PAN AMERICAN HEALTH ORGANIZATION

PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER

FEDERATIVE REPUBLIC OF BRAZIL

MINISTRY OF AGRICULTURE

INTERNATIONAL SEMINAR ON THE IMPORTANCE OF BIOTECHNOLOGY IN LIVESTOCK DEVELOPMENT

(REPORT)

GOIANIA, GOIÁS, BRAZIL, MARCH 21-23, 1988

CONTENTS

Page

APPENDICES (cont')

* * *

INTRODUCTION

In compliance with Resolution IV approved at the XIV Regular Meeting of the South American Commission for the Control of Foot-and-Mouth Disease (COSALFA), the Pan American Foot-and-Mouth Disease Center (PANAFTOSA) of the Pan American Health Organization (PAHO/WHO), the Ministry of Agriculture of Brazil and the University of Wisconsin, USA, jointly held the "International Seminar on the Importance of Biotechnology in Livestock Development". The following organizations also lent their cooperation: Secretariat of Agriculture of the State of Goiás, the Goiana Livestock and Agriculture Society, the State of Goiás Agriculture Federation and the Goiana Association of Zebu Raisers.

The seminar was held in Goiania, Brazil, on March 21-23, 1988, in response to the invitation from the Brazilian Minister of Agriculture and authorities of the State of Goiás. The state belongs to the west-central region, an ecological reserve of world importance having a constantly growing livestock activity. The seminar was held to update the attendees regarding the accomplishments and possibilities for biotechnology throughout the world, and its effects on animal health and livestock development in South America. Opportunity was given to discuss the advances and extraordinary impact it will have on nature, man and society.

The seminar was attended by the Directors of Animal-Health and Foot-and-Mouth Disease Programs in South America, observers from international agencies engaged in agriculture and health, and involved South American technicians and scientific personnel. Scientits from the University of Wisconsin, PANAFTOSA and research organizations in Brazil transmitted information of great value for the orientation and strengthening of the technological development programs of the South American countries.

The following dignitaries were present at the opening ceremonies: Dr. Henrique Hercilio Santillo, Governor of the State of Goiás; Dr. Iris Rezende Machado, Brazil's Minister of Agriculture; Dr. Pedro Paulo Assumpção, Coordinator of International Affairs and Agriculture of the Ministry of Agriculture; Dr. João Joarez Bernardes, Secretary of Agriculture and Supply of Goiás; Dr. José Magno Pato, Federal Supervisor of Agriculture in Goiás; Dr. Raúl Casas Olascoaga, Director of PANAFTOSA; Dr. Sizelizio Simões, President of the Goiana Livestock and Agriculture Society; Dr. Antenor Amorim, President of the Goiana Association of Zebu Raisers; Dr. Joe R. Held, Coordinator of the Veterinary Public Health Program of the PAHO; Dr. Thomas Yuill, Coordinator of the International Center for Disease Control of the University of Wisconsin, USA; municipal and state authorities, and representatives of the livestock and agriculture producers.

It is with deep sorrow that the Director of the Pan American Foot-and-Mouth Disease Center communicates the death of Dr. Pedro N. Acha. During his many years of work in the Pan American Health Organization, as well as during the last two years engaged with the Inter-American Institute for Agricultural Cooperation, Dr. Acha never ceased to promote and honor the veterinary profession and the control of zoonoses and to serve the countries of the Americas.

This seminar, as well as innumerable other events, always merited his interest and cooperation. His sense of friendship, responsibility and unequalled enthusiam shall never be forgotten by those who have had the good fortune to know and work alongside him.

TALK delivered by
Dr. Raúl Casas Olascoaga, Director,
Pan American Foot-and-Mouth Disease Center (PAHO/WHO)

During the "Seminar on Minerals Utilized in Agriculture and Livestock Raising", held in Goiania, Goiás, on June 2-3, 1987, Dr. Iris Rezende Machado, the Minister of Agriculture of Brazil, invited me to hold in Goiania the "International Seminar on the Importance of Biotechnology in Livestock Development" and the XV Regular Meeting of the South American Commission for the Control of Foot-and-Mouth Disease (COSALFA).

As ex officio secretary of COSALFA and on behalf of the Pan American Health Organization (PAHO), I accepted his kind and honorous invitation.

It is indeed a great honor for us to hold this seminar in Goiania, capital of the vast, prosperous State of Goiás. Together with the Pantanal of Mato Grosso, this state composes an ecological reserve of world importance, where the livestock raising activity is expanding constantly.

There are similarities and coincidences in the geographical and historical characteristics, as well as of the political and cultural development of the populations in this region, in the States of Goiás, Mato Grosso do Sul and Mato Grosso. Of foremost consideration are the substantial reserves of resources and the large cattle herds, natural parameters for the adoption of measures of control, preservation, exploitation, handling and technological applications that will encourage increased productivity and have a decisive influence on social reformulation.

Brazil's Amazon and west-central states encompass some 32 million inhabitants and approximately 55 million head of cattle. Livestock raising in the region is currently engaged largely in beef cattle and buffalo distributed throughout pastures amounting to more than seven million artificial hectares. This new herd, in on-going expansion and formation, is controlled by private enterprise and will go to both domestic and international markets. To this end, modern meatpacking and storage facilities are being implemented in the main livestock centers. It is therefore indispensable to improve the herds' health and yield substantially.

At the XIV Regular COSALFA Meeting, the member countries unanimously selected the topic of "The importance of biotechnoly in livestock development." To fulfill that resolution, we are gathered here today with this very distinguished audience, honored by the presence of persons who orient and conduct the development of livestock raising and agriculture in Brazil and

in this fine State: the Minister of Agriculture, Dr. Iris Rezende Machado and the Governor of the State of Goiás, Dr. Henrique Hercilio Santillo.

The new biotechnology, which has expanded extensively in the past twenty years, will exert a forceful impact on Nature, Man and Society. It constitutes a powerful instrument to forge great hopes for the solution of human and animal health problems, and for increasing agricultural and livestock productivity and yield.

Thanks to the initiative of Dr. Robert Paul Hanson, professor of Bacteriology and veterinary sciences at the University of Wisconsin, we have received vital cooperation from the International Center for Disease Control. Unfortunately, Dr. Hanson died suddenly on July 30th, 1987, on the job. He was a man of many and great accomplishments, who contributed to the development of livestock raising, veterinary sciences, and the training of professional personnel. His active, ever-striving personality, flowered in the creation of the International Center for Disease Control.

At this seminar, we are fortunate to have with us scientists of world-renown, who will provide us with information of great value in orienting and strengthening our technological research and development programs.

The scientific and technological gap existing today between the developed and developing countries becomes wider and wider every day, as the former move steadily ahead at dizzying speed. Biotechnology requires basic and technological research based on the availability of a critical mass of highly prepared and trained technical and scientific personnel supported by adequate, continuous funding.

The objective of our seminar is not only to obtain information for orienting our efforts, but also to strengthen joint action and work. In this sense, we urge you all to maintain a generous and sincere dialog, one which will enable us to reach our goals.

During this week, on the 24th and 25th, we shall hold the XV Regular COSALFA Meeting. We shall assess the performance of the national programs for the prevention, control and eradication of foot-and-mouth disease in 1987. And of major import will be our efforts to comply with Resolution XIII of the V Inter-American Meeting on Animal Health at the Ministerial Level, as approved by the Ministers of Agriculture of the countries of Americas. That Resolution calls on all the countries of the region to strive toward attainment of the goal of "Health for All in the Year 2000", by carrying out all action required to eradicate foot-and-mouth disease by the close of the Century. In that regard, RIMSA V urged PAHO and COSALFA to draw

up a hemispheric plan, including the mechanisms for its implementation, and to submit it to the XV Regular Meeting of COSALFA.

Nowadays, we have the knowledge and the technology to accomplish the goals of foot-and-mouth disease control and eradication. If we labor with sincerity, dedication, care and technical and operational efficacy, through efforts that include the rural producers, we can reach that so ambitious and so important goal for our South America. But the rural producers must provide active and conscious leadership, combined with the efforts of the specialized official services of state and local Ministries of Agriculture, of the veterinary profession and of the veterinary pharmaceutical industry.

It is within this framework that we promise our best efforts in offering the Pan American Health Organization's cooperation through its Pan American Foot-and-Mouth Disease Center.

In closing, let me express our gratitute to all those who have sponsored and collaborated to make this seminar and the XV COSALFA possible in this fertile soil of west-central Brazil, whose development is forged by men and women filled with love and vocation for the land and its proper agricultural use.

Thank you very much.

TALK by
His Excellency, Mr. Iris Rezende Machado
Minister of Agriculture of the
Federative Republic of Brazil

Ladies and Gentlemen,

It is with the most grateful satisfaction that I participate in this opening session of the "International Seminar on the Importance of Biotechnology in Livestock Development" and the "XV Regular Meeting of the South American Commission for the Control of Foot-and-Mouth Disease (COSALFA)."

A satisfaction that mounts as I see these two important events being held precisely in my State, whose rich lands and dynamic people --the pride of all us Brazilian and Goianians alike-- daily forge a reality in all sectors of human activity, a reality made of the vision of conquest and greatness inherited from our intrepid pathfinders, those who came to this area from the 18th century on. Their trek was arduous indeed, over the paths crossed our vast field and prairies, or along the generous meanderings of the Araguaia and Tocantins Rivers.

Greatness and conquest, yes. But we know nowadays that such ideals are not maintained without a clear perception of goals and a permanent disposition to struggle, energetically and determinedly, to overcome all obstacles.

Ladies and gentlemen, we are all together in this struggle, Brazil and its ten COSALFA partners, in constant pursuit of solutions for the problems that might affect the development of our herds. This is the solid impression that I draw from a reading of the agendas proposed for the meetings and discussions of the Seminar and the XV COSALFA.

With respect to the Seminar --intended to update the attendees about the accomplishments and possibilities of Biotechnology, and its possible effects on animal health and on livestock development in South America-- I would like to inform this illustrious gathering that the policy of my government regarding the solution of problems of tropical biology, is one that seeks to make Brazil a trusted and trusting partner in the process of producing technology. We are pursuing a context in which foreign contributions, financial or otherwise, whether arranged bilaterally or through multilateral agencies, do not collede with nor hamper the officially set objectives of scientific and technological progress. So whenever the interests are complex, or the quality of the participants may vary, we feel that the joint venture may be the best mechanism to accommodate international cooperation.

Within the Ministry of Agriculture, the importance of biotechnology and its effects in the animal sector were acknowledged in the terms of Directive (Portaria) No. 193, issued on August 25, 1987, which set up the National Commission on Agricultural and Livestock Biotechnolgy. With the participation of representatives from private industry and from the official agencies, the Commission has outlined the following goals: to formulate the agricultural and livestock biotechnological policies to be adopted by the Ministry; to establish an integrated plan of livestock and agricultural biotechnology seeking to integrate the strategies for biotechnological development and the national agricultural policies; to identify priority areas for the application of public funds; to identify the proper channels for close cooperation with other ministries; to train qualified personnel; to define systems for integrating the Ministry of Agriculture's efforts with universities, official research centers and Brazilian private initiative.

Regarding the agenda of the XV COSALFA meeting, which covers institutional and operational aspects of the on-going efforts to control and eradicate foot-and-mouth disease, we may readily recognize how relevant it is for Brazil. For our country has the world's larger cattle herd --about 127.6 million head-- and Goiás, our nation's major cattle-raising state, now accounts for 20 million head.

I would like to stress that success in the battle against foot-and-mouth disease, because of the evident impact on an overall improvement of the Nation's 'livestock assests', will generate acknowledged valuable repercussions on the main parameters of the country's socioeconomic development; on income, as it favors jobs, salaries and taxes; on wellbeing, as it generates food, raw materials and utilities; on health, in that it affects nutritional aspects while also reducing animal diseases.

Considering the foreign sector of our domestic economy, the sanitary guarantees ensuing from the FMD-control and eradication program have amply helped to raise Brazil to a position as one of the world's major exporters of beef. For the past nine years beef exports have earned an average of US$ 500 million annually.

But we have not been merely content with acknowledging the relevance of the topic and announcing working goals. We went to work, from words to actions. And we were successful, despite an overall picture that usually faces the developing countries, where the shortage of adequate funding contrasts with the magnitude and urgency of priority initiatives in the socioeconomic field. In this general picture, the government has obstinately sought to improve the domestic economy and balance the foreign accounts.

Thus, on July 27, 1987, at the World Bank Offices in Washington, D.C., my fellow Minister of Finance and I signed a loan agreement of US$ 41 million. The funds will be utilized in the "Control of Animal Diseases" project whose total cost has been set at US$ 108.4 million. Scheduled for implementation over the 1988-1993 period, the project aims to upgrade the quality and increase the yield of the beef, pig and fowl herds in 15 states of Brazil. It also aims to maintain and expand sales to the demanding international meat markets.

The project, proposed by the Ministry of Agriculture, will make it possible to strengthen and modernize the Secretariat for Animal Health Protection, the veterinary services in 15 states, and nine federal laboratories belonging to the network of the National Laboratory of Animal Reference (LANARA). Therefore, within criteria established by the Government, technical personnel will be recruited and existing personnel will receive technical and administrative training. Laboratory and field equipment will be acquired, while the physical plant at nine federal laboratories and four quarantine stations will be upgraded.

Of vital importance for the project's success is the animal health training for the rural producers who, as we well recognize, ultimately bear the major responsibility for all the work of prevention. Through personal contacts with the field veterinarians, they will acquire basic notions, orientation and educational messages to aid them in the prevention and treatment of their herds' diseases; of course, this process will ensure lower costs and increased yield. Under the coordination of the Secretariat for Animal Health Protection of the Ministry of Agriculture, this gigantic and worthy task will be conducted in the states by the respective State Secretaries of Agriculture and by the State Companies of Technical Assistance and Rural Extension (EMATERES).

Along this same line of action, on July 24, 1987, in the city of Porto Alegre, State of Rio Grande do Sul, the Ministers of Agriculture of Argentina and Uruguay, and I, cosigned an Agreement of Technical Cooperation for Control and Eradication of Foot-and-Mouth Disease. The Pan American Foot-and-Mouth Disease Center participated in the Agreement, joining with the three countries in action to be developed in the area southeast of the Plata Basin. The project will, in essence, encompass the Provinces of Corrientes and Entre Ríos in Argentina, the State of Rio Grande do Sul in Brazil, and all of Uruguay, where the livestock raising activity is of especial importance within the overall agricultural economy.

Let us consider the expressive data relating to the Foot-and-Mouth Disease Control and Eradication Agreement: it covers an area of 650,000 square kilometers, having about half a

million livestock establishments with an estimated 33 million cattle, 40 million sheep and 31 million pigs.

Another relevant aspect we should stress is that the 13.5 million inhabitants of the region comprise one of the world's highest per-capita percentage of beef consumers. Moreover, that the zone is a net exporter of beef makes it of vital national and international importance.

Through this Agreement, which will make maximum use of the technical, financial and human resources normally available in the national structures of animal health defense and control, and whose budget has been set at US$ 1.5 million for the three nations, we are finally breaking away from the traditional, unsuccessful approach of isolated, individual activity. For one of the essential characteristics of the problem area is that the livestock sector is exposed to similar and interdependent epidemiological risks.

Although the high incidence of foot-and-mouth disease in the Plata River Basin area in past decades has been substantially reduced in the 1980's, especially in Uruguay and Rio Grande do Sul, official statistics for the 1980-85 period still indicate annual physical losses of about US$ 14 million. This amount may be regarded as of minor importance. However, the private sector still requires expenditures in excess of US$ 30 million annually to ensure adequate levels of sanitary protection.

Ladies and gentlemen, we would not be gathered here today without the indispensable support and efforts of the Pan American Foot-and-Mouth Disease Center. Since its founding here in Brazil in 1951, it has developed the competent and valuable record that we all readily acknowledge, in pursuit of the continental goals set for it. On my own behalf, and as Minister of Agriculture, I would like at this time to thank the present Director Dr. Raúl Casas Olascoaga, and all of his fine team, for their dedicated and careful performance. The high technical and scientific standards they have attained and maintained have earned the admiration and prestige that the Center now deservedly ensoys in the specialized world community.

In the technical personnel and scientists from the countries, international agencies and private organizations, here gathered to carry out the very meaningful agendas of the Seminar and COSALFA XV, I recognize the evident vocation for the good fight, and the vision of conquest and greatness that I mentioned earlier. Each of them, in his or her respective area, endeavor and discipline, knows that nothing will be achieved without the energy and determination of true observers.

So I am pleased to welcome you all to Brazil, with the certainty that these two international gatherings, here in Goiania, are a benchmark in forming goals, defining strategies and honing instruments in the joint fight against foot-and-mouth disease. The Brazilian Government will continue to render its full support. So let us work together, with the same strong will and confidence in the future that have brought us this far.

Thank you very much.

PROGRAM AND PARTICIPANTS

INTERNATIONAL SEMINAR ON THE IMPORTANCE OF BIOTECHNOLOGY IN LIVESTOCK DEVELOPMENT

Goiania, Brazil, 21-23 March, 1988

PROGRAM

Monday 21

09:00-10:15 a.m. Opening session: Addresses by Dr. Raúl Casas Olascoaga and Mr. Iris Rezende Machado.

10:15-11:15 TOPIC 1. Biotechnology in 1988.

1.1 Achievements and perspectives in biotechnology: definitions, perspectives in human and veterinary medicine, the pharmaceutical industry, food industry, agricultural production and environmental protection.
Dr. Thomas M. Yuill.

11:15-12:30 p.m. TOPIC 2. Areas on research in biotechnology and application for animal health.

2.1 Biotechnological approaches to understanding pathogens and pathogenesis.
Dr. Ann Palmenberg.

02:00-03:00 2.2 Biotechnology for improved diagnosis and epidemiological surveillance.
Dr. Geoffrey Letchworth.

03:00-04:00 2.3 Biotechnology and the new generation of vaccines.
Drs. Geoffrey Letchworth, Ann Palmenberg and William Kenealy.

04:15-06:00 Panel

Tuesday 22

08:30-11:15 a.m. TOPIC 2. (cont')

2.4 Industrial microbiology, enzymatic fermentation technology and bioprocessing.
Dr. William Kenealy.

2.5 The acquired immunodeficiency syndrome (AIDS).
Dr. William Kenealy.

2.6 Applications of biotechnology to animal production.
Dr. Neal First.

11:15-12:30 p.m. Panel.

02:00-03:00 p.m. TOPIC 2. (cont')

2.7 Health issues in international genetic exchange.
Dr. T.H. Howard.

03:00-05:00 TOPIC 3. Infrastructure for the management and application of biotechnology.

3.1 Models for the integration and development of biotechnology: orientation, organization, structure, strategies, integration and organizational rules.
Dr. Thomas M. Yuill.

3.2 Possible effects of biotechnology in livestock production in Latin America.
Dr. Raúl Londoño Escobar.

05:00-06:00 Panel.

Wednesday 23

08:30-11:00 a.m. TOPIC 4. Role of PAHO in the development of biotechnology.

4.1 Technical cooperation between developed and developing countries.
Dr. Gabriel Schmunis.

4.2 The Pan American Foot-and-Mouth Disease Center, its participation in research and application of biotechnology in animal health.
Dr. Raúl Casas Olascoaga.

4.3 Normative aspects for the handling of technology. The use and safety of techniques.
Dr. Pedro N. Acha.

11:00-12:30 p.m. Panel.

02:00-05:00 Working groups: Conclusions & Recommendations.

05:00-06:00 Closing session.

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RECOMMENDATIONS

RECOMMENDATIONS

The XV Regular Meeting of the South American Commission for the Control of Foot-and-Mouth Disease (COSALFA), which was held in Goiania, Brazil, on March 24 and 25, 1988, studied and revised recommendations issued by the International Seminar on the Importance of Biotechnology in Livestock Development, held in Goiania on March 21-23, 1988, and adopted them as Commission resolutions.

RESOLUTION I

AREAS OF BIOTECHNOLOGICAL RESEARCH AND APPLICATION IN THE FIELD OF ANIMAL HEALTH

WHEREAS,

The advance of new biotechnology offers a wide perspective for national development in the field of livestock raising and animal health;

Its development and application to the livestock sector must form part of, and be coherent with, current national policy in the field of biotechnology;

There must be a permanent evaluation of the realities and perspectives of biotechnology in the field of livestock raising;

It is indispensable to have solid scientific knowledge and sufficient ability to produce and evaluate appropriate and effective technologies, thereby avoiding the waste of money, time and human and physical resources in dealing with or adopting unproven or ineffective technologies;

It is necessary to have improved and permanent coordination and integration, both nationally and internationally,

THE XV REGULAR MEETING OF COSALFA HEREBY RESOLVES:

1. To urge country's Ministry of Agriculture to create and/or incorporate, where none exists, a National Commission for coordinating the use and development of biotechnology in the field of livestock raising and animal health.

2. That Ministries of Agriculture should promote the development of biotechnology at the highest level taking care that such be done under the following conditions:

a) the integration of participating sectors --researchers, adaptors and appliers of the technology and its users;

b) intersectorial coordination;

c) multidisciplinary work, and

d) orientation of its development for solving the country's problems with the highest priority in the field of livestock raising and animal health.

3. That Ministries of Agriculture should promote and/or carry out an analysis of the situation involving the development and availability of the biotechnology that can be utilized in production and animal health, which, in turn, will serve as a planning human-resource training, infrastructure development and requests for financial resources.

4. That international agencies, which contribute to technical development in this field, pay heed to national concerns, offering support and promoting coordination and integration while avoiding the duplication of efforts.

RESOLUTION II

NEW BIOTECHNOLOGY AND EMBRYO TRANSPLANTS

WHEREAS:

New methods of biotechnology related to embryo transfer are undergoing rapid development;

These advances can be used in individual herds as well as in livestock rapid development;

Procedures described in the Manual of the International Society of Embryo Transfer, based on the Commission on Norms for the Zoonosanitary Code of the International Office of Epizootics (OIE) for the handling and washing of bovine embryos, enable the transfer of embryos to be the safest form for international trade in genetic material;

The adoption or increase in the use of embryo-transfer techniques in South American countries could serve to increase each country's livestock productivity, without neglecting other handling practices;

Many countries have import and export regulations that are not in agreement with scientific advances inasmuch as the elimination of risks is concerned, such as in the case of embryos with an intact zona pellucida and that have been properly washed and treated,

THE XV REGULAR MEETING OF COSALFA HEREBY RESOLVES:

1. To call for the revision and adaptation of current legislation for the importation and exportation of bovine embryos in order for it to be applicable and effective.

2. That semen used for embryo production must proceed from lots approved by the Ministry of Agriculture based on guidelines from the Commission on Norms for the OIE Zoosanitary Code.

3. That any firm producing embryos for export must be registered and certified by the Ministry of Agriculture of the exporting country.

4. To study the possibility of establishing in the exporting country a bank for frozen serum from potential donors of genetic material for export.

5. That, within the context of animal-health programs, countries should standardize sanitary regulations regarding the movement of genetic material.

RESOLUTION III

THE APPLICATION OF BIOTECHNOLOGY IN FOOT-AND-MOUTH DISEASE CONTROL AND THE ROLE OF THE PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER

WHEREAS:

Consideration is given to the scope and implications of RIMSA-V Resolution XIII in regard to the eradication of foot-and-mouth disease in South America;

There is a need to make use of advantages, whenever such exist, that could result from the application of new biotechnology to solving problems of vesicular diseases,

THE XV REGULAR MEETING OF COSALFA HEREBY RESOLVES:

1. That the Pan American Foot-and-Mouth Disease Center (PAFMDC) give priority to those aspects by which biotechnology can produce results in the shortest period of time based on demands posed by the goal of a regional eradication of the disease.

2. That the PAFMDC organize, maintain and place at the disposal of each country a technical and scientific databank dealing with biotechnological advances and achievements, assigning priority to data concerning vesicular diseases and subsequently to other aspects of animal health and production, according to its possibilities.

3. That the PAFMDC continue and expand joint projects with laboratories in each country, promoting and coordinating actions leading to the exchange of experience and the development of human resources.

4. That the PAFMDC continue to serve as a Reference Center, providing services and transferring technology in those areas where biotechnology contributes to solving critical problems such as diagnosis, vaccine control, the production of reagents and personnel training.

5. To request that the PAFMDC, as quickly as possible, place at the disposal of each country information concerning laws for the handling and use of products generated by new biotechnology.

6. To urge the Pan American Health Organization (PAHO) and other national and international agencies for technical and financial cooperation to support the development and incorporation of new biotechnology in those areas that can significantly contribute to eradicating foot-and-mouth disease in the region.

RESOLUTION XI

TRIBUTE TO LATE PROF. ROBERT P. HANSON

WHEREAS:

The distinguished professor Robert P. Hanson, an enthusiastic promoter of the International Seminar on the Importance of Biotechnology in Livestock Development, passed away before it was held;

During his professional career, professor Hanson was a tireless researcher and a master of veterinary science,

THE XV REGULAR MEETING OF COSALFA HEREBY RESOLVES:

To request that Dr. Raúl Casas Olascoaga, director of the Pan American Foot-and-Mouth Disease Center (PAFMDC) act on behalf of participants at the Seminar in sending the wife and relatives of Dr. Hanson and the University of Wisconsin a special message expressing our deepest sympathies and esteem.

(Approved in the Plenary Session
on March 25, 1988)

A P P E N D I C E S

1.1 ACHIEVEMENTS AND PERSPECTIVES IN BIOTECHNOLOGY: DEFINITIONS, PERSPECTIVES IN HUMAN AND VETERINARY MEDICINE, THE PHARMACEUTICAL INDUSTRY, FOOD INDUSTRY, AGRICULTURAL PRODUCTION AND ENVIRONMENTAL PROTECTION

Dr.Thomas M. Yuill, University of Wisconsin-Madison, USA

Biotechnology is a mixture of dreams and realities. What is biotechnology? Everyone has his or her own definition. The broad definition offered by the office of Technology Evaluation of the Congress of the United States of North America, which was included in the materials sent by Dr. Acha, states, "Biotechnology is any technique or method which employs living organisms to produce or modify products, improve plants or animals, or create microorganisms for particular uses." According to this definition, my grandfather was a biotechnologist with his program of dairy herd improvement in Canada, and his grandfather was also a biotechnologist with his small fermentation plant for the production of Scotch alcoholic beverages. It is obvious that biotechnology isn't something very new. Nevertheless, contemporary biotechnology with its new technical armamentarium, has the potential to bring about very significant changes in various areas of biology at an increasingly high rate of speed.

I propose in this introductory talk to provide a rapid overview of certain areas within biotechnology with the hope that we will be able to identify areas of common interest, and open lines of communication between us all to discuss and understand these areas, and the issues that relate to them.

The impacts and future perspectives of biotechnology perhaps are more clearly recognized and understood in medicine, including veterinary medicine. Biotechnology can provide a better understanding of the structure of pathogenic organisms and the functions and biological consequences that these structures and functions have. For example, the technology that permits the identification and description of specific structures is now available. Understanding the structure of viral glycoproteins, and their relation to cell receptors, can provide an understanding of the underlying mechanism of tropism of cells and tissues, and through that knowledge, insights into the susceptibility of vertebrate hosts and arthropod vectors.

Frequently, molecular virologists utilize models as a system to approach their research in understanding basic mechanisms. These models utilize virus-host or virus-vector systems, utilizing viruses representative of large medically important groups. Usually, models are aimed at virus or pathogenic agents which are well known, with a broad basis of understanding in the published literature. By utilizing well known agents, the speed of discovery is much more rapid than with agents that are relatively little understood. Moreover, many of the agents used

in these laboratory models are useful because the viruses themselves are likely to be less pathogenic for humans or animals, permitting their use in the laboratory without the requirement for a high degree of biosecurity and containment. Nevertheless, it is very important that the biotechnologists not have their viewpoint restricted by the four walls of their laboratories, in order not to lose the vision that their work should relate directly to real problems that occur in the field. Close communication is needed between individuals with the responsibility for animal health and basic scienists, so that models appropriate to animal health are selected. The familiarity with field problems is necessary in order to chose models that are most appropriate so that their application may address, sooner or later, important pathogenic agents. One must guard against the error of spending ones working life with models that ultimately are not relevant to real problems.

Biotechnology is also improving very rapidly laboratory systems for detection and diagnosis of diseases caused by pathogenic agents. Diagnostic tests such as molecular hybridization using cDNA probes to detect the presence of pathogenic agents in cells or tissues of infected animals are becoming readily available for the diagnosis of a variety of infectious and parasitic agents or diseases. Nonetheless, these tests have their problems. In some of the following talks, the speakers will provide insights into what some of these problems are.

Monoclonal antibodies are also becoming available increasingly for the diagnosis of infectious and parasitic diseases. These monocolonal antibodies, produced in mice, are useful because of their high degree of specificity. Monoclonal antibodies can be used to detect very specific epitopes possessed by given agents, or even their specific variants. Monoclonal antibodies can also be produced to common antigens shared by a group of related organisms. Either group --or species-- (variant -) specific monoclonal antibodies can be used as laboratory diagnostic standards. They can be mass produced, and are relatively easily distributed and preserved in diagnostic laboratories. They provide the advantage of flexibility in their use in immuno-fluorescence or immunoperoxidase, for the detection of virus or other pathogenic agents. As you will hear in later talks, the development and testing of monoclonal antibodies, and their certification as reliable diagnostic reagents is extremely expensive, and is not something that every diagnostic laboratory can undertake.

ELISA tests are also becoming increasingly popular for laboratory diagnosis of infectious and parasitic diseases. These tests are useful for detection of antigens or for specific antibodies.

The key factors for the use and interpretation of new tests are the same ones with which we have had to contend for any

laboratory diagnostic test. It is important to know the limits of specificity and sensitivity. Setting these limits requires effective communication between the biotechnologist, the scientist developing and adapting the technology to the specific test, diagnostic laboratory program directors, and officials responsible for animal health programs, in order to establish an adequate scientific and epidemiological base for interpretation of the tests themselves, and for the use of these data in decision making for animal health program delivery. Important questions must be dealt with such as the use to which the test results will be put, implications of false/negative or false/positive results sampling strategies in the field, and relative costs of providing the diagnostic service.

Many of the new tests, such as the ELISA, are rapid, inexpensive and relatively unsophisticated. Thus, it is possible that many of these tests will find their way directly into the hands of the producers and their employees. When this happens on a large scale, regional-or national-level epidemiological surveillance is lost, if there is not an adequate flow of information (results) from those conducting the tests and measuring the presence of infectious and parasitic diseases, to those responsible for the broader epidemiological surveillance. The potential loss of epidemiological data that could occur when producers rather than animal health laboratories carry out routine testing is a matter for serious discussion in the development of animal health policy.

Biotechnology also provides good methods that permit evaluation of pathogenic agents, and their interrelationships. For example, the evaluation of various strains of rabies virus by monoclonal antibodies has provided an objective basis to the argument that cycles of rabies transmission in bats are different from those strains maintained in dogs or other wild animals, with relatively little transmission between bats and other species (with the exception of vampire bat transmitted rabies to domestic animals). This type of information is very useful in the design of field control programs.

Analysis of nucleic acid fragments following enzymatic digestion, i.e., fingerprinting, has also been very useful in development of epidemiologic-geographic mapping of medically important viruses. Fingerprinting can provide information about the distribution of virus subtypes, and their movement in populations of vertebrate hosts. Fingerprinting can be useful in determining if reinfection by related viral subtypes is occurring within population, over the course of time, as has been done with herpes viruses, for example. Fingerprinting has been used for the evaluation Bunyaviruses (California Group viruses) and flaviviruses (Yellow Fever) and provided insights into their interrelationships and evolution. Analysis of similarities and differences in the composition and sequences of nucleic acids has provided a basis for establishing the

relationship between given pathogenic agents. Studies of nucleic acid homology again raise the eternal questions that taxonomists always face: What is the biological significance of the differences that are observed? How great a difference must be observed for agents to be classified as truly different? For example, in studies done recently and usually at our University, the homology between Mycobacterium paratuberculosis, the causative agent of Johne's disease, and M. avium are extremely small, from the point of view of their DNA homology. Although there nucleic acids are practically identical, it is clear that their epizootiological cycles of maintenance, and their relative pathogenicities, are very different.

Even within antigenically identical strains of viruses, vary small changes in nucleic acid can produce significant biological and epidemiological changes in the behavior of the virus. For example, in rabies a single change at position 333 in the genome is associated with significant changes in pathogenesis and tropisms of the virus. Thus, interpretation of changes in nucleic acids sequences must be made with caution. Sometimes large differences in nucleic acid sequences have relatively little importance in the epidemiology and pathogenesis of the agent, and at other times, small differences can be critical. An area in which biotechnology has received a great deal of attention recently, and will receive a great deal more in the future, is in the development of new vaccines. There are articles practically every day in the popular press about new vaccines produced by recombination --that is the introduction of genes from one virus which may code for specific proteins, into another vector agent or organism, such as Escherichia coli, in order to produce expressed proteins on a commercial scale. The expectation that these new techniques can rapidly provide new products must be tempered by the technical realities and difficulties that most new production systems entail, especially when laboratory technology is expanded to commercial production levels.

This technology, that is the introduction of specific protein-producing genes into vector organisms such as Vaccinia virus, has produced results that are both interesting and extremely controversial. Biotechnologists and animal health officials must never lose sight of the fact that these new products cannot be applied in a social vacuum. The social and economic acceptability of these new products must be determined before widespread use is attempted. In other cases, where the genetic sequences responsible for the development of disease in the host are well known, it has been possible to remove those genes for pathogenesis in order to produce a modified or attenuated live virus vaccine. This approach has been used in commercial form in the vaccine for Pseudorabies (Aujezsky's disease) of swine, for example. Another approach, and one which gets away from the introduction of genetically altered organisms into the field, is the development of anti-idiotypes. In this technique, a

monoclonal antibody that reacts specifically key antigens of a virus or other pathogenic agent, is used as an antigen to produce antibodies against the original antibodies. These anti-antibodies (anti-idiotypes) have a chemical structure that is very similar to that of the antigen of the original virus or pathogenic agent. Thus, they can be used in place of the original immunizing antigen. Thus, the anti-idiotypes can be used in place of the original immunizing antigen, without the risks associated with live vaccines, or the problem of producing good immune responses to killed vaccines. These vaccines must have two important characteristics: (1) they must be effective in preventing infection and disease by the pathogenic organism that they mimic, and (2) must not create problems of hypersensitivity or immune complex disease. The effectiveness of anti-idiotype vaccines will depend on the nature of the antigen that they mimic, and the type of immune response that antigen elicites and its role in host immunity to the pathogen --in many instances an area that is not well understood.

An overwhelmingly important factor in the success of vaccines in preventing disease is that those vaccines must find their way to the animal. A vaccine that remains in good condition, but always in the refrigerator or freezer, cannot produce good herd immunity if it is not applied. Unfortunately, in many instances we have been unable to convince the livestock producer of the importance of diseases in his or her animals, and the importance of accepting costs of animal health programs to receive the benefits of good immunization through vaccination. Thus we must increase our efforts in establishing contact with the producer, in order to educate him or her about the benefits that new vaccines can bring, assuming that they are effective, safe, and relatively inexpensive. We must involve the biotechnologists, related scientists, officials responsible for animal health, and field veterinarians in the education of the public in order to promote programs of animal health in the field.

We must educate government officials who have the responsibility for establishing regulations and laws relating to biotechnology. There is always the temptation to copy norms and rules from other countries, frequently without a solid scientific base or an assessment of the risks and benefits within each specific country, or each agro-ecologic region of a country, in order to establish a sound scientific base for these laws, rules and norms.

Biotechnology is becoming increasing important for the pharmaceutical industry for the production of new drugs, biologicals such as hormones, fine chemicals, and other substances. These products, however, are not without their own controversial aspects. For example, the development and biotechnological production of bovine somatotropin (bovine growth hormone) for the increase in milk production is a topic of considerable debate in several industrialized countries. It

seems likely that their use will be adopted by many milk producers. One of my concerns about the use of these products in developing and industrialized countries is that milk producers may want to use them as a substitute for good programs of management, nutrition, and genetic improvement. There is still no good vaccine or drug to substitute for good management.

Transgenic animals, that is animals that have genes introduced from other animals, including other species, potentially may improve production efficiency significantly. Assuming that these animals are well managed, under optimal conditions, biotechnology can accelerate animal production in many areas and with a variety of species. Genetic improvement has become an extremely active area in biotechnology. Embryo transfer is no longer a novel approach. A division of embryos into four or more individuals is likely to be commercially available in the near future. Soon, techniques will permit the selection of the sex of individuals, and transfer of specific genes. One might asks what is the importance of this highly technical approach to genetic manipulation for developing countries? None of this high technology will pay off if management is so inadequate that these new animals do not have the conditions required to achieve their genetic capacity. It seems to me that we also need new parameters in genetic criteria for production under tropical conditions. Sophisticated genetic technology, and the genetic base itself, is located in temperate zones of the industrialized countries, particularly for dairy cattle. It remains to be seen if this genetic material is also optimal for tropical areas.

Genetic improvement in developing countries requires reliable information. We need good data bases, herd registers, and computerized archives in order to measure and compare production of different genetic lines under the conditions of the tropics. Unfortunately there are very few record systems available for the tropics that run on microcomputers that permit good record keeping for animals in individual herds.

Perhaps one of the most promising areas in biotechnology for the improvement of animal production will be provided by agronomists, through the improvement of forages. It is clear that in many areas in the tropics, the principal limiting factor for livestock production is the availability of forages and other adequate sources of animal nutrition throughout the year. Plant biotechnology can bring improved forages that will permit the intensification of ruminant agriculture in given areas. This will bring the dual benefits of increased production, while alleviating some of the intense pressure on the natural resource base with the conversion of forests and other plant communities to pasture for extensive animal agriculture. There is hope that plant biotechnology will improve nitrogen fixation and improve soil fertility, make available forages with a high percentage of digestible protein in palatable form, while providing important byproducts such as firewood and construction materials.

Biotechnology is also expected to improve the availability and utility of single cell protein. These systems can greatly improve sources of protein for animal feeds or perhaps even serve as a source of protein for humans.

The future of biotechnology is bright with promise for the developing nations. It is absolutely indispensable that biotechnologists work closely with those involved in agricultural and rural development to create systems that bring the broadest benefits possible to rural and urban citizens alike.

2.1 BIOTECHNOLOGICAL APPROACHES TO UNDERSTANDING PATHOGENS AND PATHOGENESIS

Dr. Ann Palmenberg, University of Wisconsin-Madison, USA

This conference provides an excellent opportunity for a research scientist such as myself to share some new and exciting biotechnology discoveries with an audience which can potentially help to translate these laboratory developments into practical applications in the field. I think that it is important for you to become familiar with the approaches that are being used in high-technology laboratories, so that you are in a position to make informed decisions on which of these new developments actually represent good science, and which ones are simply attracting attention in the press.

The techniques we apply in my laboratory all come under the general heading of "biotechnology". There is nothing magical about this term. Biotechnology really just refers to those modern molecular tools that a researcher can use experimentally to dissect and analyze a biological system. For example, we use computers, nucleotide sequencing, genetic engineering, monoclonal antibodies and crystal structures in our laboratory in order to construct pictures of viruses and their life cycles, in the same way that a carpenter might use his tools to build a house. If you have an understanding of what these tool can do, then it becomes easier to understand the value of the resulting science. In my talk, I will try to show you examples of how biotechnology is being applied towards aftosa virus (FMDV), in attempts to understand how these viruses make cattle sick, and what preventive measures, like new effective vaccines, can be developed to help with this problem.

My laboratory is located in the United States and therefore we cannot work directly with aftosa. However, we can work with other similar viruses as model systems. As you will see today, many of the new biotechnology developments that we have discovered by working with the model systems are very relevant to aftosa. I hope to soon be able to bring these new discoveries directly into your laboratories here in South America as part of collaborative efforts to improve vaccines and diagnostic techniques. Much of my work is completely new and at this time has not even been published in the United States. I hope today to give you your first visual picture of the aftosa virus structure.

The aftosa viruses are members of a larger group of viruses called picornaviruses. "Pico" means small. "RNA" means the type of genetic material contained within these viruses. Other familiar members of this group include the enteroviruses (for example polio, coxsackie and hepatitis A), and the rhinoviruses

(common cold virus). Yet another subgroup of the picornaviruses are the cardioviruses. These primarily infect mice, but biologically and biochemically, they are the most closely related of all picornaviruses to aftosa. However, since all picornaviruses are very similar to each other, much of what we learn about any one member of the group is important for the other members, too. Though it may seem strange, by studying cardioviruses in mice, we can learn very significant things which are directly applicable to aftosa in cattle. For this reason, and because we are permitted to work with cardioviruses in the United States, my laboratory has extensively studied the cardioviruses as models for the more pathogenic aftosa viruses.

Over the past few years, much experimental work has focussed on the outer structure of picornaviruses. This work has involved physicists and mathematicians as well as biologists in attempts to determine and understand the protein crystalline structure of the virions. This is the part of the virus that is involved in antigenic response and binding the virus to the surface of a cell to initiate the infectious cycle. Recently, the structures of a number of different viruses were completely determined. Several of these were plant viruses, but the resolved structures now also include representative picornaviruses: polio, Mengo (one of the cardioviruses) and rhinovirus. Work is in progress on the structure of aftosa, coxsackie and hepatitis A.

Each of these viruses is made up of 60 copies of a protein subunit. For all picornaviruses, including aftosa, each one of these subunits comprises four individual proteins which can be separated from each other if the viruses are denatured. With only four proteins to make up the entire capsid structure, the picornaviruses are relatively simple biochemically and this facilitates our understanding of the crystal structure. The structures of larger DNA viruses or viruses with complicated glycoproteins on their surfaces would be much more difficult to analyze.

Figure 1 shows what the three largest proteins in the mengovirus look like. Through use of very powerful computers, we have determined which exact amino acids of each protein fit into particular portions of these structures, and by doing so, have learned which specific residues are on the surface of the virion. The surface residues are the most important parts of the virus to be considered when we are trying to make a vaccine. Antibodies cannot find or touch those parts of the virus which are beneath the surface of the particle, so we do not need to worry about them for vaccine development. To illustrate the surface residues, we have developed a special computer program which allows us to make projections showing only the surface of the virus.

Figure 2 is a "road map" of the surface of one of the subunits of a picornavirus. Sixty of these triangles would make up one

completed virus. This is the protein surface of the virus which would be exposed as the virus circulates in the blood. Each one of the tiny squares represents a separate amino acid. We can use these maps in the same manner as real road maps to identify specific locations on the surface or to find out which residues might lie next to each other. Thus we can recognize and predict the regions which the antibodies might react with. We can also identify constant regions or other sequences which might vary between serotypes. Almost all the important biological information for understanding how a virus behaves is laid out in this map and encoded in these amino acids.

The particular road map shown in Figure 2 is for a rhinovirus, one of the first crystal structures of a picornavirus to be completed. One feature that is very noticeable is that the virus surface is not smooth; it has surface topography --hills and valleys, or canyons. For example, when all the subunits are connected on a virus, we can clearly identify a deep "canyon" running round the virus and circling the five-fold axis of crystallographic symmetry. (These residues are shaded in different levels of grey in Figure 2). This deep narrow groove is common to all rhinoviruses and the amino acids that line its surface are very well conserved among all serotypes. There are at least 100 different serotypes of rhinovirus (compared with only seven for aftosa), but all maintain a similar sequence within this narrow canyon. The residues which line the canyon cannot react with antibodies because the canyon is too narrow to permit antibody entry.

The virus surface also has high projections which stick far above the surface of the canyon. We believe the deep part of the canyon contains the cellular receptors, the contact points which are used by the viruses to interact with cells. Antibodies are too large to fit into these depressions and can only touch or interact with the tops of the higher projections. Thus the high parts or "mountains" on the virus surface must define the antigenic epitopes. These are the areas one must learn about if you want to make a vaccine. In contrast, to invent an agent which could potentially react with all the viruses within a group you would have to develop some way to interact with the deeper, common areas of the surface. Picornaviruses very cleverly escape the natural immune system by putting their antigenic sites on the higher exposed parts, yet at the same time, concealing the deep common areas which are constant among the different strains.

These computer projected road maps are useful biotechnology tools, because in addition to showing which residues lie next to each other, they can also be used as topographical maps, to identify which areas are high and which are low. The specific shapes of the higher areas are predicted to vary only slightly for each serotype, and the actual differences between serotypes are therefore physically very superficial from a

crystallographic point of view. A very small change in the identity or location of a residue on the surface of one of the peaks can make a profound difference in antigenicity, for example, while the surrounding structure and sequence remains virtually unchanged. For rhino and polioviruses the particular surface areas which react with antibodies have been very carefully mapped. This was done by making a panel of monoclonal antibodies against the virus, and then growing virus in the presence of one of the antibodies. Only those viruses which develop mutations to escape the antibody can survive. Then we can sequence the mutant virus genome to determine where the mutations are located, and thus determine within the crystal structure, the exact location of the neutralization epitopes.

Figure 2 also shows residues that are hatched in color (those with stripes). These represent the amino acids that have been determined to react directly with antibodies for this particular virus. If the virus could be viewed as a whole in this figure, we would see that these residues cluster together on the surface in different regions where the subunits come together. This finding is important because to shows that one cannot just concentrate on the sequence or structure of one small piece of a capsid protein in order to mimic a natural epitope. It is the total combined conformations of several proteins acting together which forms the intact antigenic site. Thus, to develop a peptide vaccine you must be very about careful which peptide or combination of peptides you pick because it is only when you have the right topographical and sequence combination that you will effectively mimic the identical surface that the native virus would present to an antibody.

During the last five years the nucleotide sequences of many picornaviruses have been determined. Given this kind of information, it is also possible to predict what viral proteins coded for by these genomes might look like. Since there are many picornaviruses for which the virion structures have not yet been determined, we thought it might be possible to predict new structures from this sequence information alone, with the help of a computer. To do this I first made a sequence alignment for the capsid proteins of more than 100 different strains of picornaviruses. This means that I identified those sequences which most probably correspond to the known structural elements in mengo, rhino and poliovirus. This information then basically predicted where the equivalent sequences might be within the new viruses and essentially creates theoretical crystal structure for that particular sequence. Although this computer method is certainly not as accurate as an authentic X-ray structure determination, it is relatively fast (2 weeks per structure) and can be applied to many different types of strains and sequences whose parental viruses may never actually be crystallized in the laboratory.

Of course, one of the viruses we were most interested in was aftosa. Figure 3 is a computer predicted road map of aftosa, serotype A. This diagram is based strictly on computer alignment of sequences and comparative data determined from the crystal structures of rhinovirus and mengovirus. The model represents a collaborative effort between workers in my laboratory, and those in the crystallography laboratory of Dr. Michael Rossmann at Purdue University. The computer helped us cut, paste and recreate segments from the determined structures (like mengo and rhino), based on the amino acid differences between the viruses (for example, between aftosa and mengo). This surface map looks somewhat different from that of rhinovirus although the basic structural characteristics (most of which are evident beneath the surface residues) are really still there. There is a shallow depression or canyon-like feature which may be conserved among all aftosaviruses. This may potentially be the location where the virus interacts with cells to initiate an infection. There is also a high "mountain" or prominent loop region on the surface, which represents the dominant antigenic epitope. There are also other surface features like small bumps and knobs, whose functions are presently unknown.

For many years it was believed that the structure of aftosa would contain only a single antigenic determinant because only one predominant epitope had ever been discovered and characterized. Current work with peptide vaccines has centered exclusively on this region, which is called the FMDV loop. From our predicted structure, we can clearly see that this is not really the case, and that aftosa, just like all other sequenced picornaviruses, actually has four different and distinct "elevated" areas that could potentially interact with antibodies. That these theoretical epitope areas were really antigenic in the normal viruses, was discovered only recently. At the same time that same time we were developing our computer structure model, Dr. Simon Bartelling and Dr. Adri Thomas were mapping neutralization escape mutants for FMDV type A at their laboratory in the Netherlands. This work was done independent of knowing anything about our putative crystal structure. They found that the major FMDV epitope on which they were working was not just part of a continuous protein (the FMDV loop) but rather represented the juxtaposition of several different pieces of protein. We have placed their mutant amino acids on our surface map and can see that they lie in related clusters. In this way, a second and third FMDV epitopes were discovered which lie across the axis between subunits, and a fourth epitope was determined to lie near the fivefold axis at the top of the virus. (These regions are hatched in Figure 3).

It is interesting to point out that for all picornaviruses epitopes which have been mapped thus far --polio, mengo, rhino-- the same four structural regions always seem to be involved. Thus what we learn about polioviruses can be used in making better vaccines for aftosa. The important lesson to remember is that if you want to make an effective peptide vaccine you must try to "see" the virus like the cow immune system does, and include determinants for all regions, and not just the FMDV loop. If you make a synthetic or killed vaccine against only one epitope it cannot possibly be as effective as one which takes all four areas into account. In the case of polio it turns out that the largest epitope is not really the most effective within a vaccine, though we do not yet know if this will also be true for aftosa.

There are many laboratory groups that are interested in learning about how antibodies specifically interact with viruses. This kind of information is essential to the development of effective vaccines. Some recent work with rhinovirus has been very helpful in this regard. Dr. Roland Rueckert and his group at the University of Wisconsin have isolated large panels of monoclonal antibodies against rhinovirus, and have been characterizing how these antibodies react with each of the four epitope sites on the virion surface. By doing careful neutralization curves for each antibody and for each neutralization escape mutation, they found that all the antibodies that are produced in an animal can be classified into one of three groups on the basis of its binding characteristics to the virus. The type of curve can tells us which particular kind of site the antibody binds to. Some of the subclasses are very good neutralizing antibodies, some are not good neutralizing antibodies. Obviously if you want to make a good vaccine you should elicit antibodies which are good at neutralizing. Why do they react this way?

When an antibody binds to virus the two arms of the antibody must both fit down onto the surface of the virus. We already know the arms will affix on the surface "mountains" and also that the antibody is so large that if one arm fits onto one subunit, the other arm must fit a different subunit. Dr. Rueckert has discovered that the main determinant of whether an antibody will be a good neutralizer or not is the distance between he symmetrical epitopes; that is, the distance between the epitope on one subunit of the virus and the next. If the sites are very far apart the antibody has to stretch to fit and will not bind very tightly to the virus. If the sites are too close together the antibody has to squeeze its arms to fit and again will not be able to bind very tightly to the virion. The ideal distance between sites is 90-135 angstroms. The best epitopes on the surface of a virus, and therefore those epitopes that will make the best vaccines, are those that are situated such that an antibody can form a good fit onto the surface of the virion. Peptides cannot effectively mimic the correct virion configurations very well, and are therefore rather poor at

eliciting natural, strong neutralizing responses in animals. The best immunogen against aftosa, or any other picornavirus, therefore remains the native virus particle, because only the natural viruses can accurately present the correct topography and also the correct sequences for a complete protective antibody response.

It is important that you remember, that we do not necessarily need to determine the specific crystal structure for every virus we are interested in; we can predict some structures with reasonable accuracy using computers. Though we expect the actual structure for aftosa type-O to be complete in a few months, we already expect that our computer-predicted structure might be a reasonably approximation, because the neutralization mutant maps show clustering of epitopes in the same locations as for other viruses. Comparison of nucleotide and amino acid sequence information now allow us to directly apply pieces of biological information learned about one virus, or type of virus, to other viruses from the same, or even different subgroups. It is also important to remember that knowledge of a crystal structure or predicted crystal structure for a virus can give us much valuable information about the shape and distribution of the natural virion epitopes. Only with this information, is it possible to understand how antibodies specifically react with viruses, and to design safe and effective antibody therapies (vaccines) for the prevention of disease.

Until now we have been talking about what happens on the outside of the virus. The picornavirus genome is a single piece of RNA. The arrangement of coding for proteins and different functions along the RNA is the same for all picornaviruses. Virologists who work with these viruses get very excited about specific differences in the various strains, but for the most part the general biology of all these viruses is very similar. When a picornavirus infects the cell its RNA genome is inserted into the cytoplasm and ribosomes start to make protein. A ribosome starts at one end of the genome (the 5' end) and goes all the way along to the other end (see Figure 4). However, by the time this protein is only about half made, a viral protease has been formed which goes to work to cleave the nascent protein into two pieces. One of the two new proteins thus formed contains the information for making the capsid (P1 proteins). The other contains the information for the virus to replicate and also for another protease. This is a special enzyme (called 3C) is characteristic of the picornaviruses, and while all the picornaviruses encode this particular enzyme, cells do not have it. This enzyme cleaves the bigger viral protein precursors to liberate the smaller proteins which are necessary for the virus to function. A large area of research in my laboratory is directed towards this enzyme because we think it may be possible to develop drugs to specifically inhibit this protein. Such drugs would then specifically inhibit virus infection. This is clearly an area for much more work in the future.

In addition to work on the proteins of picornaviruses, I would like also tell you a little bit about some novel types of genetic engineering we are doing with these viruses, which we hope may potentially produce new ways of making attenuated vaccines. The afthosaviruses and the cardioviruses, but not the rhino or polio viruses have a peculiar and unusual piece of nucleic acid as part of their genomes. This segment, which does not code for protein, includes within it a long stretch of poly(C), a long repetition of bases. It is very unusual to find a genome sequence in cells or even other viruses with so many C's in a row. This region is located in the 5' non-coding region of the virus genome before the sequences that begin coding for protein (see Figure 5). We wanted to know why the poly(C) region is present in these viruses and why it was apparently valuable to the virus. In order to do this we needed to make a full-length cDNA clone of the virus RNA.

It has been possible to construct clone full-length copies of some RNA viruses into bacterial vectors. This kind of genetic engineering was achieved for polio and rhinoviruses several years ago. However, when similar work was tried for aftosa it was not successful. Likewise we tried for five years to make a full-length copy of a cardiovirus genome. The problem was the poly(C) region. When you make those C's into DNA and put them into a bacterial vector for amplification, every bacteria carrying the sequence is killed. Apparently, is very difficult for the bacterial enzymes to copy these sequences, and as a consequence, the biochemistry of the bacteria is disturbed and the cell dies, whenever long poly(C) tracts are included in the sequences to be cloned. Unfortunately, some of these cardio and aftosaviruses have as many as 300 C's in a row, though some also contain as few as 50. Nevertheless, with some fancy biotechnology, we finally managed to get genomic tracts of Mengo RNA which were similar the full-length sequence of mengovirus. However, we had to leave many of the C's out of the construction in order to achieve this. Our biggest construction had 13 C's as opposed to 50 in the natural Mengovirus.

Since the longer poly(C) tracts are present in the wild-type viruses, we were sure that there must be a biological reason for their existence. Therefore, we were not very optimistic that our cDNA clones with the shorter C tracts would contain infectious sequences because, technically, they were biologically "incomplete". Nevertheless, one of my graduate student took the DNA from these clones and made it back into RNA then used that RNA to transfect Hela cells. Surprisingly, we got plaques on the HeLa cells showing that even with the short poly(C) sequences the cloned Mengo genomes were infectious! This result clearly means that it should now be possible to make analogous aftosa clones with short poly(C) tracts which are also infectious. Biotechnology has taught us how to do this.

What does all this have to do with vaccine development? When we tested out recombinant Mengo viruses with the short C tracts in tissue culture, again we were surprised to find that they behaved just like the wild-type viruses. We might have predicted that these viruses would be somehow defective because of their shorter C tracts, but they were not defective. In tissue culture we did every test we could think of. The genetically engineered viruses are not temperature sensitive, they grow exactly like normal viruses, give the same growth curves and they maintain the C sequences that we engineered. The C tracts are not enlarged or changed in any way by passage in tissue culture.

Why then are the long C tracts present in the natural isolates? To test what happened with the recombinant viruses in animals, we next carried out a series of experiments in mice, the natural host for Mengo virus. If one takes wild type virus, mengo or EMC, and injects it intraperitoneally or intracerebrally into a mouse, these viruses will normally kill the animal within 2-3 days. The LD_{50} is about 1 virion particle/animal (by intracerebral injection). When we did the same experiment with our recombinant Mengoviruses with short poly(C) tracts, however, we discovered that the short C tracts seemed to have attenuated the viruses to a very great extent! Though the recombinant viruses behaved in the same manner as the natural viruses in tissue culture, they gave a completely different response in the mice. The LD_{50} for the recombinant viruses was at least 1,000,000 times higher than the for the wild-type viruses. Inoculation with the recombinant viruses did not kill the animals, but rather served to immunize them very effectively against subsequent challenge with the wild-type viruses. When we sacrificed animals receiving the recombinant virus "vaccines" and tested them for virus, we found that the genetically engineered sequences within the recombinant viruses were stable. That is, they did not revert in any way back to the wild type poly(C) lengths. The virus we got back out of the mouse was the same as we had put in.

Therefore, we believe that we have found a new method to make attenuated, stable strains of cardioviruses, through creative genetic engineering of the viral poly(C) tract. Since the aftosa viruses also contain analogous poly(C) tracts, we are confident that our method will be applicable to the aftosa viruses as well. Though much remains to be done before genetically engineered vaccines for aftosa are ready to be tested, with your cooperation we may begin this work very soon. Therefore, I hope in the near future to be able to tell you that we have successfully isolated new attenuated strains of genetically engineered aftosa virus for vaccine development testing in animals. I think that application of our fundamental laboratory research in this new and effective way represents only one example of how biotechnology can potentially be used to make better and safer vaccines for the benefit of all agricultural development, and for enhancing the lives of people.

SOME IMPORTANT REFERENCES FOR THIS WORK:

Duke, G. M. and Palmenberg, A.C. Cloning and Synthesis of Infectious Cardioviral RNAs containing Short, Discrete Poly(C) Tracts. J. Virology, submitted. (1988).

Luo, M., Rossmann, M.G. and Palmenberg, A.C. (1988). Prediction of Three-Dimensional Models for Foot-and-Mouth Disease Virus and Hepatitis A Virus. Virology, 166:503-514.

Luo, M., Vriend, G., Kamer, G., Minor, I., Arnold, E., Rossmann, M.G., Boege, U., Scraba, D.G., Duke, G. and Palmenberg, A.C. (1987). The Atomic Structure of Mengo Virus at 3.0 A Resolution. Science 235:182-191.

Page, G.S., Mosser, A.G., Hogle, J.M., Filman, D.J., Rueckert, R.R. and Chow, M. (1988). Three-Dimensional Structure of Poliovirus Serotype 1 Neutralizing Determinants. J. Virology 62:1781-1794.

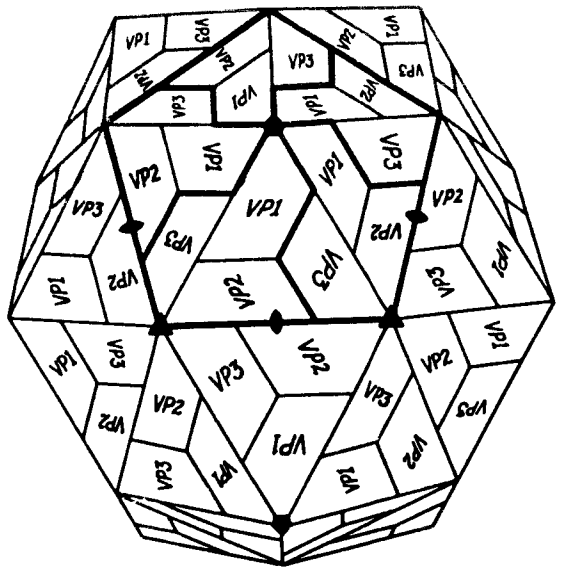
Palmenberg, A.C. (1987). Comparative Organization and Genome Structure in Picornaviruses. In: Positive Strand RNA Viruses (Brinton M.A. and Rueckert, R.R., eds.) Alan Liss, New York, p. 24-34.

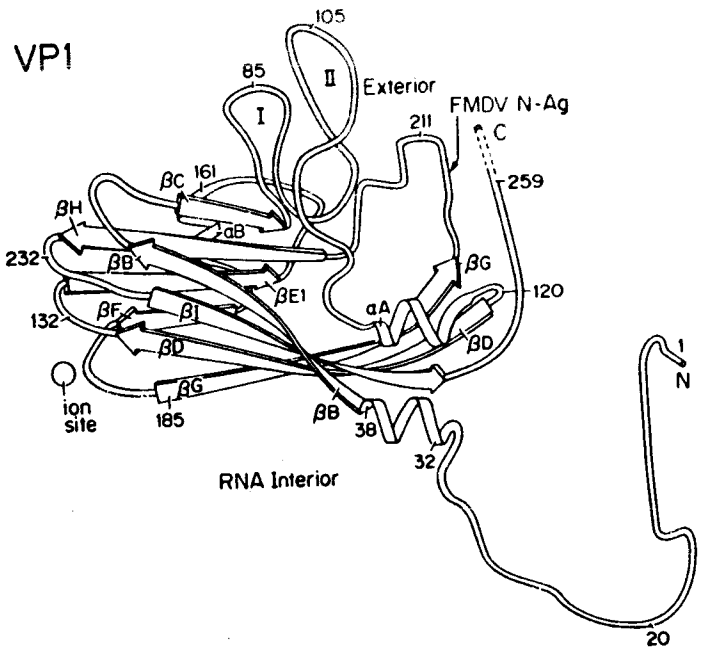
Palmenberg, A.C. (1987). Genome Organization, Translation and Processing in Picornaviruses. In: The Molecular Biology of Positive Strand RNA Viruses. (Rolands, D.J., Mahy, B.W.J. and Mayo, M., eds.). Academic Press, London, p. 1-15.

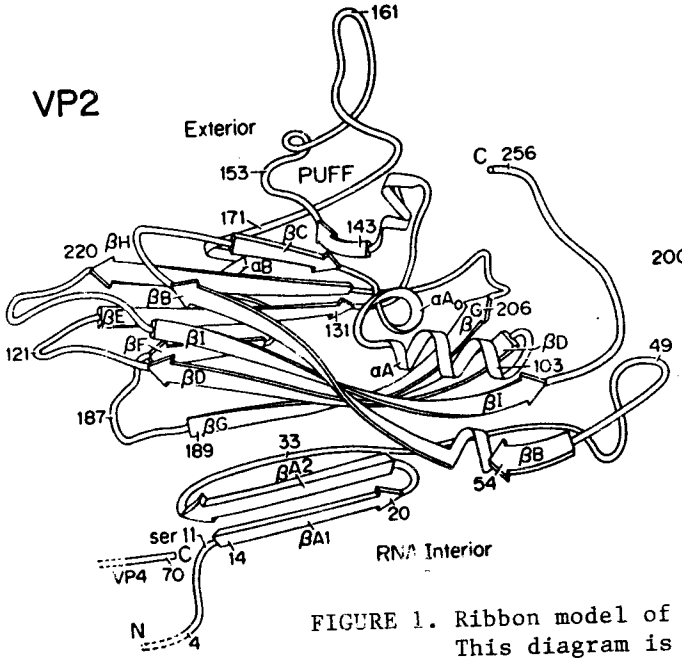
Palmenberg, A.C. (1989). Sequence Alignments of Picornaviral Capsid Proteins. In: Molecular Aspects of Picornavirus Infection and Detection. B. Semler and E. Ehrenfeld, eds. American Society for Microbiology, Washington D.C. p. 211-242.

Rossmann, M.J., Arnold, E., Erickson, J.W., Frankenberger, E.A., Griffith, J.P., Hecht, H-J., Johnson, J.E., Kamer, G., Luo, M., Mosser, A.G., Rueckert, R.R., Sherry, B. and Vriend, G. (1985). Structure of a Human Cold Virus (Rhinovirus 14) and Functional Relationship to Other Picornaviruses. Nature 317:145-153.

Rossmann, M.J. and Palmenberg, A.C. (1988). Conservation of the Putative Receptor Attachment Site in Picornaviruses. Virology 164:373-382.







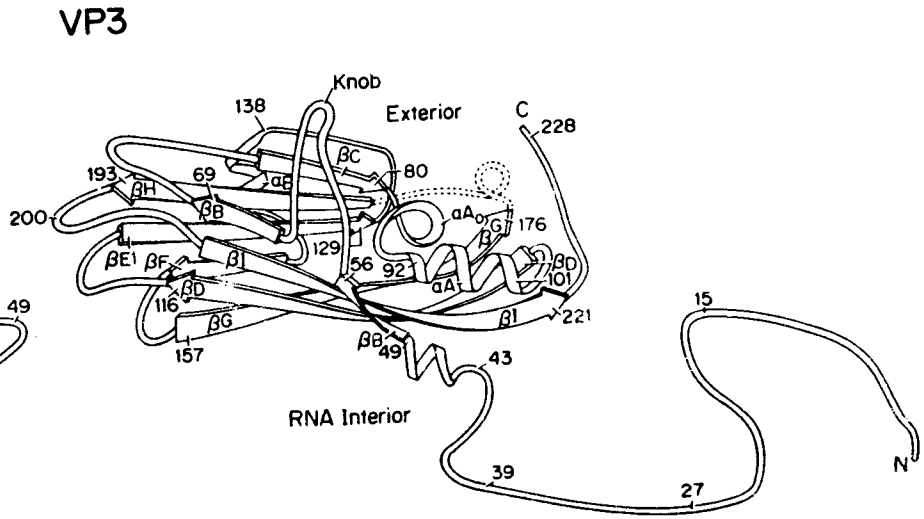


FIGURE 1. Ribbon model of the crystal structure of mengovirus. This diagram is reproduced from Luo, et al, 1988.

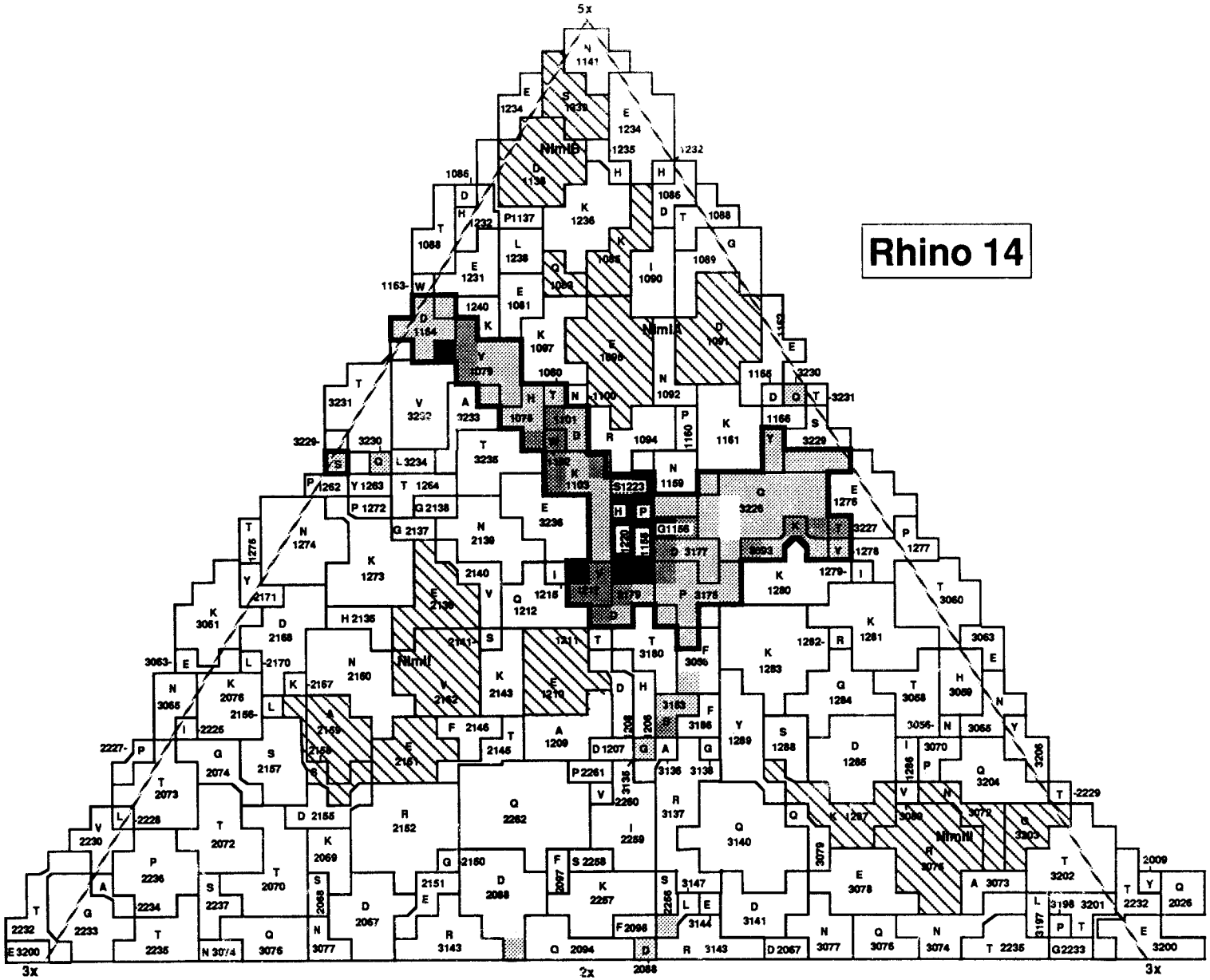


FIGURE 2. Schematic "road map" of the surface of one icosametric subunit of the rhinovirus 14 virion particle. This diagram is adapted from Rossman and Palmenberg, 1988.

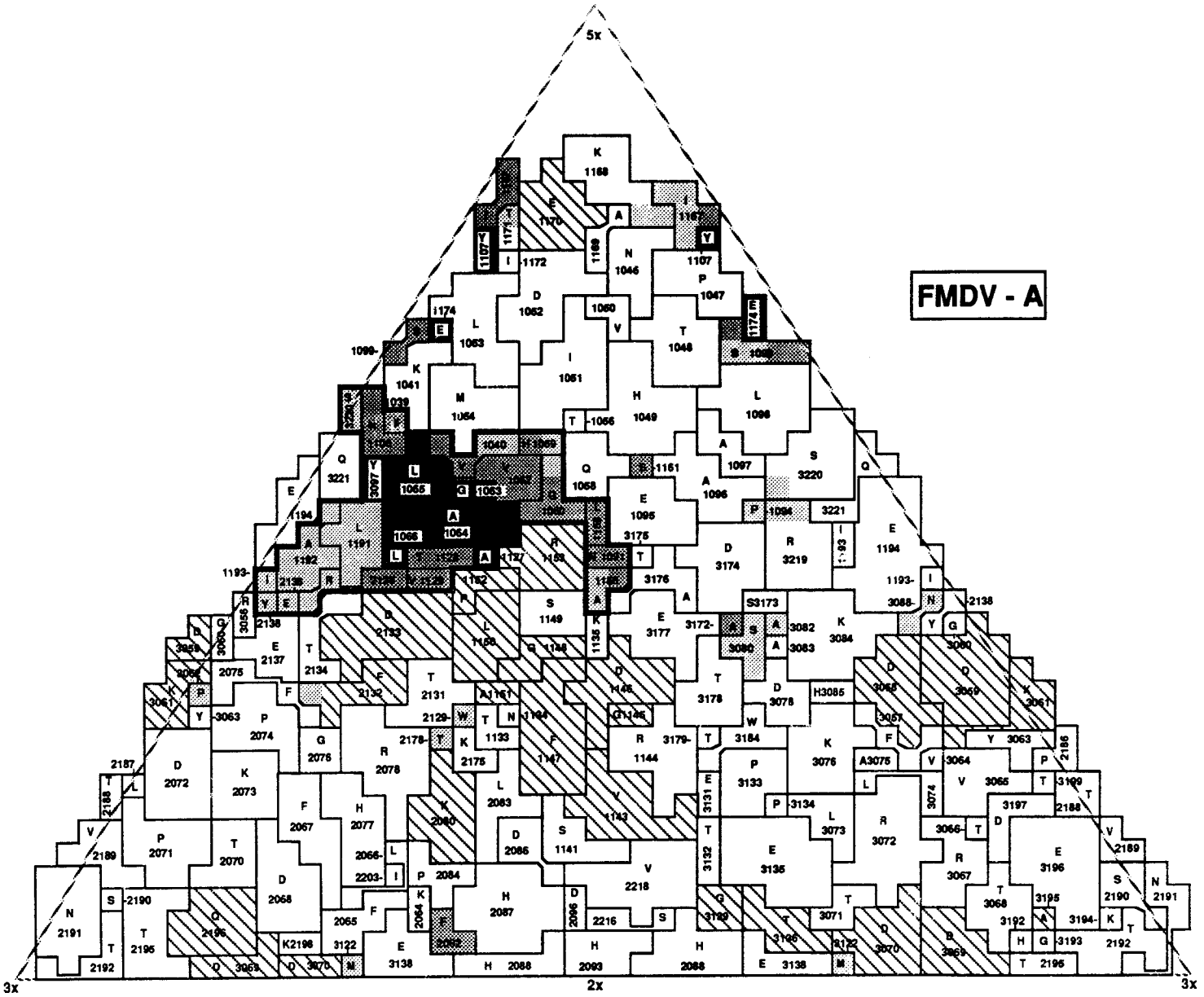


FIGURE 3. Schematic "road map" of the surface of one icosametric subunit of the aftosavirus type-O virion particle. This diagram is adapted from Lou, et al, 1988.

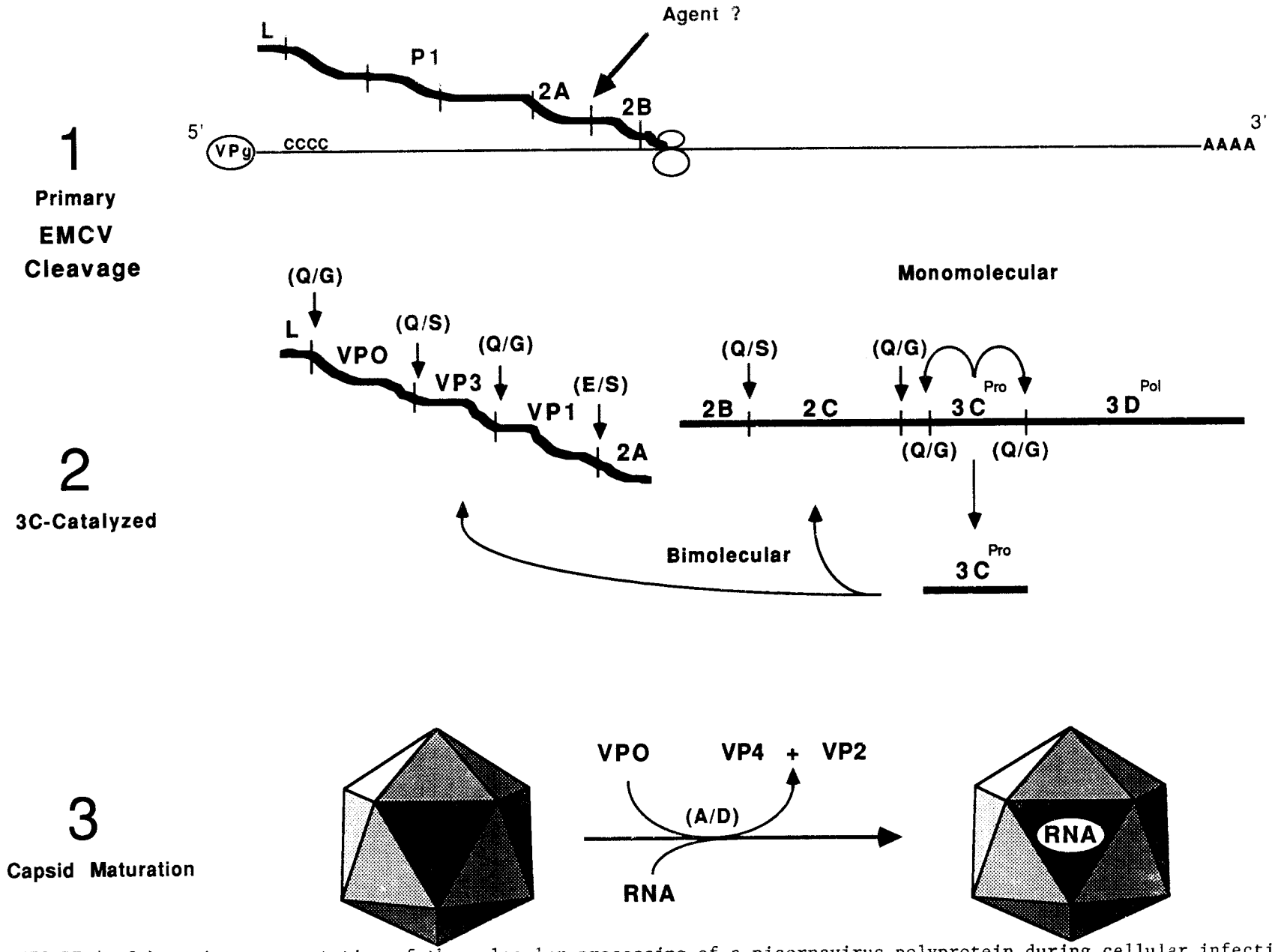


FIGURE 4. Schematic representation of the molecular processing of a picornavirus polyprotein during cellular infection.

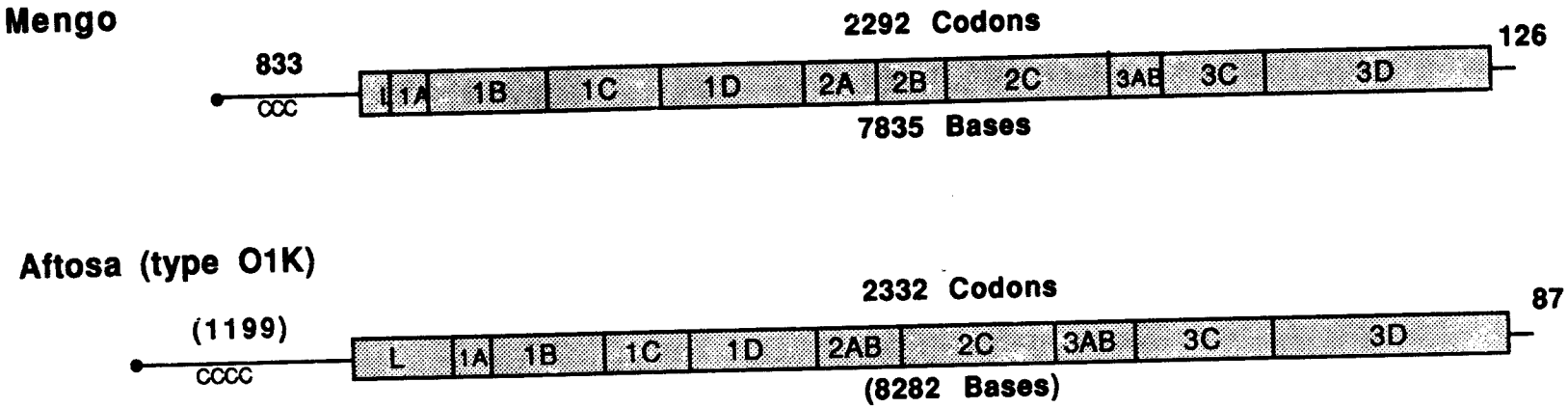


FIGURE 5. Genomic map of mengovirus showing the 5' non-coding location of the poly (C) tract which is important for attenuation of viral pathogenicity.

2.2 BIOTECHNOLOGY FOR IMPROVED DIAGNOSIS AND EPIDEMIOLOGICAL SURVEILLANCE

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Hybridomas: Hybridomas are mouse cell lines that are used to prepare virtually unlimited amounts of homogeneous and concentrated antibodies against known antigens. They can be used to identify antigens in tests where you have used antibodies in the past such as neutralization tests, ELISA, radioimmunoassay, fluorescent antibody, etc.

Let us go through the steps for hybridoma preparation (Figure 1) and point out some of the problems involved. The first thing you need to do is to immunize a mouse against whatever antigen you are interested in. If you do not have the antigen you probably will not be able to immunize the mouse. This is the first mistake people make in trying to prepare hybridomas; they forget they have not properly identified the antigen. Then you grow a mouse myeloma cell culture. Myelomas are mouse lymphocyte tumor lines. Beginning with about 100 million each of the spleen cells from the immunized mouse and the myeloma cells, you add polyethylene glycol and fuse some of the cells together. Occasionally a spleen cell fuses to a myeloma cell. Out of 200 million cells you might get 500 that fuse properly. When a myeloma cell is fused to a spleen cell, it will become resistant to a medium containing hypoxanthine, aminopterin, and thymidine (HAT). The unfused cells will die in this medium. So we start with several hundred million cells and by the time we have selected them in HAT medium we have only about 500 left. If we do the procedure correctly each of these cells will end up in a separate culture. If you are going to grow a single cell in culture, the culture conditions have to be very, very good. If there is any heavy metal, if your serum is not good, or if there is something wrong with your incubator these last 500 cells will die. Each one of these cells must be assayed to see if they are making the antibody you want. When you find the cells making the antibody you need, you reclone them. The reason you reclone them is because fused cell has twice as many chromosomes as a normal cell. So as it replicates, it starts to throw out chromosomes and the cells have lower and lower number of chromosomes. Some cells will throw out a chromosome carrying an antibody gene. You have to select the cell that has antibody genes and is producing antibody. Then you expand this to a cell line and freeze some away as a way of keeping this population alive forever. You can grow it in large cultures and achieve antibody concentrations of 10 micrograms per liter, which is quite adequate for neutralization tests, ELISA tests and other processes. Or you can put the cells back into a mouse where they will form an antibody-producing tumor. Often the antibody concentration is over 10 mg/ml, sometimes as high as 80 mg/ml. However, the mouse producing the ascitic fluid with hybridoma antibody is already immune to E. coli, lots of other bacteria, antigens in its food

and a variety of other things, so the antibody produced in ascitic fluid is not pure. It is mouse serum plus whatever you were producing with the hybridoma. If you use the ascitic fluid in diagnostic tests you must keep these impurities in mind.

Let us review a few important points about making monoclonal antibodies. Production and characterization often take 12 months. Expertise is needed in cell culture and in biochemical techniques in order to properly characterize an antibody. Hilary Koprowski made the statement that one antibody costs one man-year. After many years of making hybridomas, I agree. So if you can buy a monoclonal for $100, do it! Buy them or borrow them; it is a big investment to make your own.

There are many advantages of monoclonal antibodies. For example, they are always the same. You do not have to worry about immunizing different animals over and over again. The concentrations of antibody are very high in relation to what you will find in normal antiserum. They can be as high as 8% in ascitic fluid. The supply is virtually unlimited. The specificity is often very good and may give you clean results. They can be used with any radioactive, enzyme, or fluorescent label you want to use. But there are some disadvantages to monoclonal antibodies. The first is the difficulty of making them. Also, they may be too specific and show you some irrelevant differences between viruses. They are often quite low affinity and may not compete well with the antibodies in serum. And, they may give you some unexpected cross reactions.

> Hybridization: Nucleic acid hybridization is used to identify specific gene sequences in various specimens. It is based on the ability of homologous DNA of RNA sequences to hybridize to each other.

In order to do many of the hybridization techniques, it is necessary to clone the DNA sequences that you want to detect. The cloning is now becoming relatively straightforward but it may require years of work. Basically, you clone a piece of foreign DNA into a bacterial plasmid that contains an antibiotic resistance gene, introduce the plasmid into bacteria, and select with antibiotics for those bacterial cells that are resistant to the antibiotic. Bacteria continuing the desired plasmid are particulary easy to select if you clone your foreign DNA into the middle of a second antibiotic resistance gene, destroying this gene. These plasmids will give bacteria resistance to one antibiotic but not the second and thus can be selected easily. This same process is also used to clone messenger or viral RNA sequences if you first make a complementary DNA copy of that RNA.

Once the DNA sequence of interest has been cloned into a plasmid, large amounts of the plasmid are grown in bacteria and purified away from the bacterial DNA. Then the cloned DNA must

be labelled, usually with radioactivity. This can be done by a variety of methods (Figure 2).

(1) Nick translation is done by cutting single-stranded nicks in the double-stranded plasmid DNA, removing a few bases at each nick, and then adding radioactive bases and polymerase to repair the nicks. This process is extended down the DNA creating a long radioactive probe. This is relatively easy to do, all the required enzymes and buffers are available in commercial kits that usually, but not always, work very well.

(2) 5'end labelling is also relatively easy to do. First, the cloned DNA is cut with a restriction enzyme and then a radioactive phosphorous is attached with a kinase either at an overhanging end or a recessed end of a plasmid DNA fragment.

(3) Random primer labelling is used extensively in my laboratory. This technology is also available in kits that work very well. You begin with a single-stranded piece of your cloned DNA to which you bind the random primers from the kit. The primers will hybridize to just a few areas of the DNA and then you just extend the primers using radioactive nucleotides. This technique works very well for us, often giving 100% incorporation of the radioactive nucleotides into the probes. We get our best probes using this technique.

(4) Riboprobes are also extremely useful. To make a riboprobe, you clone the DNA of interest into a plasmid that has a T7 promoter at one end of the cloning site and an SP6 promoter at the other end. The cloned DNA is then cut with a restriction enzyme and either T6 or SP6 primers are added along with RNA polymerase and radioactive nucleotides. The primers will be extended down the cloned DNA to the cut made by the restriction enzyme. The T7 primer will make a probe from one strand of the cloned DNA, the SP6 primer will make a probe from the complementary strand. This allows you to make a single-stranded probe that will bind only to its complement. For example, you can make one probe that will bind only to viral genomic RNA and another that will bind only to viral messenger RNA. By selecting the proper restriction enzyme, you can make probes of different lengths. So the advantage of riboprobes is that you can taylor the probe to give you the results you need.

Once the radioactive probes are ready, the actual hybridization test is done with different kinds of samples. For slot blots, all you need is something containing nucleic acid. This is blotted onto a nitrocellulose filter either by filtering the fluid through the filter or just by dropping the sample on the filter. Then you bake the filter to make the nucleic acid stick to the filter, hybridize it with the radioactive probe, and autoradiograph it. If you have labelled the probe with an enzyme label, an enzyme substrate is added to show which samples hybridized with the probe.

Southern blots are used to examine DNA for sequences in common with the probe. The DNA is extracted and digested with a restriction enzyme to reduce the DNA to short fragments. These fragments are separated by size using agarose gel eletrophoresis if they are long, or by polyacrylamide gel electrophoresis if they are short. The gel is placed on a nitrocellulose filter, the fragments are transferred to the nitrocellulose and baked on. The probe is incubated on the nitrocellulose where it binds to complementary DNA sequences, and the filter is autoradiographed just like a slot blot to locate the DNA that bound to the probe.

Northern blots are a way of examining RNA for sequences in common with the probe. This technique is used to examine either viral RNA or messenger RNA. The RNA is separated by gel electrophoresis, blotted, baked, hybridized, and autoradiographed just like slot blots.

In situ hybridization is used to examine cells or tissues both for the presence and the location of particular nucleic acid sequences in cells. The cells or tissues are fixed on slides and hybridized with the probe. They are then coated with photographic emulsion and put away in the dark for days or weeks. When the emulsion is developed, the location of radioactive probe is easily seen.

The amount of time and effort needed for hybridization assays depends on where you begin. It takes a Ph.D. level scientist months or years of hard work to clone and properly characterize a single gene. The cost is US$10,000-$100,000 but can be reduced to almost nothing by the "phone clone". This is when you telephone someone who has already cloned the gene and ask them to send you their clone. This saves time and money and is what my laboratory usually does. The growth, purification, and labelling of plasmids costs about US$200 each in a laboratory that does this regularly. The actual slot blot assay costs about US$2-3 per sample, but you have to do thousands before the cost gets that low. The cost of Southern, Northern, and in situ hybridization is about $100 each if they are done routinely.

The sensitivity of nucleic acid hybridization on nitrocellulose filters is really quite low. At least 10,000 to 100,000 plaque forming units of virus are required to detect anything. The sensitivity of in situ hybridization is better since a single infected cell can be detected, but finding that single infected cell can be very difficult. It is obvious that nucleic acid hybridization will not replace tradicional virology.

In summary, slot blots are relatively simple and inexpensive. Once you have the technology working in your laboratory, technicians can do large numbers. Southern and Northern blots and in situ hybridization take more work but Southern blots will identify specific DNA sequences, Northern blots will identify

specific messenger RNA sequences or specific viral RNA, and in situ hybridization can be extremely sensitive, but all three techniques are expensive and difficult and should probably be reserved for research purposes.

> Fingerprinting: Fingerprinting is very useful for comparing closely related sequences and showing if they are different and how different they are. Figure 3 shows the fingerprint of a small RNA. The complete sequence was incubated with T1 ribonuclease which cut the sequence at each of the G's. Notice the G at the end of every single fragment. The RNA is labelled either before or after the T1 digestion and can be detected ay autoradiography. Then the fragments were sorted out by electrophoresis in one direction and chromatography in the other direction. Different techniques for separation can be used depending on the size of the fragments. You can see the spots have been nicely separated. We know exactly what has happened in this example because each one of the spots has been sequenced. If you compare the positions of spots 4, 10, and 11, for example, you see what a large different a single nucleic acid can make in the fingerprint. You can even see a difference between an AUG and a UAG. Even the sequence of the fragments is important in putting them in different places.

But if you fingerprint a viral genome 6000 bases long, there are going to be hundreds of CGs, hundreds of UAG's, etc. If one of the UAG's turns into an AAG in a virus with 6000 base pair you are not going to see it because the new AAG spot will be superimposed on an existing AAG spot. The only place you will see differences is in the big unique fragments and you will see these particularly well if one of the bases changes to or from a G. If a C changes to a G, a big spot will disappear and you may gain two smaller spots, although these may be superimposed on other small spots. The conclusion to all this is that fingerprinting can see differences in some pieces of the sequence, it cannot see differences in other parts. In fact, it analyzes differences in only about 10% of the genome. The other 90% is cut into little fragments which end up at the bottom of the gel and provide no information whatsoever. In some cases fingerprinting will see a difference between viruses that are actually different, in other cases it just will not see a difference. Even if it does see a difference, many of the differences appear to be insignificant. The time involved in fingerprinting is about one week and it requires a Ph.D. level individual to get it running and a sophisticated and careful technician to do it regularly. And it is fairly expensive even when done in large numbers. In summary therefore the advantage of fingerprinting is that it analyzes a big genome sequence in one gel; the problem is that is does not tell you the whole story since 90% of the genome is cut into uselessly small fragments.

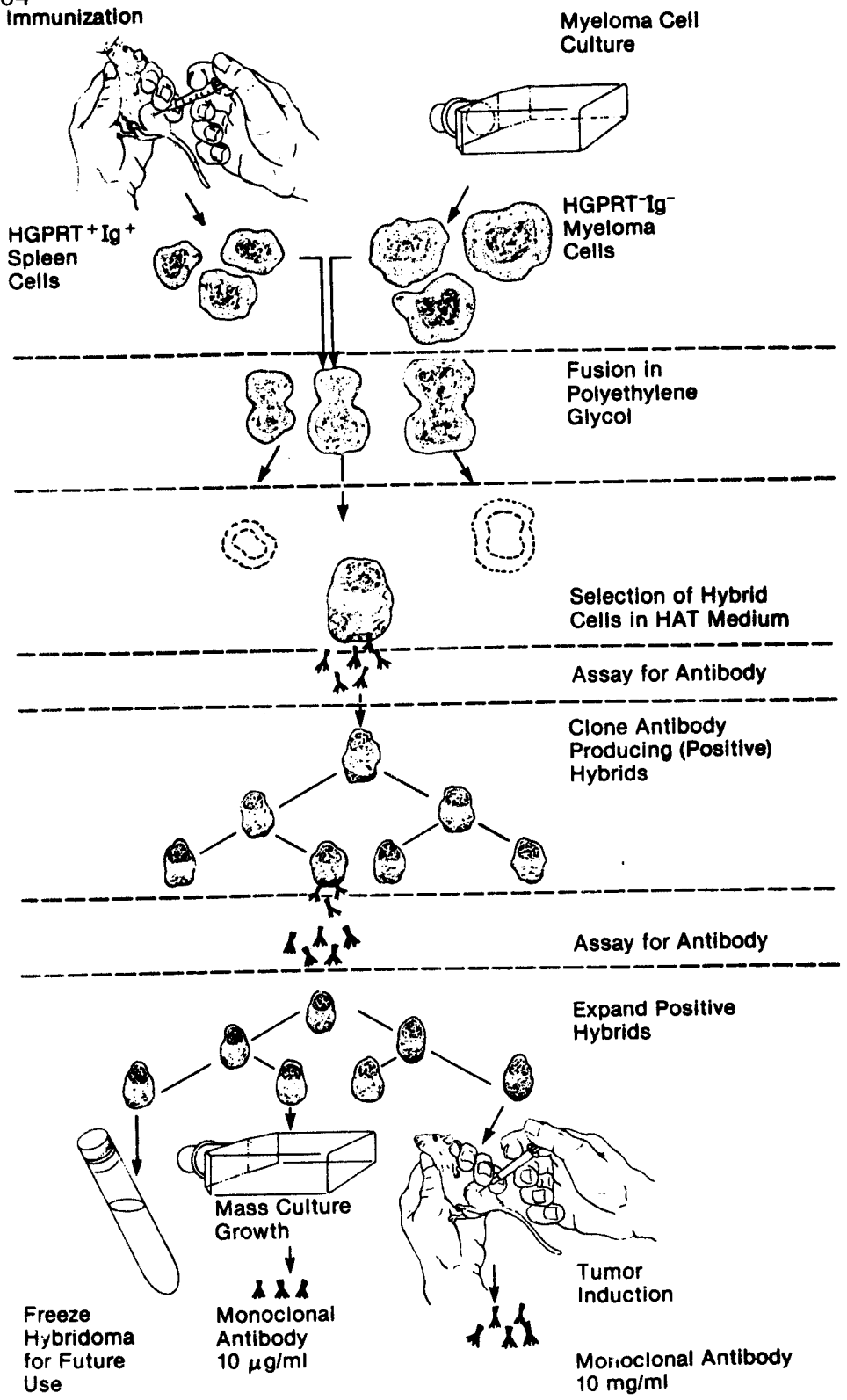


FIGURE 1. Flow chart for monoclonal antibody production.

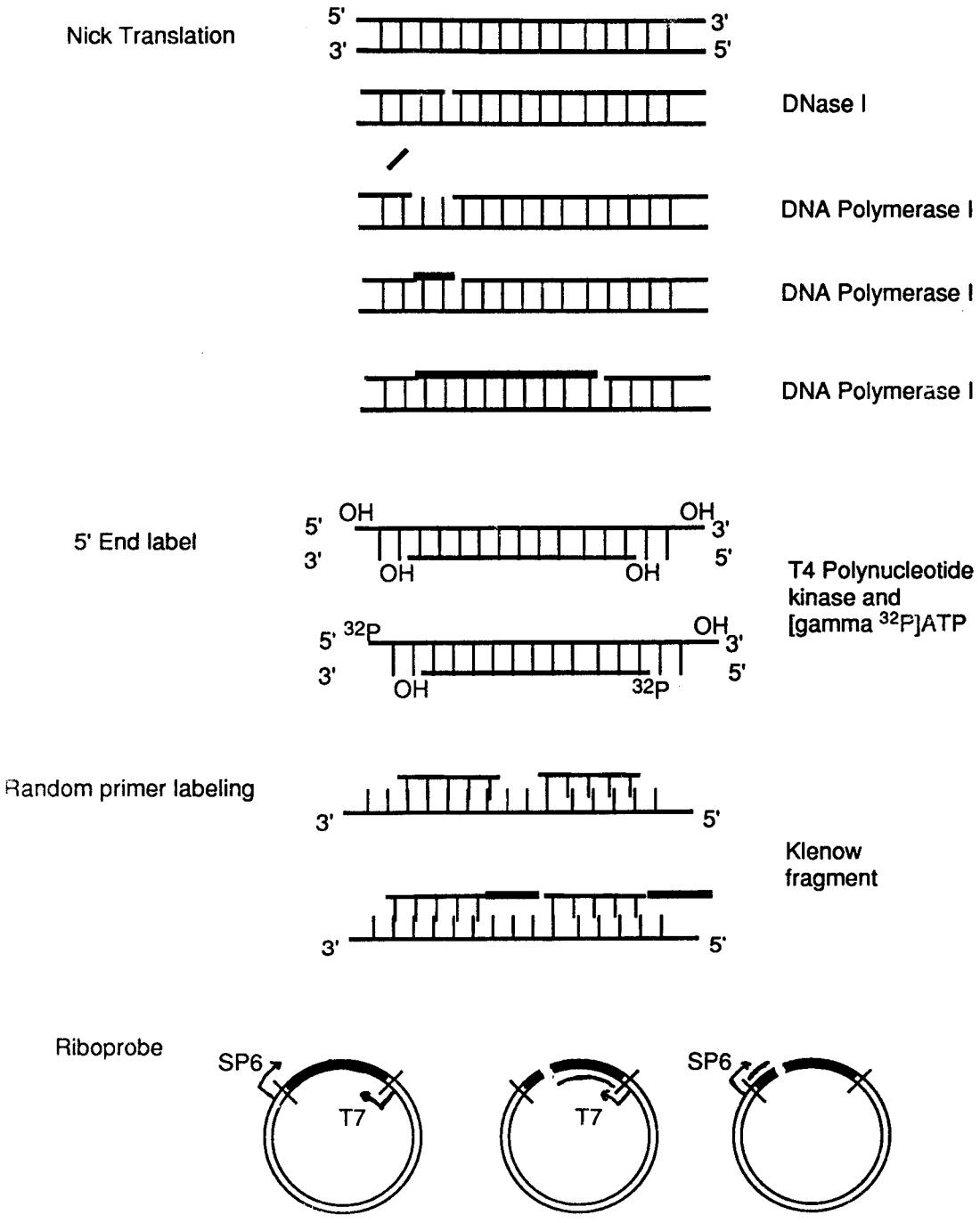
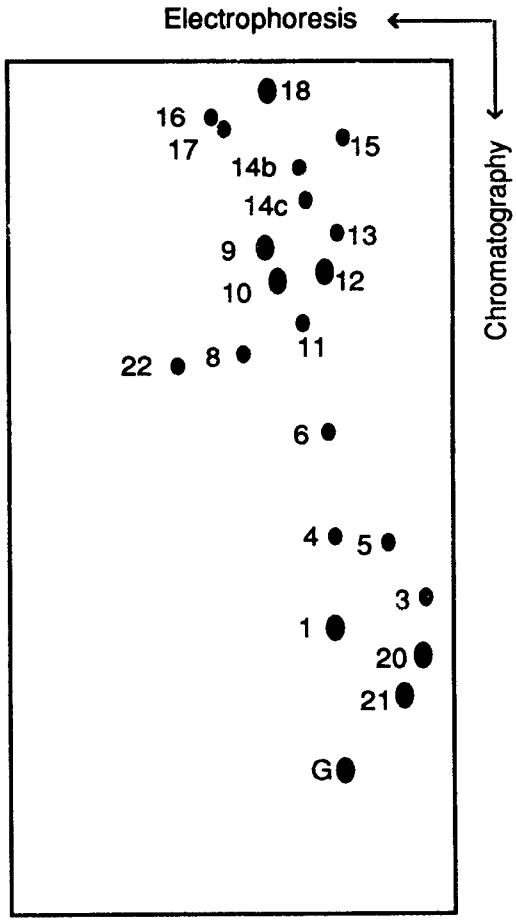


FIGURE 2. Methods for radioactively labelling DNA probes.

Spot Number	Sequence
1	AG
3	CCCG
4	AAG
5	ACCG
6	AAAG
8	CG
9	AUG
10	UAG
11	UCG
12	CCUG
13	CCUACG
14b	AAUACCG
14c	AUCCAG
15	CCACACCACCUG
16	UUAG
17	UCUG
18	AUCUCG
20	CCG
21	CG
22	pG
G	Gp



From: Cell 12:1045 (1977)

FIGURE 3. Representative T1 oligonucleotide map of a known DNA sequence.

2.3 BIOTECHNOLOGY AND THE NEW GENERATION OF VACCINES

Drs. Geoffrey Letchworth, Ann Palmenberg and
William Kenealy
University of Wisconsin-Madison, USA

Dr. Letchworth: You have all heard that biotechnology is creating simple, easy, new vaccines. We can clone the gene for basically any protein, put it into vaccinia or into a yeast and make vaccines. But it really is not all that easy.

Vaccine biology started with Jenner almost 200 years ago when he recognized that you can use an attenuated virus to immunize a host. Over the past 200 years, scientists have wanted to improve this process and to understand on an academic level what was hapenning. So let me try to break apart the process of vaccination and try to understand the process of putting an animal and a vaccine together and making an immune animal.

Animals have non-sepecific immunity (natural killer cells, interferon, polymorphonuclear leukocytes, complement, macrophages, etc.) and specific immunity (cytotoxic T lymphocytes, suppressor cells, helper T cells, and different kinds of antibody, etc.) and researchers have been looking at the interactions of each of these with viral glycoproteins, viral transactivator proteins, structural proteins, and enzymes (Figure 4). We have broken each category of vaccine protein into individual molecules and analyzed the individual peptides that make them up. The point is that we are not necessarily trying to hunt for a vaccine in which a single peptide stimulates a single immune mechanism to create an immune animal. We are trying to <u>understand</u> the whole process.

Let me show you an example of how big a mistake you can make when you try to use a single protein as a vaccine. This is a half million dollar mistake I made. There is a long list of vaccines available for infectious bovine rhinotracheitis (IBR) in the United States. It includes killed and live virus vaccines. We decided there were so many vaccines because nobody understood IBR vaccination and we thought we could use biotechnology to make a better vaccine. We thought that if we could determine which proteins in a vaccine caused a neutralizing response, we could produce large amounts of these proteins, put them into animals, and create immunity.

We found out that there are three major glycoproteins on the surface of IBR virus. All three are dimers. Two are dimers of identical molecules and the third is a dimer of one molecule embedded very deeply in the membrane linked by a disulfide bond to another protein (Figure 5). The proteins probably are configured like they are in herpes simplex virus. Glycoprotein gI looks like a "T", gIII is a fairly long spike and gIV looks like a lollipop. The IBR genome contains a number of other genes that might code for surface proteins, but these genes have not

been cloned and we cannot associate a protein with them yet. We made monoclonal antibodies directed against the three known glycoproteins. The monoclonals specifically immunoprecipitate the proteins, and identify a number of epitopes on each. They neutralize the virus and the neutralization titer is increased with complement. All three glycoproteins were targets for neutralizing monoclonal antibodies and so we thought they should be good complement in a vaccine. Does that mean they are also involved in protection of animals against infectious bovine rhinotracheitis? It is fairly easy to ask that question.

We injected huge amounts of these antibodies into cows. The cows developed neutralizing titers, higher than what we normally see in recovered animals. Then we challenged them using virulent IBR intranasally and intraconjuntivally; a fairly natural exposure. We monitored them for fever, lesions, nasal discharge, ocular discharge, conjunctivitis and coughing. When we compared viral titer in the noses of animals injected with single monoclonals or mixtures of monoclonals to the negative controls we found that they were identical. Clinical signs were identical in the animals which had huge neutralizing titers and animals that did not have neutralizing titers. None of the antibodies directed against gI, gIII of gIV provided passive protection whether the animls were given single antibodies or antibodies against all the epitopes.

Maybe the protective response against IBR virus is not mediated by antibody. Maybe it was mediated by T cells. We purified the viral glycoproteins and injected them into cattle. We used all three proteins singly and in combination. The animls developed lower titers than with a commercial killed vaccine but certainly higher titers than negative control animals. We assumed we had generated a T cell response in these animals. Immune precipitation showed us what the animals had reached to each protein. So we had shown that we could prepare purified protein, we could inject them into animals, we could get a neutralizing response, and we probably had a T cell response. Those animals were immunized against just single proteins and we could prove it. The animls had all the criteria accepted by the US government for being completely immune. When we challenged these animals they had virus in their nasal secretions starting a day after inoculation and for as long as 10-12 days. They had symptoms of disease for the same length of time and there was no difference between the immunized animals and the unimmunized controls. The vaccination with purified protein was valueless, except that the animals fulfilled the criteria of the US government for proof that the vaccine worked. Obviously something is wrong. Biotechnology has taken us half million dollars worth up a blind alley.

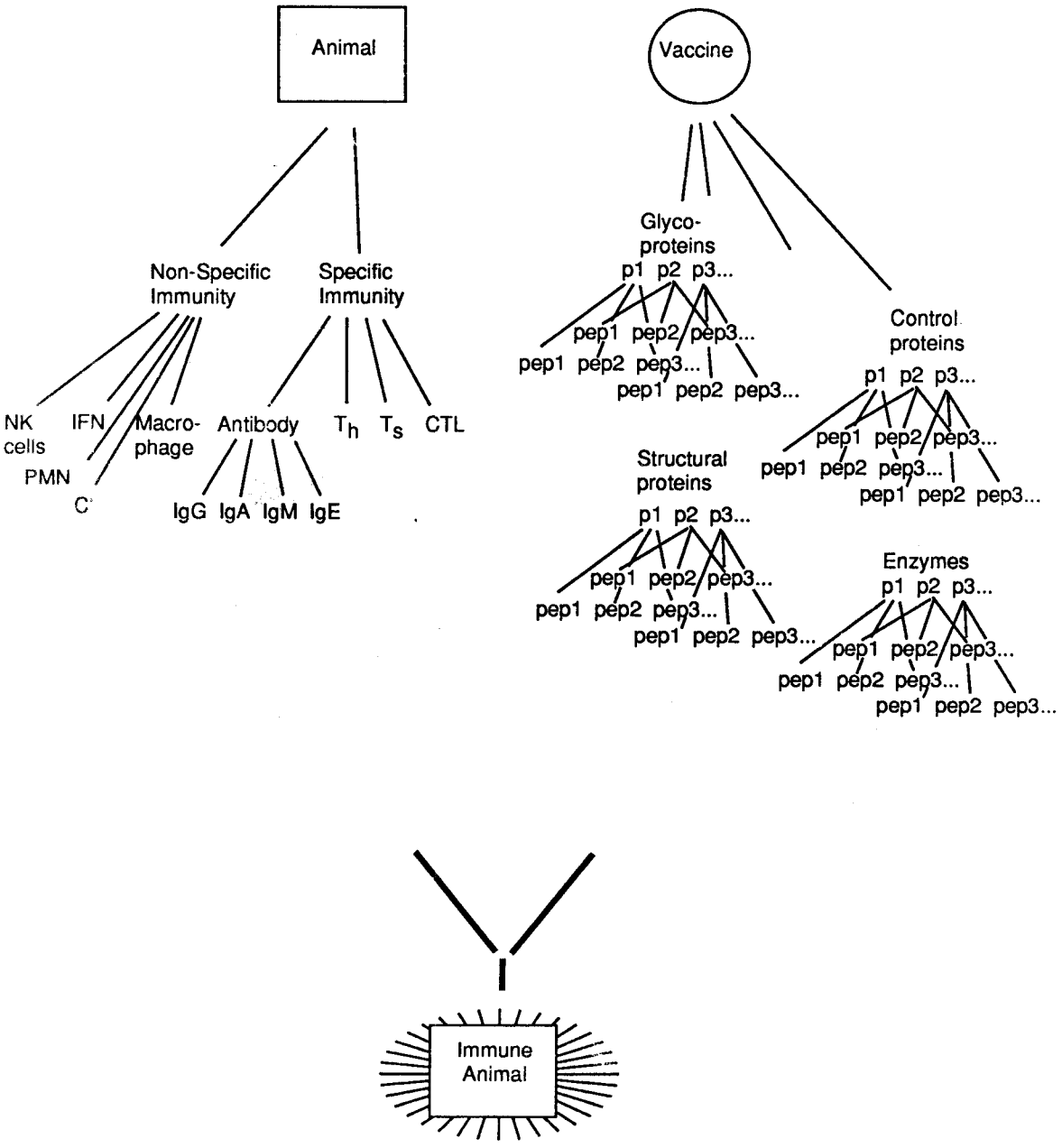


FIGURE 4. The complex specific and non-specific immune responses normally directed against each of the peptides in a large number of viral proteins that all combine to create "Immunity" in a vaccinated animal.
Abbreviations: NK = Natural killer cells, IFN = Interferon, PMN = Polymorphonuclear leukocytes, C' = Complement, Th = T helper cells, Ts = T suppressor cells, CTL = Cytotoxic T cells, p1 etc. = protein 1 etc., pepl etc. = peptide 1 etc.

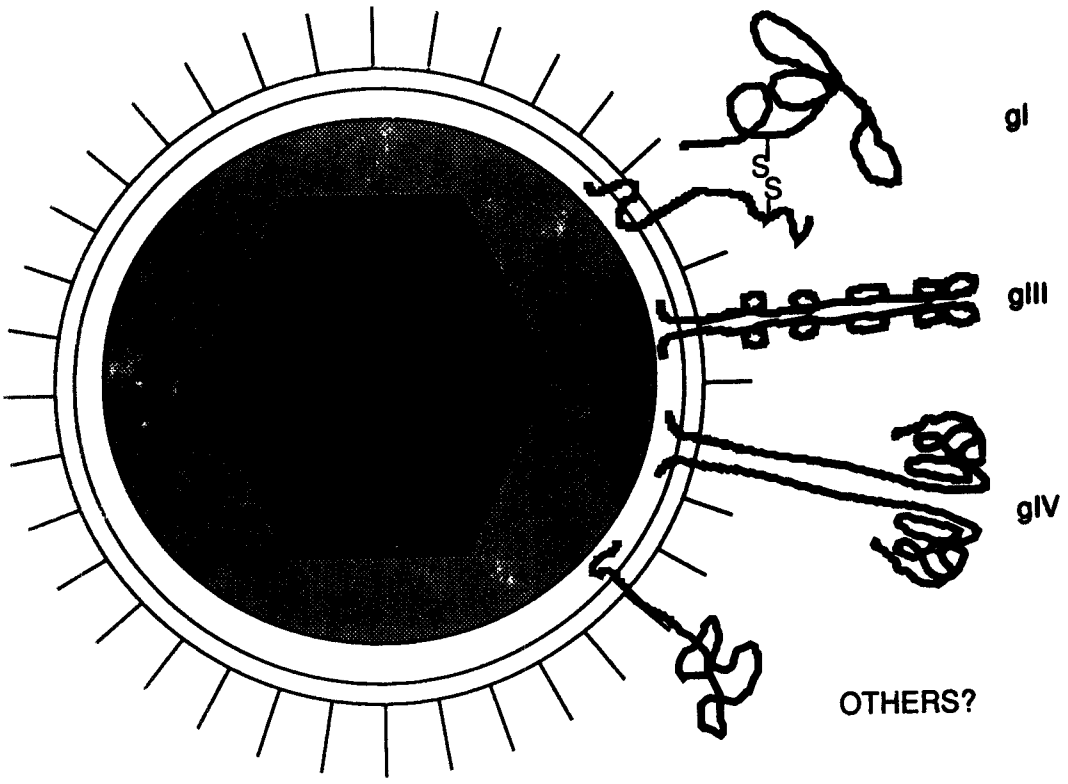


FIGURE 5. The hypothetical structure of the infectious bovine rhinotracheitis virus glycoproteins gI, gIII, and gIV.

2.4 INDUSTRIAL MICROBIOLOGY, ENZYMATIC FERMENTATION TECHNOLOGY AND BIOPROCESSING

Dr. William Kenealy, University of Wisconsin-Madison, USA

Research in industrial microbiology is primarily concerned with two areas: the cost of processes, trying to make a product for less money; and discovery research, finding new things to make products from or new products. As you work on a process to try to make something for less money and make your profits higher there are a few tools that are used and some of them have to be very finely balanced to optimize the amount of product for the least amount of money.

You may achieve cost savings by increasing the speed of the process, concentration of product or yield improvements. Many industrial processes with microorganisms give you a diluted product in an aqueous system and the problem is to get rid of the water. Here we are primarily talking about increasing the concentration of a product. Increasing the concentration of the product reduces problems of recovery and when you work up the economics of the process you can decrease the volume of the vessels required for the process. If you decrease the volume of the process you will save money on the capital investment needed to set up the plant.

Time will vary depending on the process. Sometimes you might increase the time required for the process to increase the product concentration. By waiting longer the microorganisms build up more product. Obviously if you wait longer times you will require more vessels to be operated at the same time to produce the same amount of product to meet the production goals of your plant.

One of the first things that will have to be done when approaching commercialization of a product is to estimate the amount of the product you will need (kilograms per year etc) and then project the size of plant you will have to build to come up with this much product in an efficient operation. This is when you find that increasing product concentration could bring down the volume many times and there will be significant initial savings. It is usually desirable to increase the product concentration as high as possible which lowers the volume needed, however when substrate costs are high the yield will also be of prime concern. It is quite often found that the variables of volume, concentration and yield must be optimized for an economical process.

A good way to increase profitability is to decrease turnaround time. This is the amount of time it takes you to have the entire process completed once, reclean everything and begin the next process. You can reduce this by accelerating the growth rate of the microorganisms, engineering an organism that produces faster

on induction or selection for different strains which produce faster. Again concentration and speed with which the product is produced will dictate how much volume you are going to use, and the size of the plant.

The main research areas in industrial microbiology are concerned with making a better process and finding new products. While Biotechnology has had its most significant impacts with new discoveries it has also been used to improve existing processes. Biotechnology is now offering ways to use recombinant microorganisms rather than to use strain selection and other manipulations to change the speed, yield and concentration of various processes.

Several companies have started to increase the level of enzymes in pathways leading to products. Gene amplification is used to make many copies of the genes of several of the enzymes. This leads to an increased accumulation of the product since these enzymes are able to draw more of the intermediary metabolites into the product pathway. As you increase the enzymes in the biosynthetic pathway for amino acids, you will accumulate more amino acids and build up the precursors for those amino acids.

A second manipulation that has been used is to alter the host organism and put in additional pathways that were not there. What used to take two steps and two organisms can now be done with one. An example of this is the work done by Genentech (1) with Erwinia herbicola in the production of ascorbic acid. There is a two step process to get to the precursor of ascorbic acid which employs Erwinia herbicola and a Corynebacterium species. They took the key enzyme from the latter (2,5 diketogluconic acid reductase) and put it into Erwinia herbicola. This organism will now take the glucose and make 2-keto-gulonate which is then readily converted to ascorbic acid. A two step process is converted into one using recombinant DNA technology. The time and volume of the process can both be affected in this manner.

For the rest of my talk I will concentrate on the area where Biotechnology has had its biggest impact on industrial microbiology. The bulk of the work in biotechnology and industrial microbiology is the production of a protein in an organism which does not normally produce that protein. Much of the recombinant expression can be viewed as an improvement in processes where the concentration of the product is increased. Instead of grinding organs or tissues the protein is produced in an easily manipulated organism. Recombinant expression of protein depends on several factors all of which are interrelated. The gene, expression organism, method of control over production, vessels for growth, harvest method for product, recovery and purification of product and the safety involved are all important features of recombinant protein expression.

The gene is important and obtaining the gene of interest is often highly competitive between different companies. Let us assume for the purposes of industrial microbiology that you already have a cloned gene. What are the problems you will encounter after you have that cloned gene? The first thing that should be asked is "What is the end use of cloning and expressing that gene product? Are you making an enzyme, an antigen, a vaccine? The answer to that first question may indicate the proper systems for the production of the protein.

What expression system should be used? There are a wide variety of organisms you can try. Almost all DNA cloning goes through E. coli so that is often tried first, but there are many organisms that can be used and will match the end uses of that protein better than E. coli. The end use of the protein will often indicate the best systems for the production of the protein.

The promoter that you use to control the expression of the gene is organism dependent. The goal here is to generate biomass by growing the organism under optimal conditions and then turn on the synthesis of the protein with some physiological signal. This can be by the addition of some chemical inducer, exhaustion of a nutrient or by temperature shifts.

A factor that is important in the harvest of the product is the location of the product. Is it excreted or secreted? Is it accumulated within the cell? Is it gathered around the outside of the cell? These are important considerations when you want to purify large quantities of the protein. For recovery you may need extraction methods, again is the product inside the cell? Do you have to break the cell open or can you take it from the medium?

Finally you must consider the safety precautions that you need for many organisms. I will address this later. Some of the measures are important for personal safety, others are required by regulatory agencies.

Let us consider the type of product and the most common expression system E. coli. What is the end use of the protein. Is it going to have any activity associated with it? Is it going to be an enzyme which will catalyze a reaction? Or is it going to be an inactive antigen such as a component of an ELISA test or vaccine? The protein produced by E. coli may be soluble or insoluble. The solubility may either help or hinder the purification of a given protein.

E. coli normally grows as a very short rod. When we produce interleukin 2 in E. coli we see the cells elongate as protein is accumulated in inclusion bodies (2). This is quite visible in the phase contrast microscope as black dots in a long cell or bright crystalline material (depending on the optics used). To purify this protein you will need to know where it is located in

the cell. If you break open the cells and centrifuge the suspension you will find the protein principally in the pellet, indicating interleukin 2 is a precipitated, insoluble protein. Not all proteins produced in E. coli are insoluble. When the poliovirus 3C protease was made in E. coli it was more soluble than the Interleukin-2 or beta-interferon but not as soluble as the beta-lactamase of E. coli (2). In the case of beta-lactamase produced in E. coli, the majority of the product is found in the soluble portion of the cell, but it has been reported (3) that this protein can aggregate even outside the cell and depending on the strain of E. coli used can be aggregated inside the cell (2).

In many cases the insoluble protein can be resolubilized and renatured to get activity, in others it cannot. For each protein product you will not know how soluble it is going to be until you produce it in E. coli. There are some indications that membrane spanning regions of proteins or export signal sequences of proteins may have a greater tendency to aggregate in E. coli. There is also an indication that solubility is concentration dependent. The more protein that is produced the greater likelihood of forming precipitated protein.

In each case there are pluses and minuses of having insoluble and soluble protein. These relate directly to the end use of the protein and the methods available for purification. If you are looking for an active protein and it is soluble you can employ classical purification techniques after breaking apart the cells. Many times with E. coli the protein will be soluble.

If the protein is insoluble and you want an active protein, the purification can be somewhat simpler. You can break open the cells and centrifuge the inclusion bodies and debris from the soluble protein of the cells. This will get rid of about 50% of the protein. But since the protein is insoluble and you need it active, you will need a denaturation/renaturation scheme which will entail solubilizing the protein before doing further purification on it. Often this will be done with urea or SDS or some similar denaturing agent, often with the addition of beta-mercaptoethanol or dithiothreitol to keep the protein in solution.

You will get a highly concentrated protein in the pellet fractions and when this is exposed to air disulfide bonds form in the same protein and between proteins. These disulfide bonds must be broken to keep the protein soluble and in a form in which you can purify it. Overall when you are looking for an active protein, you might get a lower specific activity of the protein that you are after because of improperly folded proteins. If the wrong disulfide bonds form the correct folding of the protein may not take place. In these cases another denaturation/renaturation scheme can be used or other expression systems may be warranted.

Two different examples of proteins we produced in E. coli are interleukin 2 and B interferon. If interleukin 2 is put in an SDS solution and the disulfide bonds reduced and the solution diluted there will be a significant increase in activity. Beta-interferon, subjected to the same steps will give almost no activity. Beta-interferon production as an insoluble fraction in E. coli is not very good when you want the activity of beta-interferon. At least part of the problem with beta-interferon is the improper disulfide bond formation that occurs upon denaturation and renaturation.

Now let us consider production of proteins that do not have an activity associated with them. This is easier to deal with because you do not have to worry so much about the condition of the protein or whether it is remaining active. If you have soluble protein you can often use affinity purification. You can make an antibody to the protein and put it on a column. Run the protein through, rinse the column several times and put a high salt concentration or dilute acetic acid through the column and come up with very good purification of the protein that reacts with your antibody.

Since E. coli expressing foreign proteins often does not make them soluble you may have only low levels of the protein of interest in the soluble fraction. An example that I worked with is the GAG proteins of human immunodeficiency virus-1 (2). They are relatively soluble proteins. In E. coli a soluble fraction is formed and the rest aggregates and precipitates. The insoluble fraction served as well as the soluble fraction as an antigen or for use in an ELISA test. Purification by breaking open the cells and centrifugation is important and gives you good initial purification. You still have to solubilize the material but you do not need to worry how to renature the protein. All you need to do is find out if it is active in the type of assay you want to use it in (an Elisa or Western blot for example).

Figure 1 shows a standard E. coli recombinant incubation. This is what most laboratories do if they want to find out if E. coli is making protein. They normally start with a frozen stock culture. If E. coli are kept in the refrigerator they can still metabolize at 0-5 C and changes in the strain occur over time. With a frozen culture the starting point is always the same. Often then what happens is that you start with a small culture and an antibiotic selection. This scheme may have to be modified if you are dealing with a protein that will be later injected into humans. You then inoculate a larger vessel and allow the cells to grow. This is the stage when you are trying to accumulate cell mass. When a certain mass has been achieved you will induce the cells to make the protein of interest. You can do this by addition or depletion of a nutrient or by temperature induction. You want to grow the cells to a high cell density first because when you turn on the protein production the cells

do not survive very long. They will just be producing protein and not continue to grow.

A standard way to observe the synthesis of a protein is by using polyacrylamide gel electrophoresis. This is usually done during the initial production of the protein to determine when the protein is being synthesized. Unfortunately it takes too long for gel electrophoresis to be used as a monitoring tool during a fermentation. You would normally correlate the time period when the protein was produced with some other kind of monitoring device easier to use than gel electrophoresis. The observation of the culture in the microscope is a commonly used tool for the correlation of protein production with an easily observed event (2).

With E. coli there is another problem and that is the stability of the clones. You normally have an antibiotic resistance marker that you would use in the first couple of steps. This is usually quite stable when E. coli is not induced to produce protein. If you induce the production of a recombinant protein the E. coli containing the plasmid will be difficult to grow. There will be a competitive advantage in growth for cells that do not have plasmids or have altered plasmids that do not allow protein accumulation. Non-producing cells are easy to differentiate from producing cells as they are small when viewed under the microscope and the cells which are producing protein are typically long and have protein inclusion bodies in them.

An example of a stable and unstable E. coli production run is shown in Figure 2. For a stable clone you would normally see that with time of incubation the cell grows. At some point where there is maximum cell growth you will want to induce the protein synthesis. The product will then accumulate. It will only accumulate to a certain extent because growth is quite limited and after a while the cells begin to die. If you have an unstable clone the initial portions of the cycle are about the same. Both cell types grow. You induce protein production and the cells which do not produce protein continue to grow. This dilutes the product and changes the percentage of protein that you are dealing with when you harvest the product. This will cause further problems in purification.

If you look at the plasmid in these non-producing cells of the unstable culture you will find one of two things. In one case the plasmid will not be there, and the antibiotics used in the selection will have been consumed by the enzymes produced by cells containing the whole plasmid (4). So cells without the antibiotic resistance can grow quite well after the antibiotics are exhausted in the fermentation vessel. Or alternatively some of the plasmids have been altered and do not produce protein. Something happens which is selective against the plasmids which produce protein. A problem which is not resolved is how the cell gets rid of all the plasmid which is able to produce protein.

So far we have talked a little about the end use of the product. The next factor to consider is the expression organism. E. coli has been most often used. Its advantage is primarily that it is easy to manipulate and produces quite large amounts of protein. The protein, with rare exceptions, is not secreted. E. coli is unable to perform post-translational modifications of the protein. It is also unable to secrete large amounts of protein. Primarily E. coli is the DNA workhorse, what you first use when assembling your plasmids. Usually it is worth trying to express the protein in E. coli. Bacillus and Streptomyces are two organisms that have been used to produce protein. Bacillus produces a bit more protein than Streptomyces does, probably because Streptomyces is not as well characterized. Their key advantage is that they secrete protein. Instead of having to look inside the cell for the protein you can find it in the medium. Both of them are unable to perform most post-translational modifications. A problem they have in common is that as they produce proteases, a lot of their natural survival depends on protein metabolism and this can be a problem if you want to accumulate protein.

There are two yeasts that are used quite a bit as expression systems, Saccharomyces cerevisiae and Pichia pastoris. They will both accumulate good amounts of protein. They will both secrete protein and perform post-translational modifications. Sometimes Saccharomyces internalizes protein depending on the pathways and signals used for expression. Saccharomyces has been used to produce a hepatitis B vaccine. The protein produced is secreted into the medium and will form the proper conformation to use as a vaccine.

Several species of filamentous fungi have been used. I think this list will grow in the future since there are several advantages in using them. Neurospora crassa, the filamentous fungus whose genetics we know most about, produces a small amount of protein. The advantage is that it secretes protein in response to the correct signal. These fungi are also able to perform post translational modifications. The basic advantage of Neurospora over other filamentous fungi is that its genetics are well characterized. This is also true for Aspergillus nidulans. This produces more protein than Neurospora but they are very similar organisms as far as protein production.

In the case of Aspergillus niger a large percentage of the protein produced is secreted. A. niger glucoamylase can be produced at levels of 20 grams/l secreted protein, which is the largest amount of protein secreted by any organism in culture. Admittedly it is a homologous protein that is secreted in that amount. If you can get a small percentage of the protein you are interested in secreted in the medium it is well worth it since the protein is easier to purify.

Another filamentous fungus that has been used is Achlya ambisexualis. It works for protein expression and has been used to produce protein using a promoter from the SV40 virus. A variety of promoters have been used, they do not necessarily have to be from the organism producing the protein, although the best results will probably be obtained with homologous promoters. Controlled promoters can be used in almost all the expression systems to turn on the protein production.

Animal cells and insect cells have both been used to produce proteins. The animal cells produce relatively small amounts of protein and can secrete them into the medium. The post translational modifications of these cells are what you really want. The proteins produced are most closely related to the native material when you use cells from the same organism that the gene came from. The post translational modifications will be virtually identical to the protein that you could produce from that organism, except that you may be able to produce it in higher amounts. The disadvantage for these two cell types is that the medium is usually quite complex. When protein is secreted into a medium which has serum in it, you will have to purify the protein away from the protein components of the serum. It is much better to have a serum free medium.

Finally we should consider insect cells. Insect viruses can be used to produce protein in these cells. The nuclear polyhedrosis virus (baculovirus) infects insect cells and signals the production of large amounts of its own protein which is called polyhedrin (5). For production, the signals for this protein have been separated and used to attach the gene for your own protein. This has become an easy system to get proteins secreted in good amounts and with post-translational modifications.

The choice of expression system will depend partly on what is available to you, but also should depend on what you want to do with the protein. If you need something very close to native material, you will have to use a system close to the natural system to produce it. If you need something that is secreted and active, you may be able to broaden your choices to other expression systems. The greatest flexibility is available when activity is not required but the protein still serves a biological function (as an antigen or immunogen).

Let us next consider a few characteristics in the growth of the organisms we are using. There are key differences between bacterial growth and animal cell growth. Bacteria will need a higher rate of oxygen transfer than animal cell growth. Most of these differences are important when selecting a vessel for the culture of the organism.

A large cell mass is needed and oxygen must be added either by increased air sparging or by supplemental oxygen mixtures. Along with this you must have the capacity to remove heat which will be generated by the organisms. This is dependent on the design of the vessel and the mixing rate. The pH control is also influenced by the mixing rate. If you only measure the pH at one spot in your mixture and the mixing is not adequate in that spot there will be a tendency either to over acidify, or become very alkaline. Mixing is therefore important to insure access to oxygen, removal of heat and control of the pH.

Another major problem when there is a high rate of aeration and high rates of agitation, is to control the foam. This is necessary to contain the culture in the vessel, avoid producing aerosols and to keep your fermentation as a single culture. When foam reaches the outflow filters the sterile integrity of the vessel may be impaired. Foam can be controlled by addition of antifoam, mechanical foam breakers and reducing the agitation or aeration rates.

When you culture animal and insect cells there are also oxygen supply problems as you start to accumulate cell mass. The rate of increase of the cell mass is never quite as great with animal cells as it is with bacterial cells but it is much more important to balance the effect of shear produced by the mixing impellers since these cells are characteristically more sensitive to being broken apart by the impeller blades than bacterial cells. Heat removal is again required, but there is less heat as the cell mass is less and there is less metabolic activity. However there is also less mixing so efficient heat removal and temperature control are needed.

Some animal cell types have different gaseous requirements. Some require carbon dioxide, which is often needed for pH control. Management in a fermenter is a little different from smaller cultures which are normally put into an incubator. The pH and oxygenation are often controlled by the gas mixture used.

Some of the problems in cell culture can be partially addressed by continuous culture. Continuous production of monoclonals is quite promising for animals cells; this is better than in batch fermentation, but most recombinant protein production is done in a batch mode.

It is often valuable to have an external monitor when you are producing protein. Gel electrophoresis may take several hours to give you a result and what you really need to know is whether you should harvest now, or one hour from now, not in several hours. ELISA assays are often used for protein activity. Again this is time consuming. If you can find some signal from one of your monitoring devices that says "protein is being produced now" it is very useful. Quite often, if you know the process well, just the optical density of the culture will tell you when

the protein is being produced. The observation of cells in the microscope can be useful. Often the oxygen uptake can be used; what is monitored is the dissolved oxygen in the fermentation as cells shift into production of protein. There is a shift in the metabolism and characteristically less oxygen is used than before protein was produced. Often there is a little "blip" in the profile which serves as an indicator.

Similarly a glucose utilization monitor can be used. This has the advantage that you only feed the amount of substrate required for the organism and eventually the oxygen uptake rate and glucose utilization will start to decline when the protein is produced mainly because cell growth has ceased. There are some more sophisticated monitors such as NADH fluorescence probe which can be used. This probe uses the normal concentrations of NADH in the cell as an indicator of growth and cell physiology. Another method similar to optical density monitoring, depends on knowing the characteristics of the run, based on gel electrophoresis and the elapsed time from addition of the inducing agent.

For recovery of the product, location is a critical factor. If the protein is inside the cell and is soluble, all you need is a cell paste from a centrifuge. This is resuspended, broken up and homogenized, the cell debris centrifuged off and then the protein purified from the supernatant. If the protein is inside the cell and aggregated, the first few steps are the same: cell paste, resuspend, homogenize, centrifuge, but then instead of dealing with the supernatant from that centrifugation, you work with the pellet and have to solubilize the protein. Repeat the centrifugation after the protein is solubilized and this removes the cell debris that is not fully solubilized. Finally you have to purify the protein from the supernatant. Here it is likely to be mixed with the solubilizing agent. If you use SDS some types of chromatography will be prohibited for subsequent purification.

If the protein is membrane bound you can sometimes purify it to a higher extent by making a cell paste, resuspending it with a reductant such as mercaptoethanol or a detergent. If this process is done in the cold sometimes you can take the protein off the outside of the cell. If it is tightly bound you will have to treat it further.

The secreted protein is what everyone tries to get in protein production. Then you can remove the cells and get the protein in the smallest volume possible so it can be handled without specialized equipment. This is usually done by salt precipitation, membrane treatments or ion exchange resins or other materials which absorb the material out of the solution. If you need large amounts of material you have to concern yourself with how large a vessel you need and whether or not the process, which you worked out so carefully in small containers,

will work in large containers. One reason to scale up would be because you need a large amount. Another would be because you need a reproducible source; one large fermentation batch and the possibility to go back to the same cells that have been kept frozen.

When you scale up the oxygen requirement of the cells is an important consideration. You must know this parameter for the growth of the cell and the formation of the product. What are the needs for oxygen supplementation? Design of the size of the vessel is an engineering concern because you have to be able to remove the heat generated by the growth of the cells. As before, temperature and pH monitoring should be adequate. With a large vessel it is important to have multiple positions at which to measure temperature and pH. Often probes fail, and replacement of a probe is very difficult if there is 2000 liters of liquid above that probe. Foam control either by physical or chemical means is important. You must know how an anti-foaming agent will affect your process, or whether just a physical foam break will do.

Figure 3 shows the growth characteristics in a test tube. There is poor oxygenation and this is limited to the amount of surface to air ratio. With a small bore large test tube you end up with very poor oxygen transfer. You do not really have pH control other than the buffer in the system. The temperature control is quite good and heat generated can be adequately removed. There is no substrate control available at this small level. Just using shake flasks provides better oxygenation as the surface to air ratio is improved. The other parameters are as limited as for the test tube culture.

Characteristically you proceed step by step, increasing the volume of your process, trying to make your process work at each step. Oxygenation control, pH control, temperature control, all become better when the small fermenter is used. Also you have opened up the possibility of substrate control. When you move into very large vessels there are usually engineering problems. There is less control of oxygenation. You cannot put in as much agitation into a large vessel without grossly exaggerating the motor required and the power needed. You may have localized zones of dead space in the mixing in large vessels. Your probes should not be located in these areas. Temperature control and mixing rate are still crucial. Outside the vessel you usually have a means of removing heat with cooled or chilled water, so inside you have to mix the contents to make sure it maintains contact with the wall that is cooling.

Finally I want to talk about biosafety. Primarily for me this means NIH Biosafety level 2 Large Scale Containment guidelines. In directing a pilot plant in Wisconsin, there are several parameters that I have to consider. If you are using organisms covered by this class, a closed system is required for volumes

over 10 liters. At the end of the closed system there must be a filter for exhaust gases. If you concentrate volume down to under 10 liters, there is still a need for primary containment, for instance under a biosafety cabinet. The operation must take place in a room with directional airflow. There must never be more air being put into the room than is being taken out as you do not want to spray organisms out of the room. You must be concerned with aerosols produced in centrifugation, or in pouring from one bottle to another.

There must be emergency plans. What happens if the vessel breaks and you have 2000 liters to get rid of? A sealed floor is necessary, with an epoxy resin that seal all the cracks which could harbor insects or infectious material.

A whole room that is under this level of biocontainment should be under negative pressure. A safety device which can be used for a large spill control is a formaldehyde generator. This is basically a hot plate which can be heated and paraformaldehyde put on it. The room is sealed and the plate heated, releasing formaldehyde to kill the organisms in the room.

We are planning such a facility with these safety features for the Department of Biochemistry at the University of Wisconsin. We will use this for recombinant bacterial and fungal cultures that permit us to work with genes from organisms that are regulated in the United States, such as foot and mouth disease. To produce recombinant antigen for that we have to work under biosafety level 2 in the United States. We have to be able to harvest and contain these organisms, basically requiring filtration and centrifugation. We will eventually construct a second area where we can work with animal and insect cells at a similar level of containment. We would also like to set up the facility so that the material produced in the facility could be used in clinical studies or field trials.

I have presented an overview of what impact biotechnology has had on industrial microbiology. The main impact is in the area of heterologous protein production. I concentrated on the steps after the cloning since obtaining a gene is often something researchers have thought through, but the steps afterwards have many problems which must be addressed. Some of these problems are best handled early before effort has been made towards developing a scheme for producing a protein which may have to be altered later because it does not fit the end use. The options available in the future will only increase.

Literature Cited

1) Anderson, S., Marks, C.B., Lazarus, R., Miller, J., Stafford, K., Seymour, J., Light, D., Rastetter, W. & Estell, D. 1985. Science 230;144-149.

2) Kenealy, W.R., Gray, J.E., Ivanoff, L.A., Tribe, D.E., Reed, D.L., Korant, B.D., & Petteway, S.R. 1987. Journ. Indust. Micro. Suppl. No. 2, 45-52.

3) Georgiou, G., Telford, J.N., Shuler, M.L. & Wilson, D. B. 1986. Appl. Environ. Micro. 52; 1157-1161.

4) Pierce, J. & Gutteridge, S. 1985. Appl. Envoron. Micro. 49; 1094-1100.

5) Luckow, V. A. & Summers, M.J. 1988. Bio/technology 6;47-55.

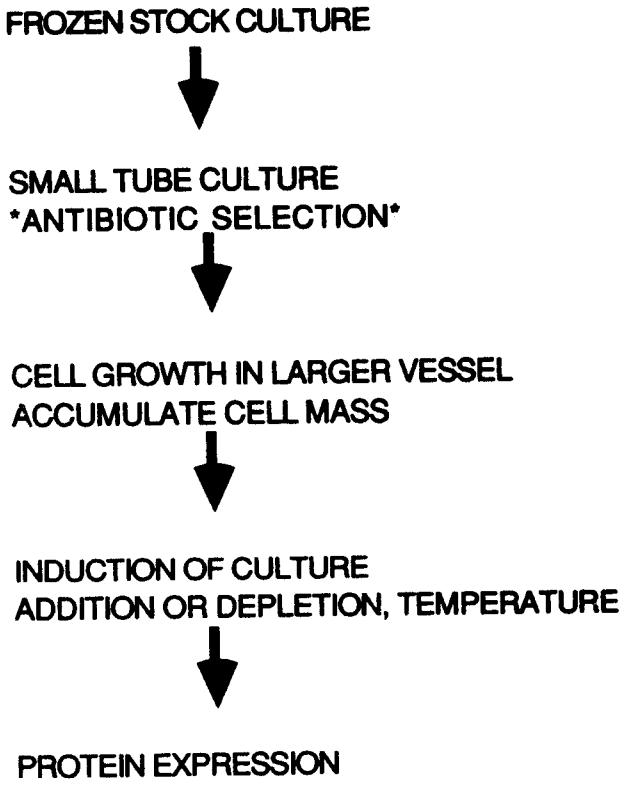


FIGURE 1. Protocol for recombinant expression of proteins in E. coli

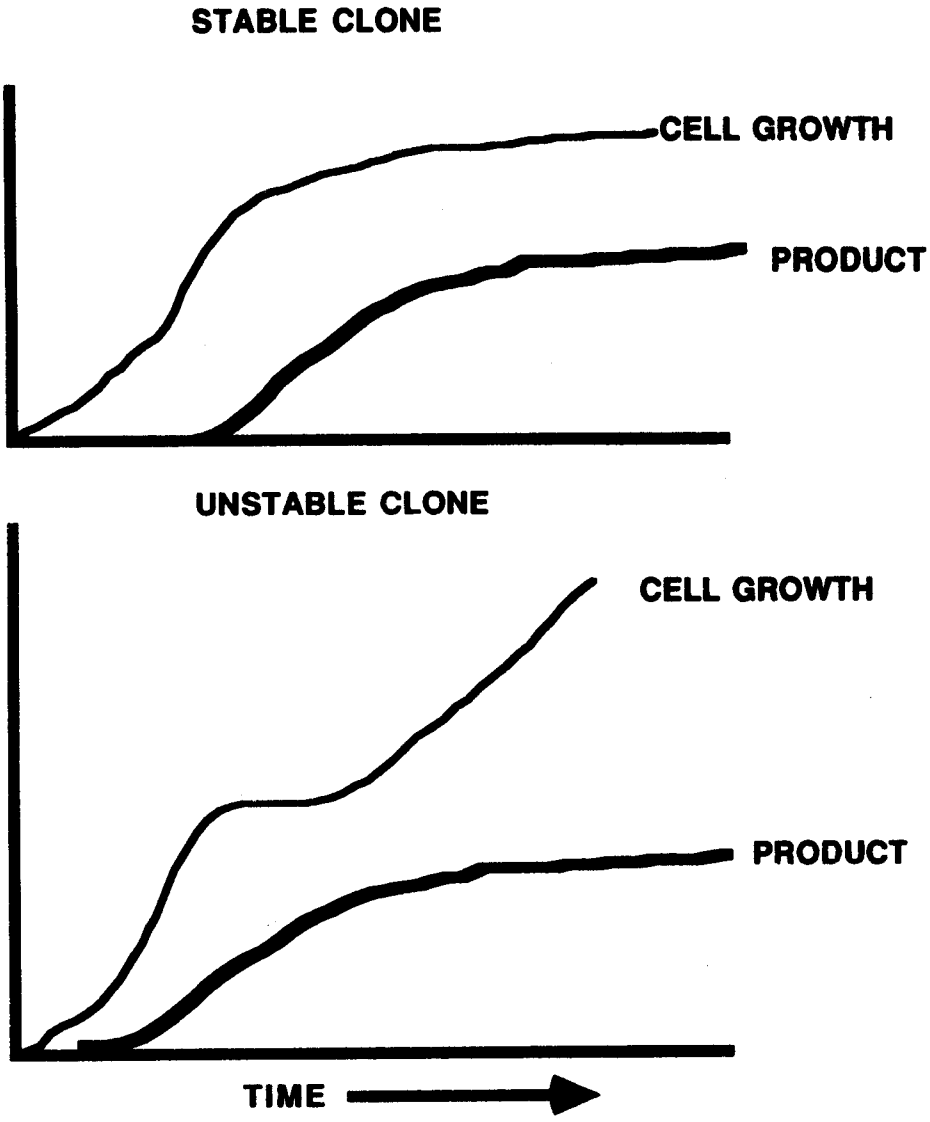
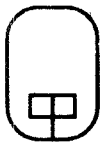


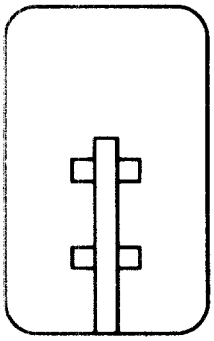
FIGURE 2. Time course of protein production for E. coli stable and unstable clones

TEST TUBE-
POOR OXYGENATION
LIMITED pH CONTROL
GOOD TEMPERATURE CONTROL
NO SUBSTRATE CONTROL

SHAKE FLASK-
BETTER OXYGENATION
LIMITED pH CONTROL
GOOD TEMPERATURE CONTROL
NO SUBSTRATE CONTROL



SMALL FERMENTER
GOOD OXYGENATION CONTROL
pH CONTROL
GOOD TEMPERATURE CONTROL
SUBSTRATE CONTROL POSSIBLE



LARGE FERMENTER
LESS OXYGEN CONTROL
pH CONTROL AND MIXING RATE
TEMPERATURE CONTROL AND MIXING RATE
SUBSTRATE CONTROL AND MIXING RATE

FIGURE 3. Characteristics of vessels used for incubation of cell producing heterologous proteins

2.5 THE ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Dr. William Kenealy, University of Wisconsin-Madison, USA

I would like to discuss the AIDS virus and some of the efforts to develop a vaccine for AIDS. I choose this topic because I have worked on diagnostics and human serology in response to the AIDS virus for three years and because it isa very good example of the approaches being used in biotechnology today.

The characteristics of AIDS virus are such that it would be dangerous to consider a killed virus or a live virus vaccine. These limitations make us go to other technologies for the development of a vaccine. We have a lot to learn before we are ready to begin making a vaccine. We need to know where the neutralizing antibodies bind and what parts of the virus can generate protective immunity.

The virus infects the T4 cells of the immune system. The T4 cell is the T helper cell and is central to the functioning of the immune system. When the T4 cell communicates with the B cell, antibodies are made. The virus after initial infection undergoes a latent period when viral DNA can be detected in the genome of the cells but no virus is produced. The virus is hidden in the cells and can be transmitted that way. People infected with the virus can pass the virus before they know they are infected.

While there are other cells that will support the virus growth the disease is primarily mediated through the T4 cell. The T4 cell is killed in a couple of ways. The growth of the virus produces a cytopathic effect which will kill the cell (1). Also the virus infected cell is producing a protein which is glycosylated and is located on the outside of the infected cell. This protein, known as GP120 is the key protein in determining the interactions of the virus with the targets of the virus. Antibody to GP120 is made in AIDS patients. It is made relatively early in infection and can bind to infected T4 cells. Antibody bound to the outside of the cell signals that there is something wrong with the cell and macrophages and other cytolytic cells recognize the cells and antibody mediated cytolysis occurs (2).

Several groups of researchers have found that GP120 comes off the infected cells and can bind to uninfected T4 cells. This takes place because there is a receptor for GP120 on the surface of the T4 cells called CD4. Another group at Duke University showed that these CD4 positive cells which have bound GP120 and antibody to GP120 are then recognized as foreign and cytolytic cells will kill them also (2). Thus the population of T4 cells, both infected and uninfected is rapidly depleted. When the AIDS virus infects T4 cells eventually all of the functions of the immune system catalyzed by the T4 cell are affected.

A major limitation in studying the virus is that there is no usable animal model for AIDS. Chimpanzees can be used but when infected with the AIDS virus they become viremic and do not develop the symptoms of AIDS. Although virus is circulating in their blood they do not become sick with immunodeficiency disease. You cannot check results of therapies, but you can check whether or not a vaccine will raise a neutralizing antibody response or generate protective immunity.

This brings us to the question of what is known about the immune response to the AIDS virus? Antibodies are normally made in the first three months after initial infection. One of the proteins that is recognized early is the GP120 I have mentioned. The gene for the envelope encodes for a GP160 protein which is cleaved to form the GP120 and GP41. GP41 is the transmembrane protein and holds GP120 on the outside of the virus or cell. Both of these proteins are important because the envelope proteins are major targets for possible vaccines. Another important protein in serological studies is the GAG protein or group antigen. It is produced first as a 55 kilodalton precursor and broken down into smaller fragments P17, P24, and P15. We also see antibodies to P17 and to the core protein P24 which binds the RNA of the virus. Antibodies also are produced against other proteins such as the reverse transcriptase.

What happens after initial infection? The antigen will build up and antibodies to the various proteins will start to rise in titer. Some people remain perfectly stable in their antibody profile and it may be 3-4 years before other changes occur. A correlation has been made where people start to contract AIDS and die when antibody to P24 decreases and P24 antigen starts to increase in the bloodstream (3,4).

This can be used as a prognostic assay, which would assess the chances of a person progressing to AIDS. The sequential pattern allows a prognosis which can tell us whether a person will come down with the disease depending on whether the titer against the core antigen is dropping, rising or remaining constant.

The first antibodies developed on infection appear to be reactive with GP120, on the exterior of the virus. These can be detected by radioimmunoprecipitation (RIP) and the binding site of at least some of these have been localized to the carboxyl end of GP120 (5). The RIP assay will detect antibodies even before a whole virus ELISA. At the same time the RIP assay detects antibodies to GP120 an ELISA assay using ENV9 (which is produced in E. coli and represents the carboxyl end of GP120 and most of the GP41) also detects the antibodies (5). This type of assay is much easier to run and is quantitative in nature. The antibodies rise fast to the region of the transmembrane protein represented by the protein P121 (60 amino acids of GP41 produced in E. coli (6),). These antibodies can be detected all the way through disease progression. We also find antibodies to core

protein developing early during infection. Antibodies are eventually made to most of the proteins of the virus.

Neutralizing antibody activity has only been correlated with antibodies to the GAG protein, GP120, GP41 and the reverse transcriptase. There are regions of the virus which elicit a large immune response but which do not seem to have any antiviral activity (such as those antibodies which bind to P121). So basically these can be considered immunodominant epitopes to which a lot of antibody is made. These antibodies bind to virus, infected cells and free protein and the immune system clears the virus or proteins from the bloodstream. If these antigens were to be present for longer periods of time there may be other antibodies developed. Antibodies that do not neutralize the virus may enhance viral entry to different target cells (7). The exact role of these antibodies in the immune response to the virus is not known and awaits further research efforts.

Now we are ready to pose the question: how do we develop a vaccine? Certainly an attenuated vaccine is a very dangerous proposition. There is the possibility of the viral genome becoming incorporated into the cells. Recombinant vaccines, where the envelope protein is made by vaccinia virus have been constructed (8). Again we do not know enough about the antibodies elicited to know if the antibodies will have an enhancing activity or an antiviral activity. Component vaccines are pretty much the same as we have just seen illustrated for bovine herpes virus. Vaccine trials are currently underway with the GP120 protein as are trials of recombinant virus vaccines. Both these trials are still at the stage of proving that they do not harm the human host; they have not yet reached the stage of assessing whether any antiviral activity is achieved. There is also the possibility of a peptide vaccine of wholly synthetic peptides. This has possibilities for AIDS as we can select regions which are able to elicit antiviral effects without the possibility of producing antibodies which will enhance the entry of virus into cells.

I want to present a quick idea of what we can expect from the different types of immunogens. If you use a whole live virus obviously you will get a humoral response to all the epitopes recognized. You will also get some cell mediated immunity which will be very effective provided the host can overcome the virus. For AIDs this is not the case, but for most other viruses, if the host survives they have all these defense functions in place. We could use whole killed virus and most of the epitopes of the live virus will be in place. Some of the cell mediated immunity would be missing. If you use a single protein expressed in a recombinant virus some epitopes will not be seen. Sometimes epitopes will not be seen. Sometimes the right epitope will not be found at all because it depends on a mixture of several different proteins to make it up. There will also be limitations

on the cell mediated immunity. A recombinant vaccinia vaccine with the exterior envelope protein, would not offer all the epitopes, but some cell mediated immunity might be found since infected cells will be making the proteins.

Recombinant proteins used as a component vaccine also have some limitations. When you make a protein in E. coli there is no glycosylation; no carbohydrate will be attached to the protein. When you make this type of protein in a mammalian cell the protein will be glycosylated. If a protective immune response is to amino acid sequences, then the proteins made in E. coli may be an effective vaccine. Likewise, chemically synthesized peptides could also be used. If the protective immune response relies on having the native configuration of the protein, including its glycosylation, presented to the immune system then the proteins produced in E. coli and synthetic peptides will do no good.

Various methods have been used to produce protein which will resemble the native protein. The group at Duke University have used insect cells to the protein with some carbohydrate attached (8). A company called Microgenesis, National Cancer Institute and another company called Repligen have all been involved in trying to produce or use the material from insect cells for vaccine trials (8). Chiron has produced GP120 in yeast however the yeast system puts on more carbohydrate than expected. Duke University and NCI have also produced the native GP120 from infected cells but purification from infected cells is more difficult since other viral proteins are present. The protein has also been made in recombinant mammalian cells by Genentech.

A variety of proteins produced in E. coli did not work. They just did not elicit any neutralizing antibody when put into animals. One of these proteins, PB1, which was made by Repligen was able to elicit neutralizing antibodies in rabbits and goats (9). The antibodies from these animals were able to neutralize the infectivity of only the virus isolate from which the protein was derived. This small section of the envelope protein was able to elicit a neutralizing response in animals which was greater than or equal to that obtained with the native GP120 or deglycosylated GP120.

One immune response we want to elicit is a neutralizing antibody. So we will first search for neutralizing epitopes. We will have to look at the other components that go into a good vaccine later. All the proteins, thus far injected into the animals have produced only a type specific response.

That is very disappointing because the AIDS virus mutates rapidly. A protein or peptide which only elicits an antibody response to one type is not going to be a good vaccine. When look at human serum we find group specific neutralization. These antibodies could arise as a result of continuous exposure to the

virus or they could be the result of mutation and neutralization of several different strains of the virus.

These antibodies may depend on a number of things. The three dimensional structure of neutralizing epitopes may be assembled from several different protein portions. Or it could be a less dominant epitope that is only seen a long time into infection. If the response was present upon initial infection it might have a protective effect. Since these antibodies are present in AIDS patients they do not do much good after infection.

What we want to define is the binding site of any neutralizing antibody preferably one that is group specific. Since the PB1 region as expressed in E. coli was able to elicit a type specific neutralizing antibody my colleagues at Dupont and Duke University and I reasoned it would be a good region to investigate using synthetic peptides. The entire sequence of GP120 was divided into regions and synthetic peptides made representing these regions (10). We concentrated on the PB1 region for our initial studies. The peptides were made overlapping by 5 amino acids so all continuous sequences of 6 consecutive amino acids would be represented.

The peptides were synthesized by a manual method called Ramps which is marketed by Dupont. We were able to synthesis 50 to 60 milligrams of a peptide very rapidly and have enough to test human serum reactivity and inject into animals to test the resulting antibodies. The peptides which covered the PB1 region are shown in Table 1. The reactivity of human HIV-1 positive sera is also shown in Table 1.

The ELISA assay results of Table 1 are from a screening assay and the peptides were fixed to the plates with a high level of glutaraldehyde. From these and similar results we decided to concentrate on peptides 1-68, 1-69, 1-73, 1-74, 1-77, 1-80, 1-81, and 1-84. We compared the reactivity of normal and HIV-1 positive serum and these results are in Table 2.

The results of Table 2 show evidence of the reactivity assayed by peptides 1-69 and 1-80 as being the result of infection with the AIDS virus HIV-1. The reactivity of HIV-1 positive serum with some of the peptides was encouraging. At least 2 of the peptides of the PB1 region were recognized. We also wanted to know if any of these peptides would elicit a neutralizing antibody in animals.

We conjugated all the peptides to carriers such as ovalbumin or keyhole limpet hemocyanin and injected the conjugates into guinea pigs. Out of all the peptides shown in Table 1 the only one which was able to elicit an antibody response which was able to neutralize the virus and block cell to cell fusion was peptide 1-69. The other peptides did not elicit a response that produced antibodies which bound to gp120 on western blots. The

neutralizing activity was unfortunately type specific and was elicited by a segment of protein that is hypervariable in virus isolates (11).

The peptides representing other sections of GP120 are being screened with HIV-1 positive serum for evidence of reactivity. Any of these peptides which show reactivity with human serum will also be tested for the ability to raise neutralizing antibodies in animals. We hope to be able to find regions of GP120 which will elicit a group specific neutralizing antibody. Even if that is found there are other important components of a vaccine which should be present. The work would have to continue on finding a way to elicit cell mediated immunity if that is required to gain protective immunity.

Overall the field of AIDS research is using the available technology as soon as it can be applied. The production of recombinant antigens for vaccines and diagnostics is a small portion of the total effort at combating AIDS. Other techniques such as DNA probes, polymerase amplification of DNA, production of anti-sense message etc are being applied to basic research problems as well as leading to possible therapeutics.

Literature Cited

1) Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) Science 220, 868-870.

2) Lyerly, H.K., T.J. Matthews, A.J. Langlois, D.P. Bolognesi and K.J. Weinhold. Proc. Natl. Acad. Sci. 84, 4601 (1987).

3) De Wolf, F., Lange, J.M.A., Houweling, J.T.M., Coutinho, R.A., Schellekens, P.T., van der Nororda, J., and Goudsmit, J. (1988) Journ. Infect. Disease 158, 615-621.

4) Kenealy, W.R., Reed, D., Cybulski, R., Tribe, D., Taylor, P., Stevens, C., Matthews, T., and Petteway, S. (1987) AIDS Res and Human Retroviruses 3, 95-105.

5) Saah, A. J., Farzadegan, H., Lee, T. H., Petteway, S. R., Rinaldo, C.R., Phair, J.P., & Fahey, J.L. (1987) III International Conf. AIDS Abstract T.3.1.

6) Chang, N.T., Chanda, P.K., Barone, A.D., McKinney, S., Rhodes, D.P., Tam, S.H., Shearman, C.W., Huang, J., Chang, T.W., Gallo, R.C., & Wong-Staal, F. (1985) Science 228; 93-96.

7) Barnes, D.M., (1988) Science 241; 533.

8) Matthews, T.J., and Bolognesi, D.P. (1988) Scientific American, October 120-127.

9) Putney, S.D., Matthews, T.J., Robey, W.G., Lynn, D.L., Robert-Guroff, M., Mueller, W.T., Langlois, A.J., Ghrayeb, J., Petteway, S.R., Weinhold, K.J., Fischinger, P.J.,Wong-Staal. F., Gallo, R.C., & Bolognesi, D. P. (1986) Science 234;1392-1395.

10) Kenealy, W.R., Matthews, T.J., Ganfield, M-C., Langlois, A.J.,Waselefsky, D.M., & Petteway, S. R. (1989) AIDS Res and Human Retrov. 5;173-182.

11) Hahn, B.H., Gonda, M.A., Shaw, G.M., Popovic, M., Hoxie, J.A., Gallo, R.C. & Wong-Staal, F. (1985) Proc. Natl. Acd. Sci. USA 82:4813-4817.

TABLE 1. Reactivity of HIV-1 positive sera with peptides representing gp120 amino acid sequences

PEPTIDE Number	Sequence	Number of Sera Reactive	% Reactive	Reactive Sera Average Absorbance ± SD
I–67	LNQSVGINCTRPNNNT	0	0	———
I–68	RPNNNTRKSIRIQRG	1	8	0.23 ± 0.00
I–69	RIQRGPGRAFVTIGK	8	67	0.79 ± 0.40
I–70	VTIGKIGNMRQAHCNI	0	0	———
I–71	QAHCNISRAKWNNTL	0	0	———
I–72	WNNTLKQIDSKLREQF	4	33	0.29 ± 0.10
I–73	KLREQFGNNKTIIFK	10	83	0.85 ± 0.44
I–74	TIIFKQSSGGDPEIV	4	33	0.34 ± 0.10
I–75	DPEIVTHSFNCGGEF	0	0	———
I–76	CGGEFFYCNSTQLFNS	0	0	———
I–77	TQLFNSTWFDSTWST	5	42	0.36 ± 0.13
I–78	STWSTKGSNNTEGSD	4	33	0.30 ± 0.12
I–79	TEGSATITLPCRIKQI	0	0	———
I–80	CRIKQIINMWQEVGK	2	17	0.22 ± 0.06
I–81	QEVGKAMYAPPISGQI	5	42	0.37 ± 0.10
I–82	PISGQIRCSSNITGL	1	8	0.21 ± 0.02
I–83	NITGLLLTRDGGNSNE	3	25	0.29 ± 0.06
I–84	GGNSNNESEIFRPGG	5	42	0.31 ± 0.06

TABLE 2. Comparison of the reactivity of HIV-1 positive and negative sera on selected peptide ELISAs

PEPTIDE Number	HIV POSITIVE SERA # Reactive sera	HIV POSITIVE SERA Average Absorbance	NORMAL # Reactive sera	NORMAL Average Absorbance
1–68	1	———	0	———
1–69	12	0.71 ± 0.49	0	———
1–73	16	0.28 ± 0.13	3	0.24 ± 0.03
1–74	0	———	0	———
1–77	0	———	0	———
1–80	14	0.34 ± 0.24	0	———
1–81	1	———	0	———
1–84	0	———	0	———

ELISAs were run as in Table 1 except the microtiter wells were blocked with diluent for 1 hour prior to use. Sera were scored as reactive if they gave an absorbance greater than 0.2. Thirty-seven HIV–1 positive sera and forty-seven normal sera were tested.

2.6 APPLICATIONS OF BIOTECHNOLOGY TO ANIMAL PRODUCTION

Dr. Neal First, University of Wisconsin-Madison, USA

I will discuss some of the newer technologies that affect embryo production and eventually might be applied to embryo transfer. We will consider embryo production in the laboratory, how to clone genes into those embryos and how to clone the embryos themselves.

Two biotechnologies that have traditionally been used with animals will be the means of delivery for newer biotechnologies in the future.

One of these is artificial insemination, which through the last 30 years, has resulted in a twofold increase in milk production. However, there are some problems in changing animals by this method. Changes are only through the bull. Secondly, it is slow. Thirdly, it does not allow the introduction of genes from other species.

The second biotechnology for animal use is embryo transfer. I believe there are more than 60 veterinarians in the Brazilian Embryo Transfer Society at this time. Embryo transfer also has some limitations. The number of embryos we can produce from a bull x cow mating are small. Secondly, every sperm-egg combination is a new embryo which is totally different from each other embryo. They are brother and sister, but they are not alike. So the expected milk production from a set or flush of embryos, may be 18,000, 20,000, 23,000, 25,000 or 30,000 pounds of milk, but only one embryo would be at each production level. If we were to make the greatest improvement in milk production, we would like them all to be the highest and without this variation.

I will be talking about three technologies. These are the development of ways to produce embryos totally in the laboratory. To clone embryos to make them identical and to transfer genes from other organisms into those embryos to change the product the cow produces or the characteristics of the cow, perhaps disease resistance.

In production of embryos in the laboratory, there are three parts. First is the question of how to mature the egg in the laboratory. The egg is taken from small follicles, perhaps even from a slaughtered animal. Second, how to mature the sperm so they are capable of fertilizing the egg in the laboratory. How to do the fertilization, and finally how to develop the fertilized egg to a stage where we could transfer it to a cow. This all has to be done totally in culture.

Maturation of the nucleus of the oocyte has been easy. Maturation of the cumulus cells around the oocyte has also been

easy. Maturation of the cytoplasm so that it is capable of causing the fertilized egg to go through development has been difficult.

In maturing or preparing the sperm to fertilize, one of our students has discovered that the oviduct contains compounds that will prepare the sperm to fertilize. We call this process "capacitation". The oviduct produces compounds called glycosaminoglycans, which cause sperm "capacitation". The natural product of the oviduct that prepares the sperm to fertilize appears to be a heparin sulfate. By administering heparin to sperm as they are in culture with the egg, over a period of 4 to 6 hours, the sperm are prepared to fertilize the egg at high frequency.

We also use a sperm swim-up separation, which involves putting the semen in a column in which the live cells will swim to the top and the dead cells will stay at the bottom. With this, we have high quality sperm removed from the top, treated with heparin and the eggs are fertilized. These eggs are harvested on different successive days. The fertilization frequency is approximately 80% and repeatable day after day.

The greatest supply of eggs for <u>in vitro</u> fertilization would result if eggs could be used from the abundant small follicles of the ovary (1-5 mm) rather than the few preovulatory follicles. When these were compared, the rate of completed fertilization where they form a pronuclear egg, a one-cell fertilized egg, was the same, but the ability to become an embryo of late stage which could be transferred into a cow was about 38% for those matured in the cow. For those matured in the laboratory it was zero. The problem was then to determine what the cow contributes to development of the egg in vivo that allows it to go through completion of embryo development and how do we change the situation in the laboratory.

We cultured the cells that the egg grows with. They are called granulosa cells of the follicle. Each of the cultures was treated with hormone: FSH, LH, estradiol. When we co-cultured with the granulosa cells, the immature eggs from small follicles were able to go through development just as well as ones matured in the cow. This granulosa cell co-culture or the use of extra cumulus cells provides a system in which we can culture embryos in the laboratory totally to a late stage of development and compatible with good pregnancy rates after transfer into recipient cows.

The next problem was how to culture the embryos <u>in vitro</u>. Here the important problem is that embryos of all species are blocked in their ability to go through development at the exact point where they change from maternal or egg control of development to the new embryo control of development, the expression of the father's genetic information begins at this time. These

transitions occur at the 2-cell stage in the mouse, the 4- to 8-cell stage in the pig, 8- to 16-cell stage in sheep and 4- to 16-cell stage in cattle. In frogs and other amphibians this transition is very late, about 4000 cells. This is important for two reasons. One, because the blocked development in culture occurs at this transition for all species. It is not that the culture fails to support development but that the oviduct does something to allow turn-on of new genetic information for these embryos, which we cannot do with just a cell culture that has media alone. The other aspect that will be important when we consider cloning embryos is that it is mostly the non-differentiated donor embryo that is useful for the cloning procedure. So a frog can produce many clones. The procedure does not work so well for mice which are differentiated after two cells.

How do we develop embryos in the laboratory? Originally, we put the embryos in agar blocks, the same agar you would use for culturing bacteria. We put these blocks in the oviduct of a sheep and let them culture in the sheep. When they developed to a late stage we transferred them into a cow. The next step was to ask what was in the oviduct of the sheep. We removed the epithelial cells from the sheep or cow oviduct and made a monolayer culture of them. Then we placed the embryos on those oviduct epithelial cells in culture; this is a co-culture. Forty-three percent became blastocysts which could be transferred into a cow and 63% went past the period of blocked development. If we cultured in medium alone, in the absence of oviduct cells then none of these would develop. Either sheep or cow epithelial cells supported embryo development. Now we had a system in the laboratory where we could culture to a morula or blastocyst stage which could be transferred into a cow to produce calves.

From this system we produced several offspring. Our first calf was born in 1986. There are other laboratories that have followed this procedure around the world. Some of them do it more efficiently than we do, in particular a laboratory in Ireland and two laboratories in Japan. The interesting part is that both countries are doing it to multiply a breed of animals and not individuals. In Japan they are using it to produce Japanese black cattle which are considered to produce tender meat. You can produce a thousand or more embryos easily in a day from ovaries of slaughtered cattle with this technology.

The next technology I want to talk about is how to clone embryos; how to make identical copies. The first approach is one which has been used here in Brazil by Dr. Roberto de Ben. It is a technology developed in Cambridge, England by Stein Willadsen. He developed it in 1982 and in 1983 and we used it for a project with American Breeders Service. The procedure used a microknife to cut the late stage embryo in two parts.

One part is left in its original casing, its zona pellucida. The other part is placed in an empty zona pellucida from an unfertilized egg. Both parts are transferred into cows. There are at least 10 embryo transfer companies in the United States now that do this regularly and with very good success. Almost identical pregnancy rates result from bisected and whole embryos. We produced about 60 calves. There were 15 twin pairs.

This system is only useful for producing two embryos. If we cut the embryo to make four or eight, then there are not enough cells in each one to form a surviving fetus after the embryo is transferred. So what is needed is a way of producing many identical embryos. We would like to produce many identicals to provide a system for performance testing and selecting clonal lines. The cloning and selection system would work as follows. If we identify female embryos and clone each to about 30, we could keep 10 frozen, and we could transfer 20 to cows to produce 10 calves and then eventually 10 new cows. After 3 years of milk production, those 10 cows, being identical, would give an estimate of the milk production of all the descendents of this line of embryos. One embryo and its descendents might produce 18,000 lb of milk, some 20,000, 23,000 and sometimes there will be one line of descendents that would all produce 30,00-0 lb. We would like to save this line and reclone to multiply to larger numbers. Now, the question is, can we do it?

Frogs have been cloned for a long time by a procedure that is very close to the one that we use for cattle. But in the frog, differentiation does not occur until the late gastrula stage at 4000 cells. So we have 4000 donor cells that are identical to use. When this is done in the frog and these are transferred into an enucleated oocyte of the frog, 62% develop back to the stage that was started with, the gastrula. Thirty-five percent become young born frogs. However, if we use differentiated tissue and test the epithelium, then very few ever develop. So there is one important principal: we must use non-differentiated or only slightly differentiated cells to begin with or we must be able to differentiate and redifferentiate.

When these experiments were tried in the mouse, they did not work. When transfer was performed between pronuclear, one-cell fertilized eggs of mice, the procedure worked and 95% became late stage embryos, blastocysts. But when any of the multicellular stages, 4-cell, 8-cell and onward, were used as donors --and that is what you would like to use-- none of them developed. The reason that they do not develop is that the mouse transitions from maternal to embryonic control of development and initiates genomic differentiation by the 2-cell stage. The second reason that it did not work was that these experiments all transferred into an enucleated fertilized egg which has no ability to reprogram a nucleus. The oocyte has some ability to do so.

We have developed a model for nuclear transfer in mammals which does work. It is very much like the model we used in frogs. We recover embryos non-surgically by flushing the uterus of the cow. These are embryos at the 20- to 60-cell stage. Let us suppose that there are 30 cells. The procedure involves removing each one of these cells individually, transferring the cell into an oocyte that has had its nucleus removed. The oocyte is waiting for the sperm to fertilize it. We aspirate out the metaphase plate and polar body of the oocyte so we remove all the nuclear material from the oocyte. In the space that is left, outside the plasma membrane of the oocyte, we deposit the donor cell. Then with electrofusion, we fuse one cell into the other and develop it back to the stage we started with, which may be 30 cells. The idea is that if we could get as many as 10 embryos produced from the 30 we started with, once through this cycle we would have 10 embryos. But the second time we did it there would be 100. The third time there would be 1,000, the fourth time 10,000. That is the idea behind cloning embryos. We have produced cows from this, but we have not attempted to produce large numbers. Our attention has been directed to improving the efficiency of each step.

There are others who have done the same work in private industry and who are attempting to produce a large number of cows. One group is Granada Genetics in Texas. The other is Allagenetics in Calgary, Canada. This same procedure has resulted in pregnancies from nuclear transfer derived embryos in cattle, sheep, rabbits and pigs. The efficiency, however, has been low.

Some of the steps that are important and contribute to efficiency are as follows: micromanipulation, cell fusion, oocyte activation, embryo culture and recloning. The entire process is at a level of about 20% efficiency.

Finally I want to talk about transferring genes into embryos. There are four reasons for transferring genes into embryos of cattle. First, we would like to transfer new genes into embryos of cattle to increase the efficiency of milk production. The gene of interest is growth hormone. If we inject growth hormone in cattle, we expect a 20% increase in efficiency and more than that in milk production. As a model for cattle we are using mice and so we are doing experiments with the growth hormone injected into mice. We are measuring milk production of the mice with milking machines on the mice. We study the effect of growth hormone on strains of mice that are high in milk production and strains low in milk production, and so we are asking the questions of importance to cattle production in mice.

Secondly we might want to introduce genes for increasing disease resistance. In mice there is evidence that genes for the major histocompatibility complex will protect the mice from some viral diseases, if introduced to make them transgenic.

We would like to change the milk or cheese production so in mice we are putting in genes for different caseins to change the kind of cheese the mouse makes --or the cow if we did it in cows. Also in cattle there is a problem with casein and the binding of proteins. The casein in cows milk is very poor for binding other proteins. Casein in goat and sheep milk is much better. So if a cow had a different casein, then we might have more cheese from the cow's milk. There are also some strains of cattle that are very poor in casein binding of proteins and those strains are avoided in cheese making.

We would like also to produce pharmaceutical products through milk. This was first discovered as a possibility last year in Scotland and at the National Institutes of Health in the United States. It is possible now because we have promoter sequences that will target a gene to the mammary gland without expression in the rest of the body. There are three useful promoters: the mouse mammary tumor virus, a actoglobulin promoter sequence and an acidic whey promoter sequence. Any one of the three, when attached to a gene of interest will cause expression of that gene only in the mammary gland.

Scientists at Edinburgh, Scotland, did a very exciting experiment. They transferred into embryos of mice and sheep the gene for clotting factor-9 with a promoter targeted to the mammary gland. The gene was expressed and the new protein produced in the milk. Clotting factor-9 can then be extracted from the milk of the sheep as a new protein. It is estimated that 100 sheep producing clotting factor-9 could produce all the clotting factor-9 that the world needs.

At the National Institutes of Health the gene for tissue plasminogen activator was put into embryos of mice and shown to be expressed in their milk.

Many pharmaceutical companies are now asking about cows that might produce milk containing tissue plasminogen activator because they want this product produced in a post translational way that perhaps only the mammary gland could do.

In cattle we microinjected the gene for thymidine kinase into a pronucleus of a 1-cell embryo and assayed the expression of additional thymidine kinase 2-cell cycles later. When we did this about 30% of the embryos expressed additional thymidine kinase above normal levels. If we carried this through to the born offspring, the efficiencies would get very bad. They would probably be less than 1%. And that is indeed the problem in microinjecting genes into embryos. The efficiencies are very bad. There are more than 400 strains of mice now produced in the world from gene microinjection. We call these transgenic. But for every egg that is injected in mice, on average in the best laboratories the success rate is about 1.3% that actually express the gene as born mice. It is even worse for domestic

animals, about 0.3% in pigs, sheep and cattle. This is not an acceptable efficiency. We estimate in cattle that if you were to put a gene into valuable embryos in the place that you would want them you would need an investment in embryos of at least $500,000. What to do about this problem? We can do it in mice. The cost is low and we can turn them over rapidly. But not in domestic animals. In spite of this, in swine there are at least 12 strains of transgenic pigs that have been produced by gene introduction and there is at least one case of born offspring that express genes in cattle, but the efficiencies are terrible.

We focus now on how to improve the efficiencies. Microinjection in mice is 1.3% efficient now. In mice if we use a replication defective viral vector to introduce genes, the efficiency of expression can be as high as 20% in the born offspring. That is a big difference. We call this a suicide virus, or replication defective virus. We are developing replication defective viral vectors for use in cattle in collaboration with Howard Temin at Wisconsin. This procedure is dependent on infecting a helper cell with an incompetent rescue virus which provides the proteins needed to rescue the defective viral vector. The resulting virion can infect once, but it is not capable of replicating because it does not have a functional encapsidation site. Such vectors have been used to infect cells of mice, hamster, sheep, goats and recently cattle. They may prove useful for infecting genes into embryos.

There is a second method for gene transfer that has been successful in mice and hamsters but not the domestic animals. This is called embryonic stem cell transfer. In mice stem cells removed from the presumptive embryonic germ cells are grown in culture in a non-transforming way, then removed from culture and chimerized into the inner cell mass of another embryo. Genes are transferred into the cells while in culture. This has resulted in offspring chimeric in their body and germ line cells for the cultured cells. With mating and selection true breeding lines derived from the cultured cells are established.

In summary, embryo production in the laboratory is being commercialized in Ireland and Japan. It may not have much use in the United States because we do more individual selection than breed selection. However if someone wants to multiply a breed it will be a useful technique.

The cloning experiments are moving rapidly in several areas and I would expect that within 2 or 3 years cloning of embryos will be commercialized by one or more commercial companies.

The gene transfer aspects will be slower in application, because even though we may have a good system next year, or 2 years from now, we will have to wait for safety approval for environmental release.

Additional Reading

First, N.L. and Barnes F.L. Symposium on Development of preimplantation embryos and their environment. Kyoto, Japan, July 14-16 (1988).

Gandolfi, F. and Moor R.M. Proceedings 11th International Congress AI and Animal Reproduction. Dublin (1988).

Murray, J.D., Nancarrow, C.D. and Ward K.A. Proceeding 11th International Congress AI and Animal Reproduction. Dublin (1988).

Rexroad, C.E. and Pursel V.G. Proceedings 11th International Congress AI and Animal Reproduction, Dublin (1988).

Simons, J.P., McClenaghan, M., Clark, A.J. Science 328, 530-532 (1987).

2.7 HEALTH ISSUES IN INTERNATIONAL GENETIC EXCHANGE

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As we anticipate the effects of the biotechnology revolution on the exchange, conservation and management of genetic resources in agriculture and the larger biosphere, it is appropriate to consider the role of veterinary medicine in the global exchange of these resources. As a first principle we must remember that biotechnology will not overcome the need for high standards of management and disease control if we are to fully utilize these new genetic resources.

I believe there are two dangers in our attitudes toward genetic manipulation. The first is a naive belief by some that the genome can be altered to create the biological equivalent of a perpetual motion machine: animals or plants that will be highly productive without fundamental husbandry and disease control. Both adaptability and disease resistance may be changed by biotechnology, but genome manipulation will not repeal the laws of energy conservation or completely upset the evolutionary balance between host and parasite. We cannot forego continuing efforts in disease control, improvement of nutritional resources, and producer education in the expectation that such efforts will be rendered unnecessary by the genetic revolution. Neither can any nation with intentions to improve its national herd ignore or abandon performance and progeny testing, since the effects of genetic engineering and asexual propagation ("cloning") are additive to the older methods, not a replacement for them.

The second danger is a more immediate one for regulatory veterinary medicine. If the fundamentally different animal health issues involved in new methods of genetic exchange and the new diagnostic capability created by biotechnology are not assimilated by veterinary medicine, obsolete attitudes and diagnostic methods will delay the benefits of such exchange and may even lead to new disease problems. Transgenic animals may represent a resource that is not limited to agriculture, as such animals could be used to produce medically important proteins. If this potential is realized, creation transgenic animals will not be limited to those parts of the world with the most immaculate animal health status. Veterinary regulators will then have to find ways to safely import these animal pharmaceutical factories, or their nations will become dependent on external sources of the new biologics.

In many respects, the animal health issues of genetic exchange have been with us for many years, which is one reason a representative of the artificial insemination (AI) industry such as me is making this presentation. A classification of the infectious diseases of concern in bovine AI was proposed in 1976

by Bartlett, et al., and with revision is still a useful organizational tool for this subject (2,13).
Freedom from agents of specific animal disease (SPF status) can be documented for semen and other forms of germ plasm using four criteria:

1. SPF by territorial status
2. SPF by repetitive immunologic or microbiologic surveillance that demonstrates freedom of the herd from infection
3. SPF by hygenic control of environmental contamination
4. SPF by addition of antibiotics or other substances

Documentation of the disease-free status of seminal donors commonly utilizes each of these levels of SPF certification. Most of the obstacles to international germ plasm movement are found at the level of national territorial or herd status, or when national control strategies for important infectious diseases are fundamentally different.

With some variations, the principles of this classification are applicable to genetic resources in all forms. What is critical to rational sanitary regulations, and the real message of this presentation, is' the need to recognize the fundamental differences among the various forms of genetic resources, and to apply the SPF criteria appropriate for the form of germ plasm being moved.

LIVE ANIMAL TRADE: TERRITORIAL AND TEST CERTIFICATIONS

Trade in living animals depends heavily on SPF certifications based upon territorial status for disease(s) within some defined political boundaries. Additionally, various immunologic or microbiologic tests of the animals to be exported are performed up to the time of embarkation and upon arrival at the destination. At some time the testing and quarantine of animals must stop, and they are released into the national herd of the recipient nation. This open-ended test schedule creates the danger that disease may be present in a latent or incubating stage, diagnostic methods may be insufficiently sensitive, or may be confounded by immunization, with the result that infected animals could escape detection. For this reason national veterinary requirements and OIE recommendations have placed great emphasis on the clinical and test histories of the herds from which animals for export originate, especially with respect to insidious diseases that are difficult to diagnose in individual animals.

A more subtle concern is the quality of the territorial and herd certifications that may be rendered. These are obviously no better than the epidemiologic surveillance upon which they are based. It is one of the paradoxes of the international animal trade that an exporter who does not perform systematic surveillance for insidious diseases such as bovine leukosis or

paratuberculosis may be able to make territorial or herd certifications that those who practice more diligent surveillance cannot. Presently, one of the obstacles to persuading more cattlemen in the USA to participate in voluntary control programs for these diseases is the discrimination of domestic and export customers if evidence of infection is found.

Greater attention should be given by importers to the intensity of suveillance behind these territorial and herd certificationse behind territorial and herd certifications. Imposition of simplistic time and distance requirements for "disease freedom" in herds or areas can be very counterproductive, especially when dealing with assembled donor herds providing germ plasm in the form of semen or embryos.

In nations that encompass tropical or warm temperate climate zones, insect vector-borne viruses have become a unique challenge to traditional regulatory practices, including both territorial certification and serologic/microbiologic certification of animals. Notable examples include akabane, bluetongue, ephemeral fever, epizootic haemorrhagic disease, Japanese encephalitis, and vesicular stomatitis viruses. Such agents move from enzootic zones into epizootic or sporadic incidence zones in cooler areas largely as the consequence of movement of infected insects by weather systems, although animal movements can also precipitate epizootics if competent vectors are present (24). In territorial certification for such diseases, political boundaries are far less important than the climate and seasonal effects that affect the suitability of the environment for the insect. These environmental attributes should receive greater recognition by regulators of animal movements.

Another paradox of the germ plasm trade is the adverse effect of these vector-borne viruses on the trade in tropically-adapted livestock breeds. The American Brahman and its derivative breeds, the Brazilian Zebu, the Barbados Black Sheep, the Australian Sahiwal and Milking Zebu all represent genetic resources whose best representatives are likely to become seropositive to one or more of these viruses if they remain in the regions of their birth. Young seronegative animals can be selected and removed to vector-free environments, but in such instances the selections are made at an age that prevents full assessment of the animal's genetic merit. It is very contradictory to the best utilization of AI or embryo transfer in genetic exchange when donors are selected because they can pass the export veterinary conditions, with secondary attention to genetic merit.

Immunization practices are an important component of perceived differences in SPF status in international trade. Two difficulties raised by use of vaccines are worthy of mention. First, traditional veterinary vaccines and serodiagnostic methods have not been compatible, since no immunologic markers or antigenic deletions are incorporated into vaccines to enable distinction between vaccinated and convalescent animals.

Consequently, regulatory barriers are often placed against the international acceptance of vaccinated animals. Second, the vaccination practices of the recipient country may create further animal health risks: poorly attenuated products or contaminating blood-borne agents such as bovine leukemia virus and bovine virus diarrhea virus can result in significant health problems among imported animals.

SEMEN AND EMBRYO TRADE: SPF METHODOLOGIES

Unfortunately, veterinary conditions for international movement of semen and embryos have often been derived with little modification from those for live animals. As we move into an era when these two forms of germ plasm will constitute the majority of international exchange, it is important that regulations include the appropriate testing and certification methods, recognizing the real differences in risk of disease transmission among these three entities.

The fundamental difference is the "time-stopping" attribute of frozen semen and embryos, which permits the application of the serologic or microbiologic tests of interest to the donors both before and after collection of the product. The sensitivity and specificity of the test method used, and the potential for spread of the specific etiologic agent should determine the frequency of such tests. For example, bovine campylobacteriosis and trichomoniasis can be transmitted rapidly within an AI center. Accordingly, surveillance for these agents should be performed at least semi-annually. In contrast, tests for an agent such as Mycobacterium paratuberculosis, that is unlikely to be transmitted among adult animals, may be done on an annual basis (7,13). Virologic and serologic surveillance for vector-borne diseases should be performed during and after the seasonal vector activity.

Unfortunately, the simplicity of this scheduling approach often gives way to the highly variable practices and requirements of individual trading partners, with the result that some donors are tested many times each year simply to conform with each customer's prescribed schedule. The simple elegance of a proof provided by negative donor or herd tests at the appropriate intervals before and after collection should be a first principle of sensible regulation of the frozen semen and embryo trade.

In this context, I will suggest that a very desirable management practice for regulators and managers of germ plasm resources is to create and maintain frozen serum banks from donor herds. Such banks can be preserved very conveniently togetner with frozen semen and embryos, and can serve to document the status of donors long after collection of the germ plasm or even death of the donor. This is especially useful if semen is stored and donors disposed of before genetic evaluations are completed. This practice is becoming more common even in large cattle-raising

countries, in order to reduce the costs of housing donors of the the less numerous dairy breeds and the beef breeds, while maintaining selection opportunities and genetic diversity.

Individual collections of semen and embryo recovery medium can be subjected to SPF controls at the microbiologic level. Microbiologic surveillance of individual seminal collections and embryo collection medium is being augmented by several new developments. Complete surveillance of all semen collected in an A.I. center for several common bovine viruses is possible by inoculation of pooled seminal specimens into seronegative calves (22). This method is not only useful for the purpose of export documentation, but the epidemiologic surveillance afforded has helped to characterize the potential of a number of viruses to contaminate bull semen under the practical conditions of commercial artificial insemination.

While the practice of virologic testing of semen, and to a lesser extent embryo recovery medium, has been practiced for years, it is now recognized that these fluids are not always the diagnostic specimen of choice. Opportunistic blood-borne viruses such as those of bluetongue and bovine virus diarrhea are more readily detected in blood specimens obtained from the donor concurrently with semen or embryos, while esophageal-pharyngeal washings are the specimen of choice for isolation of persistent foot-and-mouth disease virus (FMDV) infection (1,4,11,14,18). These diagnostic methods are very useful if serologic methods are confounded by vaccination, serologic response to non-persistent infection, or the absence of measureable immune response to a congenital infection. These techniques have been applied in both Brazil and the USA to the exchange of germ plasm from seminal donors that are seropositive for bluetongue or have been vaccinated against FMDV.

A wider spectrum of bacteria than ever before is now within the control of seminal antibiotic treatment. Addition of the appropriate antibiotics to both raw semen and extender will augment control of both specific and non-specific bacterial pathogens (16,17,25). However, very significant interactions between extenders and antibiotics do occur, so the effectiveness of each combination of antibiotic and extender must be tested (15). Recently completed research suggests that in the future this technique could be further augmented by the addition of specific bovine immune gobulin to seminal extenders for additional control of specific viruses (23).

In the case of embryos, the repeated washing of embryos which have intact zonae pellucidae will remove most viruses (12). Further treatment with dilute trypsin will remove viruses such as bovine herpesvirus-1 and vesicular stomatitis virus that can adhere to the zona pellucida (26,27). Because of this intrinsic safety factor, it is evident that embryo transfer technology is likely to replace live animals for germ plasm exchange between

those nations where high risk infections such as foot-and-mouth disease, bovine leukosis, and infectious bovine rhinotracheitis have presented trade obstacles.

FUTURE DEVELOPMENTS IN INTERNATIONAL GENETIC EXCHANGE

NEW DIAGNOSTIC METHODS AND VETERINARY BIOLOGICS

The effects of the biotechnology revolution in veterinary diagnostics are just beginning to become evident. Many of our traditional serodiagnostic methods have been employed in domestic disease surveillance and control programs because of their low cost and ease of application to large numbers of animals. Unfortunately, some of these methods are poorly standardized within and among the nations of the world. For example, the standard leptospirosis microagglutination-lysis test depends upon living antigens that must be individually prepared and standardized, are a biohazard, and can be overgrown by saprophytic leptospires (10). Even some of the better standardized techniques, such as those employing immunodiffusion, can be insensitive and somewhat subjective, especially when used for serodiagnosis of non-persistent infections or infections by agents which produce serological cross-reactions (3,8,19).

ELISA (Enzyme-linked immunosorbent assay) technology has been known for over 20 years, but is just beginning to enter veterinary diagnostic laboratories. In addition to improved sensitivity and the capacity many samples, this method lends itself to automation and objective interpretation, enabling the laboratory to devote greater resources to quality control (9). Further, the development of genetically engineered vaccines with compatible ELISA diagnostics has already assumed a major role in the regulatory control of pseudorabies and may eventually permit diagnosticians to discriminate between vaccination and natural infection with several animal viruses.

In addition, the ELISA technology is being extended into the hands of veterinary practictioners, farmers, and milk processors in the form of rapid field tests for milk progesterone or antibiotic contamination. One can envision "plane-side" or "ship-side" serologic tests for common infectious agents to reduce shipment and laboratory delays and the resulting disease transmission risks in international livestock trade.

Manipulation of the genomes of viruses employed in vaccines will lessen the risk of reactivation of latent infections or reversion to virulence in vaccinated animals. Since viruses attenuated by specific laboratory deletions can be further marked by the addition of new genes that permit their convenient identification, the acceptance of vaccination and vaccinated animals in the international germ plasm trade may be enhanced. The development of completely non-living vaccines derived from

individual virus proteins and enhanced in effectiveness by new adjuvants or immune modulators will further reduce the perceived risks associated with vaccination.

At least one veterinary biological presently marketed in the USA for prevention of colibacillosis in calves is a monoclonal antibody (mouse immune globulin) preparation. The biological safety of monocolonal antibody preparations for humans has been reviewed and may require similar attention from veterinary medicine (6). These products may have some potential for the subsequent confounding of serologic tests of animals so "immunized" against mouse IgG, to the extent the mouse is the source of antigens for such tests.

Despite improved methods, a major obstacle to germ plasm exchange will remain unless the problem of international standardization is attacked at the same time these new technologies are developed. It has occured to me that animals in AI centers represent a unique resource for diagnostic veterinary medicine that is presently under-utilized. Nowhere else are there animals that have been repeatedly subjected to serologic and microbiologic surveillance over long periods. Depending on national and AI center status and immunization practices, reliable histories for both positive and negative animals can be found. The AI center with which I am associated has cooperated with a number of laboratories and investigators to furnish reference specimens, and I encourage you to also consider your AI centers a resource for this purpose.

It seems likely that in the future greater emphasis will be placed on virus isolation, or specialized serology, instead of the traditional techniques of serodiagnosis. I have already mentioned the use of virus isolation for diagnosis of bluetongue infection. Of even wider importance is the acceptance of FMDV-vaccinated bulls for semen export to the USA, based on their negative seroresponse to the infection-associated or core antigen of FMDV. Virologic diagnosis is likely to replace serologic diagnosis in control of at least two important diseases which are now known to have greater potential for dissemination by persistently infected, seronegative animals. Such animals are responsible for the spread of non-cytopathogenic bovine (BVD) and ovine (border disease) pestivirus infections. These animals excrete virus in many body fluids, including semen, and dams transmit the virus vertically to the fetus (1,18,21). It can be expected that requirements for BVDV isolation from blood will be appearing in veterinary requirements for live animals and seminal donors, and that bovine blood products utilized in embryo recovery, manipulation and storage will also have to be proven free of this and other common viral contaminants (12,28).

EMBRYO MANIPULATIONS AND TRANSFER

The rapidly advancing science of manipulation of pre-implantation embryos for such purposes as sex determination, gene insertion, or asexual propagation ("cloning") has fundamentally changed the position of embryo transfer in international exhange. The genetic power of embryo transfer, which is presently low, will increase fundamentally as the embryo genotypes being exchanged are sexed, performance tested, and in some cases enhanced by addition of new genes. However, all these manipulations require that the zona pellucida, the sanctum sanctorum of infectious disease control, must be breached. It is obvious that none of these procedures can be safely performed without first washing the embryos free of potential contaminants, and that precautions must be taken to assure that the embryo media, including blood products, are free of contamination. In the case of cloning the laboratory hygiene and freedom from in vitro contamination after collection will surely be far more important than the status of the genetic parents that may be removed from the embryos at hand by many years and successive cryogenic storage and re-cloning. It is also possible that in the near future biocides will be found that are compatible with in vitro manipulations of embryos as well as the processing of spermatozoa: such materials might be synthetic or derived from natural systems.

One further responsibility of veterinary medicine in embryo transfer genetic exchange deserves attention. We have perhaps been less attentive to concerns about the disease status of recipient dams than should have been the case. It is true that the recipient dam, especially in the tropics, can imbue her foster offspring with resistance to enzootic diseases that imported adults acquire only at risk of great economic loss. However, attention must be paid to certain chronic infections that can be transmitted across the placenta, during parturition, or via colostrum and milk. There are at least three important bovine infectious diseases that have the potential to transmitted from recipient dam to the calf in these ways: bovine leukosis, paratuberculosis, and persistent BVDV infection (5,7,21). Rigorous testing and qualification of recipient dams is as important to successful disease-free genetic exchange as the transfer technology itself. While the surgical transfer of embryos is no longer widely practiced, the potential for instrument-borne transfer of infectious blood among recipient animals at the time of transfer should be a concern if this method is used.

FINAL COMMENT

Finally, those of us involved in the commercial germ plasm trade and in regulatory veterinary medicine must remember a role that is fully as important as the enhancement of productive efficiency in animal agriculture. The knowledge of disease prevention that we develop while dealing with the gametes and embryos of

domestic animal species is vital to the conservation of the diversity of genetic resources in the larger world. The techniques of modern biotechnology and cryobiology can be applied not only to preservation of "minor" breeds and species of domestic animals but also to animal species under threat of extinction due to human encroachment and habitat loss (20). If both the production and conservation potentials of the biotechnology revolution are used to their fullest extent, we will have properly served both our own species and our fellow passengers on this small planet.

REFERENCES

1 Barlow, R.M., P.F. Nettleton, A.C. Gardiner. 1986. Persistent bovine virus diarrhea infection in a bull. Vet. Rec. 118:321-324.

2 Bartlett, D.E., L.L. Larson, W.G. Parker, et al. 1976. Specific- pathogen-free frozen bovine semen: a goal? Proceedings, Sixth Technical Conference on Reproduction and Artificial Insemination, National Association of Animal Breeders, pp. 11-22.

3 Bowen, R.A. 1987. Serologic responses of calves to sequential infections with epizootic hemorrhagic disease virus serotypes. Am. J. Vet. Res. 48:1449-1452.

4 Bowen, R.A,, T.H. Howard, K.W. Entwhistle, et al. 1983. Seminal shedding of bluetongue virus in experimentally infected mature bulls. Am. J, Vet. Res. 44:2268-2270.

5 Burny, A., C. Bruck, H. Chantrene, et al. 1980. Bovine leukemia virus: molecular biology and epidemiology. In: Viral Oncology, G. Klein ed., New York: Raven Press, pp. 231-289.

6 Carthew, P. 1986. Is rodent virus contamination of monoclonal antibody preparations for use in human therapy a hazard? J. Gen. Virol, 67:963-974.

7 Chiodini, R.J., H.J. Van Kruiningen, R.S. Merkal. 1984. Ruminant paratuberculosis (Johne's disease): the current status and future prospects. Cornell Vet. 74:218-262.

8 Della-Porta, A.J., I.M. Parsonson, D.A. McPhee. 1985. Problems in the interpretation of diagnostic tests due to cross-reactions between orbiviruses and broad serological responses in animals. In: Bluetongue and Related Orbiviruses, L. Barber and M. Jochim, eds. New York: A.R. Liss, pp. 445-453.

9 deSavigny, D., A. Voller. 1980. The communication of ELISA data from laboratory to clinician. J. Immunoassay 1:105-128.

10 Ellinghausen, H.C. 1979. Laboratory practices involving the leptospiral microscopic agglutination microtiter test. Proceedings, United States Animal Health Assocation, pp. 163-179.

11 Graves, J.H. 1979. Foot-and-mouth disease. A constant threat to U.S. livestock. J. Am. Vet. Med. Assoc. 174:174-176.

12 Hare, W.C.D. 1985. Diseases transmissible by semen and embryo transfer techniques. Technical Series No. 4, Office International des Epizooties, pp. 34-58.

13 Howard, T.H. 1986. CSS sire health: present and future. Proceedings, Eleventh Technical Conference on Reproduction and Artificial Insemination, National Association of Animal Breeders, pp. 19-26.

14 Howard, T.H., R.A. Bowen, B.W. Pickett. 1985. Isolation of bluetongue virus from bull semen. In: Bluetongue and Related Orbiviruses. L. Barber and M. Jochim, eds. New York: A.R. Liss, pp. 127-134.

15 Howard, T.H., L.A. Vasquez, R.P. Amann. 1981. Antibiotic control of Campylobacter fetus by three extenders of bovine semen. J. Dairy Sci. 65:1596-1600.

16 Lorton, S.P., J.J. Sullivan, B. Bean, et al. 1988. A new antibiotic combination for frozen bovine semen. 2. Evaluation of seminal quality. Theriogenology, in press.

17 Lorton, S.P, J.J. Sullivan, B. Bean, et al. 1988. A new antibiotic combination for frozen bovine semen. 3. Evaluation of fertility. Theriogenology, in press.

18 McClurkin, A.W., M.F. Cor:ia, R.C. Cutlip. 1979. Reproductive performance of apparently healthy cattle persistently infected with bovine virus diarrhea virus. J. Am. Vet. Med. Assoc. 174:1116-1119.

19 Monke, D.R., W,D. Hueston, J.W. Call. 1986. Veterinary management of an artificial insemination center containing bluetongue seropositive bulls. Proceedings, United States Animal Health Association, pp. 139-143.

20 Office of Technology Assessment, Congress of the United States. 1987. Technologies to Maintain Biological Diversity, pp. 137-165.

21 Roeder, P.L., J.W. Harkness. 1986. BVD virus infection: prospects for control. Vet. Rec. 118:143-147.

22 Schultz, R.D,, L.S. Adams, G. Letchworth, et al. 1982. A method to test large numbers of bovine semen samples for viral contamination and results of a study using this method. Theriogenology 17:115-123.

23 Schultz, R,D., M. Kaproth, B. Bean. 1988. Immunoextension: a method to eliminate viral infectivity in contaminated semen. Accepted for presentation, 11th International Congress on Animal Reproduction and Artificial Insemination.

24 Sellers, R.F. 1980. Weather, host and vector-their interplay in the spread of insect-borne animal virus diseases. J. Hyg., Camb. 85:65-102.

3.1 MODELS FOR THE INTEGRATION AND DEVELOPMENT OF BIOTECHNOLOGY: ORIENTATION, ORGANIZATION, STRUCTURE, STRATEGIES, INTEGRATION AND ORGANIZATIONAL RULES

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The area of biotechnology, as is now obvious after a day and a half presentations here, is extremely broad. Perhaps this field is so broad that every country cannot become involved in all areas. This suggests that each country must first identify its needs in order to establish its priorities to meet those needs. The establishment of needs and programmatic responses in biotechnology should be a continuous, interactive system.

I suggest that it is desirable to think of biotechnology as a spectrum ranging from basic science, to its eventual application. The flow of information across that spectrum must totally interconnected. One must resist the tendency to divide science into compartmentalized activities, such as the separation of research into basic and applied. This division is artificial. It is clear that we need a nucleus of basic science connected with those who adapt and apply it to specific questions in specific programs. Basic science is essentially a bank of information in biotechnology; currently, this area is extremely productive and active. At this time, the mass of basic information doubles every twenty months. That is to say, the knowledge that we had three years ago although perhaps not completely obsolete, has been supplanted by more contemporary information in biotechnology. Basic science requires a high scientific and technical level. It requires sophisticated equipment, a good infrastructure and adequate funding.

Adaptation, or adaptive science is fundamentally the use of information from the basic sciences in the development of techniques or specific methods. This transfer of information implies that those involved in the application have ready access to information as it emerges from the basic sciences. One example might be the development of a slot-blot diagnostic test to detect the virus of infectious bovine rhinotracheitis in nasal secretions. The techniques applied come from advances made in the basic sciences.

The ultimate application is the use of the developed technique or method in routine form. That is the transfer of information from the basic science through the adaptive process to its application and everyday use, such as a diagnostic laboratory which utilizes slot-blots for the routine diagnosis of infectious bovine rhinotracheitis virus. The scientific and technical level of the personnel involved in the adaptive process are practically the same as those involved in basic science itself. The scientific requirements of those involved in the final application perhaps are somewhat different, depending on the level of sophistication that the particular technology

requires. If the ultimate application is simply the utilization of kits purchased commercially, obviously one does not require a high level of laboratory sophistication and understanding of the basic science involved. For this reason, the identification of the objectives of programs established by governments must fit the aims and needs of those programs. Definition of the ultimate needs of animal health programs must be the first step in establishing and focusing biotechnology research and developing its applications. There are 122 developing countries in the world today, each one of which is in a different stage of development, with different problems. This suggests that there is no single formula or model for the establishment of biotechnology and the kinds of capabilities that must be developed. Each country must face its own needs and take into account its own capabilities in order to develop its unique program in biotechnology.

Since no institutions can maintain activity at all levels across the broad range of what is biotechnology today, there is an absolute necessity that there be effective linkages between scientists and institutions that have activity in biotechnology. For example, if a university in an industrial country is working in basic science, it is necessary that its results flow to applied (perhaps national) laboratories where it can be adapted and later transferred to users for its ultimate routine application. Each unit must receive mutual scientific feed-back and benefit from this linkage in order to function as an interactive system.

Many international laboratories have a wide range of activities, as does the Pan American Center for Foot-and-Mouth Disease. These centers must be regional in nature, and serve as a mechanism to bring together human and financial resources and avoid costly and unnecessary duplication. International centers can also facilitate administration and operation of logistic systems which at times, pose an enormous problem for individual countries.

Comprehensive universities generally have two missions--teaching at the pre and postgraduate level and a responsibility for investigation at both basic and adaptive levels. However, few universities have the responsibility for the application of their research findings, with the possible exception of those which also have responsibility for laboratory diagnostic activities in their respective areas for both animal and plant diseases. In my opinion, these universities in the industrialized countries have not been utilized as effectively as they could be in the international arena, for the transfer and application of appropriate technology.

National laboratories, obviously, need a clear definition of their mission and objectives. They require an ongoing system which permits evaluation of their activities to determine where

they are and where they are going. Since every institution tends to undergo a constant change in personnel, it is easy for a group in transition to lose its vision and forget why it exists. Laboratories do not exist for the satisfaction of the scientists. Laboratories exist to serve the public and the producers.

Given the spectrum covered by biotechnology, and the large numbers of individuals and institutions involved in it, how can one assure the proper interconnections in a functional system? Various mechanisms exist to maintain the flow of information within the system, and it maybe worthwhile to quickly analyze where we are in this regard. Training is an important ingredient. The provision of post-graduate level training of developing world scientists in laboratories in the industrialized countries is very routine, and a mechanism of scientific capacitation with which we are all familiar.

I suggest, however, that for this training to be relevant to the country of origin of these trainees, students should do at least part of their research in their own home environment. Although this is often difficult, it is very important that we attempt to provide this opportunity to our trainees. The same principle should be applied to postdoctoral fellows who might do part of their training in their home countries, and part in host laboratories in the industrialized countries. It is desirable for scientists from developing countries to become familiar with the laboratories in the industrialized world to become better acquainted with the latest techniques, methods and with their scientific counterparts. I do not believe, however, that we have yet found the most effective method for postdoctoral training or professional refresher experiences that will permit maximally effective transfer of technology, in an adequate and appropriate form, from the industrialized country to those countries in development, accomplishing linkage to the basic sciences.

Today, commercial companies are becoming increasing involved in biotechnology. One must not forget that fundamentally commercial concerns have one long-term objective -- to generate income in order to be able to survive economically and to grow. These companies have owners or share-holders whose goals are profitability. The drive for profitability frequently generates short-term activities. This drive for short term gains per se is not bad nor immoral, it is just limited. Nevertheless, there are good opportunities within biotechnology that permit an interconnection between commercial companies and scientists in universities and other areas of the noncommercial sector for information exchange and mutual collaboration. Great care must be taken to ensure that mutual benefits accrue to both parties.

At times university-private sector collaboration is problematic. The free flow of information can be restricted if information relates to a commercial product and results in proprietary

secrecy. The issue of confidentiality of proprietary information requires that universities as well as national and international laboratories and investigators collaborating with commercial companies develop policies and a philosophy to guide these relationships. For example, in our case at the University of Wisconsin, any professor or student who enters into a contractual relationship with a commercial company must have the freedom to publish results, although perhaps with prior notification of the article to be published and its inspection by scientists and other employees of the company for short period of time prior to submission. It is essential that commercial interests not be in a position to block publication or dissemination of research findings. If absolute secrecy is required, it is better that the commercial companies carry out their own investigations. University-private sector links can and do provide opportunities, economic benefits and an interchange of information and viewpoints that can be very interesting. Generally, commercial companies are interested in adaptation and application, and can nicely compliment the basic science focus of many academic investigators, and provide a useful flow of information and funds between institutions and investigators.

National animal health programs and their diagnostic laboratories play an important role within the spectrum of biotechnology research and application. Animal health programs can promote the adaptation and application of basic science, most notably in the flow of new information and techniques for field use in animal health programs.

Since national health programs cannot be significantly involved in all areas of biotechnology for all the important animal diseases, it is necessary to carefully identify and reassess priorities periodically. Diagnostic laboratories have two objectives. One is to provide service to the producer or the field veterinary practitioner, and the other is epidemiologic surveillance and monitoring. The system must function interactively, providing feedback. Development of this fully interactive system, with the diagnostic laboratories as the hub, has been a problem in some countries. The diagnostic lab must establish its norms of operation. Adequate levels of test sensitivity and specificity must be established. An adequate quality control must be established for the tests that are carried out. Who shall be responsible for quality control? What happens to the results? Who provides the reagents? There must be a clear but mutually supporting definition of responsibilities between the national veterinary research laboratories, and their national veterinary diagnostic laboratory counterparts.

Many scientists working in basic sciences frequently think that their responsibility ends when the results are published in a scientific journal, and the reprints are carefully put away in the files of the library. It is necessary that this

responsibility does not end there, but rather steps must be taken to assure that these results find their way to diagnostic laboratories and into the field. But who has this responsibility to ensure tha the information arrives where it is needed and in a form that is useful? There must be a strong, interactive connection between research laboratories and diagnostic and field service groups, to provide a good flow of the latest biotechnological information, its application for disease control, such as diagnostic tests, help with the initiation of these tests, and assistance with their quality control.

What is the relationship of the agricultural producer and biotechnology? It is, afterall, the producers for whom we work. They and other taxpayers pay the cost of the laboratory and our salaries. If our information is not of service to them we are not meeting our responsibilities.

Biotechnology brings with it the possibility of delivering the capability of diagnostic testing into the hands of producers and field veterinarians. Many of these tests are extremely easy to carry out. This capability brings with it great concern that epidemiological surveillance will be lost if official laboratories are not involved in routine diagnostic testing. Diagnostic tests in the hands of producers also has implications in terms of the regulation of animal movement for the control of disease. The challenge we face is maintaining adequate surveillance of diseases that occur in populations, and of controlling disease through regulatory disease testing of animals and animal materials destined for movement.

Each country must define its national policy relating to biotechnology, identifying its responsibilities and facing the realities of what it takes to mount productive efforts, especially in meeting the costs. The benefits must outweigh the costs. A given country seriously interested in embarking on biotechnology at any level must face a series of realities, obstacles, and limitations which must be overcome. The key element, above all, is personnel. Those scientists and technologists involved in biotechnology must maintain a high technical level, and one that is appropriate for the particular sector or focus of their activity. Although they may be involved in one relatively small sector, for example, adaptation research for viral diagnosis, they must be knowledgeable about progress being made along the whole research continuum, from very basic discoveries, through adaptive research, to its application in the field or diagnostic laboratory. This is difficult because the mass of biological information is doubling every twenty months. Therefore, it becomes necessary for biotechnology programs to incorporate training. There must be a continuous upgrading in order to maintain scientists abreast with their fields. Training can be of short or longterm. Scientists must be life-ling students. They must have easy access to the information produced by other groups in the same or related

areas. They must have available complete libraries, which have become extremely costly to support. They must participate in congresses and meetings. As Dr. Ann Palmenberg stated yesterday, data presented at meetings that has been gathered within the last eight weeks can be considered current. If one waits for the publication of scientific articles in international journals, the data that appear there are old, and possibly obsolete. The process of data analysis, organization, scientific review and finally its appearance in print is a process that can easily consume two years. During that time the field has moved ahead swiftly.

Congresses, meetings, and formal talks, and perhaps more importantly, informal talks and communications among colleagues, constitute a mechanisms of communication that are particularly important and effective in maintaining scientific currency. Scientific visits to other laboratories, although an activity difficult to obtain support for, have become an absolute necessity. These visits are not merely "scientific tourism". They are really a true and effective mechanism for communication. Although these kinds of scientific visits are expensive, loss of contact with colleagues working in similar areas is more costly still, with the attendant risk that scientists might inadvertently duplicate experiments and efforts, with a real loss of precious resources and time. We must do a more effective job in convincing scientific administrators that funds spent for effective communication do not represent a cost, rather they are an investment. Personnel management in biotechnology must be demanding. Personnel must be adequately compensated and rewarded for what they do. At the same time, it is reasonable to expect them to produce a great deal. Biotechnology groups can not bear the cost of sustaining individuals who are not productive. Maintaining productivity and scientific currency is more than a full time job. It requires productive work during the day and constant study of contemporary literature on nights and weekends. The time demands do not permit work at other jobs in one's "free time". If there are not adequate resources for the exchange of information and scientific communication, and for adequate compensation of technologically skilled personnel working at a high scientific level, it is not worth contemplating embarking on efforts in biotechnology. It is essential to maintain continuity within the biotechnology laboratory. Scientists must be shielded from political influence and change. The biotechnology laboratory cannot be productive if personnel change every time there is a change in the government. It is simply not feasible to enter into the vicious cycle of training and retraining every three to five years.

The purchase and maintenance of expensive scientific equipment is essential in the modern biotechnology laboratory. Equipment budgets can easily consume $100,000 annually, or much more. If one is to embark on biotechnology, this equipment is essential.

If there is not a qualified group to plan the laboratory and help select the equipment within a country, it becomes absolutely necessary to obtain that expertise from outside. The consultancy costs are more than made up for by the money saved in the purchase of only that equipment which is necessary, avoiding excessive purchases or obtaining equipment that is inadequate to support the methods and objectives of the laboratory. Laboratory design, function, and equipment must be a reflection of the programs of the laboratory, and is every bit as important as the training of the laboratory personnel. Reagents are also a critical area within biotechnology. Systems for agile purchase and rapid delivery are absolutely indispensable for a biotechnology laboratory to function. If it is not possible to organize this type of logistical support, biotechnology programs should not be considered.

Purchase orders which requires six months, or perhaps even a year to process and fill, asphyxiate good science and discourage scientists. Enzymes which are retained by customs in bonded warehouses for three months at 40^{o}C represent a loss of precious resources and time. Governments must decide if it is more important to maintain strict customs control of importation, or to provide more flexible systems for rapid logistical support of those materials that are essential to biotechnology. There can be no compromise.

Physical laboratory space is also an important consideration. Both the actual size of space available, as well as its quality, are important. Biosafety is a consideration that becomes more important daily. Pathogenic organisms, particularly those exotic to the country in which the work is being done, must be confined strictly to the laboratory. Employees must be protected from exposure to zoonotic agents, as well as chemical, toxic and carcinogenic substances.

The administrative unit of biotechnology laboratories and programs must be supportive and encourage a high level of production. Unfortunately, postgraduate masters or PhD level training programs, although scientifically excellent, almost never provide trainees from other countries with the administrative skills necessary to administer a scientific laboratory effectively. International students, trained in the industrial countries return to their home laboratories well prepared scientifically. Frequently, in 3-5 years, they find themselves with significant administrative responsibility, but without any preparation to carry out the duties now expected of them. This is, in my opinion a situation which those of us providing postgraduate training in universities in the industrialized countries must confront, and be willing to provide our international trainees with special programs in laboratory and science administration.

Another important consideration is equipment maintenance. We all have seen situations in various laboratories where work has been

suspended because of the unavailability of a key replacement part the cost of which may be very low, but where no one knows what the problem is or how to fix it. A maintenance program is essential for any biotechnology lab. The shortage of specialists in scientific equipment maintenance and repair is a lament that we all have heard throughout our scientific activities in Latin America. In my opinion this calls for the development of an international program for maintenance, with availability of a variety of technicians specialized in different types of equipment to provide both routine and emergency service to biotechnology programs. Attention also has to be paid to the problems with electrical energy and water. Both must be continuously available in the biotechnology laboratory.

In my opinion successful transfer of biotechnology rests on three broad important points:

(1) Each developing country must establish its own policy and organizational approach in order to become involved in and successfully utilize biotechnology.

(2) Institutions in the industrialized countries as well as international agencies and donors such as the World Bank, Inter-American Development Bank, foundations and others should encourage the development of biotechnology not as a series of isolated efforts, in small pieces, but rather as broad, integrated systems.

(3) The biotechnology network must achieve rapid, easy communication and cooperation. This network is an urgent necessity that will bring benefits to all the participants, and should led to close cooperation in research and development projects of mutual interest. I believe that it is possible to attract scientists to participate in these kinds of collaborative programs involving personnel from industrialized working with those in developing countries. All participants must accrue benefits from their efforts. Continuity is the key to achieving successful, longterm interactions. Short term training, as isolated, unrepeated events, can bring more problems than benefits. Visitors interrupt the normal working rhythm of the laboratory, the benefits to the host participants are limited, and they absorb a great deal of time and resources. Nevertheless, short-term visits can be an initial step in the achievement of cooperative long-term efforts between counterpart scientists. The collaboration becomes much more attractive for the scientists and institutions involved with joint participation in the laboratories of both scientists. A series of problems must be solved if collaborative research and training programs are to be developed and maintained. Who will organize them? Who will pay for them? It is my hope that through contacts such as those that we have made during this symposium, it may be possible to find those paths that will lead us forward together, cooperatively.

3.2 POSSIBLE EFFECTS OF BIOTECHNOLOGY ON LIVESTOCK PRODUCTION IN LATIN AMERICA

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It has been a great satisfaction for me to have participated with the group from the University of Wisconsin, and to cooperate with the Pan American Foot-and-Mouth-Disease Center, in this magnificent idea. On this opportunity, I would like to thank Dr. Raúl Casas Olascoaga and his colleagues for their invitation to participate, and to congratulate them for this event. I am certain that it will be regarded as a landmark in the area of biotechnological development in South America, and that it will undoubtedly have an influence throughout the entire Third World.

The preceding talks and discussions have shown that there will be a period of total change in the area of livestock production. There are changes underway in both the objectives of the livestock industry and the systems of livestock production. Our countries, our directing entities, the animal health directors and the representatives of the international agencies must all work toward the organization of a new cattle and livestock scenario.

Biotechnology necessarily brings with it an environmental impact. Almost always, when we hear the words "environmental impact" we immediately think of negative effects. However, we will have a positive environmental impact when we manage to release all the enormous natural resources nowadays bound up in the traditionally utilized model of livestock procuction. Another series of positive environmental efects will ensue from the controls that biotechnology can exert on contamination. For example, there are biological controls already available to treat and utilize milk serum, thus increasingly improving the situation of water and soil contamination, problems presently reaching the alarm stage even in outlying areas of the cities. Such problems are affecting livestock production and, obviously, the quality of life in our urban areas.

In the field of human nutrition experiments are underway with single-cell proteins. The production of single-cell protein will quickly increase, as is happening in some countries, and it will become a serious competitor to livestock production and its meat and dairy products as a new source of food for mankind. Likewise, single-cell protein can also become a great resource for animal feeding, produced at low costs if industrial fermentation enters the scene with cheap substrates like sugarcane and the yucca plant, both very abundant in most of our countries. Therefore, if these raw materials are directed toward a higher-grade production, with a higher aggregate value, they

may well exert a weighty impact on livestock production in the very near future.

Likewise, biotechnology has and will have a profound influence on the field of animal health, an aspect that has been on of the major causes of the low rates of livestock production and yield throughout the tropical world. After 27 years of professional experience, I feel that we veterinarians have an increasingly greater challenge in the field of animal production and specifically in the field of animal health, which is the greatest of our responsabilities. At present, the human nutrition rates in Latin America are lower than those recorded 20 years ago. In absolute terms, perhaps, more milk and meat are being produced; but in relative terms the consumption rates have declined. Of course, there are some exceptions to this assertion, and they enable us to remain optimistic. Let me cite the example of my country, Colombia: in the last ten years, per-capita annual consumption of meat dropped from 25 kilos to 17 kilos. This obviously shows that livestock production has not kept pace with demographic growth. And that the livestock production model is out of step with the nation's macroeconomic model of development. Although it is a problem affected by the macroeconomy, the responsibility nevertheless rests on our shoulders. Our people see it that way and we must accept it that way. Solutions for animal health problems are sought only from veterinarians; therefore, we are the ones ultimately held responsible for meat and milk production.

New models of cattle production, with modern systems of nutrition, must be developed. Very little progress has been made in feed production. The soils of Brazil --itself almost a continent-- well, we see them covered today with the same pastures and grasses they had 40 or 50 years ago. Evolutions has been slight; we went from <u>chopín</u> and <u>yaragua</u> to <u>braquiaria</u>. On the other hand, the green revolution brought enormous advances in the production of cereals. The production of leguminous forage crops has also advanced very little. Greater research in this field is required, in the use of nitrogen-fixing bacteria, so that the livestock production model is not so isolated from the agricultural production model. Biotechnology is the science that has to unit livestock raising and agriculture, just as was expressed by the Brazilian Minister of Agriculture at the opening of this Seminar.

Good nutrition without good health is impossible. We learned that premiss when we entered the university, but we do not practice it when we plan and carry out livestock-production related programs. We cannot have health with undernourished animal, with a weak or defenseless animal; we cannot improve birth rates nor lower the slaughtering age if we do not integrate the entire productive system.

In the health field we are always overjoyed with the expectations of the next saving, miraculous vaccine that is going to do away with foot-and-mouth disease and the other livestock-decimating deseases. But the greater danger and the major problem are to be found in the slight and inefficient use of the existing vaccines. New vaccines, we should remember, require a slow-moving process demanding a lot of time and money, and the efforts of not a few scientists and researchers.

Consequently, with deficient mutrition and health, the genetic advances are either not going to be possible or will not find economic application in our sphere. Without good nutrition, we cannot expect good genetic expression, nor can we expect that the genetic advances attained by Dr. Neal First and other researchers around the world, find application in our circumstances. What are we going to do with health- and nutrition-demanding animals, the product of an embryo transplant? In this case, the transplant carries us toward ruination, due to its high cost. If we do not prepare enough, the scientists and technological advances are going to burn our hands, because we will not have the possibility of making use of them. Even more common is it to see that much of the semen we are importing, and which is still a small market in relative terms, cannot realize its real genetic goal by fecundating cows in our countries. In this way we are throwing money away, and the semen producers are wasting a potentially enormous market.

According to the ABS, semen exports last year were on the order of two million doses for the underdeveloped world. What are two million doses a year of semen for the entire underdeveloped world? Brazil alone could be a potential market of more than 10 million doses annually! Due to low coverage or lack of expression, the genetic improvement programs are not yielding the expected results. The averages of livestock production in Latin America continue being very low; with a slaughter age of four years, a birth-rate of 50% and 1600 liters/milk/cow/year, we cannot produce economically. Nevertheless, we go on thinking that the problems of production will be resolved with the semen or the embryo, without giving thought to health and feeding. I believe we cannot go on thinking in an isolated manner.

I do not mean to attack scientific advances, but rather the facile, quick solutions. We should think of a harmonious development of all the components of animal production, so we do not fall on our faces again, or suffer a new frustration. For this could be fatal, at the moment, for all socioeconomic development in Latin America.

When we study the case of the growth hormone utilized with success in other countries and regions, like in the State of Wisconsin, we note that they also have surpluses of corn and alfalfa, among other feed crops. In those regions, the scientists labor to make use of the surplus alfalfa, corn and

other cereals. When the researchers utilize hormones that induce increases of 15%, 20%, and 25% in milk production, it is because they have a feed response capacity, and the certainty that the money that goes into the cow's mouth comes out through the udder, but with greater aggregate value. The animals treated with the growth hormone have to be well fed, or we are just spending our money foolishly, and make our production more expensive. As a consequence, yield and profitability drop and destroy companies needed for feeding our people. And credibility in technology and in the scientific community is lost.

Naturally, in this case, too, health will be damaged.If we are talking about using animal not adapted to the tropics, and from which, in addition, we are requiring much more due to the use of drugs, their health will be more affected than if the native "criollo" mestizo breeds are used. And, of course, the production will also be negatively affected. If we do not treat the whole problem in an integrated, whole manner, we may just see a collapse in livestock production.

In the near future, international trade in cattle and meat will see increased concern and demands on health. The slight commerce that we have nowadays is going to be very restricted by international standards. We must prepare ourselves to respond affirmatively, and set up a good system of diagnosis, epidemiological surveillance and vaccine production. In these areas there is a lot of room for better usage of biotechnology, with sanitary applications, in surveillance system and vaccine production and control. I still think that for a long time yet we will go on producing vaccines and with the present cell culture systems. But we have to perfect them, using biotechnology to improve the production of the current vaccines. In the specific case of foot-and-mouth disease, a good vaccine is definitive for controlling the disease, reducing the timespan between vaccinations and lowering the cost of vaccination campaign.

Europe has practically controlled foot-and-mouth disease in some parts, and eradicated it in others, with cell-culture vaccine. In Latin America, there are similar cases, cases in which the worthiness of oil-adjuvant cell-culture produced vaccine has been shown: like in Rio Grande do Sul, Brazil, and Sopó, in the Savannah around Bogotá, Colombia. The Savannah of the Bogotá had ourbreaks for 240 months, uninterruptedly, until October 1985. From that date on, following the massive, systematic vaccination of all the region's livestock using oil-adjuvant vaccine administered over several consecutive cycles, foot-and-mouth disease has been brought under control. Modern systems utilizing biotechnology to improve the quality were used in producing the vaccine, which become the <u>par excellence</u> instrument to carry out the campaign and develop a methodology that could be extrapolated for application in other regions.

For some time we have known that E. coli acts as a factory for the production of peptides. Likewise, the cow is becoming a medicine-producing factory. Therefore, this gives us a new purpose or end for production, and a change in the livestock industry's direction and objectives. But if we do not know the biotechnological bases, then we will not be able to make use of the immense potential of Latin America's herd. South America possesses one of the world's major livestock population. About 250 million head of cattle, and the still fortunate availability of land, enable biotechnology programs to be idealized as technically and economically feasible for maximum use of resources. This, without the restrictions that, for one reason or another, have made tropical livestock raising a difficult livestock endeavor and have produced a low-quality life for our people.

And moreover, we are witnessing how a resource that until just a short while ago was abundant, is now becoming a factor of restrinction: the renewable natural resources. Until just a few years ago we had abundant lands and water, but with the natural tropical limitations on the possibility of adapting breeds of high genetic potential. These conditions fomented the extensive, extractive livestock-production model, that is, the model that depredated the renewable natural resources.

We know that the livestock production model must keep in mind the proper use of the natural resources, capital and the human resource. The Latin American livestock model utilizes little capital and little manpower, but consumes natural resources excessively. The model begins when the woodlands are cut down and burned, short-range marginal crops are planted, the land is burned again and, when the crops are harvested, the pasture is sown. Then the ranchers start cyclical burning to control pests and weeds. The result is the extensive model of livestock raising that absorbs very little labor and generates one of the major problems today confronting the Latin America nations: the rural exodus and its immediate consequences, social insurgence. But, why this occur? Because of the overall poverty in the third world: by the fact that we do not have the required capital. As consequence, the livestock production model in Latin America is a model that makes an abstraction of capital: We utilize natural resources and human red cells, which because of their initial abundance, become the two main inputs for livestock production in Latin America.

The advances of biotechnology provide a magnificent opportunity to structure new models of livestock production for the third world in general. The fact in itself of having an extensive and decapitalized livestock production model makes it a "detechnified" model. If we do not have appropriate technology, we cannot employ intensive exploitation with appropriate use of resources.

When we observe the livestock production units in the developed countries, we see that they are really factories with machines for cultivating, conserving, processing and storing grains and feed crops, housing for the animals and animals of a high genetic value. All required capital is justified because there exists a balance of resources for a high productivity. We, however, lack capital. But perhaps more so, we lack the appropiate technology. So that we must do research, and seek to change to a more technified model if, as technicians, we want to be truly useful. We can make use of biotechnology to produce more foodstuff, preserve our renewable natural resources, and upgrade our qualify of life.

Biotechnology is an economic tool in intensive models of production; let us not try to use it in extensive models because we will discredit it. If we are going to use vaccines having a high incorporated cost, improved genetic material, growth hormones, then we need integral models of livestock production, integrated into the macroeconomic model of development of our countries. As Dr. Thomas Yuill said a few minutes ago, each one of them, each country, according to its level of development and its possibilities and needs, designs its models of development, because I do not think that any country, not even the smallest in South America, can produce with only a single model. A series of dynamic models, that can be permanently adapted, is necessary. We must renew our models, and we must change that old colonial model of production that will be 500 years old just four years from now. The model existing today has few variations. I do not think that this is too much to ask, and I am sure that there is enough response capability to make that change.

If we want to make use of the good possibilities that biotechnology holds up to us, we must be more dynamic and less prosy in incorporating technology to our models. Fortunately, we still have renewable natural resourses, some renewable and others that can be conserved. We have that vast Amazon region, which is a reserve for the entire world. Its conservation is a commitment and an obligation for the countries of the Amazon basin and for the rest of the world. Maybe through the conservation of the natural renewable resources we have a bridge leading to symbiotic systems between the developed North and the developing South. The North needs the tropical lung and the South needs the biotechnology.

Luckily there are still points that unit us, points with which the egoistical "I want to come out on top" mentality disappears, because we all "come on top", we all win. It is true that troughout life we are in the struggle for money, because it is necessary and we have to earn it. So let us work for it together, for all of us. I believe that there are technological niches for biotechnical application that can unit us, enabling us to make use of the very valuable basic research existing for

many years in the north, research to which we shall not have access without common purpose. To duplicate it would be the worst of errors, because we would be achieving it after it became obsolete in the developed countries.

So that we have to utilize bridges of union such as, for example, through conservation of the natural resources: some of them are on the verge of exhaustion, others remain intact.

In Latin America we have a really economically important livestock herd, but with low production. Therefore, it has high potential for improvement. We have to produce our own genetics in the tropics, not bring in genetic material. The professors at the University of Wisconsin are of the opinion that much of the success of the livestock business in the US resides in their having improved their own livestock in their own environment. From the moment that they stopped importing cattle and imported only small numbers or genetic material, like a germoplasma, they began working on animals from Wisconsin for Wisconsin, from California for California, from Arizona for Arizona. Through that strategy they have achieved indicators such as nine months of age for the first servicing, first calving at 18 months, slaughter of that cow at 20 months the highest possible price because of her age. Likewise, lactation has reached 30,000 liters. That highly productive genetic material was made for the appropiate environment.

Let us copy their example. Let us work our environments, bearing in mind our temperature limits of 40°C and 90% humidity at sea level; in our mountains, dry climates, temperatures of 7°C and altitudes of 4000 meters above sea level. We ourselves have to work on this, but we also need the help of technical personnel and researchers from the developed countries, to help us in this task. A technified Latin American herd will not represent any competition for the developed countries, simply because they do not sell us meat. Our people need the protein that meat provides. The developed countries only sell us a little milk, which is not significant in our countries. And the majority of it comes as free aid. On the other hand, a technified herd does indeed mean the possibility of new markets for the developed countries, due to the high use of machinery and equipment in crop raising, railroads, communications, highways, diagnostic services and laboratories. I feel that this also represents a possibility of symbiosis that we should make use of; this is another bridge of union.

The development of biotechnology for livestock production is limited by the number of technical personnel. In Latin America, we have a group of technically qualified cadre that, albeit not mumerous, is an important core group. I think that this core group could be better utilized to prepare sufficient human resources. To achieve transcendent biotechnological programs we must seek massive training and qualifying programs. Perhaps it

was just yesterday, one of the speakers here mentioned that we can no longer go on with the system of contracting 4 or 5 consultants per year per country, at a cost of $20,000 dollars monthly per consultant: we have to look for alternatives in cooperation. There is a good store of goodwill in the scientific community, and we could seek ways to prepare our technical personnel. Although undoubtedly they would have to go to the USA and Europe, they could conduct a large portion of their specialization in their native countries, with greater benefits and at lower costs.

There is also the magnificent possibility of utilizing the interest of post-doctoral personnel, or retired persons, to work in the tropics. They are persons who have developed important programs in private companies or universities in the USA and Europe, and now want to aid humanity in their fields. And biothecnology, at all levels, must be massified and demystified. We must not speak of it only at the maester's or doctoral levels; it must be applied and disseminated at the assistance level, and even in the field, for the livestock raisers who will be the users. When those raisers have to use the diagnostic system, for example, they should know what biotechnology is and what it is for, just as they now do with computers. We must do away with the fear of biotechnology, and that fear is abolished through knowledge.

In addition to the low number of technical personnel, we also face a limiting factor in that the physical and human resources are widespread, unitegrated, hampered by a lack of communication. And, what is worse, we have no pertinent legislation, nor organization for biosecurity and development. People in general are fearful of biotechnology, although we have actually lived with it for centures, since thousands of years ago. When we hear people talking of biotechnology, they are thinking only of transgenic life, of the escape of recombinants, of the monstruos animal that flees from the laboratory and wipes out all humankind.

Tomorrow you will attend a documented, most useful talk by Dr. Pedro Acha, dealing with biosecurity. It is time we had a biotechnology program with national plans and a continental plan of developement and application, including biosecurity. We can not afford to continue as islands among countries, or even within the countries themselves, maintaining a number of expensive personnnel or equipment, underutilized because they are dispersed, isolated or even purposeless. There are many such examples in the countries of South America, of valuable equipment still sitting around in crates, unused. If we unite that equipmemt based on purpose, and if we put them to work in a net work, we will also rescue many idle technical personnel frustrated by the lack of projects or usefulness. We must seek national and regional purposes and goals, and organize to

achieve the goals. This requires no capital, just decisiveness and discipline.

It is not too late to get started. Despite its great development, the USA only set up its National Biotechnology Commission some 10 or 15 years ago. In its short lifespan, that Commission established the agro-livestock program and the livestock subprogram. The budget increased tenfold since its founding. The Commission also established technical criteria that served as the basis for the legislation now governing biosecurity in the USA. The legislation prevented enormous problems in biotechnological development, problems born of fear, problems that could have slowed down or braked research activities. The creation of the national biotechnology commissions are an example to be imitated immediately; thus, the livestock program in biotechnology should be a proposal we take with us from this Seminar. I think that the international agencies like the PAHO and its Centers CEPANZO and PANAFTOSA, the ICA and the World Bank, as well as the Inter-American Development Bank and others like it, can fulfill a function of leadership in the organization of the national commissions and the continental network.

The control and eradication of foot-and-moth disease can go on being a banner for us to rally around, as we seek a national purpose under the control of this disease. We can utilize biotechnology for the development of animal production, in an integral way. Foot-and-mouth disease, if correctly used, can indeed produce.

I feel that our countries must not get lost in debating whether or not we enter the age of biotechnological development. I do not see any option; we have no alternative. Without biotechnology, our livestock industry would lose all competitiveness. We would lose our meat exports and would have to tolerate third countries invading our domestic markets with those products, because the only thing that our countries cannot tolerate is hunger and undernourishment among people. Therefore, at some time or another, they will have to open their doors to the offer of meat and milk and other proteins, at prices surely much lower than our own.

The alternative left to us veterinarians is to lose the function for which we have studied and labored. The possibilities remaining for us, especially over time, are few. The gross national product of our countries has a high agri-livestock component in general, and a high livestock factor in particular. If the GNP declines, its drop will have national repercussions; it will mean a national catastrophe, with our immediate responsibility, we, the veterinarians... And we veterinarians have to show the highest governmental levels the livestock production situation, the alternative and the limits of our responsability. If we do not accomplish even primary development, how can we ever attain industrial developement? If

the nutrition rates and animal health rates decline too, for this same reason, how can we ever control de zoonoses? We will not be able to, and rabies will again assail us. It is closer than you think, do not kid yourselves. Brucellosis is again threatening. Can you then achieve our purposes with livestock production? No. In order to achieve them we have to find a way to orient the countries to set up working groups and biotechnology plans. To me, seems clear that it has become absolutely indispensable to make the change or changes required in the livestock production model. Obviously not by dealing abstractly with the situation and the macroeconomic development programs of our countries and the region, but by integrating with them so they can march on in a harmonious fashion. Let us set up integral programs of production with their own good genetics, and with good nutrition. We are tired of just looking at tables and charts of food ingredients and nutritional requirements. Rather, let us ask: Where can we acquire the food that that nutrition requires?

I insist that one thing is absolutely indispensable if we are to succeed: to handle animal health and food production within a managerial concept that encompasses both the macroeconomic and microeconomic levels. We have to have all the social, technical and economic elements completely integrated with the very clear objective that we are working for nutrition and human health. We have to put that milk and that meat at everyone's reach, make them accessible to our populations, enable that nutrition and health to become a means toward a better quality of life for all the inhabitants of our countries.

I believe that biotechnology with an application in animal production, from an integral standpoint over the short, medium and long range, not as the full isolated solution that suddenly emerges, is a solution to our problem. But to get results, we have to work hard, very hard. That is the only way to prevent slipping backwards again. In your hands, as health directors members of the international agencies, and especially of the PAHO and the IICA, --in your hands rests the enormous responsibility of improving animal health and production.

Thank you.

4.1 TECHNICAL COOPERATION BETWEEN DEVELOPED AND DEVELOPING COUNTRIES

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The term "Biotechnology" encompasses any procedure that uses living organisms or their products to produce or modify substances, improve species, etc. Consequently, it is a very attractive area in that it pursues the production of goods and services by using biological systems.

The unfavorable economic situation facing most of the countries in the region obliges them to develop technologies having low related costs. Therefore, biotechnological development is being considered by various countries as a feasible alternative to stimulate socioeconomic development.

Biotechnology is also attractive because the process of development can occur on different levels. Between the empirical knowledge of the traditional biotechnologies and the basic knowledge sustained by molecular biology and biochemistry, there exists a full array of biotechnological possibilities, ranging from the low cost to the intermediate or appropriate. In the first case, the criterion of identification is economic. In the second, it is the level complexity; and in the third, its cultural and social acceptance. Thus the term "appropriate biotechnology" defines that which enables a specific goal to be attained within a given environmental and socioeconomic context, using local resources, techniques and personnel.

Several factors such as the revolution produced by the development of the recombinant DNA technique, the presumed or real economic importance, the need for multidisciplinary involvement --as well as the participation of actors from various sectors, which facilitate alliances at the moment of decision-making--, have given "biotechnology" an almost magical connotation. It means something different for each audience, precisely what that audience wants or is expecting to hear. As a corollary, there is no United Nations agency that does not intend to respond to the demand of its specific clientele, or which is not dealing with the matter in some way or another.

In order to avoid redundancy in this discussion, I shall restrict myself to pointing out some strategic factors that have been identified as bottlenecks capable of influencing integrated development in that field. I shall attempt to determine the role of the international agencies in that context.

In the second part, I shall describe the concrete initiatives that are underway in the region.

1. STATE FUNDS FOR BASIC AND APPLIED RESEARCH

The development of biotechnology depends to a large extent on a solid basis of scientific support provided by basic research and, likewise, on the capacity of developing the knowledge required to meet specific needs. This latter is the goal of applied research.

"Generic applied research" appears as the bridge between these two types of research; its objective is to solve the general problems associated with the use of a technology in an industry. Examples would be the development of bioreactors, better knowledge of the genetics and biochemistry of industrially used microorganisms, etc. Generally speaking, the funding of basic research and "applied generic research" is the government's responsibility, either because in the final instance they contribute to the public interest or because they are too risky and expensive for individual companies. The harmonious development of these three types of research does not occur without financing for this activity.

2. AVAILABILITY OF HUMAN RESOURCES AND TRAINING

Human capital is the strategic element for the development of the new technologies, including biotechnology, which depends on the availability of several types of professionals: biologists, immunologists, microbiologists, biochemists, bioprocess engineers, enzymologists and others. In general, the availability of this personnel, and their training and inclusion in the effort, are directly related to the preceding factor, that is, the availability of funding.

3. STATE POLICIES AND PROGRAMS IN THE FIELD OF BIOTECHNOLOGY

The development of the new technologies depends on the concerted effort of several factors, including the State, private companies, universities, adequate funding, and so forth. The State's role is extremely relevant in Latin America, given the weakness of the other sectors regarding the capacity of initiative for scientific and technological development.

The comparative advantages for the development of biotechnology have to be generated through state policies; as we've seen, this depends essentially on their support to industry and development in this field. The capability for gathering, developing and adapting knowledge depend on the existence of a minimal technological base which, in turn, should result largely from state policies. The political instruments wielded by the state --such as public spending, legislative instruments, economic policies, industrial and wage policies, market regulating, and others-- should be coherent in pursuit of this goal. These policies should have a multisectorial character; it

is hardly wise to undertake the development of new technologies through a single sector.

4. UNIVERSITY-INDUSTRY RELATIONSHIPS

The development of biotechnology inspired new forms of relationship between university and industry. A possible model is the funding of research carried out at universities with support from industry. However, it is probable that this type of support will decline as those companies develop their own in-house research capability and the line between basic and applied research is more clearly traced.

Another possibility is that the approach may come about through the use of a campus where university institutes or departments and companies are close to one another; in this respect, geographical proximity may stimulate the exchange. Thus the solution of research and development problems in industry would be related to the cooperation coming from university scientists and technologists.

Although the tightening of relationships between industry and the universities is an important element in transfering technology from the research laboratory to industry, and may promote an interaction that can be beneficial to both parties, some problems are worth indicating. If the required mechanisms are not defined, the possibilities for immediate profit could become the basic criterion for the selection of lines of research. Furthermore, the environment open to the free circulation of ideas and the widespread dissemination of the results produced --which is the very essence of university life-- could be jeopardized by the interests of the companies that finance the research activities. Such companies might seek to control the results and impede their dissemination until they have earned a return on their investment through some form of commercialization.

The reality in the Latin American countries is that the ties between the productive sector and the universities are extremely weak. Efforts were made in the 1960's to create a scientific infrastructure and strengthen research groups in research institute and universities. It was expected that the infrastructure would respond to the demands of the productive sector and reduce the traditional gap between the two sectors. But the gap not only remained; it also widened due to the fact that the models for development favored the importing of industry and the transfer of technique from abroad. The State --responsible for the expansion of the supply of science and technology-- has endured as its main source of demand and funding; this situation explains the vulnerability of the system of science and technology vis-à-vis the decline in public spending.

The researcher in Latin America remained apart from industry, protecting his knowledge-producing "virginity" in a context in which the industrial sector's intentions to use that knowledge were very limited. The present economic reality forces the researcher to approach the productive sector not only to produce knowledge, but also at times in order to survive. We believe that this reality --painful for some-- will increase in the future. Especially because there are already examples (such as in Brazil's microelectronics industry) that indicate how research support, when associated with suitable industrial, fiscal, credit and market-regulating policies, can create effective possibilities for closer cooperation between universities and industry. Which is to say, conditions for development with greater autonomy.

5. STATE POLICIES AND INTERNATIONAL RELATIONS WITH REGARD TO TECHNOLOGY TRANSFER, INVESTMENTS AND COMMERCIALIZATION

These policies become crucial elements in the development of technological and productive capacity.

A rather well-known position is the one that, generally speaking, the obstacles to achieving access are smaller in the initial phase of the development of new technologies than they are when the technologies reach maturity. Thus the adoption of immediate action to make use of this initial phase of the development would provide greater possibilities for success in dominating them and a reassessment of the technological gap.

The problem is that the viability of this approach depends on (1) factors of a political nature (interests of the several actors, especially the State, domestic companies and foreign companies); (2) factors of an economic nature (size of the national market which makes minimal production scales viable, possibility of competence in the international marketplace --costs, quality, etc., investment capacitiy); and (3) factors of a technical nature (scientific and technological capability to develop and adapt technologies).

Importation is generally the main access road to technology in Latin America. But so that this does not mean additional dependence, the transfer of technology must be favored by defining policies for regulating investments, contracts for acquisition of technological packages, and associations between domestic and foreign companies. This can not be separated from the existence of a market, and from how access to that market is regulated.

This access is vital in determining the viability of a given product's economic return. Whether the access is opened or closed will consequently be the critical element that could signal a given sector's survival.

6. FINANCING AND FISCAL INCENTIVES FOR COMPANIES ACTIVE IN THE FIELD

The availability of risk capital for setting up new companies, and the fiscal incentives provided by the government to stimulate the formation of capital and industrial development in the private sector, are vital for biotechnological development.

Risk capital is very limited in Latin America. Moreover, a large portion of the company is used to having its risk subsidized by the State through fiscal incentives.

7. LEGISLATION REGARDING THE PROTECTION OF HEALTH, SECURITY, ENVIRONMENT AND INTELLECTUAL PROPORTY

The laws regulating research, production and commercialization activities related to biotechnology have been the subject of heated discussions. It is a polemical topic, because the possible consequences of genetically managed organisms being released into the environment are unknown. Although the current trend seems to be oriented toward relaxing the restrictions initially adopted, this legislation can become a kind of customs barrier to imports and, therefore, an instrument controlling the access to the internal market. Depending on its more or less liberal character, it can make a country attractive or unattractive to foreign companies thinking about setting up there.

By the same token, the laws regulating the ownership of inventions and discoveries in this field should be the subject of review, from the standpoint of protecting the researcher and the domestic companies.

8. DECISION-MAKING PROCESS AND SOCIAL PARTICIPATION

The review of the economic and social development models of the Latin American countries, within whose framework scientific and technological development assume an increasingly more meaningful importance, depends on the performance of distinct sectors of the society. The widening of the consensus that encourages political support of decision-making in this field requires the dissemination of reliable information and the opening of channels of participation. Through a participatory process, the roles of the State, private enterprise, universities, unions and other actors in biotechnological development can be more clearly defined.

Within the context of these factors the international agencies are able to intervene in biotechnology. Due to the fact that the austerity affecting the countries also occurs at the level of these agencies, the actions were above all promotion-oriented, each agency within the boundaries of its specific clientele.

Curiously, several of those agencies were already conducting timely "leading-edge" actions with a biotechnological content. Biotechnology is the selection of rhizobium strains for nitrogen fixation, as well as the development of ADN probes for discovering virus in potatoes. Joining together these slightly "wasted" actions, several agencies found themselves with a biotechnology "program" that incorporated classic biotechnology as well as the "new" technology. On the other hand the countries, due to their interest, pushed the agencies to promote each of the mentioned topics. Thus the topics were discussed and rediscussed at meetings organized by the PAHO, WHO, UNDP, UNIDO, IICA, UNESCO, etc. The agencies provided the forum that led to the exchange of experiences and, in some cases, became the basis for joint programs.

Despite the financial difficulties, reaserch and development projects were also conducted with financing from several agencies. The following were the projects in the area of health:

(a) The TDR annually supports projects whose subjects range from molecular biology applied to the diagnosis of tropical diseases and/or the characterization of their etiological agents, to field assay of the toxin of Thuringiensis or Esphaericus bacilla, or toxin of a nematode such as the Romanomermis culcivorax, for the biological control of vectors.

This program is further attractive in that it funds projects that support institutional infrastructure, always and whenever those projects are conducted around a research project. More that ten institutions have been thus benefited, of which three have projects that contemplate the production and testing of monoclonals, development of DNA probes for diagnosis, and/or production of antigens through techniques involving recombinant DNA. Support was also extended to courses on the production of monoclonal antibodies in Brazil, another on the functioning of cell membranes in Mexico and one on molecular biology techniques also in Brazil.

(b) Another WHO program that is concerned with the subject is the vaccine program. It is directed toward supporting the development and production of vaccines, from the standpoint of the characterization of functional antigens, as well as their production by recombinant DNA techniques, or peptides synthesis. The main target diseases are the diarrheas, tuberculosis, polio and the acute respiratory diseases.

(c) UNDP/UNESCO/UNIDO. From the health viewpoint, the project pursues the development of diagnostic and/or immunizing methods. This program has been a great encouragement for the region; it went into operation by means of National Groups established to define its priority lines and the local capability to conduct them. The projects were rendered compatible in subsequent meetings with the countries and

therefore became joint projects. They were reviewed by ad hoc technical committees, which favors the possibility of obtaining maximum quality from them.

The budget for the five-year project was set at four million dollars. Startup began in March 1987, and more than two million dollars have already been granted. This indicates the funding will be insufficient. This program unites both scientific development and the possibility of conducting the product evaluation in the field as part of the project.

(d) Last, we have the PAHO/WHO. The PAHO already had a series on biotechnological activities through its programs focusing on Veterinary Public Health, Laboratory, Production, Control of Vaccines, etc. It was decided in 1986 to give a new push to the effort.

In January, 1987, a meeting was held with experts from Argentina, Brazil, Costa Rica, Cuba, Mexico and Venezuela. The group's objective was to set the guidelines for promoting a combined effort of technical cooperation among countries, in pursuit of integrated development of biotechnology. The scope would cover from research and development to production. The effort would take various elements into consideration.

To achieve this goal, two types of action were undertaken. The first would involve support to the research and development infrastructure of selected institutions of the participating countries. The second would seek to secure results over the medium term, at the latest.

In the first case --institutional development-- it was concluded that certain priority areas must be developed if a research infrastructure is to exist in fact. Those priority areas are: information systems; development of human resources; definition of policies, legislation and standards; development of an infrastructure of physical plant and equipment that really permits the development of a product and its industrial production.

Taking as a model the chain that could give rise to the development of a product with the use of molecular biology and monoclonal antibodies, certain strategic developments were identified. Those critical technologies, without which it would be very difficult to advance, were scale production of monoclonal antibodies, scale production of recombinant proteins and synthesis of peptides.

Moreover, there were other developments that, although more opportune, were also required by some institutions. They included the thesis of oligonucleotides; nonradioactive marking of biomolecules; processing of data on sequences of nucleics and proteins.

Of course, the teaching of the above-mentioned techniques does not solve the present situation of shortage. Consequently, other elements will be required if we are to begin moving out of the backward situation in which we find ourselves.

Once again, this involves the training of human resources, but now over the longer term in specific processes (6 to 12 months), up to the doctorate. This would mean three years, in industrial microbiology and biochemical technology. In the first case, these would be on fermenters and pilot plant, and in the second, these would discuss supports and purification of biomolecules.

The second type of action to which we referred above were those that produce results over the medium term. By consensus of the group of experts, it was decided that the central topic would have to be the development of diagnostic methods for diseases transmitted through transfusion.

On the one hand, there is a real need, evident in the number of transfusions made annually and in the high cost of the frequently imported reagents. The financial burden on the countries is substantial. On the other hand, I believe that the members of the group were influenced by the situation existing in the region with regard to AIDS and the ensuing political pressures exerted on the health system.

Therefore the developments considered as priority were: developments of diagnostic methods for Chagas disease, malaria, hepatitis and AIDS.

To date, nine projects received funding. The philosophy is that maximum use must be made of the existing materials, whether recombinant antigens, monoclonals, etc. As far as possible, the process will end with a product that can be transferable to the industry.

Whereas one example is enough, lets see what the idea was with respect to the type of project that should be supported in the case of AIDS: they would cover from virus isolation and characterization to the pilot production and testing of the kit.

It is still too early to say if the PAHO project in relation to biotechnology will be a success. However, although it has been underway for only one year, some activities have been accomplished: a project was conducted for support to institutional infrastructure in Argentina, Brazil, Costa Rica, Cuba, Mexico and Venezuela, and will serve as a basis for a proposal to be submitted to funding agencies; in cooperation with the IICA, a meeting was held about guidelines for biosafety and biosecurity standards in work with recombinant DNA; three projects were funded, focusing on isolation of the AIDS virus in Argentina, Brazil and Mexico. One of the projects was for making serum panels for AIDS; one assays recombinant protein for AIDS

diagnosis; and three, for testing monoclonal antibodies for malaria diagnosis by means of circulating antigens; and one that assays monoclonal antibodies for hepatitis diagnosis by means of the classic ELISA technique or through the ELISA dot method.

I would like to say, in closing, that what the international agencies are doing is a drop of water in a large barrel, in comparison with what some countries are doing. This is especially true of Argentina, Brazil, Cuba, Mexico and also Venezuela. The biotechnological development that we will see in the next ten years will be largely based on the action of those countries, with a small catalytic function on the part of the international organizations.

4.2 THE PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER, ITS PARTICIPATION IN RESEARCH AND APPLICATION OF BIOTECHNOLOGY IN ANIMAL HEALTH

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The Pan American Foot-and-Mouth Disease Center (PANAFTOSA) of the Pan American Health Organization (PAHO) is an intergovernmental technical cooperation institution that acts in strick coordination with the countries in the Pan-American spectrum. It is the agency that provides coordination, and catalyzes and supports all the actions of the national foot-and-mouth disease prevention, control and eradication programs. PANAFTOSA is an integral part of the PAHO Veterinary Public Health national program. On the regional scene its dynamic and firm presence constitutes almost the sole contribution in its specific area of action.

The Center's priority mid-range goal --which governed its founding-- was to develop in the Region's countries an animal health structure that would curtail the effects of foot-and-mouth disease, to the point of completely eliminating the impact on animal production and preventing the risk of spreading to countries then free of the disease.

It is worth noting that although all of North America, the Caribbean, Central America and Panama are free of foot-and-mouth disease, the region runs the permanent risk of having the disease, brought into it. Fortunately, the countries' efforts, together with coordination and support from international agencies, have enabled all that virus-free area to remain free of foot-and-mouth disease. This fact in itself is a great sanitary conquest.

It is calculated that losses, plus public and private expenses occasioned by the disease, amount to an annual average of approximately 510 million dollars.

The Center's goals may be summarized as follows;

- To cooperate with the countries affected by foot-and-mouth disease in organizing and developing their national and regional programs for the control and eradication of the disease.

- To cooperate with the Region's countries in raising the productive capacity of their domestic animal through improvements in animal health, likewise seeking to increase the internal supply and promote international trade in animals and products of animal origin.

- To encourage the countries affected by foot-and-mouth disease to intensify and strengthen their control programs, with the goal of achieving the disease's eradication within the framework of the Hemispheric Program for the Eradication of Foot-and-Mouth Disease.

- To promote the development of prevention and animal quarantine systems, to avoid the introduction of exotic diseases while preventing and controlling the spread of the existent diseases.

- To support the countries of the foot-and-mouth disease-free areas so they intensify and strengthen their programs for the prevention of the disease and other exotic diseases.

These objectives are built into the policies of the Technical Cooperation for Developing Countries (TCDC) and into the mandates of the Directing Bodies of the PAHO and the consulting and advising organs like the Inter-American Meeting on Animal Health at the Ministerial Level (RIMSA) and the South American Commission for the Control of Foot-and-Mouth Disease (COSALFA).

The following basic strategies in technical cooperation have been used to develop these policies: the mobilization of resources, the training of human resources, the direct services, the supporting research, and the dissemination of information.

(a) Mobilization of Resources

The Center plays an important role in the coordination and mobilization of financial, institutional and human resources. This strategy will tend to grow with the increased capacity and infrastructure of the countries. The mobilization of extra-budgetary financial resources for the implementation of priority projects, the setting up of networks of scientific institutions and diagnostic laboratories, as well as laboratories for the quality control of inputs --all this constitutes instruments of great interest for the integration, exchange of information, transfer of appropriate technology and developement of research programs.

(b) Human Resources

Up to 1987, more than 3500 professionals from all the countries of the Region had participated in the Center's training programs designed to train and upgrade animal-health personnel. The training programs include courses, both national and inter-tional seminars, individual training, practical exercises in anti-foot-and-mouth disease activities, etc. The strategy aims to develop self-confidence and self-reliance among the national personnel, with PANAFTOSA's role one of cooperation, coordination, support and liaison.

The training activities have led to the formation of a core of veterinary professionals and auxiliary staff possessing the basic knowledge about the disciplines and the methods necessary to conduct national foot-and-mouth disease prevention and control programs. The training of a group of more than 200 professionals in animal-health planning has been one of the major relevant measures in developing human resources for the joint action of the Pan American Zoonoses Center (CEPANZO) and PANAFTOSA. And PANAFTOSA's courses in epidemiology, strategic planning and scheduling have provided an exceptional conceptual framework for the development of animal-health programs and veterinary public health programs.

(c) Direct Services

The strategy of direct services for the Center's technical cooperation with the action seeking prevention, control and eradication of foot-and-mouth disease tries to develop, in the countries themselves, the resources and structures required for solving the animal-health problems. The priority is focused on the vesicular diseases that exert repercussions on socioeconomic development. The Center renders its technical assistance action through the following facets:

- development of national infrastructure for the solution of problems in the animal-health area;
- technological transfer based on its supporting research program;
- services and supplies, reference reagents and vaccines;
- assistance for solving emerging problems.

This function is carried out from the headquarters and by means of consultants who operate permanently in the national foot-and-mouth disease programs. Their main functions are technical advisory services in planning, conducting and evaluating the programs, consultations and coordination and advisory services to laboratories, as well as preparation and development of diagnostic and vaccine-production projects.

(d) Supporting Research and Technology Transfer

The Center's research activities form the basis for improving the advisory services to the countries in the fight against foot-and-mouth disease and contributing to their technological development. The nature of the research projects, and the growing participation of the national laboratories in some of those projects, have led to the almost immediate application of their results; this has formed a good example of technical cooperation among developing countries. The Argentina Animal Virology Center (CEVAN) and the Molecular Biology Center (CBM), Madrid, Spain, were incorporated several years ago into the network of national laboratories that coordinate their activities and work together.

The Center's research program has been directed toward solving the problems that emerge during the carrying out of the foot-and-mouth disease prevention and control programs, and has been made commensurate with the new technological developments. For example, PANAFTOSA has worked in close coordination with the University of Wisconsin Research and Development Center; is expected that the coming years will see important results for the Latin American and Caribbean countries.

The main areas of research may be summarized as follows:

- vesicular diseases diagnosis and reference;
- development, perfecting and adaptation of virological and serological diagnosis techniques of practical and standardized applicability, for use by the national laboratories;
- development of methods for rationalizing foot-and-mouth disease-control programs;
- development and perfecting of techniques for epidemiological characterization, risk characterization and endemism;
- production and distribution of viruses, immune and hyperimmune sera, antigens and other reference reagents;
- development of foot-and-mouth disease vaccine-production and control methods;
- breeding, raising and handling laboratory animals.

(e) Dissemination of Information

The Latin American programs suffer from a great lack of scientific and technical information. As an activity that is complementary to its training programs, PANAFTOSA has developed information activities. In this regard it has undertaken and conducted the relevant distribution in the countries of scientific works published in its Bulletin, monographs, manuals and technical and didactic reports, and the weekly and monthly epidemiological reports that make up the informative instrument of the continental epidemiological surveillance system. It is highly essential to continue intensifying the action undertaken in this area of gathering, managing and disseminating kowledge.

The Results

The main results of the Center's technical cooperation activities, in compliance with its goals, may be appreciated through an analysis of the projects in vesicular diseases diagnosis, production and control of foot-and-mouth disease vaccine, epidemiological surveillance and information system, development of the planning, organization and execution of foot-and-mouth disease control at the national level, and regional coordination.

- Diagnosis of Vesicular Diseases

The first goal was to create the facilities for the etiological diagnosis of the disease, first at the Center, then to transfer the methodology gradually to the countries. To this end standardized diagnosis methods were established, the supply of reagents was ensured, and professional personnel from all the countries were trained. Then cooperation was focused on establishing and organizing the national laboratories until they composed a network of laboratories in the Americas.

With cooperation from the countries and through virus identification and classification studies, the Center built up a store of complete information on the distribution of foot-and-mouth disease and vesicular stomatitis virus types, subtypes and strains. Serological and immunological studies on each strain have made it possible to select those strains which have the stability and coverage characteristics required for the production and control of foot-and-mouth disease vaccine.

By request of the South American countries at the II Inter-American Meeting at the Ministerial Level on Foot-and-mouth Disease and other Zoonoses (RICAZ-II), held in Rio de Janeiro in 1969, it has been recognized as the coordinator of diagnosis in the Americas, by the World Reference Laboratory (WRL) and by the United Nations Food and Agriculture Organization (FAO). This agreement was recognized by the International Office of Epizooties (OIE) in 1960, and the FAO included the Center on the list of vesicular diseases reference laboratories in 1984.

As a technical cooperation agency, the Center keeps up to date on the technological developments that contributes to improving the efficiency and efficacy of the foot-and-mouth disease control and prevention programs. In the particular case of the new biotechnology, the Center worked with important entities like Argentina's CEVAN and Spain's Molecular Biology Center for quick adaptation and development of the limiting end endonucleases techniques, DNA probes, monoclonal antibodies and use of recombinant DNA for the production of diagnosis reagents like VIA. Important modern techniques like ELISA and fingerprinting were also incorporated.

In this initial phase the Center's personnel were trained, the laboratories were organized and the corresponding equipment was acquired. In accordance with the technical cooperation procedures, the training of the countries' personnel has been undertaken and applied joint research activities are underway.

- Production and Control of Foot-and-Mouth Disease Vaccine

In response to the countries concern for improving the quality of foot-and-mouth disease vaccine, a section was set up for the production of vaccine used in training the countries' personnel

in preparing emergency vaccines and for studies of the coverage of South American vaccine strains.

As the needs of technical cooperation grew daily, a pilot plant was set up in 1972 through a Cooperative Agreement involving the Brazilian Government, the Inter-American Development Bank and the PAHO. The Pilot Plant developed technological research into different methods of antigen production --Frenkel, BHK, cells, suckling rabbits. The development of oil-adjuvant vaccine was also undertaken.

Beginning in 1978 the pilot plant underwent modification and was converted into an industrial pilot unit for the cell-culture production of antigens. Other reasons included meeting training necessities, continuing the studies involving development and transfer of technology in the production and control of oil-adjuvant vaccines, and producing vaccines for emergency situations. Further goals focused on studies in demonstration areas and priority programs in conjunction with the countries' official services. The plant enabled technological development to continue on the production of antigen, first-order inactivants and production of a fluid emulsion that would be easy to administer in massive vaccinations.

The following are among the Center's most relevant accomplishments:

- development of atenuated live-virus vaccine, which fulfilled a stage and is still being used in Venezuela;
- transfer of the Frenkel method technology and cell cultures to private and official laboratories in the area;
- development of first-order inactivants, method presently utilized in the majority of the vaccine production laboratories in the South American countries and in some European labs;
- production of high quality antigens in roux bottles with tank-cultivated suspension cells;
- industrialize the production of FMD simple emulsion oil vaccine in Freund's incomplete adjuvant;
- select and research the vaccine production strains currently used in the preparation of foot-and-mouth disease vaccines in South America;
- develop new vaccine formulations with various adjuvants and emulsifiers;
- provide vaccines for pilot areas, and demonstrate the characteristics, handling and application of those vaccines in the majority of the South American countries;
- maintain a strategic vaccine bank for disease-free areas;
- prepare emergency vaccines with special strains;
- hold regular courses and hold in-service training for technical personnel of the official and private laboratories in South America and in other countries of the Region;

- prepare reference vaccines for the governments of the countries;
- provide advisory services to the countries regarding vaccine production, by setting quality control standards, designing the production plants, aiding in equipment acquisition and procurement, and in the overall operation of a production laboratory.

Oil-adjuvant foot-and-mouth disease vaccine. With the start of systematic foot-and-mouth disease-control programs in the 1970's, the ecological conditions of the South American countries made it evident that the programs required a vaccine that would confer a long-lasting immunity than the saponin aluminum-hydroxide vaccine. The vaccine would be used in cattle production zones of difficult access where the deficient infrastructure made it impossible to round up the animals on a periodic basis.

In 1968 PANAFTOSA therefore undertook laboratory studies for the production of oil-adjuvant vaccine. The base studies were the preliminary studies that had been conducted at the Plum Island Animal Diseases Center (PIADC) in the USA. The projects for field application of the vaccine got underway in 1972.

The laboratory studies led to the standardization of the production methods on an industrial scale, mainly antigen quality and antigen inactivation, and to the preparation of an emulsion based on oil adjuvant in an easily applied fluid state.

Immunity studies showed that six months of protection was reached in animals vaccinated for the first time, with 12-months protection for revaccinated adult animals. Based on that information, Argentina and Brazil cooperated in broader joint studies on vaccine application; the results were similar to the initial observations.

Based on those results, PANAFTOSA has cooperated with various countries in the use of foot-and-mouth disease vaccine in demonstration areas that include diverse latitudes, breeds and types of animals. The Center presently supplies the vaccine to the governments of Bolivia, Brazil, Ecuador, Paraguay, Uruguay and Venezuela. The production represents less than one percent of the commercial foot-and-mouth disease vaccine produced in South America.

With technical cooperation from PANAFTOSA, two pilot plants were developed in Brazil. One is at the Federal laboratory of the Ministry of Agriculture in Campinas (LARA), and the second at the Desiderio Finamor Veterinary Research Institute (IPVDF) of the Secretariat of Agriculture of the State Rio Grande do Sul. The technology was transferred to those laboratories and, since 1983, they have produced high-potency oil-adjuvant vaccine with their own resources.

The Center is currently rendering cooperation to Colombia and Venezuela for the production of this type of foot-and-mouth disease vaccine. In both countries the Center collaborates in the design, planning and implementation of laboratory methods for industrial production of oil-adjuvant vaccine.

The Center has trained private and official personnel in all the South America countries suffering from foot-and-mouth disease.

The control of foot-and-mouth disease vaccine. The industrial production and massive application of foot-and-mouth disease vaccine required a mechanism that would ensure the consumer of adequate product qualilty. Except for Chile and Bolivia, the Center was a direct and active participant in the development of the units and definition of the quality-control protocols of the vaccine existing in all the countries of the affected area. That role led the countries, in 1979, to name PANAFTOSA as the Reference Center for Foot-and-Mouth Disease Quality Control.

The supporting research in the vaccine control area focused on developing methods that would enable the vaccines' potency and efficiency to be estimated, taking into account the real conditions of the national programs and of the control laboratories.

Beginning in 1982, with PIADC cooporation, the Center began controlling the quality of the antigens by means of determining the mass of the 140S antigen and the integrity of the viral polypeptides in polyacrilamide gel (PAGE). This technology is transmitted to the countries through in-service training, seminars and consulting services, involving both private companies and the official sector.

- The Epidemiological Surveillance and Information Systems

When, in the 1960's the systematic foot-and-mouth disease control and eradication programs got underway in the countries of South America, with loans from the IDB and funding from the countries themselves, two factors became evident: the lack of knowledge about the epidemiological behavior of the disease, and the consequent lack of systematic information. PANAFTOSA played a fundamental role in promoting the initiative of the IDB's cooperation in financing the development of the infrastructure of the veterinary services and of the animal-health programs, mainly those dedicated to fighting foot-and-mouth disease.

By request of the countries, the Center rendered technical cooperation for the organization and implementation of an epidemiological surveillance and information system for the vesicular diseases. The development encompassed three stages: 1. Period of organization of the national systems, including personnel training (1971-1977); 2. Period of advances in epidemilogical knowledge of foot-and-mouth disease, and full

operation, at both national and continental levels, their expansion and the dissemination of information (1978-1982); 3. Period of use of the information in the preparation of new policies and strategies commensurate with the geographic behavior of foot-and-mouth disease and with the regional specialization of the economic forms of livestock production.

The Center coordinates the national systems by means of a continental mechanism that provides all the possible information to the region's countries, to other countries and to international agencies and entities interested in the subject.

The action in the area of information systems has, in the last few years, focused on expanding those systems to cover the FMD control programs' administrative and economic aspects. Likewise, other animal diseases have been included within the spectrum of focus, such as hog cholera, with the cooperation of the Inter-American Institute for Agricultural Cooperation (IICA).

- Development of the Planning, Organization and Execution of Control and Eradication at the National and Regional Levels

Based on improved awareness and knowledge of the epidemiology of foot-and-mouth disease, the Center has been able to cooperate better with the countries in assessing and redirecting the programs in line with differentiated FMD-control strategies according to the disease's predominant ecosystems. In this regard two subregional projects have been structured: the one encompassing the Plata River Basin countries, which includes the Argentine Mesopotamia, the state of Rio Grande do Sul in Brazil, and all of Uruguay; and the second, encompassing the Andean Subregion countries. Based on the ecological and socioeconomic aspects, the latter includes seven zonal sub-projects.

As an indispensable step towards aligning the foot-and-mouth disease control and eradication programs with the socioeconomic, geographic and ecological conditions in the Region, personnel have been added and trained in the methodology of strategic planning. Outstanding was the pioneering endeavor conducted jointly with CEPANZO, whereby PANAFTOSA helped train more than 200 planners, an undertaking that bore important fruit for the contries of the South and Central Americas.

- Regional Coordination

As part of the strategy of mobilizing resources and motivating the programs of Technical Cooperation among Developing Countries, the Center supported the countries in creating the COSALFA and acts as the Commission's secretariat.

The Commission's primary objective is the coordination, scheduling, follow-through and assessment of the programs as a complement of support to the countries for their establishment

of cooperative agreements in the border areas. The COSALFA, an instrument of technical cooperation among developing countries, is of great value for the activities of coordinating, integrating, monitoring and assessing the sanitary programs.

Activities Developed by PANAFTOSA in the Biotechnology Area

The Center implemented a viral biochemical section and, in 1982, in close cooperation with Argentina's CEVAN, trained personnel and broadened its activities to the molecular biology of the foot-and-mouth disease virus. In other cooperative projects involving Spain's CBM, personnel were trained and a laboratory for monoclonal antibodies production was organized.

The projects' main emphasis was on the utilization of the biotechnological methodology for the diagnosis of the FMD viruses active in the South American countries.

The projects carried out to date may be summarized as follows:

1. Biochemical characterization of foot-and-mouth disease virus strains

In cooperation with the CEVAN, studies focused on the structure of the genoma and of the polypeptide of the foot-and-mouth disease viruses of epidemiological importance, and for the production of vaccine, by means of the fingerprinting technique (electrophoresis in mono and bidimensional gels) and isoelectro-focusing. The information obtained is considered of interest to complement other tests, to update the vaccine strains, to control the genetic stability of the strains during vaccine production, to establish the possible origin of outbreaks caused by viruses present in vaccines, and to monitor new virus strains in the field.

The techniques are routinely used to analyze new strains appearing in the field, such as: The C type virus that showed up in Argentina and Uruguay in 1984 and 1985; the A Sabana-Col/85 virus that affected the central part of Colombia; the A-81 Arg/87 virus that affected Argentina, Uruguay and Rio Grande do Sul state in Brazil. The biochemical characterization of a C type virus --C3 Resende-- attenuated for cattle by a series of passes in chicken embryo.

The analysis of the strains originating in the countries showed the presence of mutants and variations in the size of poly C, but encountered no relation between this variation and the modification of the virulence of the active virus during the passes.

2. Studies of foot-and-mouth disease viruses in carrier animals, by the DNA probe technique (dot blot)

This experiment was conducted in cooperation with the Spanish CBM, and used probang samples taken from cattle carriers of C3 virus kept in the Center's isolation units. Intracellular viral RNA was detected in samples negative to virus isolation. The samples taken from pigs were negative, which reaffirmed that the carrier state is not present among those animals.

3. Direct sequencing of the viral RNA

The use of the sequence determination technique for the gene region, which codifies through the VP1 protein, is of great importance for the study of field strains because the alterations in the sequence of the nucleotides can induce changes in the amino acids in the primary structure of the molecule and, consequently, immunological changes. This study was undertaken in cooperation with the CEVAN (Argentina) and the CBM (Spain).

4. Expression of the RNA polymerase (VIA) of the foot-and-mouth disease virus in E. coli

Obtaining the VIA antigen by genetic engineering techniques has the following advantages: production of large quantities of antigen, high purity, biological safety by not handling viruses, elimination of crossed reactions with cell components, etc.

The RNA polymerase of the foot-and-mouth disease virus was initially expressed in E. coli. Recombining complementary DNA of a C type virus was utilized; this cDNA was handled in vitro to produce a recombining plasmid capable of expressing the VIA antigen. The VIA protein was expressed in E. coli, and it was possible to recognize it with specific bovine sera.

5. Production of monoclonal antibodies

The laboratory area organized for the production of hybridomas and monoclonal antibodies against foot-and-mouth disease virus was remodeled in 1985. Concurrently, preliminary fusions were prepared in order to study the technique, determine the exact equipment and reagents needs, and train the personnel who would be reponsible for preparing the monoclonal antibodies. The method for obtaining and separating the stable positive hybridomas was defined in early 1986, with the cooperation of the CBM of Spain. In 1986 and 1987, 21 fusions with spleens from mice inoculated with the C3 Indaial, 01 Campos and A Venceslau vaccine strains, and VIA antigen, were performed.

In 1986-87 several grantees were trained in monoclonal antibodies production, selection and characterization techniques. The trainees were from Brazil, the Argentine

National Institute of Agrolivestock Technology (INTA), the Chilean Agriculture and Livestock Service (SAG), and Uruguay's Directorate of Foot-and-Mouth Disease Control (DILFA).

Plans for the immediate future include expanding our bank of monoclonal antibodies against C serotypes, by cloning the positives not studied against C3 Indaial that are frozen, and through new fusions with animals innoculated with other C subtypes.

Also with the cooperation of Argentina's INTA and Spain's CBM, studies will continue on selection and characterization of the hybridomas and monoclonal antibodies already prepared with the O1 Campos, A24 Cruzeiro and A Venceslau viruses. Efforts will also proceed to obtain monoclonal against the VIA antigen.

Based on the foregoing, the Center will organize the bank and reference center for hybridomas and supernatants and ascitic liquids with monoclonal antibodies against the strains of foot-and-mouth disease viruses. Concurrently, steps will be initiated to coordinate the production of monoclonals against selected strains of the FMD virus, with the countries laboratories, in order to avoid the duplication of efforts and to share the reagents obtained. The stable hybridomas obtained in this way could be incorporated into the hybridomas reference bank.

Final Remarks

Over the years, PANAFTOSA has developed a mechanism of technical cooperation with the countries that leads to the generation, transfer and adoption of the available technology on the different aspects related to the control and eradication of foot-and-mouth disease. That technical cooperation moves on a two-way path from the Center to the countries and vice-versa.

With regard to the new biotechnology, the Center has adopted and transferred the technology on diagnostic methods applicable to the vesicular diseases and will continue emphasizing the production of reference reagents for distribution to the national latoratories. These efforts have been made possible through the cooperation set up with other research centers, such as the United States' PIADC, Argentina's CEVAN and Spain's CBM.

As reference laboratory and as Secretariat of the COSALFA, and with support from the sanitary priorities and policies of RIMSA, the Center is in ongoing contact with the countries' network of research and diagnosis laboratories. This mechanism enables the Center to work with them in an integrated way, rapidly transferring the new technological developments that are useful for the control and eradication programs.

With regard to the high technology vaccines, and based on the experience acquired in developing the oil-adjuvant vaccines, PANAFTOSA can actively participate in experimental assessment and field projects involving these new products.

4.3 NORMATIVE ASPECTS FOR THE HANDLING OF TECHNOLOGY. THE USE AND SAFETY OF TECHNIQUES

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I would like to begin by thanking the Pan American Foot-and-Mouth Disease Center and the organizing committee for their kind invitation to me personally and to the Institute to participate in this seminar, at which I am the last speaker of the working sessions.

I sincerely believe that the presentations we have heard during the past three days have been of the highest level and of major interest; I would say that some of them have been an updating of enormous importance to us, reflecting what is going on today in the new technology.

I am going to discuss biotechnological regulations and, above all, their application in the field of veterinary medicine, of animal health.

Following the dynamic trajectory of the biotechnology market in animal health, and the effect that the matters dealing with regulations could have today, I would like to analyze briefly the policies and authority related to the regulating of the biological products prepared through the new biotechnology for use in veterinary medicine.

I will take up some problems derived from the persistent myths that surround biotechnology, discuss the commercial and agricultural repercussions of biotechnology, and cover in detail some procedures and experiences regarding the authorization to use biological products. Finally, I will discuss what work we are doing and carrying out at this moment through cooperation with the Pan American Health Organization (PAHO), the Inter-American Institute for Agricultural Cooperation (IICA), the Organization of American States (OAS) and the International Office of Epizooties (OIE). Through those organizations, an Inter-American study group was set up early this year; it has prepared, discussed and issued some guidelines on the use and safety of the recombinant DNA technology (rDNA).

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* IICA, PAHO/WHO, OAS, OIE. Guidelines for the use and safety of genetic engineering techniques or recombinant DNA technology. Washington, D.C., IICA, 1988. 134p. (Miscellaneous Publications Series/IICA, No. DRE/U3A/88/001). ISSN 0534-5391).

With respect to the regulations, I have passed out to you all an extract based on guidelines currently followed by the Veterinary Service. The regulations are known by the abbreviation APHIS, and are issued by the United States Department of Agriculture. I have selected this Veterinary Service because the current regulation it uses is the law on sera and vaccines dating back to 1913. That is the law existing at this time, because the products of the new biotechnology do not require a special body of regulations.

To put in simple terms, the regulations should encourage a climate in which the innovations, development and commercialization of new agricultural products derived from the new biotechnology are promoted while, concurrently, a regulatory policy that reduces the real or potential risks is put into practice in a responsible manner. Moreover, you as regulatory authorities, and we scientists, we should all recognize that these products must not only be innocuous and efficacious, but that the public should feel confident in that innocuity.

The biological veterinary products produced by methods that employ the rDNA should, presently, be evaluated case by case. The same strict standard and norms required to authorize the use of biological substances produced by conventional methods should be utilized in determining the innocuity purity, potency and efficacy. Later on I shall cover this classification scheme, when we review the part concerning quality control.

MYTHS AND REALITIES OF REGULATING BIOTECHNOLOGY

When we tried to establish regulation in this field, we discovered that one of the main difficulties brought about by the emergence of the new biotechnology has to do with perception as well as with the reality. The new technology's potential can be achieved only if we correct the erroneous concepts and mitigate or eliminate the harmful effects of the myths introduced and nourished by the incorrect use of the general terms "biotechnology".

The myths must be separated from the reality if a correct regulatory policy is to be effectively administered. Only through this separation of myth from reality can we explain to the public, in due fashion, the fundamental reason why there must be a regulatory policy. In my opinion, we have been too passive, or perhaps we have been too concerned upon reacting to the risks observed, instead of dedicating our energies to an objective and clear-cut discussion of what the new technology truly implies, and what can be expected of the various biotechnological processes.

At this seminar we have spoken of the importance of the new technology. The opportunities are enormous. Well then, why should the public fear it? It seems to me that there are two

reasons. First, because everything that is unknown causes an a priori fear. And second, because this new biotechnology, in its concepts and way of execution, is extremely complex and therefore, hard for the layman to grasp and to understand. In the past, many authorities were convinced that the fears and anxieties related to the biotechnology presented to the public were due to the lack of information, and that the scientists were incapable of explaining the antecedents, the status or the limitations of their new discoveries to the common citizen, in clear, understanding words. Scientists have always demonstrated their results through the scientific process, that is, according to a detailed, systematic analysis. Obviously, this method omits the common man.

Consequently, several years ago it seemed reasonable to try to do everything possible to correct that situation, by using information campaigns that would enable the principles and achievements of biotechnology to be explained in attractive, simple terms to the common man. It seems totally reasonable that starting with that information, we could clearly see the singular opportunities that the new biotechnology offers, and that the possible disadvantages are relatively insignificant in comparison with the benefits.

In recent years a large number of information campaigns have been conducted and television programs aired, and high-quality tapes and books on biotechnology have been produced. In the major and popular magazines, you have all seen articles on biotechnology; many of them have attempted to explain the new terms of that new language that is biotechnology. I promised myself, for example, to try to make up a glossary of terms of this new biotechnological language in Spanish, because the terms are new in English also (see: "Guidelines for the use and safety of genetic ingeneering techniques or recombinant DNA technology." p.113-133). So then, how can we intend to have the common man read that information, understand it, assimilate it or, on the other hand, undergo the effects of a "stress" of the cognitive type because he fails to understand what it is that we are trying to tell him or what is the message that we are producing for him.

The quantity of information necessary to capture the importance of biotechnology require an intellectual effort that many persons are not willing to make. Moreover, in many cases, the benefits of biotechnology have either been exaggerated or "promoted" beyond their reach. Therefore, in order to avoid the stress of the excess of information, it is easier and simpler to follow those who say that one should simply reject everything new, everything incomprehensible. Moreover, when even the experts are incapable of telling us precisely, in a few words, where the new biotechnology will take us! So then we'll say "The world is already in bad straits, so why should we make matters worse?"

The result is that the public adheres to many myths in order to form an opinion about complex matters. Paradoxically, the pertinent authorities must recognize that, as the complexity of the matter to be regulated grows, the public will become more suspecting and more distrustful of the regulatory process than of the matter itself. Nowadays, few matters are so badly defined as biotechnology. Some persons defend agricultural biotechnology as the solution for world hunger. Others warn that it should not be allowed to become as example of homicidal science, and still others consider the process as simply one more tool in the geneticists' toolbox.

The first of myths surrounding biotechnology is that the process is something distinct or homogeneous, and that one of its corollaries would be that there exists a simple biotechnology industry. This type of reasoning is simple and easy, but inexact. The term has become a tiring load for the academic media, the private sector and the government. Biotechnology is not a single entity, but rather an empowering technology with broad applications in diverse aspects of industry and commerce.

The way we emply the term nowadays, biotechnology includes many different applications: it is the development of hybridomas for the procuction of monoclonal antibodies employed in therapeutic diagnosis; it is the use of DNA technology for vaccine production, like the anti-hepatitis B vaccine in yeast, the rabies vaccine in vaccinia virus, enterleucine 2 in E. coli; the introduction of larger concentrations of reserve protein in soybean, and also the recombinant technology for the preparation of new microbial plaguicides. In short, it is an array of activities that can not be simple identified under the term "biotechnology".

In the first meeting, Dr. Yuill mentioned precisely the definition that is most used in speaking of biotechnology: That it is the application of biological systems and organisms to technical and industrial processes. This definition, to me, means that biotechnology encompasses the diverse processes that we have explained or have tried to mention. As a consequence, the processes and products of biotechnology are so diverse and have so little in common that it is extremely difficult to generalize about them. Perhaps a more valid and descriptive characteristic of biotechnology would be to say that it is a group of diverse biological processes that lead to good practices of innocuous manufacture for the environment, this perhaps being the most outstanding point.

A second myth is that biotechnology is a new phenomenon. As we have already heard in this meeting room, it didn't suddenly fall at our feet. This technology has been around for thousands of years. Yesterday I mentioned that some six thousand years before Christ the Summerians and the Babylonians used yeast to make alcohol and beer. This new biotechnology has been with us only

since 1953, when the double DNA helix was identified, and it continues to advance with important facts. But it is not a process that we could classify as a new biotechnology, but rather as new techniques and new procedures.

The interest of the governmental authorities and of the financial community has grown as the success of this technology has moved ahead. The result of such particular attention has been the gradual extension of the definition of biotechnology to include various techniques that have been employed for various decades without deserving special consideration. But today, we acknowledge them as very special. For example, the products derived from ultraviolet chemical lights, mutagenesis, the hybrid plants and the micro-organism produced by means of genetic exchange, are very often regarded as objects that should be submitted to new degrees of regulation, related to this new technology, when actually they are processes that have existed for several years. This change is due to various factors, but I feel that what causes most unnecessary and growing concern by the public is the use of a single inexact term to describe these activities. We ought to find a means to describe the products that will be submitted to regulation in the light of the specific properties that led to their study, without just simply applying the term biotechnology. Thus, biotechnology, including its subdivision of genetic engineering, is not the monolith nor a new structure, and the newest techniques are obviously extensions of the older ones. Nevertheless, an erroneous concept about the new biotechnology persists, and efforts motivate dealing with it as if it were a special case.

In meetings such as the present one, we should all take the opportunity of cooperating in the eradication of these myths and of considering the new biotechnology within the pertinent perspective.

COMMERCIAL AND AGRICULTURE EFFECTS

Biotechnology will have its major immediate effect on the products of the commercial sector, like the pharmaceutical and farm products in general. Whereas it directly affects matters basic to man, such as the production of food, health assistance and the availability of energy, the repercussions are in reality felt today around the world. Interesting research on agricultural applications are presently underway to intensify animal productivity and to help feed the world's population.

Another great opportunity has to do with the control of plagues and the pathogenic effects that affect animals and plants. We will soon have more and better products to diagnose, prevent and treat the animal diseases, improve their breeds and even create others.

Nowadays, some 450 companies around the world use these new technologies to produce animal-care products. Eight percent of them are chemical or pharmaceutical companies of importance, seven percent are not classed as veterinary products companies but do fit the definition of the animal-health industry, and 85 percent are small biotechnological companies or independent research institutes.

The number of veterinary products that can be made using processes related to the new biotechnology is indeed striking. The array extends from therapeutic and biological products to transgenic animals and products for specific diagnosis.

The interferons are a group of molecules, similar to hormones, that have received much attention from the biotechnology industry. As glycoproteins they have the effect of regulating the body's immune response. The interferons have shown promise in preventing viroses and it has been proven that they are efficacious in controlling certain classes of infections and of cancer. However, it was possible to confirm these affirmations clinically only genetic engineering enabled researchers to obtain a large quantity of interferon.

NEW VACCINES

One of the most important repercussions of biotechnology lies in the new generation of vaccines that has begun to emerge. They are very different from the conventional vaccines prepared with complete agents. Although the vaccines with dead or attenuated live agentus are very efficacious against many diseases, they sometimes produce secondary allergic reactions and cases of acute or slowly progressive disease.

At this point I would like to address, for a minute what Dr. Beçak of the Butantan Institute said yesterday about the need for vaccines and for a clear understanding of their new vaccines, especially in the field of veterinary medicine. Attenuated vaccines, viral vaccines or inactivated bacterial vaccines, subunit vaccines, in short, all of this is currently a reality in the field of veterinary medicine. Nevertheless, this does not means that we are going to displace vaccines that we now need for use in large campaigns, vaccines that have fully proven their practical usefulness in the eradication of a disease. You have the case of Chile, where the eradication of foot-and-mouth disease was based on broad and systematic vaccination.

Today we also have complete germ vaccines, like the FMD virus vaccine. Experiments conducted at Plum Island with a vaccine prepared from VP1 subunits of the virus demonstrated that that vaccine was a thousand times weaker in immunizing capacity than the inactivated vaccine presently produced. This is so because we haven't yet reached that stage of advancement of being able to develop vaccines that move from the experimental phase to the

pilot phase, as Dr. Schmunis said, and then to industrial phase, at the moment when we have the large campaigns underway.

Let us not forget that in the sanitary campaigns, the biological product never amounts to more than 4 or 5 percent of the campaign costs. But the importance lies in the fact that the 95% is contingent on a good quality biological product, that is, on a vaccine that affords effective immunization, confers lasting protection, is low cost, and is easy to administer. For me, these are the characteristics of a good vaccine, whether produced through genetic engineering techniques or any other.

I would say that our experience in Latin America has been rather serious in some vaccines. And I believe that Dr. Santiago Rengifo --may he rest in peace-- the great Colombian sanitarian, at no time said: "What is happening in Latin America is that the live-virus vaccines are dead, and the dead-virus vaccines are alive, and that is why our immunizations have failed".

Not only do the new vaccines resolve many of these problems, but they also offer the possibility of vaccinating people and animals against a wider spectrum of the disease. These new vaccines are innocuous, stable and efficacious. These subunit vaccines can be produced in abundant quantities, in some cases at low cost, and they make up the vaccines of the future. The advantages of these biologically or organically synthesized vaccines are the safety of production, their innocuity, their storability and their distribution. This means that the vaccines produced in this way possess advantages that are obviously very attractive to any sanitarian, or any other person involved in this type of activity.

Work is underway on multiple subtype formulations. In the future we shall have possible advantages of lower cost and easily quantifiable costs, which is already underway in diagnosis. For example, with a mixture of antigens and mechanisms to ensure slow freeing for absorption a much more constant immunization. In this case vaccines derived from recombinant substances are being prepared for diseases like vesicular stomatitis, blue tongue, anaplasmosis, swine and canine parvovirus, bovine papiloma, fowl plaque, influenza, rabies, feline leukemia, rinderpest, Rift valley fever --just to name a few. So this is not something that may come one day in the future, but rather something that is already in the experimental stage. I'm not telling you that it is in the industrial production phase, but in many cases some of the vaccines are in the pilot phase.

Of course, it is more difficult to produce vaccine against bacterial and parasitical pathogenic agents. Nevertheless, genetic engineering techniques for the preparation of protein vaccines have been applied successfully against bacterial diseases. An example: one or more of the European countries have produced a cloned vaccine to protect animals against the diarrhea caused by E. coli.

The use of new technological procedures for the production of veterinary biological substances is in constant expansion nowadays. The vaccines prepared through genetic engineering methods find their greatest potential for acceptance in the veterinary products market. As Dr. Beçak pointed out yesterday, this is fundamentally due to the fact that the production of biologicals for veterinary use finds a much broader market than the vaccines produced for human use. In our countries, above all, the government is the major buyer and consumer of those products.

The vaccines prepared in this sense are basically oriented toward lowering the exorbitant economic ravages exerted by animal diseases on the livestock industry. It is estimated that losses amount to some 80 billion dollars annually worldwide. For example, the world market for foot-and-mouth disease vaccine is greater than that of any other vaccine, including products for human usage. Nowadays, more than a billion dollars are spent annually on conventional vaccines against foot-and-mouth disease.

Because they are not sensitive to temperatures, the new subunit vaccines are more appropriate for use in the countries where they can be stored and handled without loss of efficacy. This refers to the problems faced in the cold chain; as we have heard at this seminar, these subunit vaccines could bring great advantages to our programs in the tropical temperatures.

OTHER ASPECTS OF VETERINARY BIOTECHNOLOGY

I would like to refer to something that was presented by Dr. First in this room: sex determination in embryos. The polyclonal antibodies are yielding good results for examination and confirmation of pregnancy. The ones conducted with monoclonal antibodies for this last purpose, and the fertility examinations, will see greater specificity and doubtlessly will be more attractive to everybody.

Growth hormones, produced by microorganisms obtained through genetic engineering techniques, also have a great potential in agriculture. The production of milk from cows injected with growth hormones has been seen to increase up to 40%. It is estimated that the market for those hormones in the USA is some 100,000 kilograms, at a cost of 40 dollars per gram.

Several recent studies have predicted that in the next twenty years the sales of biotechnological products for use in human health and in agriculture will reach a total of 20 billion dollars in the USA and perhaps up to 40 billion in the rest of the world.

REGULATORY ASPECTS

I now want to deal with the regulatory part and with the authorization for the products. The biological products obtained with genetic engineering techniques can be classed in three broad categories (Figure 1). The division is based on the biological characterists of the new products and on the matters related to their innocuity.

The first category encompasses inactivated vaccines obtained from rDNA, bacterines, toxoids, virus subunits and bacterial subunits. These products present no risk to the environment nor cause new or particular concern with respect to their innocuity. This category also includes the products prepared with monoclonal antibodies, whether they are used for therapeutical or prophylactic purposes or as components of diagnostic kits.

The second category includes products that contain live microorganisms that have undergone modification by adding or suppressing one or more of their genes. The suppressed genes can alter their virulence, the oncogenicity, the enzimatic activity or other chemical functions. The added genes can result in the expression of new immunizing antigens or in the production of new biochemical subproducts like betagalactosidasis. It is important that the added or suppressed genes not jeopardize the characteristics of innocuity of the microorganisms. It is hoped that this will improve in the majority of the cases and, therefore, will not pose a threat to man or to the other animal species, or above all, to the environment.

The third category includes products that employ vectors. Live vectors to transport foreign genes obtained from recombinant material that codify the immunizing antigens and/or others that stimulate the immune system. The live vectors can carry various foreign genes obtained from the recombinant material, since they carry large quantities of genetic information. They are also efficient upon infecting and immunizing the animal species that serve as the objective. Thanks to these properties, the recombinant substances, for example those prepared with vaccinia virus, have become a very popular material for vaccine preparation programs.

In addition to vaccinia, other vectors presently submitted to evaluation by authorized organizations and other research organizations, include the bovine papillomavirus, the adenoviruses, the herpes viruses, the baculoviruses and the yeasts.

PROCEDURES FOR AUTHORIZATION OF THE USE OF PRODUCTS

The general requirements for the authorization of use are summarized in the Figure 2. The products that employ modern biotechnological procedures, like rDNA, chemical synthesis or hybridoma technology, are dealt with in a fashion similar to

those prepared by conventional techniques and need special evaluations to determine the potency and the stability as well as other supplementary tests to ensure innocuity especially when live microorganism are concerned.

When a request is for a given class of products about which a set of data already exists, for example like the case of monoclonal antibodies of hybridomas, then the review process is much simpler. In the case of monoclonal antibodies, for example, their specificity and potency are compared with similar polyclonal antibodies; those characteristics should be equal.

To aid in maintaining production uniformity, authorized institutions are asked to prepare the master or standard materials for the production of a batch; this is defined as the master seed or base seed. The master seeds of microorganisms are selected and identified through a specific number of passes and the authorized institutions keep them permanently on hand as is done with the rest of the conventional vaccines.

The master seeds of products obtained with rDNA will consist of a plasmid or a virus that carries an inserted gene. The "built-up" plasmid is introduced into the appropriate eukaryotic or prokariotic system selected for the vaccine production. Likewise, the genomic rDNA can be directly transferred to various cells from mammals; in these cases the transfected cell is considered as the master seed. Figure 3 shows clearly how the proposed master seed is processed, above all when the rDNA technology is utilized. I repeat that this is an adaptation, not a new regulation, but rather an adaptation of the existing process.

The rDNA master seeds are characterized by prossessing a bacterial plasmid building map that contains the new gene. One must have the basic data on the procedures followed with the DNA to isolate, purify and identify the genetic material of a source and the modification employed to insert this material in a new host.

The data obtained on material isolation, the clon, proliferation, selection of single cells, would remain in the power of the authorized requesting institutions. Here we respect the right to confidentially; I'm not talking about the right to patent but rather about confidentiality. Obviously, in any civilized country, that belongs to the person who is making an investment in product.

However, in general the ingredients of animal origin utilized for purposes of production should be adjusted to the accepted standards of purity and quality established for any biological product. The primary cells and the cell lines employed for the production of a master seed or a vaccine should follow the regulations established for these cell lines.

I will have to be shown that all the cell substrates are free of bacteria, fungi, mycoplasma, viruses and other foreign agents. For purposes of genetic stability the cell lines will have to be characterized and the karyotype determined. Tomogenicity and oncogenicity tests will have to be conducted if there are indications that the cell may cause the formation of malignant tumors.

You are all aware of this and keep it in mind. I bring up again to stress the fact that we are not making a regulation above the other, but rather an adaption to the process.

The manufacturer should select production standards that include procedures to ensure the consistency and recovery of the specific antigenic material. The procecures of recovery should include the removal of excessive concentrations of antibiotics and of undesirable fermentation products.

Figures 4 and 5 show the proposed characterization of the rDNA master seed in the built plasmid; Figure 6 shows the surveillance procedures of the test during the process, that is, the surveillance process that is carried out jointly by the controlling entity and the entity in charge of the production, the former being the governmental agency responsible for control.

As shown in Figure 6, evaluation of the finished product requires the same tests as are conducted for any biological product, with the exception of point 5. Here we have to analyze the genetic expression in a very clear fashion, especially through high-yield liquid chromatography, peptide mapping, polyacrylamide gel analysis and determination of molecular weight. These procedures are the ones presently used by the veterinary services.

Up to the moment, the APHIS veterinary service of the United States Department of Agriculture has approved five bacterines, two products of a therapeutic nature and eleven diagnostic kits in Category I. In Category II, it has approved one vaccine, which as you know is the pseudo rabies vaccine for Aujeszki's disease (Figure 7). All these are approved products that are currently in circulation on the market.

Another aspect within this regulation is the creation of genetic probes as diagnostic instruments. DNA alignment is another technique that has left the research laboratories to be transformed into a simple <u>in vitro</u> test. The lines of genetic material constituted by DNA will mutually align even in the presence of a large number of distinct noncomplementary DNA molecules. This is the definition that is found in the guidelines of the National Health Institutes, and they are the guidelines used as the basis by a study group formed to prepare the biosafety guidelines that are to be published and

distributed in all our countries. We have taken the aspects of very clearly stated definitions in these guidelines that were reviewed by 36 scientists from the region at the Costa Rica meeting and will be submitted for the consideration of each country for their possible application.

I don't want to dwell any longer on reviewing all the principles for approval of diagnostic probes, but I do want to take up some aspects that are already occurring in our countries. I mention especially the diagnostic kits.

With the advent of these new high-tech processes, like the hybridomas that produce monoclonal antibodies and DNA inhibition, new reagents have become available for diagnosis of a great variety of animal diseases, including fowl diseases.

The new diagnostic kits must be carefully assessed and compared with the normal methods to ensure that they show a sensitivity, specificity and reproducibility equal to or better than those of the normal methods.

The diagnostic examinations employed in veterinary medicine, and derived from biotechnological processes, vary in complexity. They encompass a gamut from complicated tests employing automatic equipment, to simple assays with immersion sticks for use in the field. However, the growing trend is to use simple diagnostic kits that combine specificity and sensitivity with speed and economy. This is quite natural, I think that it is important. We have to rely on quick-use diagnostic kits, not only because of the importance of the diagnosis, but also because they provide an economic importance to which I am going to refer.

No matter which diagnostic procedure is chosen by veterinary doctors, or those selected and acknowledged as official examinations by the animal-health authorities, significant results will be attained only when the tests are conducted according to certain criteria that I am now going to stress: the tests selected must be appropriate for the particular circumstances; the quality of the sample employed for the test must be assured; the factors that may interfere in the results must be understood, as well as the test's specificity and principle; the person responsible for conducting the test must have the required skill to do so and, when necessary, to interpret the results. I want to stress all these criteria because in this room, in a kindly fashion, Dr. Tom Yuill said that even the cows themselves are going to be able to perform their own diagnosis. But that was not the goal. I believe that the object with which we must take care is that the cow NOT perform the diagnosis, because then they are going to deceive us.

That diagnosis must be duly conducted, not only with a simple technique, but for the most important part, which is the interpretation of the result of that test. Currently they are used in the field, and I am convinced that they are very useful and practical. I have no qualms that the good livestock raiser, the man who practices it well, should use the technique, because I feel certain that he knows how to use it and is going to interpret it and the result is going to be useful. But when we start to use this commercial aspect of the sale of the diagnosis --and I diagnose everything that I have-- then care must be taken with this procedure. For example, the simple tests conducted by the veterinarian, or by the rancher, are very attractive, very good. However, we should remember that they have limits, restrictions, that they can give us crossed reactions, maybe even false positives or false negatives. We can not use this type of tests when the government, for example in the animal-health program, has an official diagnosis test and we feel inclined to change it, to use our kits, our special diagnostic kits. In short, this is one of the aspects that I did want to cover among the diagnostic aspects.

The production of diagnostic reagents is another of the problems that we have to cover in the regulatory part. As new and better diagnostic tests become available, a difficulty emerges not only for the regulatory authority but also for the producer of the diagnostic reagents. The difficulty lies in conducting economic assessments on the production costs savings achieved by the manufacturers by using products obtained through the new technologies. Studies should go beyond the traditional documentation of the losses caused by the disease and of the resulting cost savings, and should include such intangible but important aspects as the greater administrative efficiency and the direct savings resulting from the quick and definitive diagnosis that does away with the need to adopt long quarantine measures.

At this time there exist antigens and diagnostic media so specific, practical and simple that they can reduce those systems of bipaired sera tests or 30 to 40-day actions. To reduce this aspect cuts a rather high cost factor when we talk of the commercialization of animals or animal products. Then those tests also have a component of economic valuation different from that possessed by only a single diagnostic test. For example in Brucella, where in there are developments that can emerge at any moment.

In this regard we are formulating the biosafety guidelines for the use of this biotechnology, and we are attempting to ensure a broad distribution. With the Pan American Health Organization we have undertaken consideration of the recommendations made by the study group, distribution of the guidelines to all the countries, and preparation of biosafety training and qualifying modules to facilitate the work in laboratories and institutions.

Finally, we hope to receive support and decisive contribution from all of you in formulating, now, other guidelines that will go beyond the rDNA guidelines to include other biotechnological technologies, especially in the field of agriculture.

Thank you very much for your attention.

NOTE: The above is a transcription of notes taken and recordings made during the Seminar.

FIGURE 1. Categories of biological substances obtained through biotechnological processes

Category	
I.	Vaccines derived from inactivated recombinant DNA, bacterines, bacterial toxoids, virus subunits or bacterial subunits.
II.	Modified live micro-organisms adding or suppressing one or more genes.
III.	Live vectors carrying foreign genes derived drom recombinant substances that codify the immnunizing antigens or other stimulants of the immune system.

FIGURE 2. General requirements for authorization of the use of products

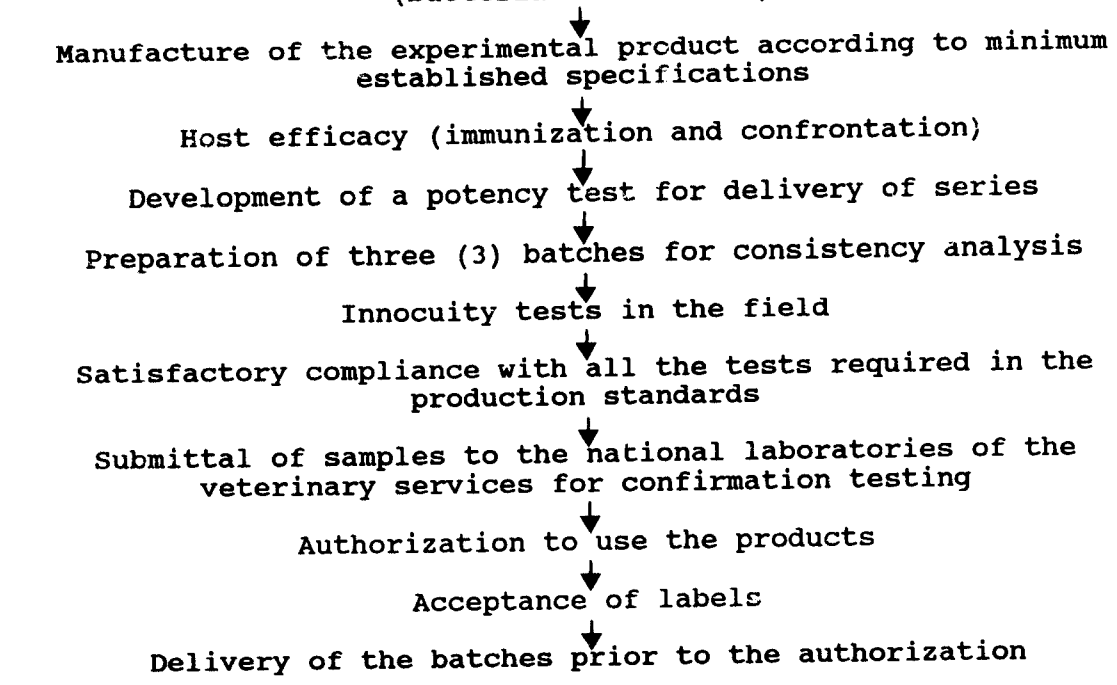

FIGURE 3. Master seed proposed for the products derived from rDNA (Policy of the United States Department of Agriculture for authorization of the usage of products)

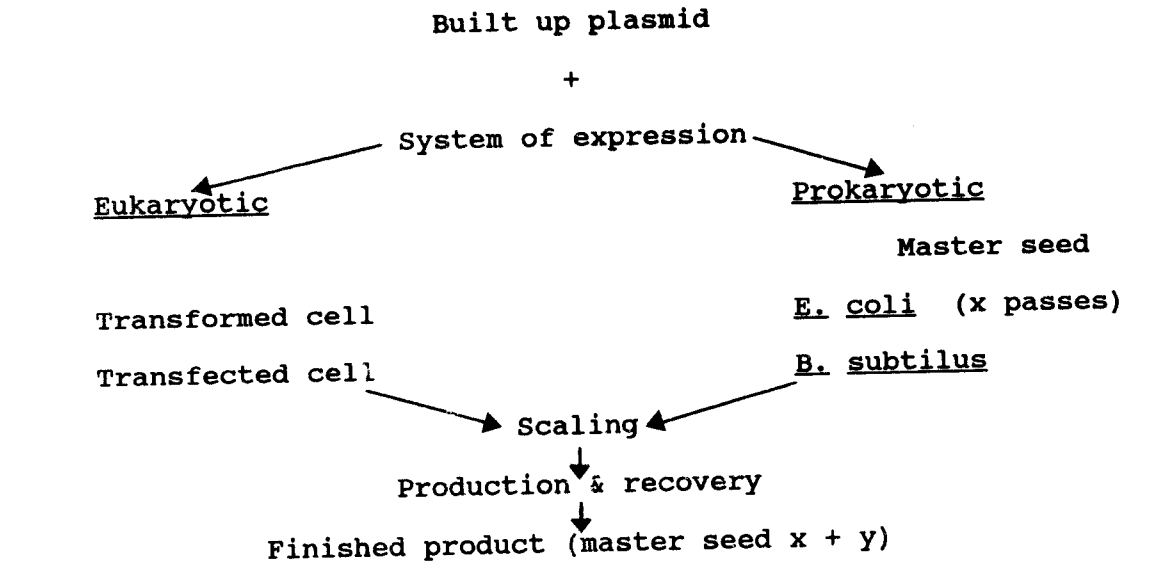

FIGURE 4. Proposed characterization of the rDNA master seed

Built up plasmid

1. Nucleotide sequencing
2. Analysis in polyacrylamide gel
3. Pharmacoresistence
4. Mapping of the enzymatic restriction
5. Description and locating of iniciators and promoters

FIGURE 5. Test surveillance procedures during the process

1. Growth index
2. Gel mapping (dodecylic sodium sulfate) (electrophoresis)
3. Resistance to the antibiotics
4. Metabolic markers
5. Molecular weight
6. Activity
7. Protein expressed in percentage

FIGURE 6. Evaluation of the finished product

1.	Purity	- Normal applicable procedures
2.	Potency	- Correlation of the efficacy test with the procedure in vivo or in vitro
3.	Efficacy	- Normal data on the immunization and confrontation of the host animal
4.	Innocuity	- Expanded programs regarding laboratory and field tests
5.	Genetic expression	- Partial analysis of sequences High-yield liquid chromatography tests Peptide mapping Polyacrylamide gel analysis Determination of the molecular weight

FIGURE 7. Authorized products prepared through biotechnological processes

Category I

Class of product	Number
Bacterial	5
Therapeutic	2
Diagnostic kits	11

Category II

Vaccine	1

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