of calcium ions which changes the pH of the sperm.

embryo loss. In mice and humans. platelet activating factor is known to be an early pregnancy reccognition signal. Preliminary data exist to suggest that it may play a role in early pregnancy in sheep and cattle (18).

Embryo Cloning

Multiple copies of a mammalian embryo were first produced by physically splitting an early embryo into halves. giving rise to identical twins (18). If the embryo) is dividemore than twice, however, few offsprings survive. Thus, no more than four indentical animals can be produced by splitting, and generally only two empryos are produced by this method. This procedure is already used in the cattle empryo transfer industry nearly doubling the number of offspring produced.

A more efficient and promising method of producing multiple copies of an embryo is a technique called nuclear transplantation. Basically, the procudure involves the transfer of a nucleus from a donor embryo into an immature egg whose own nucleus has been removed. The recipient egg cell is activated by exposuure to an electric pulse, allowed to develop into a multicelled embryo, and then used as a donor in subsequent nuclei.ir transplantations to generate multiple clones. This procedure (outlined in figure 3-2) has been used successfully with cattle, sheep, and swine. This technique has already produced hundreds of embryos that have been successfully carried to term in cattle. and recloning has resulted in as many as eight calves from one embryo (29).

The value of this technique is enhanced by the ability to transfer nuclei successfully from frozen embryos into eggs whose nuclei havc been removed. Conception rates obtained after transfer of embryos produced by neculear transplantation are varible, but rates us high as 50 percent have been obtained. However, embryo losses after transfer are higher than normal, resulting in actual pregnancy rates ranging from 15 to 33 percent (18). Combining the techniques of in vitro fertilization. embryo cloning, and artificial estrous cycle regulation can result in major changes in livestock breeding and in the rates of genetic improvement.

Embryo and Sperm Sexing

The availability of a technique to preselect the sex of the progeny holds great economic potential for the livestock industry. In the diary industry, females are the major income producers. while in the beef industry, males area economically more valuable. Until recently, no methods existed that provided the degree of separation needed for commercial use. However, recent advances in the seperation of the X and Y sperm, and sexing of the embryo have been made.

It has long been a goal of mammalian phsiologists to develop a method to effectively separte X and Y chromosome-bearing sperm to control the sex of the offspring. Most sperm seperation techniques are based on potential differenccs in the size and density of the two sperm types.[10] These methods, however have met with little success (41).

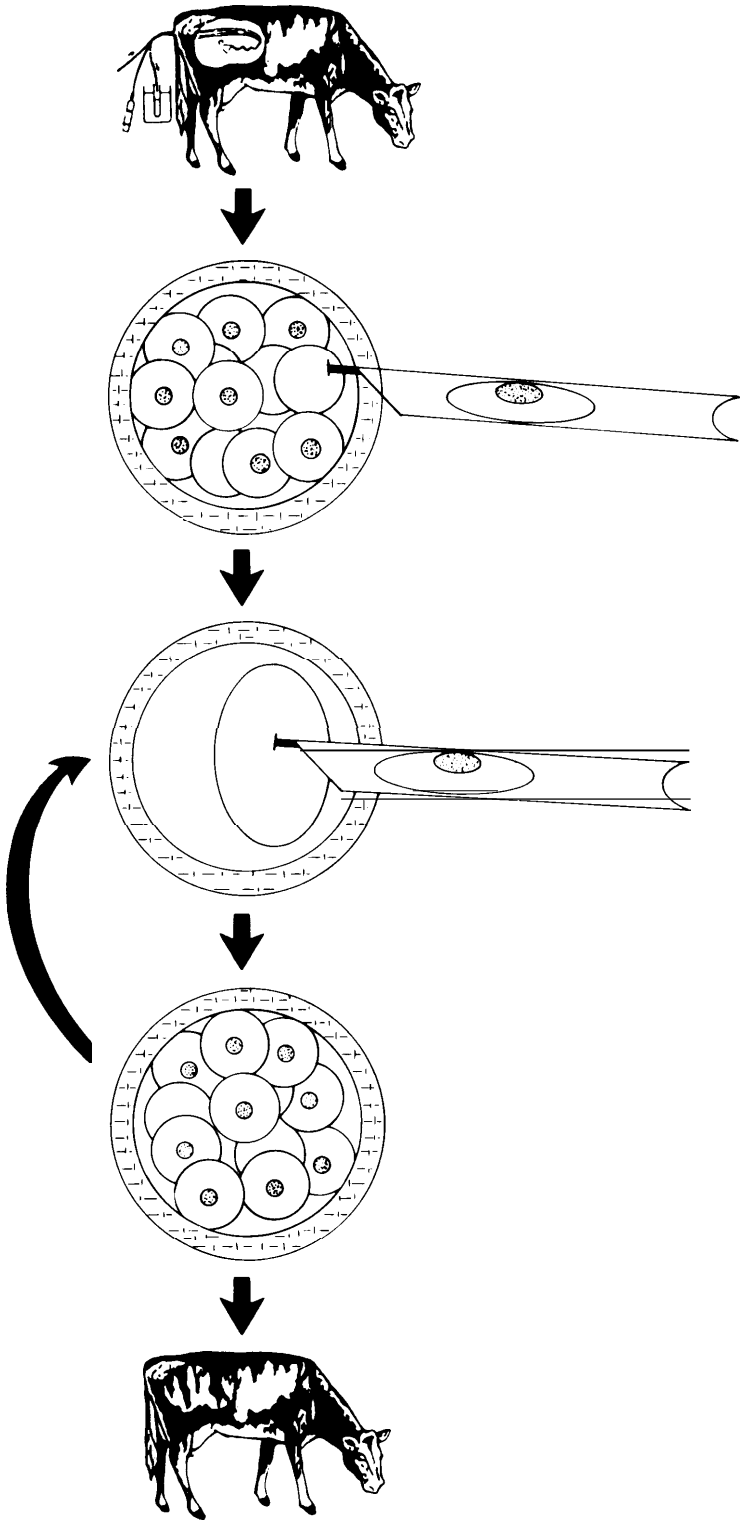
Development of cell-sorting techniques based on the differences in sperm size and Fluorescence of sperm DNA (flow cytometric measurements) has provied the first effective mehod to sort the sperm cellls. Johnsson et al. (22) recenently reported successful serperation of intact viable X and Y chromosome-bearing sperm using this method. Although the differece in DNA contents of the X and Y chromosome-bearing sperm in rabbits amounts to only about 3 percent. 94 percent of the rabbits (does) inseminated with X-bearing sorted sperm produced females and 81 percent of the does inseminated with Y-bearing sorted sperm produced males. This method has been used to separate X and Y bearing intact sperm of cattle. swine, and sheep with greater than 80-percent accuracy (2). Commercial use of this process is limited. at present. by the number of sperm that can be sorted per hour and by increased embryo mortality observed in the embryos produced after insemination with the sorted sperm. Neither of these factors is thought to represent an insurmountable difficulty.

The most accurate method of sexing embryos is to create a picture of the number, size, and shape of' the chromosomes contained in the embryonic cells, a process called karyotyping. However. this method requires removal of about half of the cells of early stage embryos, which decreases embryo viability and limits the number of embryos that can be transferred. Another method uses antibodies[11] to defect proteins (antigens) unique to male embryos. This method is not damaging to the embryos and encouraging results have been obtained in one laboratory; however, the technique yields variable results and has not been widely adopted (18).

[10] Methods used are differential sedimentation techniques including differential velocity sedimentation, free-tJow electrophoresis, and convection counter-stream ing galvanization.

[11] The antibodies are attached (labeled) to a flourescent compound to allow for detection.

Figure 3-2—Nuclear Transplantation



SOURCE: Office of Technology Assessment adapted from R S Prather and N L First. Cloning Embryos by Nuclear Transfer, Genetic Engomeeromg of *Animals,* W Hansel and B J Weir (eds.), *Journal of Reproduction and Fertility Ltd .* Cambridge, UK, 1990, pp 125-134

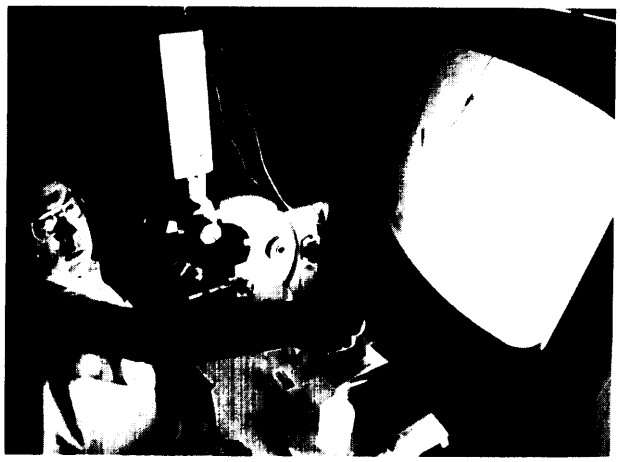


Photo credit: U.S. Department of Agriculture, Agricultural Research Service,

Animal physiologist checks swine sperm cells on video monitor to evaluate their motility, a procedure that precedes laser X-Y sperm separation.

More recently, the sex of bovine and porcine embryos has been determined by untempting to match fragments of DNA that are contained only on Y (male) chromosomes with the same DNA fragments in the embryo. Due to its chemical structure, a fragment of DNA will combine with a second DNA fragment that has a corresponding nucleic acid sequence. Therefore, a fragment of DNA that is specific to males can be used as a probe to identify male DNA fragments in the embryo. Combined with technologies that produce multiple copies of the DNA fragments, this method determines the sex of the embryo using only a few cells. It is rapid (about 6 hrs) and extremely accurate (up to 95 percent). but may be overtaken by the rapidly developing capability to separate X and Y chromosome-bearing sperm (18).

TRANSGENIC ANIMALS

The new reproductive technologies of superovulation, in vitro egg maturation and fertilization, nuclear transplantation. and embryo sexing can, and are being used to upgrade livestock herds. When these technologies are combined with recombinant DNA technologies (the identification, isolation, and transfer of selected genes), it becomes possible to produce animals containing foreign DNA **in** their germ lines (transgenic animals). (See figure 3-3.)

The tools of biotechnology provide the opportunity to develop transgenic livestock that contain genes coding for improved growth charticteristics, lactational performance, and resistance to disease and stress. Transgenic animals have human medical implications as well. It may be feasible to produce important pharmaceuticals in livestock. Only certain human drugs can be chemically synthesized or produced by bacteria, because some compounds undergo modifications after the protein has been produced (referred to as post-translational modifications). Animals are capable of performing these modifications. but bacteria are not. Transgenic animals can also serve as powerful research tools to understand genetic and physiological functions, and provide a model system with which to study human disease.

The production of transgenic animals is inextricably linked to the new reproductive technologics discussed in the previous section. lndeed, it is impossible to produce animals containing foreign DNA in their germlines without first manipulating the embryo and transferring it to a recipient animal.

Process of Creating Transgenic Animals

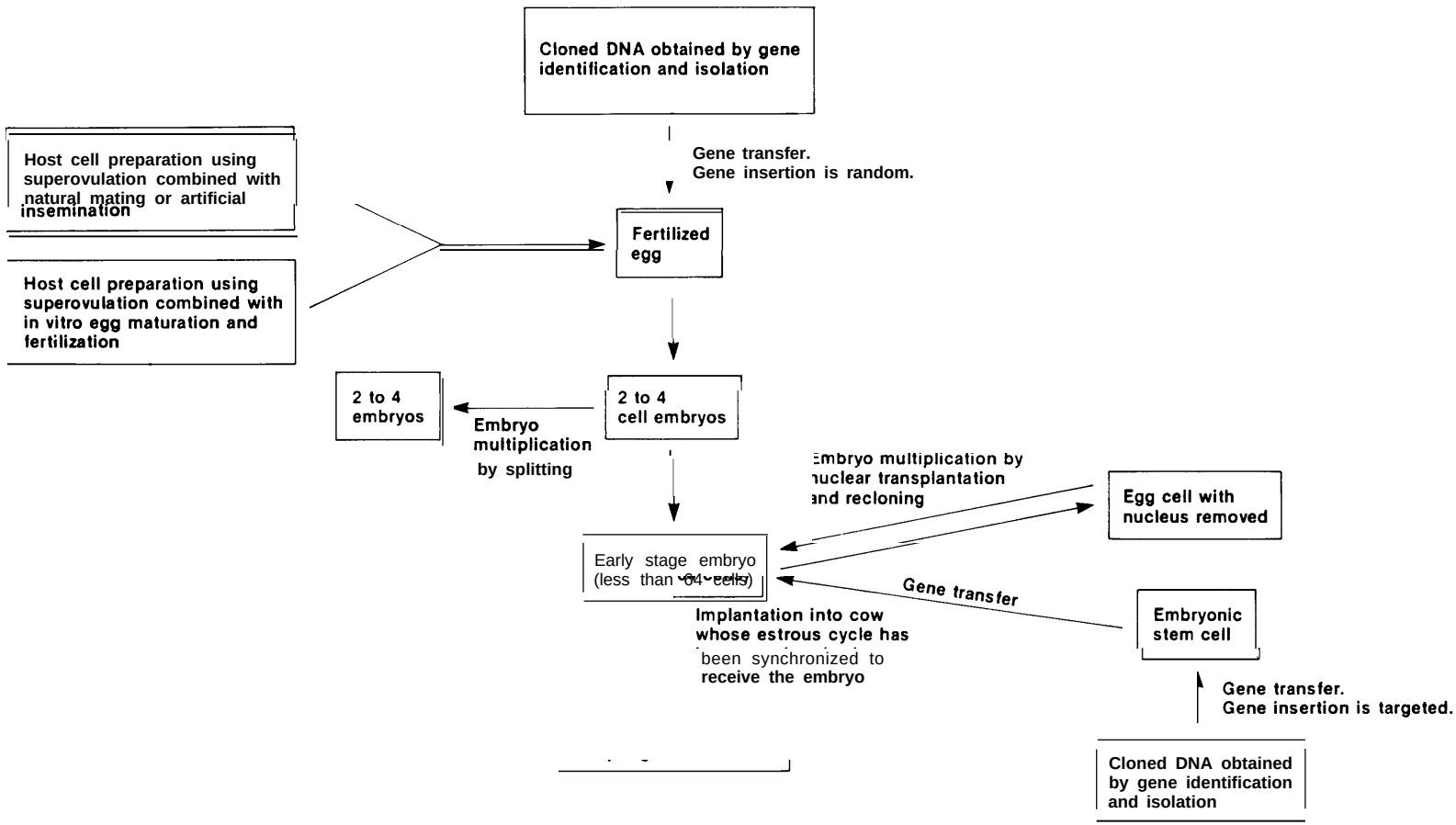
The process of making a transgenic organism is similar for plants and animals, and many of the tools and methodologies used are the same. As in plants, to create transgenic animals. the gene being transferred must first be identified and purified. Appropriate mechanisms (vector or nonvector) must then be found to transfer the gene into the animal cell, and appropriate regulatory sequences must be included to ensure proper expression of the gene. Unlike plant cells that are regenerated into whole plants by tissue culturing techniques, animal embryos (with the exception of fish) must be transferred to surrogate mothers for development and birth.

Gene Identification and Purification

The methods used to isolate and purify animal genes for transfer are the same as those used in plants, and have been described in detail in chapter 2. The method described in chapter 2 is the creation and screening of genomic libraries, libraries of DNA fragments that contain all of the genetic material of the chromosomes. An alternative approach is to create what is called a complementary DNA (cDNA) library. This method can also be used in plants, and it is frequently used in animals.

Genes are composed of DNA, and they code for proteins. But, before the protein is constructed, several intermediate steps occur. The DNA of the gene is first transcribed and processed into another compound called messenger ribonucleic acid (mRNA). It is the mRNA that serves as the actual template for the production of

Figure 3-3—Reproductive Technologies Used To Produce Transgenic Animals



SOURCE: Off Ice of Technology Assessment. adapted from J P Simons and R.B. Land, Transgenic *Livestock, J. Reprod Fert. Suppl. 34:237–250.* **1987**

proteins. Messenger RNA is not identical to the genomic DNA. This is because there are sequences of DNA contained within the gene that do not code for protein. After the DNA of the gene is transcribed to mRNA, these noncoding regions are snipped out and thrown away. Thus, the mRNA contains the coding regions, but not the noncoding regions of the genomic DNA.

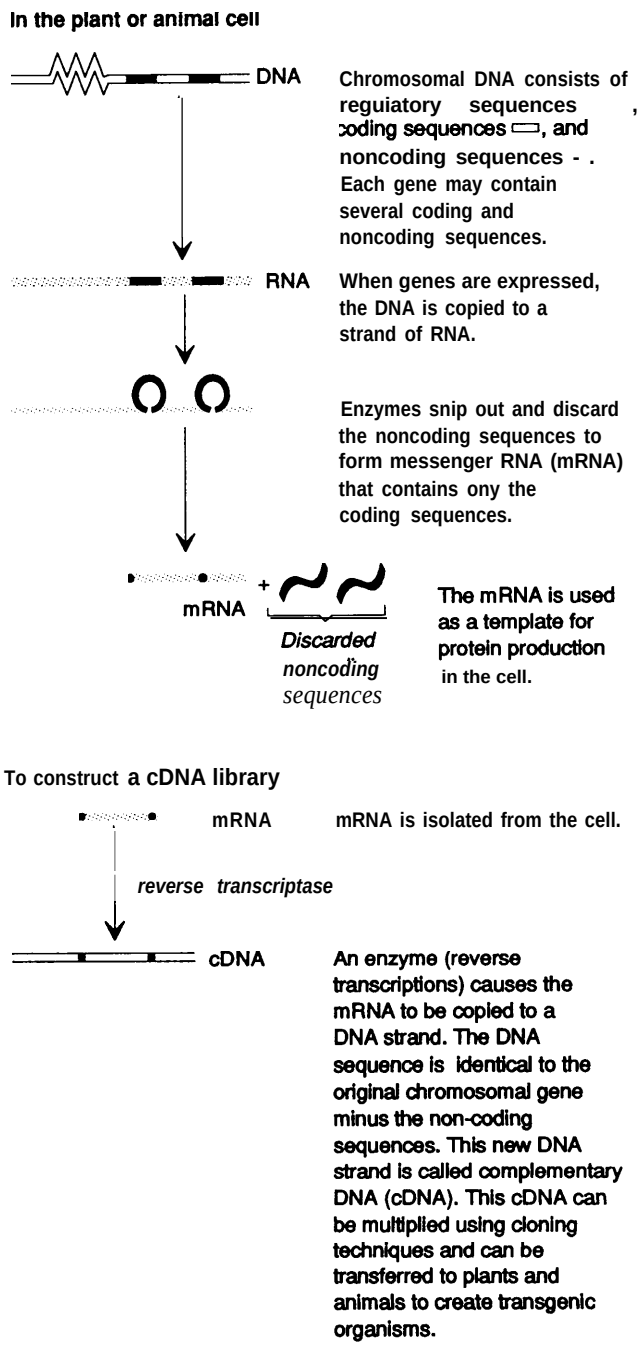
Special enzymes exist that can use the mRNA as a template to create DNA that has a complementary sequence to the mRNA. This new DNA is called complementary DNA (cDNA). It is identical to the sequence of the genomic DNA with the exception that, like the mRNA from which it was derived, it contains the protein coding regions, but not the noncoding regions of the genomic DNA (see figure 3-4). Thus. a library of cDNA sequences can be constructed from mRNA rather than the chromosomal DNA used to construct genomic libraries.

The mRNA that serves as the protein template for the desired gene can be obtained from tissues that express high levels of the protein. For example. if one wanted to find the gene that produces insulin, a reasonable approach would be to extract the mRNA from the pancreas, which produces very high levels of insulin. This high level of insulin production means that there is a significant amount of mRNA for insulin. Also, because the pancreas is specialized for insulin production, mRNA for other proteins, say for example, somatotropin, may not be present in large quantities. Thus, the use of cDNA libraries decreases the amount of genetic material that must be searched to identify the gene of interest. The process of looking for a particular gene is tantamount to looking for a needle in a haystack. Use of a cDNA library, as opposed to a genomic library, provides a smaller haystack that must be searched.

It might seem at first glance that the best method to use would be to construct cDNA libraries rather than genomic libraries. However, limits exist to the use of cDNA libraries. To construct both cDNA and genomic libraries, it is important to know the structure, sequence, and function of the protein for which one is trying to isolate the gene that codes for it. The lack of knowledge concerning the sequence and function of important proteins is the major constraint to the isolation and purification of the genes coding for those proteins.

Additionally, construction of a cDNA library is easiest when tissues exist in the organism that specialize in the high-level production of the protein coded for by the gene that is being isolated. This method does not offer significant advantages when the protein is produced in low quantities by nearly every cell in the organism.

Figure 3-4—Construction of a cDNA Library



SOURCE: Office of Technology Assessment, 1992

Also, evidence exists that genes that do not contain the noncoding regions do not function as well as genes that contain the noncoding sequences (5, 7, 33). While the functions of the noncoding sequences are not known

with certainty, they may play some role in the regulation and expression of the gene itself. Therefore, incorporating cDNA genes that do not contain the noncoding regions into transgenic animals results in the genes not being expressed as well as a genomic gene. Unfortunately, many of the animal genes that have been isolated and purified are cDNA genes rather than genomic genes. Thus, the tradeoff is that it may be easier to isolate and purify cDNA genes than genomic genes, but they don't work as well when used to create a transgenic organism (5, 7, 33).

Gene Transfer

Once an animal gene has been purified, it must be transferred to the host animal cell. Genes can be transferred using direct transfer methods (e.g., microinjection, electroporation, chemical) or vectors (i.e., viruses). The first transgenic animals created were mice in 1980 (37). Since then, transgenic cattle, sheep, swine, poultry, and fish have been produced.

The most common method used to produce transgenic animals is microinjection. This method involves directly injecting cloned DNA into a fertilized egg. [12] The cytoplasm of cow and pig embryos is opaque, and the embryos must first be centrifuged to locate the nucleus; otherwise the procedure for cows and pigs is similar to that **used** in mice, rabbits, and sheep (36). Fish embryos are surrounded by a tough membrane called a chorion, and this membrane first must be removed before DNA can be injected. Even with the removal of the chorion, the nuclei are not visible and so the DNA is injected into the cytoplasm. Injection into the cytoplasm rather than the nucleus requires greater amounts of DNA (34).

Other direct transfer methods attempted include the use of short electrical pulses (electroporation), or chemicals to make cell membranes permeable to the passage of large molecules **such** as DNA. These approaches have been used with sperm as well as eggs. The possibility of using sperm as a method to incorporate new genes into a species is an exciting prospect. One research group has reported using this method successfully to create transgenic mice that passed the new gene on to their offspring (27). Other researchers, however, have not yet been able to duplicate this result.

The use of electroporation methods in fish have resulted in up to 40 percent of the embryos becoming transgenic and this approach may be far more useful in fish than microinjection. Another approach being attempted in fish is the use of liposomes, vesicles contained in the phospholipid layer of cell membranes, as a means to encapsulate foreign DNA for entry into the cell. This method has not yet yielded any successes (34).

Poultry reproduction is significantly different from that of other livestock species. By the time the fertilized egg is layed, the developing embryo may already contain as many as 60,000 cells. This precludes using the microinjection technique because the number of cells that might incorporate the injected DNA could be small. Additionally, only some of the cells that incorporate the foreign DNA will express it. Attempts have been made to inject DNA directly into unfertilized eggs still in the ovary, **but** this method did not yield any transgenic offspring (24).

As a result of the deficiencies of direct gene transfer methods in poultry, a vector system has been developed. The most commonly used vector is a retrovirus. The gene that is to be transferred can be incorporated into the retrovirus. The host animal cell can then be infected with the retrovirus incorporating the new gene. Retroviruses are attractive vectors because only a single copy of the virus is integrated into a chromosomal site. Retroviruses also tend to be either species specific or to infect only a few closely related species.

Two types of retroviral vectors have been developed. Replication-competent retroviruses are those that are capable of self-replicating. These viruses have been successfully used in chickens. One-day-old embryos were infected with the retrovirus and transgenic chickens were hatched. Furthermore, the virus successfully infected germ line (sex) cells, and the new gene was passed on to the transgenic animals' offspring (24).

Replication-defective viruses lack the genes necessary for self-replication. These viruses cannot reproduce without the presence of a helper vector. The retrovirus is engineered in such a way that it contains all of the normal viral genes except those needed to package its own genetic material. The helper vector (also engineered) possesses the genes needed for packing retroviral genetic material, but does not include the other viral genes (i. e., genes that enable it to infect cells and cause virulence). Introduction of the retrovirus and the helper vector into host cells provides all of the elements needed to enable the retrovirus carrying the desired gene to infect and incorporate that gene into the host chromosomes. This method is considered safer than using replication-com-

[12] Specifically, the DNA is injected into the male pronucleus of the fertilized egg. The pronuclei are the egg and sperm nuclei present after the sperm penetrates the egg membrane.

petent ectroviruscs because the rcplictition-detective ectrovirus can only be infective and spread to other cells if the helper vector is present. However, there is a small possibility that the helper vector nd replicaton-defective retrovirus might recombine to form a replication-competent retrovirus. Additionally the DNA sequences carried by replication-defective retoviruses are not incorproated in the germ lnes of hickens. hence they are not passed to the offspring. Improved replication-defective retrovirus vectors are needed (2-I).

A number of transgenic cattle, pigs, sheep, chickens. and fish have been produced using direct transfer methods (almost exclusively microinjection) and viral vector methods. However, these techniques have several limi - tations. Microinjection techniques are expensive to use and the efficiency of transgenic animal production is very low. For a transgenic animal to be created. embryos must survive the physical manipulation and inflection of' DNA. must incorporate the DNA into their chromosomes, and must express the gene product. The percentage of microinjected embryos that actually results in transgenic animals is low, ranging, for example. from (). I to 4.45 percent in sheep and from 0.3 to 1.73 percent in swine (36. 38). The low rate of efficiency limits the study of transgenic livestock because of the high number of donor and recipient females that must be maintained tot- experimentation. Efficiency rates are much higher in fish, ranging from 35 to 80 percent, because fish undergo external fertilization and do not require in vitro culturing of the embryos and transfer to surrogate mothers.

Microinjection techniques are not only inefficient methods of creating transgenic animals. but they also do not provide any control over where the new gene is incorporated into the gernome (26). The site of gene incorporation is random. which also occurs with retroviruses. Because the site of incorporation influences gene expression, random insertion causes reduced control over the ability of researchers to control expression levels.

Because of these deficiencies. alternatives to viral vectors and microinjection are being sought. A promising new method for gcnerating transgenic animals has recently been developed in mice and may be applicable to other mammals. This new technique uses stern cells derived from an embryo. Stem cells are normally undifferentiated, that is. they do not become specialized tissue cells such as muscle, brain, liver cells, ect. Howe\'er. stem cells retain their ability to become specitilized cells under the proper stimuli (i.e., they are pluripotent). [13] Stem cells can be used as vectors to introduce selected genes into a host embryo. This method hits several significant advantages over microinjection methods. the most profound of which is that it is possible to insert DNA at specific, predetermined sites within the genomc of the stern cells (18). Targeted insertion is possible bccause stern cells have an intrinsic ability to recombine similar (homologous) DNA sequences. which results in the replacement of an endogenous gene with the desired gene. Stem cells can also be tested in vitro to ensure that integration of the new gene has occurred before these cells are transferred to a developing embryo.

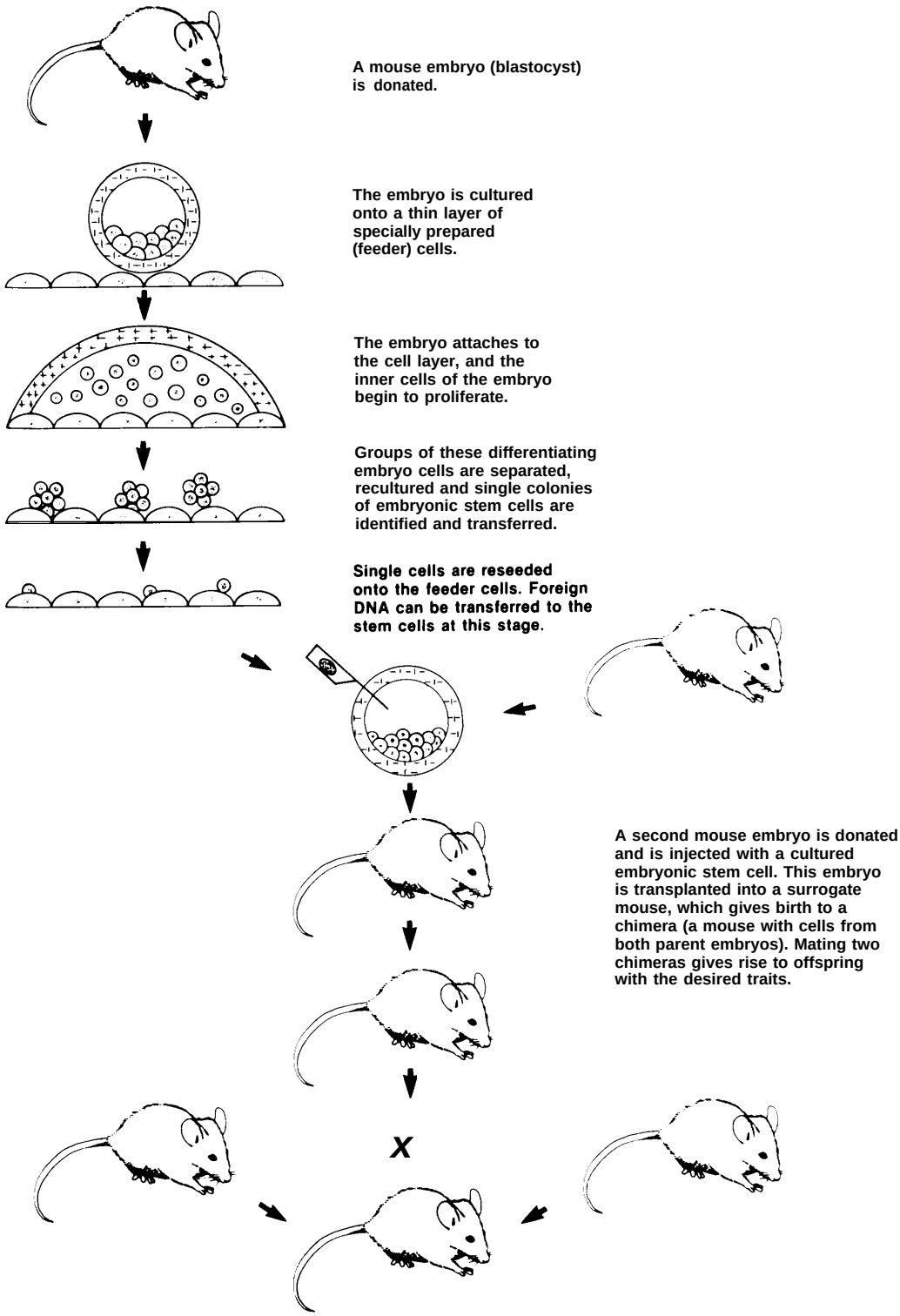
To isolate stem cells (see figure 3-5), an early stage embryo is cultured on a monolayer of specially prepared cells. The proliferating embryo cells are recultured until individual sterns cells can be isolated. These individual stem cells can then be cultured indefinitely. At this stage. DNA sequences containing desired genes can be inserted into the stem cells.[14] A geneticallly transformed stem cell is then microinjected into an immature embryo to produce a chimera, an organism that contains cells from more than one source. It the stem cells are incorporated into the germ lines of these chimeric animals, then these animals can be interbred to obtain offspring homozygous for the desired trait (18).

Use of the stem cell method will make it possible to produce a broad range of transgenic animals that could not be produced economically using direct microinjection or viral vectors. Targeted gene insertion also has the significant advantage of allowing host animal genes to be inactivated or removed and replaced with modified forms of the genes, such as ones that are expresses at a higher level, have new patterns of tissue-specific expression, or have a modified biological activity.

A host organism's endogenous genes can be inactivated by targeting an insertion into an essential region of the gene. This fact is of particular interest to the livestock industry, because inactivation of genes that have inhibitory physiological effects is likely to result in improvement in a number of productive traits. For example, bovine somatostatin is a hormone that inhibits bovine somatotropin production; inactivation of this gene would

[13] Pluripotency help make stem cells attractive vectors of DNA transfer. While in tissue culture, DNA can easily be inserted into stem cells. When stem cells are injected into an early stage embryo. the conditions for tissue specialization are present, and stem cells undergo the normal tissue development that occurs as the embryo develops during pregnancy. Thus. using stem cells provides an efficient means to transfer DNA.
[14] Methods used include viral infection and use of an electric pulse to make cell membranes leaky (electroporation).

Figure 3-5—Gene Transfer Using Embryo Stem Cell Culture



SOURCE: M.R. Capecchi, "The New **Mouse Genetics: Altering** the **Genome** by Gene Targeting," *Trends in Genetics 5:70–76, 1989*

result in increased endogenous somatotropin secretion and, presumably, increased milk production and more efficient growth. If successful, this technology could be used in lieu of administering bST exogenously to increase milk production. The genes controlling the production of inhibin, the ovarian hormone that reduces ovulation rate, provide another example of potential tin-gets for deactivation. The ability to inactivate genes also provides a powerful research tool for the study of the function of genes in vivo.

Stem cells have been isolated in mice and hamsters and possibly rabbits. There are reports that stem cells have also been isolated for swine (18). Progress is being made in isolating stem cells in sheep, and much research is being conducted to isolate bokvine stem cells, but to date, this has not been accomplished. There has been no documentation of embryonic stem cells being isolated from poulty ". However. in a similar type of procedure. 1-day-old embryonic cells from chickens have been isolated and introduced into immtaure embryos of other chickens. About I I percent of the resulting embryos were chimeric, and one embryo developed to hatching (24). Stern cells have not been isolated in fish (34).

Promotors and Gene Expression

The expression of new genes in transgenic animals is poorly regulated. Apprprate levels of gene expression are important. because overexpression can lead to impaired health in the transgenic animal. Better understanding is needed of how to turn genes on and off when desired; of how to regulate the level of gene expression: and of how to direct the expression of the gene to specific tissues at different stages of development. At the present time the factors that cause genes to have tissue and developmental specificity are not well understood.

Currently, fewer than 10 promotors or regulatory sefquences have been used to direct gene expression in transgenic live stock. Most of these promotors are derived from mice or viruses. The most commonly used promotor is the mouse metallothionein promoter, which is responsive to dietary stimulation by heavy metals such as zinc. Three promotors are being examined for their abilite to direct gene expression in mammary glands. A fourth promotor directs expression primarily to the liver.

It may be desirable to use promotors derved from the same species that is receiving the new gene. Evidencc euggests, for example, that using a mouse promotor sequence in pigs results in somewhat different gene expression than use of the same promotor in a mouse (18. 36).

Levels of gene expression do not aways correlate with the number of gene copies incorporated into the chromosome of a transgenic animal. This suggests that the site of the incorporate ion of the new gene in the host chromosome also affects gene expression. Given that embryonic stem cell procedures still require considerable development before directed insertion can occur. some researchers are examining methods to control gene expression independently of the site of integration. Research is focusing on regulatory elements that allow the new genes to provide their own environment for expression. [15]

Transgenic Poultry

Research emphasis has been given to improving growth and disease resistance. Bovine somatotropin has been transferred to chickens and increased the mass of the chicken. The envelope gene of avian leukemia virus has also been transferred to chickens and the cellss that expressed this gene have been shown to be resistant to subsequent infection with the same strain of vrirus (24).

Research is being conducted by USDA Agricultural Research Service and universities in the United States. as well as by a limited number of private firms. It is interesting to note that most of' the funding for transgenic poultry research conducted in the United States is being supplied by other countries (mainly Canada and France). Commercial availability will take 7 to 12 years after the production of an adequate number of transgenic fonder male chickens.

Transgenic Swine

Several genes have been successfully transferred into pigs, including those for somatotropin. human growth hormone releasing factor (hGRF). human insulin-like growth factor--l (hIGF-I), mouse MX (to investigate resistance to respiratory discuses), mouse whey acidic protein (WAP) (to investigate mammary- specific expression, and light and heavy beta chains for antibodies to produce specific immunoglobulns (36). With swine. as with other livestock species. researchers are focusing on improving growth. increasing disease resistance. and producing high-value pharmaceutical products.

[15] Such elements would function in a manner similar to that of dominant regulatory elements (DRE). When the injected DNA contains DREs, gene expression levels independent of the tissue and numbers of copies of the gene that was incorporated, were obtained. The "A-element," which seems to be a chromatin binding site, may permit genes to be expressed independently of the local environment into which they integrate (37).

Photo credit: U.S. Department *of* Agrculture, *Agricultural Research Service.*

Rooster on left was injected with genes of avian leukosis virus when it was a 1-day-old embryo. Roosters in center and on right are of two succeeding generations which directly inherited those virus genes.

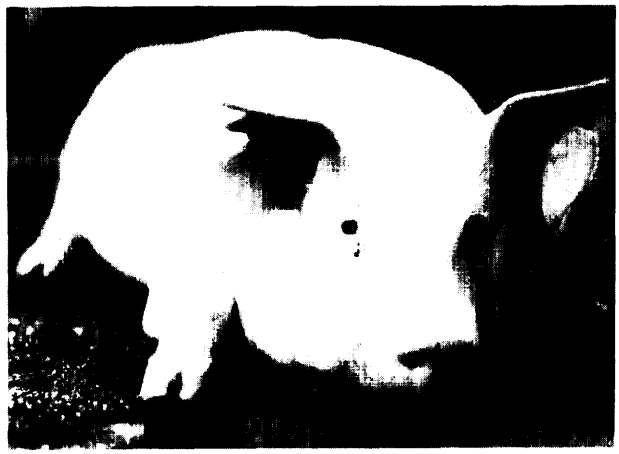


Photo credit: Mark Lyons

Transgenic pig at DNX research facility born with the capability to make human hemoglobin.

Somatotropin transferred to pigs has been shown to increase feed efficiency, enhance meat quality, reduce carcass fat, and increase the rate of gain. When fed a high-protein diet, transgenic pigs containing somatotropin genes gained weight nearly 17 percent taster than controls. and showed up to 18 percent greater feed efficiency. Backfat was significantly reduced and meat was leaner (36). Transgenic pigs that expressed the somatotropin gene passed that expression on to their offspring. Offspring that contain the somatotropin and who were fathered by boars that expressed the gene also expressed the sornatotropin gene. The offspring containing somatotropin genes who were sired by boars that did not express the somatotropin gene, also did not express the gene. This suggests that the stability and functioning of the gene are the same in the parent and offspring (36).

Pigs that continuously expressed high levels of somatotropin experienced significant health problems including lameness, susceptibility to stress. peptic ulcers, and reproductive problems. Animals that incorporated the somatotropin gene but did not express it, or that expressed it at low levels did not display these health problems (36).

Researchers are interested in improving disease resistance. Genes that confer resistance have not been isolated. Attempts to transfer genes that code for antibodies to compounds contained on the surface of selected bacteria and internal parasites are being made (28.51). Also, genes of the Class 1 Major Histocompatibility Complex [16] have been cloned. It may also be possible to induce immunity to specific viral diseases by transferring genes from the virus to the pig. This method has been used successfully in chickens and may also be applicable to other livestock species (36).

Attempts are being made to produce rare. medically important proteins in pigs. A U.S. firm (DNX) has announced that it has **successfully produced human hemoglobin in pigs. Transgenic swine research is** being conducted by the Agricultural Research Service, a few universities, and the private sector. The American Red Cross is also interested in the production of blood proteins in livestock. Commercial availability of transgenic pigs is not expected before the year 2000, and it is likely that the first transgenic pigs marketed will be used to produce pharmaceutical products. Additionally, pigs have a strikingly human-like physiology, and because of this, transgenic pigs are currently being developed to serve as a model system to understand and treat gastrointestinal cancers.

Transgenic Ruminants

The first transgenic ruminant to be successfully produced was a lamb, followed by goats and cattle. In cre-

[16] The major histocompatibility complex is a chromosomal region that contains several genes involved in regulating immune response.

ating transgenic ruminants. greatest research emphasis has been to improve growth characteristics (i. e., rate of weight gain, feed efficiency. and carcass composition). to produce valuable pharmaceutical products, and to enhance disease resistance.

Genes coding for somatotropin and somatotropin releasing factor (GRF) have been purified and transferred to sheep. While the genes have been successfully transferred and expressed. control of the level and timing of expression has not been achieved. Sormtotropin levels in sheep have varied from a low of 40 nanograms (ng)/ milliliter (ml) to over I ().000 rig/ml (31, 37). Extreme overexpression of somatotropin can lead to serious health problems in sheep. such as diabetes (39). In the future, researchers would like to alter the composition of milk and meat for improved processing characteristics, for higher nutrition. for less fat. and to alter the types of fat contained.

Another major research area involves transferring genes that code for the production of valuable pharmaceuticals. Production of blood clotting factors (factors VIII and 1X), tissue plasminogen activator (TPA. used to dissolve blood clots that cause heart attacks), erythropoietin (used to treat bone marrow side effects resulting from AIDS treatment). and o-l-antitrypsin (AAT. used to treat emphysema are being investagated. A U.S. firm (Genzyme). in conjunction with Tufts University. has successfully produced TPA in goats (13.14). A Scottish firm (Pharmaceutical Prteins. Inc) has produced AAT in sheep. and is conducting research to produce Factors VII and IX and crythropoietin (30. 52). Transgenic cows producing high levels of pharmaceuticals in their milk have not yet been reported, but these animals are under development in a number of public and private laboratories. For example. a joint U.S. and Dutch group (GenPharm International. Gene Pharming Europe BV, and two Dutch Universities) has successfully produced tramsgenic cattle incorporating the human lactoferrin (which has antibiotic propertics) gene in the genome (25).

Attempts tire being made to identify promotors that espress gene products only in milk. Research is being conducted on whey acid protein. a protein only found in milk. to identify the promotor that directs the synthesis of' this protein. The goat (3-cascin promotor is also being used (14). Once appropriate promotors are found. the high levels of U.S. milk production coupled with the ease of milk collection may make this production method more cost effectove than the cell culture systems currently used in the production of certain pharmaceutical proteins.

Enhanced disease resistance is another focus of research. Diseases that may be potentially controlled by the production of transgenic organisms include progressive pneumonia in sheep. and caprine arthritis-encephalitis in goats. The introduction of preformed antibodies have been shown to provide resistance to specific infections in mice and the antibody gene antiphosphoryrlcholine has been inserted in sheep (28). Researchers are also attempting to insert viral envelope genes that could possibly lead to enhanced resistance to viral infections.

Researchers in Australia are attempting to increase wool production in sheep. Currently, wool production is limited by the amount of cysteine contained in and absorbed from the diet. Researchers are transferring bacterial genes that code for enzymes that produce cysteinc from sulfur in the diet (37).

Research to produce transgenic ruminants is limited due to the high cost of' the research. Research is conducted primarily in the United States by the Agricultural Research Service. a handful of universities. and a few private sector firms. and in Austrulia, Great Britain, and the Netherlands. It is not expected that transgenic ruminants will be commercially available before the turn of the century.

Transgenic Fish

Several species of transgenic fish have been produced. including rainbow trout, salmon, common carp. loach. catfish, tilapia, goldfish, zebrafish. and medaka. Several genes have been transferred to fish. including human. bovine, and trout somatotropin; genes that confer antibiotic resistance; and fish antifreeze protein genes (34).

Transgenic fish containing the trout somatotropin gene grew 22 percent more than controls. and transmitted this increased growth rate to their offspring (34). Some species of fish produce a novel set of proteins that allow them to withstand extremely cold water without freezing. These antifreeze proteins are produced year round by fish living in polar regions, and during the winter in fish living in temperate regions. The antifreeze genes in several species have been purified. Antifreeze protein genes from winter flounder have been transferred to salmon. Expres- s ion levels of the gene were low. however. and protection against freezing was not achicved (34).

Research Needs

While significant advances in transgenic animal production have been made, it is unlikely that transgenic animals will be commercially available before the end

Photo credit: Thomas Chen, University of Maryland

Resultant transgenic carp with trout somatotropin incorporated into some but not all of their cells. The PI (middle) and F1 (top) transgenic carp are on average, 22 percent larger than their nontransgenic siblings (bottom).

of the 1990s at the earliest. The ability to produce transgenic livestock possessing traits of economic value is currently limited by the absence of embryo stem cell technology, the lack of appropriate gene expression promotors, and the lack of knowledge about the physiological consequences of specific gene expressions. While the techniques for isolating and sequencing animal genes are relatively well developed, understanding of the functions of the genes has lagged. Analysis of gene function is complicated by the fact that many traits are controlled by multiple genes. Thus, manipulation of such traits will require detailed understanding of these genes and of their interactions. Ultimately, identification and understanding the physiology of the major genes controlling growth and lactation, reproduction, and disease and stress resistance in animals is needed. An active genome mapping program could enhance these developments.

ANIMAL HEALTH TECHNOLOGIES

Improvements in animal health will provide considerable cost savings to the livestock industry. Biotechnology is rapidly acquiring a prominent place in veterinary medical research. New vaccines and diagnostic kits are being developed to detect and prevent a variety of major livestock diseases.

Vaccines

Vaccines are agents that stimulate an effective immune response without causing disease. Traditional methods of vaccine development have involved killing or modifying pathogenic organisms to reduce the potential for disease while preserving that pathogens' ability to induce an immune response. Biotechnology is being used to create new vaccines. Approaches used include deleting or inactivating the genes in a pathogen that cause disease, and inserting into a vector genes that cause an immune response to a pathogen. Synthetic peptides are also being produced that stimulate the immune response.

Gene Deletion Vaccines

Gene deletion techniques have been used to develop both viral and bacterial vaccines. The first gene deletion viral vaccine to be approved and released for commercial use was the pseudorabies virus vaccine for swine. initially, the removal of a single gene reduced the virulence of the virus. Since then, other genes have been deleted with a continuing reduction of virulence. Chickens that have been inoculated with recombinant avian leukosis virus (ALV) developed antibodies to the virus without developing the disease. Methods to decrease the virulity of live viruses lead to more effective vaccines because live virus vaccines stimulate the immune response more effectively than do killed virus vaccines (32).

Bacterial vaccines have also been produced. Esche*richia coli* that lack certain genes. for example, have been shown to provide protection against gram-negative bacterial infections in cattle and swine. Live Salmonella modified to prevent reproduction in vivo have also proven to be an effective vaccine for cattle (32).

Most gene deletion viral vaccines will not be available before 1995 with the exception of the pseudorabies vaccine, which is already available, and possibly the rabies and rinderpest vaccines, which are currently undergoing field trials.

Photo credit U.S. Department of Agriculture, Agricultral Research Service

Molecular biologists analyze DNA sequence reactions of a gene detection vaccine made from a modified bacterium.

Vectored Vaccines

New vaccines are also being created using vectors. Development involves deleting disease-causing genes from the vector if it is a pathogenic organism, or using a nonpathogenic vector. Genes that code for protective antigens produced by pathogens can be inserted into a vector. Inoculation of the animal with the recombinant vector stimulates an immune response to the inserted genes and confers protection against the pathogen. Pathogen surface protein genes are most commonly inserted into the vector. Inoculation of the animal stimulates production of antibodies to these surface proteins. When an animal is infected with the pathogen, it already recognizes that pathogen and produces antibodies against it. As an example, recombinant vaccines have been developed against the coat protein of a bacterial pathogen of the genus *Vibrio,* in fish.

The most commonly used vector is the *Vaccinia* virus. *Vaccinia* viruses are used because they are easy and relatively cheap to manufacture, large enough to accommodate the insertion of many new genes (1). and stable without refrigeration. A single inoculation can induce immunity, and the recipient produces the bulk of the vaccine, eliminating the need for large vaccine factories. *Vaccinia* viruses also stimulate more than one type of immune response (i. e., they stimulate both B and T lymphocytes). However, there are disadvantages to using vaccinia virises: they have a wide host range (including humans), and could infect species other than target species; it is possible that they can revert to a virulent form; they cannot be administered orally; and they may pose a risk to immunosuppressed recipients. *Vaccinia* hosts have been used to produce vaccines against rinderpest (cattle), rift valley fever (sheep), Venezuelan equine encephalitis, bovine leukemia, rabies (cattle). vesicular stomatitis (cattle), avian influenza, avian infectious bronchitis, and respiratory syncytial disease (1, 32).

Fowlpox virus is also being used as a vaccine vector. This virus cannot replicate in humans and is being used as a carrier for genes of pathogens that cause the poultry diseases of Newcastle disease, Marek's disease, bursal disease, coccidiosis, avian influenza, and avian infectious bronchitis. Raccoon poxvirus is being developed as a carrier for rabies. In fish, vaccines to control infectious haematopoietic necrosis virus (IHNV), a devastating viral disease of trout and salmon, are being developed by inserting coat protein genes into vectors. Other genetically engineered virus vectors that are in the early stages of development include avirulent adenoviruses, herpesviruses, murine and avian retroviruses, and bovine papillomavirus (1, 32).

Bacterial vectors are also being developed. *Escherichia coli* and *Bacillus subtilis* are being used to produce antigenic proteins. They can be used to produce antibodies to *Theileria annulata* (a tick-borne parasite of cattle and sheep), coccidia in poultry, anaplasma (a parasite of cattle), and cysticercosis (a tapeworm in ruminants and swine). Pili genes from Bacteroides nodosus, the cause of foot rot in sheep, have been cloned into *Pseudomonas aeruginosa,* and have been shown to be an effective vaccine for foot rot (1, 32).

Natural and Synthetic Peptides

A number of animal species are known to produce small peptides associated with white blood cells and that are effective in destroying bacteria, fungi, and enveloped viruses. Such peptides, referred to as antimicrobial peptides, include defensins in mammals, bovine nubopeptides in cattle, magainins from frogs, and cecropins from moths. Some of the smaller peptides have been synthe-

sized and appear to have biologic activity similar to that of the natural peptides, and could be used in a manner similar to antibiotics. The genetically engineered protein lysostaphin, which kills *Staphylococcus aureus,* has reportedly achieved cure rates as high as 80 percent for mastitis in some clinical trials (1). Commercial development will take 5 to 10 years.

Synthetic peptides can be constructed to stimulate an immune response in animals. Small fragments of proteins that are homologous to proteins coded for by the foot and mouth disease virus have been used to stimulate an immune response to that disease in cattle and pigs. Synthetic peptides have been **used to inhibit critical functions of lentiviruses in** sheep. Administration of a viral surface protein elicited production of an antibody and provided protection in fish. Commercial availability is not likely until the end of the decade.

Monoclinal Antibodies To Confer Passive Immunity

Monoclinal antibodies can be used to provide passive immunity to disease-causing microorganisms. They generally act not by stimulating the immune response of the animal itself, but rather by providing exogenous antibodies to the pathogen. Because monoclinal antibodies are specific to one antigen, they may provide only weak immunity to pathogens that have more than one immunogenic region of their surfaces.

Certain strains of the bacteria *Escherichia coli* cause diarrhea in newborn calves. For diarrhea to occur, the bacteria must attach to the walls of the intestines. Attachments occur via cilia-like projections, called pili, that cover the surface of the bacteria. Monoclinal antibodies specific to the attachment proteins on the pili prevent attachment of the bacteria to the intestinal wall and prevent calves from getting diarrhea. A product currently on the market for diarrhea prevention in calves is Genecol-99 (50). Monoclinal antibodies specific for bluetongue also have been shown to protect sheep from this virus in trials.

In addition to monoclonal antibodies, antisense agents can also provide passive immunity. Antisense agents can be synthesized and used as drugs, or used to block viral genes. They are very sensitive, but are susceptible to enzymatic degradation A delivery is a problem (I).

Immunomodulators

Immunomodulators are hormone-like molecules that play a role in coordinating immune defenses to infectious agents, cancer, and autoimmune diseases. They act to boost or accentuate the immune response. Some of these molecules, the lymphokines, for example, are produced by white blood cells. Other immunomodulators, the cytokines, for example, are produced by other body cells. Two classes of lymphokines, the interleukins and the interferon, have been the focus of research attention.

Interleukins are compounds that transmit signals between white blood cells. These signals help to stimulate the proliferation of disease-fighting white blood cells and the production of antibodies. Interferon induce the expression of class 11 histocompatibility antigens (define) and enhance their activity.

Several interleukins and interferon have been identified in mammals, and the genes encoding some of these compounds have been isolated and cloned into bacteria (e.g., bovine alpha, beta and gamma interferon, bovine interleukin-2) (32). Lymphokines are being tested as adjuvants to boost immune responses to poorly immunogenic vaccines. For example, interleukin genes and genes for compounds that cause immune responses in animals (antigens) are being inserted together into viral or bacterial vaccines. This combination may enhance the immune response of the animal and lead to increased protection against the antigen.

Recombinant interleukins produced in bacteria or other expression vectors may also be used therapeutically to assist in overcoming certain infections. For example, recombinantly produced interleukin-2 is being tested as a control for shipping fever and mastitis in cows. Mechanisms by which these regulatory proteins modulate immune response are now being investigated in domestic animals. Biotechnology is being used to identify and repilicate these compounds so that their function can be investigated.

Diagnostics

Safe, accurate, rapid, inexpensive, and easy-to-use diagnostic procedures are critical to the livestock industry at virtually all points in the production process. Examples of diagnostic tests include pregnancy tests and assays for pathogenic organisms. Many currently used diagnostic tests are costly, time consuming. and labor intensive, and some still require the use of aninml assay systems. Monoclinal antibodies and nucleic acid hybridization probes can be used to produce simpler, easily automated. and highly sensitive and specific diagnostic procedures.

Antibodies are proteins produced by the body in response to foreign chemical substances. Monoclonal antibodies are produced by a cell line expressing only a single antibody type. They are the primary tools for bio-

technology-based diagnostics. At least 15 different rapid diagnostic tests based on monoclinal antibodies are on the market or will be soon (table 3-4). These tests are highly specific and most lend themselves to automation, potentially allowing their application in mass screening systems for disease surveillance and control. Some of the tests have been adapted to field use and can be used by veterinarians or producers. The rapid commercialization of these products is having a significant impact on animal health management and disease control.

Monoclinal antibodies are also being used in enzyme-linked-immunoabsorbent-assay (ELISA) systems to provide sensitive, quantitative blood assays of toxins, hormones, chemicals (e. g., pesticide and antibiotic residues), and a variety of antigens including microbial agents. Many of these tests are commercially available. In some instances monoclinal antibody diagnostics have been used to replace bioassays such as mouse inoculation tests.

The high specificity of monoclinal antibodies has generally been felt to make them less useful than polyclonal antibodies in initial screenings for diseases that have many serotypes. However, an ELISA kit containing just two monoclinal antibodies was able to detect 800 different *Salmonella* strains, so it may be possible that diagnostic kits containing just a few monoclinal antibodies could be useful for initial screening of pathogens (l).

Nucleic acid hybridization can also be used to diagnose the presence of microbes and parasites (table 3-5). Such assays rely on the bonding of a specific DNA or RNA segments (the probe) to complementary RNA or DNA fragments in a test sample. The probe is attached to (labeled by) a radioactive compound or to a color compound to allow for detection. DNA probes are most commen. The development of RNA probes is very recent, and they are used to detect RNA viruses.

The major limitation of nucleic acid hybridization is inadequate signal strength. The amount of target nucleic acid present in some samples may be too small to emit a signal the probe can detect. The polymerase chain reaction technique (PCR) (see ch. 2) can be used to amplify the amount of target DNA present and improve the ability of the probe to detect its presence. Similarly, bacteriophage replicase systems can be used to amplify the RNA present in a sample.

Currently, the most reliable probes are those that are radioactively labeled. Use of these probes requires expensive equipment and trained technicians. thus precluding their use in the field. Alternative calorimetric techniques currently in development will replace the radioactively labeled probes and make the use of this technology more commercially attractive (32).

The advantage that nucleic acid probes have over traditional diagnostic techniques is speed. Conventional tests for anaplasmosis and Johne's disease (an intestinal disease in ruminants), for example, require about 6 and 14

Table 3-4—Diagnostic Monoclinal Antibody Kits

Avian leukosis
Avian reovirus
Bluetongue
Bovine virus diarrhea
Canine parvovirus
Coccidiosis
Episotic hemorrhagic disease
Equine infectious anemia
Feline infectious peritonitis
Feline leukemia[a]
Feline T-lymphotropic lentivirus
Feline T-lymphotropic lentivirus (Feline leukemia
Mastitis
Pseudorabies[a]
Rotavirus gastroenteritis
Trichinosis

[a]More than one company has a kit on the market

SOURCE: Office of Technology Assessment, 1992.

Table 3-5—Pathogens for Which Diagnostic Kits Using Nucleic Acid Probes Are Available

Viruses
Bluetongue
Bovine coronavirus
Bovine leukosls
Bovine virus diarrhea
Equine encephalosis
Foot and mouth disease
Infectious bovine rhinotracheitis
Porcine coronavirus
Porcine parvovirus
Rabies
Rotavirus
Bacteria
Anaplasma marginale
Campy lobacter
Enterotoxigenic Escherichia coli
Leptospira
Mycobacterium
Mycoplasma
Salmonella
Shigella
Parasites
Babesia bovis
Eimeria tenella
Eperythrozoon suis
Hammondia hammondi
Theileria parva
Toxoplasma gondii
Tritrichomonas foetus
Trypanosoma brucei brucei
Trypanosoma congolense

SOURCE: Office of Technology Assessment, 1992.

weeks, respectively, to confirm the presence of the pathogen. This much time allows for interim spread of disease. With DNA probes the presence of these pathogens can be confirmed within a few hours.

Restriction Fragment Length Polymorphism maps (RFLPs) can also be used for diagnostic purposes. This procedure has been used to distinguish different strains of African swine fever virus and has shown that equine herpesvirus- 1 can infect and cause abortion in cows under natural conditions.

Research to develop diagnostic kits using biotechnology is being conducted in both the private and public sector. Currently, several diagnostics kits are commercially available. Development time to bring new diagnostic kits to market ranges from 2 to 5 years. Generally, less time is required to develop monoclinal antibody kits than nucleic acid probes.

FOOD PROCESSING APPLICATIONS

The processing of animal products into foods also will be affected by biotechnology developments. Americans consume many meat and dairy products that are fermented; genetically engineered fermentation starter cultures are being developed for these products.

Starter cultures are living microorganisms used to produce fermented products such as cheese, yogurt, butter, buttermilk, sour cream, salami, and sausages. Culture organisms have been safely consumed by humans for centuries and serve as ideal hosts for the production of these natural foods. The metabolic properties of these organisms directly affect the properties of the food product, including flavor and nutritional content. In order to improve various properties of food products, food microbiologists attempt to manipulate the traits of the microorganisms, primarily through mutation and selection. The cloning and gene transfer systems developed in the 1980s are being used to construct strains with improved metabolic properties more rapidly and precisely than is possible with traditional methods. The development in this decade of new strains with precise biochemical traits will have an impact on several aspects of fermentation, including production economics, shelf-life, safety, nutritional content, consumer acceptance, and waste management (19).

Although much of the current work to develop new strains of microorganisms has focused on the use of E. *coli* and other nonfood microorganisms, there are distinct advantages to engineering starter cultures for producing high-value foods. For example, construction of cultures resistant to attack by viral infection will impact processing costs by eliminating waste. Cloning of the genes responsible for ripening of aged cheeses can decrease storage costs by accelerating ripening. Production of natural preservatives, such as nisin (effective in inhibiting foodborne pathogens and spoilage organisms), will help ensure the safety and extend the shelf life of fermented meat and dairy products. Starter strains engineered to mimic the function of nitrates could reduce the use of these compounds in cured meats.

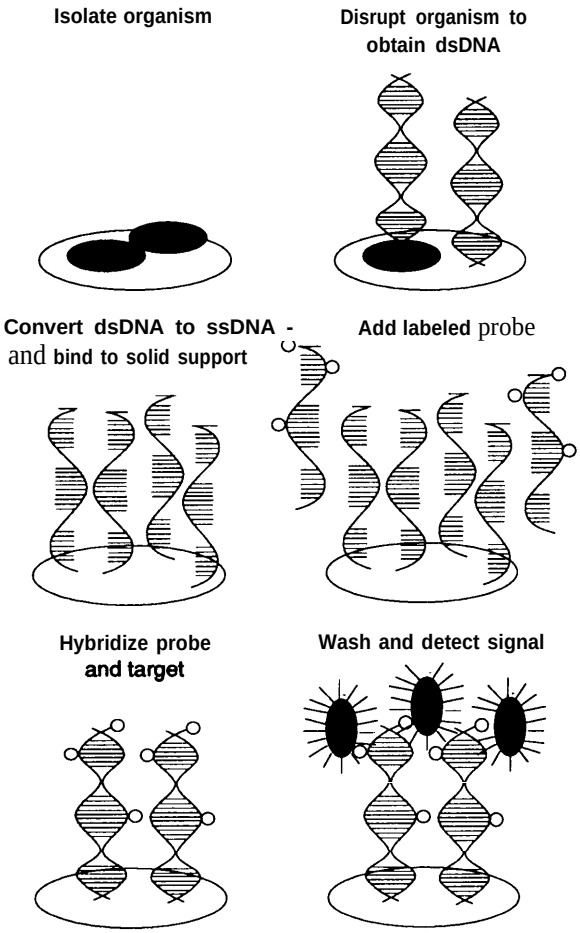
Cloning of the gene(s) responsible for enzymatic reduction of cholesterol or modification of the degree of saturation of meat and milk fat will improve the nutritional quality of fermented products. The ability to engineer strains capable of producing enhanced flavors or natural stabilizers will influence consumer acceptance of fermented dairy foods. Enzymes, which are added to the curd to accelerate ripening, or to produce dairy products acceptable for digestion by lactose-intolerant individuals, will also be produced more economically by engineered microorganisms (19).

A genetically engineered version of the enzyme preparation rennet, which is normally extracted from the forestomach of calves, has recently been approved by FDA for use in cheese manufacturing (See ch. 10).

Processing of animal products generates many wastes such as blood, bone, collagen, shells, fish parts, and milk whey. Bacteria and yeast strains engineered to convert these waste products into useful products could decrease the cost and problems associated with their disposal. For example, engineered yeast strains are capable of fermenting the lactose in whey to value-added products, such as vitamin C, biofuels such as ethanol and methanol, or pharmaceuticals. Whey protein could potentially be used to produce specialty chemicals with biotechnology.

Biotechnology products can be used to monitor animal products for food safety. DNA probes and monoclinal antibodies can be used to analyze raw materials, ingredients, and finished products for pathogenic organisms, bacterial or fungal toxins, chemical contaminants (i.e., pesticides, heavy metals), and biological contaminants (i.e., hormones, enzymes) (figure 3-6). Detection kits are commercially available. For example, kits are available to monitor several pesticides and antibiotics. Kits are also available to detect Salmonella. Animal cell cultures may partially replace whole animal systems to test for acute toxicity. Biosensors may be used to monitor food processing, packaging, transportation, and storage (19).

Figure 3-6—Basic Steps in a DNA-Probe Hybridization Assay



Organisms present in a food product are trapped on filters and disrupted to obtain double-stranded DNA. Following denaturation of the DNA to single strands, the labeled probe is allowed to hybridize with target DNA. Hybridization can be detected by a number of methods.

SOURCE: *Journal of Food Protection 54(4):387–401, 1991*

SUMMARY

Biotechnology will offer many new opportunities to alter the manner in which livestock is produced in the United States. New products are being developed to enhance feed efficiency, improve livestock reproductive performance, and enhance herd health management. Producers, food processors, and consumers all potentially may benefit from these new products.

Several new products are under development to enhance the feed efficiency and growth of meat-producing animals, and to increase milk yields in lactating animals. Increased feed efficiency could significantly decrease the cost of producing livestock. New growth promotants result in meat that is far leaner than that which is produced naturally, a benefit to consumers who desire less fat in their diets. Three new products (bST, pST, and beta-agonists) currently are undergoing FDA review for use in livestock production. Additionally, traditional growth promotants, such as steroids and antimicrobial agents, continue to be improved.

New reproductive technologies offer producers the opportunity to rapidly upgrade herd quality by selecting and incorporating desired traits at a faster rate than could be accomplished with traditional breeding. It is now possible to induce superior females to shed large numbers of eggs, and then to fertilize those eggs in vitro with the sperm of superior males. The embryos may be implanted into surrogate mothers whose estrus cycle has been synchronized to accept the embryo. Cloned embryos are currently marketed, and more efficient methods of embryo production are being developed. Advances in embryo and sperm sexing will allow livestock producers to choose the sex of the progeny and to breed for animals of highest value (e. g., females in dairy, males in beef production).

Eventually, transgenic livestock will be commercially available. Efforts are under way to produce transgenic livestock with improved production characteristics such as enhanced disease resistance, leaner carcasses. and faster growth. However, the first transgenic livestock will most likely be animals that produce high-value pharmaceuticals in their milk. Several firms have successfully produced such transgenic animals; however, commercialization is not likely to occur before the end of this decade.

New vaccines, therapeutics, and diagnostic kits will improve the ability of livestock producers to manage herd health. Several vaccines and diagnostic kits are commercially available, and more are under development.

The food processing industry will also be affected. New enzymes and starter cultures for cheese and dairy manufacturing, and meat processing are being produced with biotechnology. One genetically modified enzyme preparation, chymosin. has been approved as generally regarded as safe (GRAS) by FDA for use in cheese making. Biotechnology can be used to improve the safety of food products through the development of nucleic acid probes and monoclinal antibodies to detect the presence of microorganisms, chemicals, heavy metals, and other contaminants in food products. Additionally, new methods to manage processing waste products. such as whey, are under development.

Despite the potential opportunities offered by biotechnology, these technologies are not without controversy. Concerns have been raised about the effects of these technologies on farm survival and structure, food safety, animal welfare, and the environment. Additionally, many of these technologies will place a premium on farm management skills, and thus may not be appropriate for all farmers. These issues are discussed in more detail in the following chapters.

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