Expanding Research Horizons

T. Ross Wilkinson

The enhancement of agricultural research through biotechnology will undoubtedly benefit not only the producer but also the agricultural industry and society in general. The term biotechnology conjures up a spectrum of meanings or definitions from people and will provoke a response as to its merits or short-comings independent of scientific knowledge on the subject. One individual wrote that, "The word biotechnology probably has no equal in meaning so much or so little to so many." No matter how people define biotechnology, the horizons have changed as a result of this technology. Scientists are able to carry out experiments in the laboratory and field which they previously considered not possible. However, many of my colleagues who have been associated with biological research longer than I have will argue vehemently that biotechnology is a new name for a well known discipline, i.e. genetics.

In my thinking, the basis for the excitement in biotechnology is that our horizons or potential through research has been expanded. So often our potential is held in check because of conservative thinking, scientific beliefs, status of technological developments, etc. As a basic scientist with expertise in microbiology, I was indoctrinated with scientific beliefs that genetic material can not be transferred from one species to another and especially not between plants and animals. This breakthrough was so shocking to the scientific community and society in general, that a research moratorium on recombinant DNA (genetic engineering) was put into place in 1974 until acceptable guidelines could be established to address risk and the need for containment. Two years later, strict guidelines were put into place. Subsequently, risk assessment has been an on-going topic of discussion and research guidelines have been appropriately modified. At NDSU, the Institutional Biosafety Committee, chaired by Dr. David Berryhill, oversees recombinant DNA research on campus (see Berryhill's article in this issue.).

The forecast for major accomplishments through biotechnology-related research is still valid, but the time frame needs adjustment. However, there have been immediate benefits to the scientific community as biotechnology developed and expanded into many disciplines. In my opinion, some of the major benefits in agricultural research include; 1) elimination of artificial barriers in genetic-related research which restricted the utilization and transfer of desireable traits in plant and animal species; 2) acceleration in the development and application of technology at the cellular and molecular levels; 3) integration of basic and applied disciplines to foster team efforts in solving problems in agriculture, whether it be through the establishment of biotechnology centers or through the hiring of molecular scientists in departments of animal science, agronomy, plant pathology, horiculture, etc.; and 4) involvement of the public to enhance their awareness, understanding and support of efforts in research and development. After all, the primary goal of research sponsored through the Agricultural Experiment Station utilizing state and federal funds is to bring about benefits to the producer and consumer through effective and efficient utilization of resources.

The impact of biotechnology has certainly been felt on the NDSU campus and especially in desciplines associated with the Agricultural Experiment Station. Over the last four to six years, a special effort has been made by the Station to hire scientists who have expertise not only in the traditional agricultural disciplines but also extensive knowledge in the basic science disciplines, such as chemistry, mathematics, botany, statistics, molecular biology, genetics, etc. The integration of these scientists into the various agricultural departments has expanded our research potential, maximized utilization of human and facility resources, and broadened funding resources through non-traditional granting agencies such as NSF, NIH, DOE, etc. However, such integration does not come about without strains on existing budgets, traditional expectations of research program outputs and academic programs.

A further integration between agricultural related disciplines has been accomplished through the establishment of the Agricultural Biotechnology Center (ABC) in July 1987. The ABC is a multidisciplinary research unit administered within the North Dakota Agricultural Experiment Station (AES). This unit is composed of scientists and support personnel assigned to existing departments within the AES.

The Agricultural Biotechnology Center facilitates the interdisciplinary research approach in addressing agricultural problems utilizing biotechnology. The integration of discipline expertise at various levels of research and development provides scientists with opportunities for achievements not realized in traditional programs. The Center's activities complement the research programs in the discipline-oriented departments within the Agricultural Experiment Station. The benefactors of the ABC research and development programs are the North Dakota producer, consumer and the agricultural industry.

The overall goal of the Agricultural Biotechnology Center is to promote plant, animal and microbial biotechnology research. The Center coordinates the integration of human resources along with providing access to critical equipment and facilities. Research and development projects are selec-

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ted for support by the Center to enhance their productivity and benefit to the agricultural industry.

The Center strives to become self-supporting through contributions and grants from public and private agencies. Linkages will be established with industry to enhance research funding and expertise. The Center promotes effective communication between scientists, encourages interdisciplinary cooperation, provides an environment for creativity and strengthens grant competitiveness. The Center enhances the public awareness and understanding of biotechnology research. The scientific community has an obligation to keep the public informed on advances in biotechnology and its impact on social, economic, environmental and related issues.

Scientists involved in biotechnology-related research in agriculture represent a variety of disciplines. The inter-relationship of these disciplines is illustrated in Figure 1. One of the goals of the ABC is to not only encourage maximum utilization of human expertise but to provide a forum whereby animal and plant scientists along with the basic scientists will exchange ideas and share expertise. Such interaction has been shown to be very beneficial.

The focus of animal and plant biotechnology research in the North Dakota Agricultural Experiment Station is consistent with those goals established on a national basis at the 1987 USDA Biotechnology Challenge Forum. Their high priority goals included conservation of natural resources, reducing input cost in farm production, improving production efficiency and crop protection, enhancing market value, ensuring quality of farm products, reducing barriers to effective marketing, and promoting good nutrition.

Research projects in agriculture involving biotechnology are located in various disciplines aligned with the Agricultural Experiment Station and as such represent a community of scientists who collectively make up the research staff of the Agricultural Biotechnology Center. The following comments on the focus of research efforts in biotechnology are from scientists representing various disciplines:

Crop and Weed Sciences

Comments provided by **Dr. A. Earl Foster**, Professor and Chairman

Plant breeding as a means to improve cultivated plants has been ongoing in this department for many years. The first crops to receive plant breeding efforts in North Dakota were hard red spring wheat, durum wheat, barley, and corn. The advances made in yielding ability, resistance to lodging, resistance to diseases, earlier maturity, and improved quality have been very dramatic. Statewide average

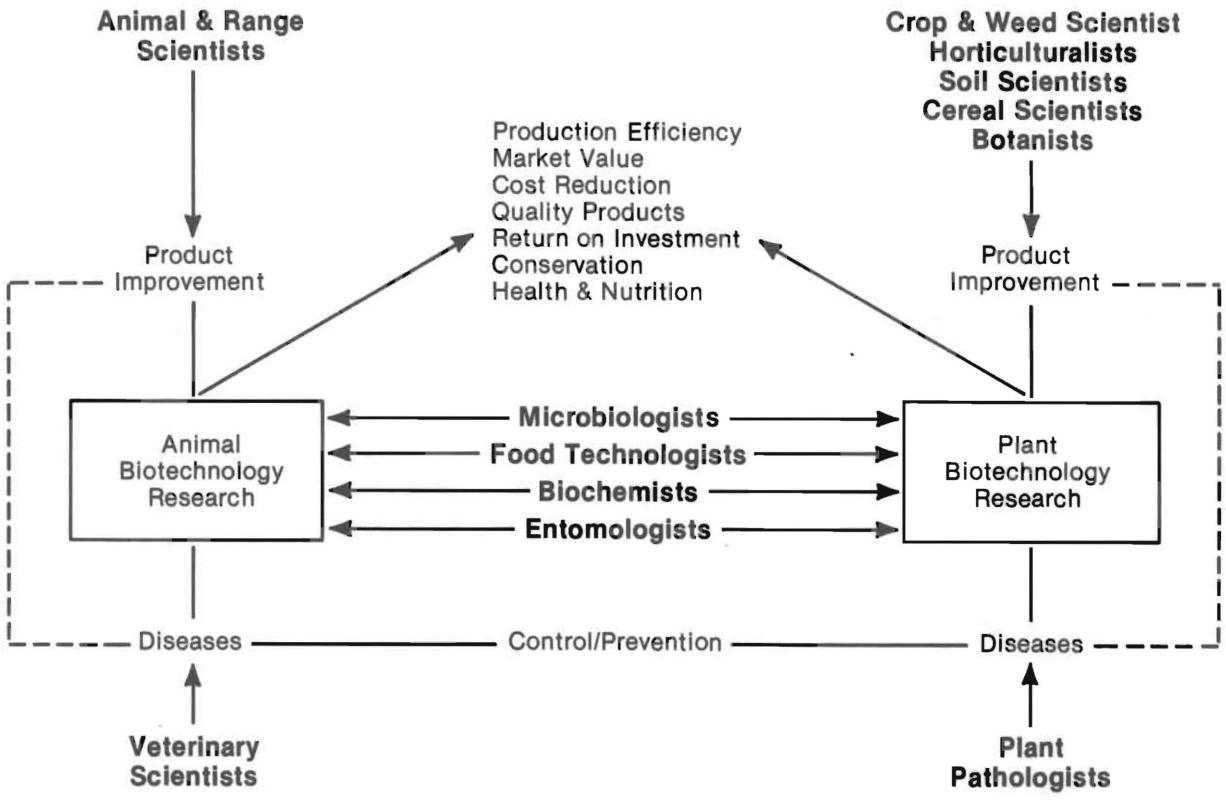

Figure 1. Relationship of disciplines involved in agricultural biotechnology research.

yields of most crops have more than doubled in the past 30 years. A large portion of this yield increase can be attributed directly to plant breeding efforts, although contributions also come from increased use of fertilizer and from ther improved cultural practices.

Recently, the concept of biotechnology was introduced. Many claims were made about how this new science would affect our lives, specifically in the areas of new types of plants and animals. As might be expected, the development of new plant and animal forms turned out to be much more complex than some people originally thought. The identification and transfer of specific genetic information is very difficult and time consuming. It is also very expensive, and many research organizations do not have the resources to subsidize massive efforts.

It now seems that plant breeders can utilize biotechnological techniques as another tool in development of new, improved varieties of crop plants. Plant breeders and biotechnologists will need to cooperate to identify those characteristics that would be desirable in new varieties. These characteristics might include: resistance to a specific herbicide, resistance to a specific disease (it could be resistance to a single race of one disease, such as for stem or leaf rust of wheat), resistance to a specific insect, salt tolerance, heat or cold tolerance, winterhardiness, and ability to germinate at low temperatures in the spring. In most cases, it will be necessary to identify sources of these genes, and these sources likely will be in some other species. Next, the biotechnologist must be able to isolate the specific piece of the chromosome that carries the gene for the desired character. This gene will need to be transferred to a single cell of the desired variety, which will be regenerated to form a new plant. The new plant must be evaluated to determine what effect the transferred gene will have. The transferred gene then can be manipulated by the plant breeder and transferred to other varieties. Obviously, the transfer of genes from one species to another using biotechnological techniques is difficult and will be time consuming. Many of the techniques of gene transfer and plant regeneration are yet to be worked out.

Some of the present biotechnology related activities of faculty in the Crop and Weed Sciences Department are as follows:

1. Callus tissue of dry edible beans will be cultured, and regenerated plants will be evaluated for resistance to *Sclerotinia sclerotiorum*. This is one of the major diseases of dry edible beans.

2. Identification of winterhardy winter wheats during early stages of testing is difficult. Several isozymes will be evaluated for their association with cold tolerance. If such an association is found, winter hardiness could be predicted on the basis of a chemical laboratory procedure.

3. *In vitro* culture techniques will be used with developing corn kernels to evaluate the effect of changing culture conditions on rate and duration of grain fill. Results from these studies will complement and confirm ongoing field experiments aimed at examining the grain fill characteristics of corn.

4. Regenerated plants from two spring wheat varieties are being evaluated for improved resistance to drought stress. This research by Dr. Deckard is described in detail elsewhere in this publication. Although this technique is being utilized on wheat, it should be possible to use similar procedures with other crops.

5. Dry edible bean plants regenerated from callus tissue have been different from the original variety. Changes in plant height, maturity date, and disease resistance have been observed and are being evaluated further. This research is part of that being conducted by Dr. McClean, and it is described in more detail in another article.

6. Tissue culture techniques were used to regenerate durum wheat plants from embryo derived callus. Some varieties produce more regenerated plants than others. However, no changes were observed in plants derived from the regeneration process. Additional study is needed to increase regeneration efficiency.

7. As indicated earlier, regeneration of plants through cell culture techniques is an integral part of producing plants with changed characteristics. Various techniques are being evaluated to develop efficient processes for regeneration of cereal crop, flax, and rape plants from single cells.

8. The type of genetic changes and their frequency of occurrence during cell culture are affected by culture management, duration of culture, media components, crop, and variety. These "management" techniques are being studied in conjunction with experiments conducted in number 7.

9. Beneficial genetic variants from cell culture of various crop species will be selected and evaluated for herbicide tolerance, drought tolerance, low and high temperature tolerance, nitrogen source, light intensity, nutritional quality, and productivity.

Horticulture and Forestry

Comments provided by **Dr. Arthur A. Boe**,
Professor and Chairman

A big part of what is referred to as biotechnology today was developed in horticulture and plant physiology labs over the past fifty years. This effort was generally referred to as tissue culture. The challenge of growing plant cells and tissues while separated from the plant was a difficult one indeed. Real progress was to come only after a growing medium was found that would support the growth of the cells. Once the necessary ingredients for the medium were found, the application of tissue culture to the commercial production of plant was possible.

Today plant tissue culture is used in a variety of technologies in agriculture production and research.

One of the first applicaitons of a tissue culture-like system was in the production of orchid seedlings. In this case the orchid seed, which has little or no stored food reserve, is sown under sterile conditions on a solidified medium containing all of the requirements for the seedling's growth and development. Once the seedlings are large enough, they can be transplanted to a suitable soil medium under greenhouse conditions.

A similar system is also used by plant breeders and geneticists who are trying to cross plant species or strains that are incompatible and that usually will not produce viable seeds from the cross. In this case the embryo is removed from the developing seed a few days after fertilization and

allowed to complete its growth on a sterile medium in a test tube. This is called embryo rescue and has been used successfully to transfer desired characteristics into agricultural crops.

By far the most extensive use of tissue culture has been in plant propagation. This is referred to as tissue culture propagation or plant micropropagation. The techniques have been perfected for the propagation of hundreds of species and strains of plants using micropropagation. Micropropagation is used extensively for the propagation of horticultural plants, and to date billions of plants of several hundred species have been propagated by this means.

There are some very specific reasons for selecting micropropagation as the means for muliplying a specific plant. Probably the first reason is the rapidity at which plants can be multiplied using this technique, and because it is a form of asexual reproduction, each plant will be identical to the next.

In the process of micropropagation, a small piece of the parent plant, usually a growing point, is sterilized and placed on a sterile medium that contains all of the nutrients and growth factors required for growth and development. Once on the medium some of the cells in the tissue begin to grow and divide. These dividing cells will differentiate into new growing tips. Most culture will in a matter of three or four weeks produce 10 or more new plants. Some species, such as African violet, may produce dozens of plants from a single culture. Often the cultures are cut up and recultured several times in the propagation process. This makes it possible for a single stem tip to produce hundreds or even thousands of daughter plants, each one exactly the same as the mother plant.

Usually the new plants as they are removed from the cultures do not have roots. A separate process, either under tissue culture conditions or in the greenhouse, is used to root the plants.

When virus-infected plants are propagated asexually, the new plants will also be infected with virus. If, however, the plant is grown at elevated temperatures and only a millimeter or two of the growing tip is used to micropropogate the new plants, they will often be free of viruses. This technique is used extensively in agriculture today to reap the benefit of virus free plants. Some of the crops that depend on having the plants freed from virus are potatoes, strawberries, apples, grapes, raspberries and many more.

In the case of potatoes, virtually every potato that is marketed in the United States has been produced by plants that are only five or six generations away from the test tube. Here in the Red River Valley the State Seed Department is responsible for maintaining the seed potato program. They start cultures of virus free plants and propagate them under tissue culture conditions. All of the cultures are monitored for virus infection using the ELIZA tests specific for the viruses. The plants that are produced in the test tubes are planted in greenhouses where they produce mini-tubers. These small tubers are sent to seed producers where they can be reproduced under conditions isolated from other potatoes. In the case of North Dakota, these areas are near Beach and Cando. The potatoes are reproduced and multiplied and after a couple of generations sold to the potato seed producers in the Red River Valley, who in turn will use them to produce certified seed.

This is a continuous process with virus free stock being fed into the system yearly. This insures a perpetual supply of virus free seed for the Red River Valley's potato producers. The use of virus free seed potatoes gives the producer a yield and quality advantage and adds to the profitability of growing potatoes.

There are many ways that tissue culture can be used as a research tool. One of these is for the development of new genetic materials for use as varieties or for use by plant breeders in developing new varieties.

Tissue cultures can be grown under conditions that will cause mutations to occur in some of the cells as they divide to form new plants. These mutations may have qualities that are desirable for agriculture production and that can be bred into varieties. Since these mutations occur at the cellular level, they are called somaclonal variants. These mutant plants can be further propagated asexually or from seed produced by the plant.

Another way that tissue culture can be used as a tool of the plant breeder is by fusing the cells of two different plants together and thus combining the genetic materials from the two plants into a new plant. This is called protoplast fusion and is accomplished through a not-so-simple process of removing the cell walls from the leaf cells of the two plants and fusing them under culture conditions.

Once the cells are fused, their genetic components are combined and the cell begins to divide and eventually forms the new plant.

In the Department of Horticulture and Forestry at NDSU, projects are in progress that related to shelterbelt tree improvement using tissue culture techniques to select for resistance to diseases and environmental stress. Tissue culture is also being used to produce potato germplasm that will be used to bridge the genetics of wild potato species with the cultivated potato. This is being done using a number of techniques, including protoplast fusions. The large base of genetic characteristics found in the wild species of potato should serve to improve potato varieties in a number of ways, including disease and insect resistance, improved yield, improved processing quality and frost and drought resistance.

Other research in the Department of Horticulture and Forestry is studying the variation or mutation that occurs spontaneously in tissue cultures. For the plant propagator, these are undesirable, while the plant breeder is interested in them as new genetic traits. Techniques are being developed to induce mutations in cultures as a means of expanding the genetic base of crop plants and possible finding new varieties.

Tissue culture and related biotechnology are tools that can be used by scientists in conjunction with existing techniques to expand the possiblities in plant propagation and variety development. The use of these tools will undoubtedly add to the productivity of crop agriculture of the future.

In separate articles Dr. Gerald Tuskan discusses the application of biotechnology to shelterbelts and Drs. Shelley Jansky and Mark Ehlenfeldt discuss the application of biotechnology to potato breeding.

Plant Pathology

Comments provided by **Dr. Glen D. Statler,**
Professor and Acting Chairman, and **Dr. Gary A. Secor,**
Associate Professor

Biotechnology research will be an important thrust of research in plant pathology programs throughout the next decade and into the 21st Century. The Department of Plant Pathology at NDSU has had and will continue to have a strong commitment to research in biotechnology. The main emphasis of research in plant pathology is the development of and screening for resistance to plant disease.

The study of the flax hypocotyl system as a method of tissue culture is being investigated by Statler. Hypocotyl segments are taken at different times after shoot removal and placed on a nutrient agar medium using sterile techniques. This system is being used in our lab by Paul Enger to develop rust resistant flax through naturally occurring somaclonal variation. Chemical mutants are also being used on callus tissue in an attempt to develop resistant cultivars through mutations. Several mutations for disease resistance have been developed using these techniques.

Much of the increased research from federal and private sources have focused on the potential utilization of genes for disease resistance (Sequeira 1986). Certainly, the development and utilization of genes for disease resistance has great potential for agriculture. In fact, the development of crop cultivars for disease resistance has been one of the most successful methods of controlling plant disease. However, many plant species grown for crop production have a limited number of genes for resistance. Other species have no known genes for resistance to certain diseases. In still other cases resistance may be overcome by the appearance of new races of the pathogen. Therefore, the intelligent deployment of genes for resistance must be practiced so that genes for resistance are not lost by successive mutant races of the pathogen. It follows that new or unusual sources of resistance developed by new techniques in biotechnology could vastly improve our pool of genes for resistance. More importantly, a vast amount of formerly untapped resistance called nonhost resistance (i.e. corn is resistant to wheat rust) or alien germplasm could be available to future scientists by new techniques of gene transfer which could allow genes from other species to be moved to crop plants (Kiesling, 1988).

Gene transfer should reduce the time involved in the development of resistant cultivars even when using traditional genes for resistance. Gene transfer could also facilitate the use of previously difficult sources of resistance for breeders. These sources include such things as slow rusting, polygenic resistance, partial resistance, field resistance and tolerance. The combination of major gene resistance with slow rusting cultivars should increase the durable life of the cultivar. However, McCabe et al. (1988) pointed out that the transfer of genes from one organism to another represents the most difficult and labor intensive application of biotechnological techniques in agriculture but is probably the least developed system.

Once a desired gene for resistance is transferred to a suitable crop plant, it must be tested against populations of the parasite for which it was developed. This will require traditional pathologists to test the new cultivars. Freistadt (1988) recently voiced concerns of reduced funding of traditional programs since biotechnology programs are so expensive. He stressed the importance of leaving traditional programs in place so that the benefits of biotechnology programs can be utilized soon after development.

It is important to remember that disease resistance must be located and identified before it is transferred to a crop plant (Kiesling, 1988). The identification of genes for resistance requires intelligent use of selective pathogenicity so that effective genes are located and transferred. It would be of little value, for example, to transfer a gene that had already been overcome by a mutant race of the pathogen.

Pathologists will be forced to develop unique tester systems to test novel resistances to ensure that new sources of resistance are in advance lines. We have had some experience testing resistance genes for which there were no tester races in the U.S. flax rust population. We developed new rust races in our lab through fungal genetics to test advance lines to be sure breeders still had these unique resistances in advance lines (Statler et al., 1981).

The identification of genes for resistance by traditional methods is time consuming and costly. It involves screening to identify the gene, then crossing the plant containing the gene with all known genes, and finally testing for segregation in the F_2 generation. This may also be done by selective pathogenicity; that is, inoculating with races virulent on all but one of the genes involved. However, correct races with specific pathogenicity are difficult or impossible to find.

We currently have a cooperative program with Dr. Sparks in biochemistry designed to identify genes in flax responsible for rust resistance. This research involves the identification of DNA regions containing a rust resistance gene by comparing restriction fragment length polymorphism (RFLP) between resistant and susceptible flax plants. Restriction fragment length polymorphisms between the two plant types may be an ideal way to identify the DNA region that contains the resistant gene. Once genes have been identified, future plans include the transfer of genes from rust resistant to rust susceptible plants. The benefits of this research are that techniques could be developed to detect new or unusual sources of resistance by genetic engineering then identify these through RFLP techniques.

The development and identification of resistance genes through RFLP techniques could shorten breeding time since fewer or perhaps no rust screenings would be necessary to develop a resistant variety. Gene transfer and identification could allow for gene conservation, and by using gene manipulation more than one gene could be used in new cultivars, decreasing a chance for a mutation to virulence by the pathogen and extending the expected duration of the cultivar.

The program initiated by Dr. Secor in biotech started out about 1980 by trying to regenerate potato plants from single cells (protoplasts) and determine how it affects resulting plants. It turns out that after many trials, we were able to regenerate many clones of the cv Russet Burbank. These clones all came from leaf cells of the same plants. Their clones, plus a number from Dr. Jim Shepard, who was then at Kansas State, were evaluated in the field. Interestingly, the clones were all different from each other. They varied in such things as vine morphology, tuber yield, and maturity. This phenomenon in called somoclonal variability and has since been documented in several other crops. This naturally occurring variability in populations of cells forms the basis of much of the biotech, and can be capitalized on because we can work with millions of cells in the lab.

Following this initial success, we began to expand our potato biotech program. Dr. Raymond Taylor, who is now a post docotoral researcher in our group (funded by Red River Valley Potato Growers Association), developed techniques to regenerate other varieties and techniques for screening cell cultures for soft rot resistance in the lab. Dr. Taylor along with his technician Cheryl Ruby have continued to look at clones of a chipping potato variety, Crystal, for an improved modified clone. Field and lab tests have led to identification of a clone that yields as well as the original Crystal, but with better soft rot resistance, bruise resistance, and the ability to make better chips following storage. The processing work was done in cooperation with Paul Orr at the USDA Potato Research Lab in Grand Forks. We are also working with a major food company for development of potato cultivars resistant to physiological storage disorders using cell culture screening techniques.

In addition to finding beneficial germplasm through naturally occurring somaclonal variability from regenerated single cells, screening techniques can be utilized with cell culture to identify and study resistance to disease. Because we can grow large numbers of plant cells on synthetic media in a small space in the lab, we can expose the cells to stresses in order to select cells resistant to that stress. A good example of one kind of stress is disease. Many disease-causing organisms produce a toxin which is often the substance responsible for the disease. Toxins can be separated and added to the media used to grow plant cells. The resulting interaction of the cells to the toxin can be used to study how disease occurs, to identify the reaction (susceptible/resistant) of varieties or cell selections to disease, or to find new sources of resistance by finding resistant cells in a susceptible population.

Early research was conducted in cooperation with Dr. Jim Venette of our department with funding from the Northarvest Bean Growers Association, using phaseolatoxin, the toxin produced by *Pseudomonas syringae* pv *phaseolicola*, the cause of halo blight of beans. This toxin was active and could be incorporated into media used to grow bean cell cultures. The mass of growing cells, called a callus or calli, reacted to the toxin in the growth medium just like whole plants inoculated with the bacteria. Callus from resistant varieties was resistant and callus from susceptible varieties was susceptible. Cell cultures could be used to predict the disease reaction in the lab. These results suggest that a callus screening system can identify bean cultivars resistant to halo blight.

Building on this success, we have done similar research using the edible beans-white mold (Sclerotinia) system. Chris Hartman, who is finishing a PhD degree in this department, has shown that calli of resistant genotypes and species were also resistant when grown on media containing toxic filtrates of Sclerotinia. We are continuing this work in cooperation with Dr. Ken Grafton, Crop and Weed Science, and his PhD student, Phil Miklas, looking at improved screening techniques for white mold resistance using cell culture as a means of identifying resistant germplasm. The heritability of this resistance will also be investigated.

A graduate student in our department, Fereshteh Saghaei, is using cell culture of potatoes to determine the presence of a toxin produced by *Corynebacterium sepedonicum*, the cause of potato ring rot. There is little evidence for the production or nonproduction of a toxin, and symptom expression varies by variety. Using cell cultures of resistant and susceptible varieties as markers, we are determining whether this bacterium produces a biologically active toxin and the nature of that toxin. The reaction of calli grown on cell free extracts of *C. sepdonicum* (toxin) is being investigated. This procedure could be used to understand the mechanism of pathogenicity and be useful in identifying the susceptibility of advanced selections prior to release.

Similar kinds of research are being conducted in the new USDA Northern Crops Science Lab on sunflower and sugar beet with technical assistance of Vicki Gustafson. Sunflower cell culture biotech work is cooperative with Dr. Tom Gulya, USDA Plant Pathologist, and Dr. Jerry Miller, USDA sunflower breeder, with support and cooperation from Cargill, Inc. We have tested 50 sunflower inbreds for regeneration ability from cell culture. Four of these produced plantlets. Work is continuing on screening for an improving regeneration. It appears that ability to regenerate is under genetic control, and experiments we have conducted have shown it to be transmitted to progeny. We have experimented with three diseases using cell culture-toxin systems for identifying resistance and identifying resistant inbred lines: *Phoma macdonaldii*, the cause of black stem in cooperation with Pat Donald, a PhD student here; *Sclerotinia*, the cause of white mold, and *Phomopsis*, the cause of brown gray spot. The last disease is important primarily in Europe, but does occur in the U.S.

We have developed an international connection with the country of Yugoslavia for application of biotechnology for Phomopsis control. Dr. Stevan Masirevic has been a visiting scientist here, and has found that Phomopsis produces a toxin and is potentially useful for screening cell cultures for identification of resistant germplasm. We are continuing the scientific exchange.

Using research support from the Sugar Beet Research and Education Board of Minnesota and North Dakota, Dr. Bill Bugbee and Secor are cooperating on a similar biotechnology study to identify sugar beet cultivars resistant to Rhizoctonia. This fungus causes a root rot for which there is little, if any, control. Because the fungus produces enzymes that cause the disease, we are attempting to find sugar beet cells resistant to these enzymes using cell culture techniques. Hopefully, plants regenerated from these resistant cells will remain resistant to the disease.

The ultimate step in biotechnology is using new methods to transform plants by addition of alien genes. We are excited because we have initiated two studies that will allow us to develop systems to move genes into crop plants. Both of these systems employ the bacterium *Agrobacterium* as the gene vector. We are developing a gene transfer system using our sunflower cell culture system in conjunction with private industry. A second active program is a cooperative research project with Dr. Jesse Jaynes, a biochemist at Louisiana Sate University. The work is being conducted by Chris Onstad, a graduate student in our department with funding through the National Potato Council by the USDA-ARS. Dr. Jaynes has engineered an *Agrobacterium* to contain some naturally occurring genes from a moth. These genes produce a protein which lyses (kills) a broad spectrum of bacteria, including those infecting plants. Because *Agrobacterium* infects many plant species, including potato, and *Agrobacterium* can act as a vector to transfer genes, we are attempting to transfer the anti-bacterial genes to potato via our tissue culture system we have developed. The main questions to be answered are if the genes will successfully transfer, will they be operational, and will they cause the potato plant to be resistant to bacterial diseases. Since Drs. Neil Gudmestad and Secor both have active programs on bacterial disease control of potato, this would be a novel

way of obtaining plants resistant to bacterial disease, specifically *Erwinia* soft rot and ring rot. This is an exciting concept which has far reaching ramifications.

Dr. Neil Gudmestad in our department in cooperation with Dr. Arland Oleson and Dr. Bob Sparks of the biochemistry department have developed a unique assay to potentially detect small numbers of bacteria which can contaminate seed potatoes. The assay, which is still under development and testing, utilizes small pieces of plasmid DNA of the bacteria to detect otherwise immeasurable numbers of bacteria in seed potatoes. Small pieces of DNA called RFLPs, can also be used to differentiate strains of bacteria and are being tested as a sensitive probe. Infested seed lots can then be discarded before disease occurs.

The impact of this research, indeed the whole area of biotechnology on agriculture research, is truly great. These new research tools allow us to do things only talked about 10 years ago. Innovative technology in biology will remove many of the limits of scientific progress. With continued, and hopefully increased support from state, federal, private and industrial sources, we will continue to conduct research for all the people of North Dakota and the nation. The practical application of biotechnology has great potential for economical development in the state.

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Botany

Comments provided by **Dr. Donald S. Galitz,** Professor and Chairman

Biotechnology carries with it certain connotations, one of which is that suddenly here is a marvelous new methodology to be used in plant and animal research. Rather, it is the application of a series of techniques that have gradually evolved in biology, chemistry and physics which allow us to approach long standing biological problems from a different perspective and in extended dimensions. The faculty of the Botany/Biology Department have stayed abreast of new technologies through the years and have adapted many of these in their research as they have provided opportunities to obtain answers to persistant questions of biological significance.

Studies of the effects of water deficits on nitrate reduction and assimilation by intact crops and range plants had been conducted by Dr. Galitz for some time. However, direct effects of water stress on utilization of nitrogen by plants were impossible to determine because of indirect effects of other environmental factors. The use of cell suspension cultures was adopted because cells could be suspended in a liquid medium of known water potential. In this way the effects of internal water stress on nitrogen metabolism by cells was possible. Subsequently cell and tissue cultures have been used in studies of the biochemical differences in several biotypes of leafy spurge which demonstrate different levels of sensitivity to herbicide treatment. Cell and tissue cultures have also been used in studies on the hormonal regulation of crown and root buds of leafy spurge and to determine the role of plant phytochromes in the cold hardening of underground spurge tissues.

Over the past decade new biochemical techniques and instrumentation have made it possible to look at cellular and molecular processes in greater detail. Dr. Duysen has been interested in the effects of water deficits on the development and functioning of the photosynthetic apparatus in crop plants. To understand these effects, current cellular and molecular techniques are utilized to obtain evidence for the developmental steps in chloroplast biogenesis. More specifically, investigations have been directed at unraveling the complex processes in the development of the light-harvesting pigment-protein complex and the role of a degrading factor. To do this the pigment-protein complex is extracted and degraded to its various subunits at various stages of development under different treatments. Each of these subunits is regulated by nuclear genes and some by cytoplasmic genes. Through tissue culture this group is investigating the possible transfer of cytoplasmic genes from one source of tissue to another for the purpose of modifying its expression and thus regulating the photosynthetic apparatus in wheat.

Dr. Freeman has been a collaborator on these photosynthetic studies. Both scanning and transmission electron microscopy studies of the development of the chloroplast and the thylakoid membranes within it have enhanced the understanding of the structure-function relationships in the photosynthetic apparatus during development and under stress. Electron microscopy allows resolution far beyond the powers of magnification possible by traditional light microscopy. With this capability viral-like particles have been identified in the cytoplasm and nucleus in durum wheat and the morphological characterization of the instar stages of the Russian wheat aphid is being worked out.

Dr. Fawley has focused his research interest in the comparative biochemistry and evolution of the light-harvesting pigment-protein complexes in green and other algae. These molecular studies involve the isolation and characterization of the plant pigments and the organization of the photosynthetic apparatus of the lower plant forms.

Entomology

Comments provided by **Dr. Craig R. Roseland,** Assistant Professor, and **Dr. Gary J. Brewer,** Assistant Professor

Biotechnological solutions to contemporary agricultural problems have begun a new era in which answers to entomological problems can be elaborated upon with strategies that would have been unthinkable in previous years. For example, one new approach is the alteration of plant genomes so that a plant expresses a constitutive insect specific protein toxin. Recombinant DNA technology is being pursued by several commercial interests to achieve these ends. In fact, tomato hornworm-resistant tomatoes engineered with *Bacillus thuringiensis* toxin have been successfully demonstrated in field plantings by the Monsanto Company. The toxicant principles employed in these biotechnological efforts have been well-described and are in commercial production as conventional microbiological pesticides. While the payoffs may arrive in the near future, the richness of the plant as an integral player in the continuous battle of plants to survive herbivory is neglected with this type of biotechnological goal. Selection of plants that resist insect infestation should proceed from an understanding of the chemical details of the plant and then should build on that specialized knowledge.

Because there is only a limited range of susceptiblility for the known protein toxins specific to insects, it is appropriate to look for additional toxic or deterent principles specific to a given crop pest. All plants possess some mechanisms to resist or inhibit insect feeding, although many obviously do not completely deter their own pest species. The endogenous defenses of agricultural crop plants such as the sunflower need to be carefully examined in order to find some that might be amenable to enhancement. The alternatives include classical selection of variants or enhancing the expression of a specific constituent with further recombinant DNA techniques.

Classical selection is accomplished by observing decreased damage by insects within a variety or in a population and subsequently breeding the exceptional plants. This strategy requires years of effort. Classical selection with chemical criteria rather than damage criteria however should greatly reduce the time that is required to discover potentially resistant varieties. The presumption requires that mortality can be demonstrated as a result of a specific constituent. Selecting individual plants with higher levels of an appropriate defensive constituent by means of a straightforward chemical assay would therefore appropriately discern individuals and later populations of plants expressing the desired properties. Both constitutive and induced-response defenses could be analyzed.

A second strategy, that of engineering transgenic plants that synthesize more of the desired toxin or deterrent, would be another possible direction for the selection of insect resistant plants. Again the demonstration of an effective concentration of defensive chemical would allow the genetic enhancement of that product. While it is doubtful that it would be possible to insert multiple copies of the specialized gene that makes the product toxicant without a major investigation of sunflower biochemistry and internal regulation, it should be possible to enhance the number of copies of identified genes which are precursors to the desired end product.

Sunflowers that are under stress from fungal infections synthesize in high quantity two coumarins ayapin and scopoletin. Roseland and associates have shown that insects such as sunflower beetles and thrips also turn on the synthesis of these coumarins. Furthermore we have shown that the sunflower beetle is deterred from feeding or oviposition by high levels of coumarins. Thus selection of plants with highly inducible coumarins would be useful to agricultural production of sunflower. A collaborator at South Dakota State University, Dr. Anne Espinasse, is attempting to select varieties of sunflower by means of *in vitro* culture. This technique introduces genetic variability by chromosomal changes in the cultured cells. We will analyze the possible content of coumarins by both thin layer chromatography and HPLC. Although the levels of coumarins in the callus cultures may be too low to detect, we will also analyze the newly regenerated shoots of the callus clones. A second source of variability in the genetic base of the callus will be introduced by mutagenesis of the callus cultures. Finally, additional genomic variation will be available within various lines and cultivars of commercial and public sunflower releases.

The current research efforts of Roseland's project are directed toward demonstrating the means by which sunflower defensive responses are induced and transmitted within the plant. For example, we wish to know whether the response is a general one or whether it is confined to only certain leaves of the plant. We also wish to discern whether the plant remains primed to respond indefinitely after insect attacks or whether the response is of a shorter term. The precision of the signal that initiates synthesis of coumarin is also in question: does insect attack specifically initiate the plant response or is insect attack along with other stress, say bacterial secondary infection, required as well? We are also comparing the level of deterrence provided by induced plants with that of control plants using sunflower beetle as the assay insect and thrips as the inducing insect.

From other studies we know that terpenoid chemicals in sunflowers also provide feeding deterrence to lepidoptera that damage the florets of developing sunflower. We are attempting to quantitate the levels of these terpenoids expecially the sesquiterpenoid lactones on one hundred varieties of sunflower and analyzing the biological effects of these on insects in collaboration with Dr. Gary Brewer of this Department. In some of these same varieties we are also determining the genetic variability within the line to suggest whether there is a basis to select for high levels of plant allelochemicals. Presently, we are chemically identifying the structures of these complex defensive compounds.

The chemical diversity of "secondary chemicals" synthesized by sunflowers should not go unexploited. We know that other chemicals such as acetylenics and flavonoids exist in high concentration but we have no idea of their biological relevance to the defense of sunflower. Fractionation of sunflower extracts will yield subsets of these which we will assay for biological activity. As some of these larger fractions are found to have interesting properties, we can further fractionate and isolate active principals and identify and analyze them in various assay protocols. As more details of the natural defenses are discovered, recombinant DNA technology firms will become more deeply interested in the potential of sunflower as at least an outlet to demonstrate the feasibility of engineered plants in agriculture. The potential for some collaborative work on creating transgenic sunflower will thereby be actualized, providing yet another direction for the genetic improvement of sunflower as a North Dakota agronomic crop.

Brewer is doing research on the insecticidal bacteria *Bacillus thuringiensis* (Bt). It produces an endotoxin which is specific to certain insect groups. Bt has been successfully used to control many Lepidopteran (moth and butterfly) pests in various crops. Recently, new Bt strains have been isolated with different endotoxins which effectively control other insect groups, such as Diptera (flies) or Coleoptera (beetles). Available techniques make it technically feasible to transfer the Bt endotoxin genes to plants. Sunflower, which is plagued with several lepidopteran and coleopteran pests, is a candidate for receipt of Bt gene(s). Whether it is economically feasible will depend on, in part, which pests are potentially controlable by transgenic sunflower expressing the Bt gene.

Testing to determine the susceptibility of the sunflower moth and beetle and other sunflower pests to Bt is underway. This will determine the effectiveness of various strains of Bt, their endotoxins, and genetically engineered Bt toxins.

Phytochemically, sunflower is complex, producing many secondary compounds, including flavonoids and phenols. Some phenols inhibit the activity of Bt. It needs to be determined whether phenols will also inhibit the action of natural and engineered endotoxins.

Results will identify Bt strains and toxins effective against the various insect pests of sunflower and aid in predicting their response in combination with other plant factors. This will provide necessary information for the biotechnology industry to make an informed judgement whether to bioengineer sunflower for resistance to insects.

Future research efforts will involve Bt and other potential gene transfer candidates and their interaction with conventional sunflower resistance.

Microbiology

Comments provided by **Dr. David A. Gabrielson,**
Associate Professor and Interim Chairman

The field of Microbiology is at the center of the biotechnology issue in terms of both basic and applied research. To help demonstrate this point, I would like to share the following thought;

1942 Dr. Selman Waksman, winner of a Nobel Prize for the discovery of streptomycin: "There is no field of human endeavor, whether it be in industry or agriculture, or in the preparation of food or in connection with the problems of shelter or clothing, or in the conservation of human and animal health and the combating of disease, where the microbe does not play an important and often dominant role."

In our department, Dr. Berryhill is looking at the large plasmid profiles of various strains of *Rhizobium* spp. to try to determine if these characteristics can be used to either identify or improve the nitrogen fixing capacity of his strains. His work has also been the basis for grant support to study the possibility of improving the nitrogen fixing capacity of *Rhizobium* strains from Latin American countries. He is also part of a group of scientists at NDSU that are using restriction enzyme maps of plant mitochondrial DNA to evaluate the lineage, phenotype and genotype of various plant germplasms. The work that Dr. Berryhill is doing has tremendous potential to benefit both agriculture and the general public. This basic research could well result in significantly improved plant hardiness, make it easier and faster to select for the desired characteristics needed for plant breeding, and lower the cost of production. This area of activity will grow to become more multidisciplinary and eventually move from the area of basic science into the applied areas. There is already evidence for this trend in the number of "new" organisms that are being looked at for release into the environment.

Dr. Funke has studied the microbial ecology of stripmined land in efforts to help revegetate this land and return it to production agriculture. This research has been in cooperation with the NDSU Land Reclamation Research Center at Mandan. Microbes that participate in the nitrogen cycle are an essential element of reclaimed land, as are the mycorrhizal fungi. He has also studied the control of molds in insect rearing media, which is important when one considers that the field of insect tissue culture is just beginning to take off because of many of the unique biological applications for these cell lines.

Dr. Struble's efforts focused on looking at the microbial ecology of no-till farming. The push toward no-till systems was and still is driven by the desire to reduce production costs and control soil erosion. However, the trade-off is that increased chemical use is necessary in the form of herbicides and changes in the microbial ecology of the soil. Dr. Struble's work is necessary to find if changes in tillage systems affect soil fertility or increase the likelihood of plant disease. Dr. Struble is also part of a consortium of scientists that are looking at the ecology of grazing systems to look for ways to improve the management of grasslands. He is active in the groups that are looking at alternative crops and sustainable agriculture in North Dakota. He has recently been successful in obtaining funding from the soybean growers association to begin some preliminary investigations on soybean-rhizobia interactions. Dr. Struble and Dr. Berryhill have begun looking at ways to identify and recover released, recombinant, microorganisms from the soil. These studies are essential to the development of policies which will allow the wide-spread use of laboratory-engineered microbes for improved agriculture production.

Dr. Glass is interested in the physiology of anaerobic rumen bacteria. Currently, his research is concerned with the mechanisms responsible for transport and metabolism of sugars by rumen cellulolytic bacteria. These bacteria are responsible for degrading the cellulose of plant tissue and thereby enable ruminants to use forage as feed. The transport and metabolism of glucose and cellobiose are the final steps in the degradation of cellulose by rumen bacteria. Basic knowledge of these transport mechanisms is essential to understand what cellular factors control the production and activity of cellulase, the enzyme responsible for cellulose breakdown. This knowledge together with that on the characteristics of cellulase enzyme systems and combined with data on the genetic systems of rumen bacteria will provide the foundation on which a rational approach to the genetic engineering of cellulolytic rumen bacteria can be done. Such an approach should lead to development of new bacterial strains with an increased capability for cellulose digestion

resulting in a general increase in feed efficiency for the ruminant animal.

The development of a subunit vaccine against *Toxoplasma gondii,* the analysis of bacterial mechanisms to escape phagocytic destruction, and the use of probiotic feed additives to improve animal thriftiness have been undertaken by Gabrielson.

The development of a safe and effective vaccine against toxoplasmosis has been a desirable goal to help decrease losses that may occur in the livestock breeding industry. The vaccine would also be a valuable tool to improve human health if domestic cats were vaccinated. This study indicates that a potential exists for the development of a subunit vaccine against toxoplasmosis. We were able to protect mice completely against this disease and our preliminary work suggests that the same product will also protect cats.

In separate articles of this issue, Dr. Berryhill discusses his work on plasmoid diversity within bean rhizobia, and Drs. Struble and Funke discuss biotechnology and soil microbiology.

Biochemistry

Comments provided by **Dr. Allan G. Fischer,**
Professor and Dean of Science and Mathematics

Research presently being conducted in the Biochemistry Department by Drs. Fleeker, Killilea, Oleson and Sparks are all biotechnology-related. Dr. Oleson's laboratory is studying the molecular biology of plant pathogenic microbes and using this information in the applied area called DNA probe technology. Dr. Spark's research group is investigating the mechanisms involved in gene expression of ornithine decarboxylase, an enzyme controlling the synthesis of polyamines, in both insect embryos and flax plants. Dr. Killilea's research centers on the isolation and characterization of protein kinases and phosphatases, enzymes responsible for the metabolic regulation of cellular processes in response to hormonal and neural stimulation. Dr. Fleeker is developing monoclonal antibody-based immunoassays that can be done quickly in the laboratory and in the field; these methods are used to measure small amounts of pesticides and other materials. These research programs utilize numerous biotechnological methods, including recombinant DNA and gene cloning, probe technology, hybridoma generation, enzyme purification, and mechanistic studies, to provide new approaches to agricultural research.

A study of the molecular genetics of the potato bacterial ring rot pathogen, is a good example of a basic research effort that has yielded important practical applications. It involves scientists from the Departments of Biochemistry and Plant Pathology: Dr. Arland Oleson (project leader), Hope Olson (Chemist I), Li You and Jill Fahrlander (graduate students) are from Biochemistry, and Dr. Neil Gudmestad and Paul Henningson (Microbiologist I) are from Plant Pathology. The bacterium under study, *Clavibacter michiganense sepedonicum,* causes one of the most devastating diseases of potato, and state seed certification agencies have applied a zero tolerance rating for this pathogen. The disease is termed ring rot because of the characteristic necrosis of the vascular ring of infected tubers. The organism also attacks other vascular tissues of the plant and causes extensive wilting. More subtle, latent forms of the disease also exist that are difficult to detect. Basic research on the ring rot pathogen has revealed the presence of a plasmid, a small, supplemental piece of genetic information, in almost all strains of the bacterium. A molecular probe using this plasmid has been developed for detection of the pathogen and differentiation of strains of the pathogen. Studies have revealed conditions for dot-blot DNA hybridization under which the probe will interact solely with the ring rot pathogen, and applied work is continuing that will permit the detection of the very low levels of the bacterium that are present in latently infected potato plants.

The molecular probe has also been found to permit specific strains of the ring rot pathogen to be identified by means of a genetic fingerprinting method. This method, termed RFLP analysis, will be of great value in epidemiological studies on the origin and spread of potato ring rot outbreaks, and it is anticipated that this method will aid in the resolution of litigation involving ring rot in the potato industry.

Dr. Robert Sparks along with Dr. Oleson, Dr. Killilea and Dr. Leopold (USDA) is trying to gain an understanding of the mechanisms of controlled gene expression of the enzyme ornithine decarboxylase (ODC) during the development of insect embryos. This enzyme regulates the biosynthesis of the polyamines, putescine, spermidine and spermine. These three polyamine molecules are intimtely involved in the control of insect development and are required for RNA and DNA synthesis, cell growth and division. Embryos of house flies are particularly suitable for the study of developmentally regulated gene expression because large numbers of embryos are easily obtained and they can be synchronized in their developmental processes.

The results of this study will provide a basic understanding of the control of gene expression during development, and will provide greater insight into the developmental biology and possibly the ecology of all dipteran insects. For example, in some cases effective coordinated pest management might depend on the availability of specific genetic and developmental information to determine when, if and how control action should be taken. Basic knowledge of mechanisms of gene control in insect embryogenesis may allow development of new management strategies with highly specific growth inhibitors.

With the collaboration of Dr. Glen Statler in the Plant Pathology Department, Dr. Sparks is concerned with the role of ornithine decarboxylase gene expression in two species of flax plants, rust resistant and rust sensitive. There are two major objectives of this project: (1) to identify and isolate the gene(s) responsible for rust resistance so that these genes can be transferred by asexual biotechnologies to rust sensitive plants; and (2) to clone and characterize the genes for ODC so that we can gain an understanding of ODC regulation in flax plants. Chang Sung is a graduate student in Biochemistry, associated with the project.

The efficiency of food production by farm animals is closely related to the cellular regulation of carbohydrates, fat and protein metabolism during pregnancy, growth and lactation. At the cellular level it is now appreciated that many

hormones reversibly modify complex metabolic processes by switching on/off certain key enzyme activities by phosphorylation/desphosphorylation reactions catalyzed by protein kinases and phosphatases respectively. Much of this research has been carried out on tissues from laboratory animals. Little reserch effort has been focused on systems in red muscle from food-producing animals, but, the little work that has been reported indicates that differences do exist between different muscle types and between species. It is also now apparent that hormones may regulate a variety of different metabolic processes via the same protein kinases and phosphatases. Thus by studying and characterizing these enzymes using one metabolic process it will be possible to later extend the studies to other processes.

The focus of current work by Dr. Killilea centers on the elucidation of the regulation of metabolism of pig muscle glycogen, the storage form of carbohydrate in these tissues. Later the studies will be extended to the regulation of fat and protein metabolism.

Besides laying the foundation for later research, the study of pig muscle glycogen metabolism is also important in that glycogen levels and its metabolism play a role in determining the quality of pork in "stress-resistant" pigs and is associated with the porcine stress syndrome (PSS) and pale, soft and exudative pork (PSE), two related problems seen in "stress-susceptible" pigs. These conditions are due to an inherited trait associated with the production of pigs with rapid growth rates, high efficiency and superior muscling. Thus, if the genetic effect could be identified and then controlled by processes such as genetic engineering, a return to the production of this type of animal would be very desirable. (Recent approaches using partitioning agents and growth hormone treatment to improve efficiency may also cause a return to high incidences of PSS and PSE-type problems.)

A detailed understanding of the regulation of metabolic processes could lead to means of manipulating these processes to achieve greater animal efficiency. Monoclonal antibodies, for example, could be used to block key regulatory proteins and recombinant-DNA techniques could be used to either correct genetic defects or to introduce new regulatory proteins into cells. These types of approaches could possibly be used to decrease fat deposition by adipose tissue (in these and other farm animals) and so increase growth efficiency. Similarly, a decrease in protein turnover might stimulate muscle growth since the rate of protein degradation is as high as 75% the rate of protein synthesis in animals. Manipulation of control of glycogen metabolism could result in the overall enhancement and standardization of the quality of fresh and processed pork products.

During the last 25 years, there has been a concern among agriculturalists due to pesticides in our environment. However a greater emphasis has been seen in the last couple years as contamination of groundwater becomes a problem. The initial work of Dr. Fleeker on 2,4-D metabolism and other herbicides by chromatography showed these methods to be slow and poorly adaptable to large samples. Therefore Dr. Fleeker's goal is to develop quick tests for chemicals of agricultural importance based on antibodies which bind the particular chemical to be measured. These tests are called competitive binding assays or more commonly immunoassays. Current projects underway are: Development of a commercial immunoassay for daminozide, a plant growth regulator, in order to detect this substance in apples (ImmunoSystems). His laboratory has developed the antigen and is attempting to raise the antibody. Development of a 10-minute assay for aldicarb (Temik) in groundwater in cooperation with Dr. M. Low, Minot State University. In the past his lab in conjunction with Penn State University developed an immunoassay to aldicarb, however the test has the drawback of requiring a long incubation period, and now the focus is with Dr. Lowe to reduce the incubation period. To test the reliability of a commercial immunoassay for triazine herbicides using gas chromatography to confirm the results.

Animal and Range Sciences

Comments provided by **Clayton N. Haugse,** Professor and Chairman, and **Dr. C.S. Park,** Professor

"Long term goals of biotechnology may or will result in (1) fewer chemicals used in crop and livestock production, (2) new crop varieties which may benefit the livestock industry, (3) better efficiency of food production by livestock, (4) reduction of commodity surpluses, (5) better farm living, and (6) in meeting of human food demands." (ND Farm Research Bulletin, Vol. 46, No. 1, 1988)

Drs. Dale Redmer, Lawrence Reynolds and James Tilton have discussed in a separate article of this issue the importance of improving reproductive efficiency in livestock through a better understanding of the reproductive process.

The regulation of mammary gene expression is being studied by Park. Compensatory growth, a rate of growth faster than normal, occurs in a number of animal species when previously marginal or under-fed animals are realimented on a higher nutritional level. Compensatory growth has a profound influence upon animal growth, pregnancy and subsequent lactation, yet little is known about how compensatory growth regulates these body functions and processes. Ongoing long-term research efforts in our laboratory have been on the role of a compensatory growth pattern in the regulation of animal growth, mammogenesis, and lactation. The main thrust of current research centers on testing hypotheses raised by our compensatory growth model. This model, outlined in Figure 1, postulates that a change in nutrient density may alter hormone secretion and enzyme activity in such a way as to modulate mammary gene expression: rate of cell replication, transcription, and translation. We hope to test these hypotheses through detailed examination of the regulatory factors thought to be critical to mammogenesis such as growth hormone and RNA polymerase as well as through studying mammary gene function in acinar cell culture via amino acid uptake, protein secretion, and cytoplasmic and pre-nuclear mRNAs of milk protein.

The significance of this research is the direct applicability of the findings to the enhancement of the efficiency of animal growth and lactation. Our study will be useful in the development of a simple and cost-effect method of improving overall efficiency of production in dairy, beef and swine operations. This study will provide opportunities for the

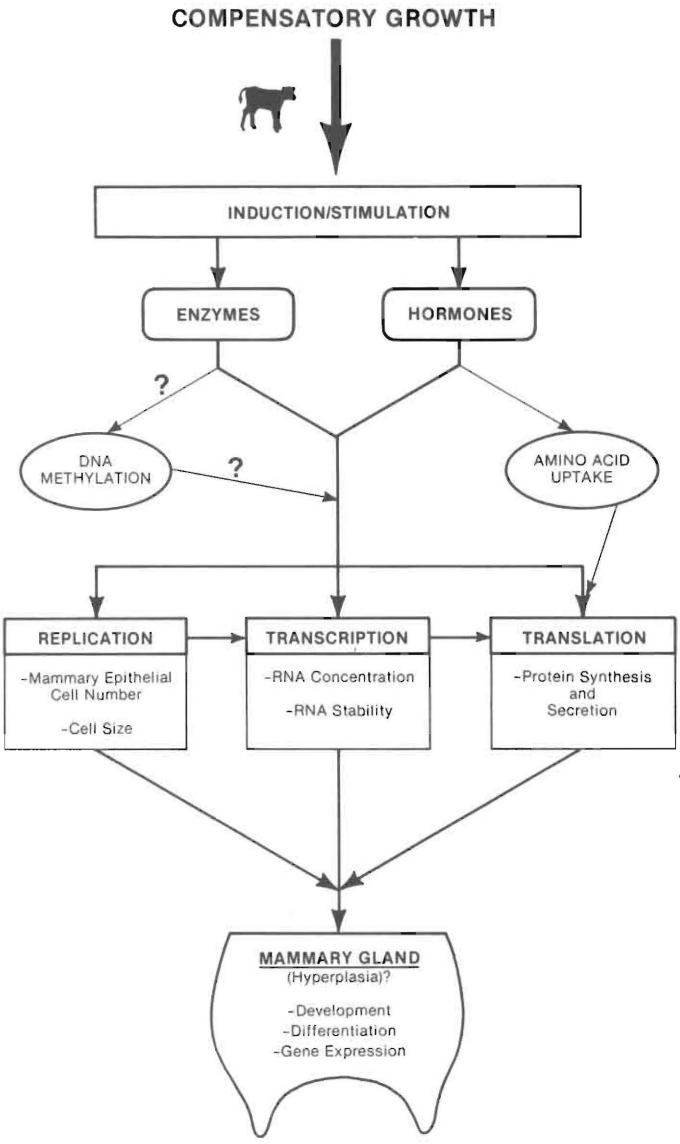

Figure 1. A model postulating the regulatory role of compensatory growth in mammary differentiation.

evaluation of the long-term, overall influences of compensatory growth and nutrient interactions on efficiency and longevity of lactation, reproductive performance, pregnancy, and survivability of progeny.

The data on cellular and molecular functions of the mammary gland obtained from this research will contribute to research directed at improving the quality of milk for nutritional and industrial purposes. Basic data on gene modulation is essential for the future development of an effective model for gene transfer experiments (e.g. transgenic cow) which may allow for the future reduction of lactose content in milk and the production of interferons or other blood factors in place of the usual case in or whey in milk.

Veterinary Science

Comments provided by **Dr. M. Herbert Smith,** Professor and Chairman

The biotechnology related research in the Department of Veterinary Science includes several projects. The bovine viral diarrhea virus research is discussed in a separate article by Dr. Berry. Biotechnological approaches and techniques are being used to study the pathogenesis of BVDV induced disease. Research on the anti-idiotype antibody vaccine against bovine brucellosis is in initial stages of development by Dr. Nemat Khansari. The anti-idiotype vaccine would be entirely safe to use since it does not involve living organisms as do present vaccines. The response to the vaccine in cattle could also be identified as unique and different from field strain response. This would overcome a major problem in identifying vaccinates from animals naturally infected.

Khansari is also involved in the study of the reversal of immunological functions due to aging recombinant somatotropic hormone, the development of a herpes virus specific cytotoxic T-cell using monoclonal antibody techniques, and the development of immunomodulating substances for use in cattle. Immunomodulatory substances incorporated in vaccines would produce a much more efficacious product and be of direct and significant benefit to the livestock producers.

Continued from page 2

sciences in order to expand their knowledge base and to enhance their career opportunities. At the present time, there are approximately 50 students who have declared a major in biotechnology.

The uniqueness of this program of study is its sound interdisciplinary course requirements, its practical application through on-the-job experience and its potenial of new career opportunities for our undergraduates. Even though the academic program is very demanding, the payoff is excellent in terms of professional career development and employment opportunities. North Dakotans will benefit from this program as an investment, not only in their children's education, but possibly in their state's economic development.