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About the Institute
The Hunt Institute for Botanical Documentation, a research division of Carnegie Mellon University, specializes in the history of botany and all aspects of plant science and serves the international scientific community through research and documentation. To this end, the Institute acquires and maintains authoritative collections of books, plant images, manuscripts, portraits and data files, and provides publications and other modes of information service. The Institute meets the reference needs of botanists, biologists, historians, conservationists, librarians, bibliographers and the public at large, especially those concerned with any aspect of the North American flora.

Hunt Institute was dedicated in 1961 as the Rachel McMasters Miller Hunt Botanical Library, an international center for bibliographical research and service in the interests of botany and horticulture, as well as a center for the study of all aspects of the history of the plant sciences. By 1971 the Library's activities had so diversified that the name was changed to Hunt Institute for Botanical Documentation. Growth in collections and research projects led to the establishment of four programmatic departments: Archives, Art, Bibliography and the Library.

Important cutting

D. Information input

Data requirements -

1. For endangered species what is known of the species, its habitats, genetic diversity -

See data from Baniszchke.

Contents -

A. Intro. - Problems - Scope, Detail of approach
3 categories explanation

B - Description of state of germplasm resources for each of the following -

1. Natural Ecosystems -
2. Economically important organisms (4 subcategories - wild, folk collections, organized maintenance)
3. Specialized collections -
a) genetic b) medical uses
c) pollutant monitors
d others - zoos, aquaria, botanic gardens

C. Problems of Assessment -

C - Preserv. probl.
1) administrators
2) technical -

D - Information mgmt

E. Challenges

F. Summary

G. Citations

Get Trenn Williams
supplement to
Biblio. of Pl. Genetic
Resources -

Write section on
marginally important
economic crops -
address problems of
C -

Waltzing NRC - D - Information Mgmt.

1. Introduction -
A - Info/data diff. & requirements -
Published lists may be useful references -
" literature well in hand by BIOSIS/NAL/MEDLARS
others in other countries -
CODATA Check w/ Rita Col well-groups / data manuals.
Raw data -

2. B - Concepts of info "mgmt" -
All types of data processing systems -

NRC - 3/12 -

Robert Jenkins - Director, Nature Conservancy - History of N.C. 1926

"The Noah of the Conservancy movement":

Systematically identify lands important to conserv. of species

>1000 K acres of land -

owns 700 preserves - in US, Caribbean, Mexico

Maintain ecological inventories, data banks -

State & national heritage programs -

1 - Estabd. ongoing inventories

10 or 11 with State govmts now.

ecosystem classifications as an indicator of plant / animal diversity -

coarse filter — if can presume at least one major community of each type, would preserve diversity -

fine filter — but need special categories for "special" species.

The categories are useful as something on which to hang data -

Jenkins believes in centralized & diversified permanent data centers -

The "living atlas".

Botanical Garden functions

1. Horticulture (Floriculture)
   Temperate zone
   Tropical / tender herbaceous } display, seed exchange, research.

2. Systematics research -
   Regional - Herbaria sometimes
   National - " "
   International - Herbaria

3. Other research -
   Mycology / plant pathology -
   Physiology
   Genetics / Plant Breeding

4. Teaching
   Formal courses (University related)
   Public education -

5. Publication + Library -
   Ranging from popular to scientific

Botanical Garden - relationships -

Public (government support)

Semi private - partial support from local/state govt.)

Private - private income/endowment -

United States -

National support

The National Arboretum

State support, local (city) govt -

New York

California - Davis, San Francisco, - Berkeley -

Chicago -

Denver - Denver B.G. (mostly private, some city).

Private -

Mass. - Arnold Arb.

Missouri - St. Louis B.G.

Illinois - Morton Arboretum.

Pennsylvania - Longwood Gardens -

?? Alabama - Bellingrath -

University / College -

Range of different levels -

From: "Preserves" - with no program of conservation

To : formal collections, research -

V

~~① Wait for Sprague comments~~
~~② Insert into Kingman.~~

B2. Economically Important Organisms.

- ✓i. Crop plants
- ii Livestock
- iii Fish
- ✓iv Forests
- v Pests
- ✓vi Organisms used in industrial production
- ✓vii Drug Plants
- ✓viii LDC's food plants.
- viii Amphibia.

ECONOMICALLY IMPORTANT COLLECTIONS OF ORGANISMS

Crop plants currently used in the U.S. and the developed world, both for food and fiber, range plants

The United States and the world face many challenges in the future. Study after study has indicated that if the human family expects to feed its burgeoning numbers, "We have to find in the next 25 yers, food for as many people again as we have been able to develop in the whole history of man 'til now" (1975). In addition to this humanitarian aspect, bountiful and secure agricultural production is essential for quality of life and economic prosperity of the nation.

Throughout the history of cultivation, plants judged to be superiod were saved for propagating subsequent crops, and this doubtless sometimes consisted of chance and manmade hybrids. This activity led to a large number of "folk" varieties in all parts of the world. Great genetic variability existed within and among these varieties; moreover, that portion of species not chosen for cultivation generally survived in nature, because the pressure of human population and advanced agricultural technology did not destroy their natural habitats. In this century the circumstances that existed for so long began to change and continue to change rapidly.

Professional plant breeding based on the rediscovery of Mendel's laws and the development of the chromosome theory of heredity began 60 or 70 years ago. Applocation of these scientific principles led to modern crop varieties generally very uniform and specialized for yield, quality, and adaptation to specific environments. The continuing release of improved varieties and the adoption of advanced

production technologies have resulted in remarkable increases in agricultural productivity. The immediate advantages of these modern varieties over "folk" varieties led to their widescale adoption in this country and in other parts of the world. Many old varieties and land races were abandoned, and the erosion of plant genetic resources accelerated. Additionally the world population exploded from something over a billion to 4 billion people. The industrial revolution coupled with population pressure disturbed the natural habitats of many species and genetic resources in the wild shrunk as did that in cultivation. The important crops began to have a narrowed germplasm base.

Responsible agricultural leaders in this country and abroad have recognized for many years that genetic variability of crops was being reduced and that valuable plant genetic resources were thereby being lost. The southern corn leaf blight epidemic of 1970 aroused a sense of urgency in the nation. Conservation and proper utilization of plant genetic resources were activities of first importance to continued wellbeing.

Loss in the 1970 corn leaf fungal blight reached 50% in some states and 15% nationally. This threat to a major crop created so much alarm that the National Research Council appointed a committee on Genetic Vulnerability of Major Crops to consider (1) what caused the corn blight epidemic in 1970, (2) how vulnerable are our crops to attack by pests, and (3) what should be done to hold losses to low levels and reduce the probability of epidemics? The report "Genetic Vulnerability of Major Crops," was issued in 1972. The chapter on "The Challenge of Genetic Vulnerability," says, in part, "Two points are clear: (a) vulnerability stems from genetic uniformity; and (b) some American crops are on this basis highly vulnerable.

This disturbing uniformity is not due to chance alone. The forces that produced it are powerful and they are varied. They pose a severe dilemma for the sciences that society holds responsible for its agriculture. How can society have the uniformity it demands without the hazards of epidemics to the crops that an expanding population must have?"

A partial answer to the above question was provided in "A Special Report by an Ad Hoc Subcommittee of the Agricultural Research Policty Advisory Committee (ARPAC)," entitled "Recommended Actions and Policies for Minimizing the Genetic Vulnerability of our Major Crops (1973). The USDA Secretary's Memorandum No. 1875, dated July 3, 1975, established the National Plant Genetics Resources Board to advise the Secretary of Agriculture on national needs for the assembly, description, maintenance, and effective utilization of living resources in plant improvement programs. The Board recognizes the importance of conserving and utilizing plant genetic resources, if agricultural scientists are to have the genetic diversity to meet current and future problems. The Board is preparing an analysis of the status of crop germplasm resources in the United States.

The Board established in 1976 a liaison with the International Board for Plant Genetic Resources. The IBPGR was established by the Consultative Group on International Agricultural Research (CGIAR). when? Its mission is to ensure that genetic variability in economic species of plants is conserved so that it can be used by plant breeders, and by research workers interested in the evolution of cultivated plants and of agriculture itself. To this end it is expected to develop international collaboration among the members of a global network of institutions active in the exploration, collection, conservation, documentation and use of plant genetic resources. It accepts that the volume and duration of its support will depend primarily on the practical and scientific results of its work.

Categories for Crop Germplasm Maintenance

Plant germplasm in the broadest sense, includes all living plants capable of reproduction. Most species, particularly the noncultivated ones, will be left to their own devices for survival. They will continue

to evolve or become extinct. This category includes wild relatives of and noncultivated forms of the crop species.

A second category are those species that are sufficiently useful to merit some protection or cultivation. These constitute the "folk" varieties and "dooryard" plants, and may be of greatest importance in the centers of diversity (Harlan?).

A third category is those plants that have been aseembled by scientists, or amateur botanists. It is not unusual for an individual, members of a research station, or employees of commercial seed companies to accumulate extensive germplasm collections beyond their immediate needs. Most are willing to share their stocks with others. This is an informal germplasm system since there is no overall coordination nor designation of responsibility for its continued maintenance. Although this informal system is very valuable, it is difficult to know what stocks are available. It is vulnerable to losses as people retire and as administrators reevaluate priorities.

Crop plant scientists do not believe that the first three categories i.e. the wild, the "folk" varieties, and that held by individual scientists or institutions are adequate to meet the crop germplasm needs. A more formal fourth category has evolved over the years. It is currently known as the National Plant Germplasm System (in press). This system recognizes the fact that the U.S. derives most of its food and fiber crops from plants that were originally introduced from other countries. As such a coordinated program of introduction, evaluation and maintenance of germplasm is mandatory. Some crops are known to have very little genetic diversity, and the wild and primitive varieties should be assembled for use in breeding disease and insect resistance.

The organization of the national system is outlined in (in press). Although considered fully

operational and well established there may be areas that need further attention. One such aspect is the identification, funding and clarification of duties of the persons and institutions chosen as curators for specific groups of crop plants. The National Plant Germplasm Committee advises the system, coordinates activities and reviews present and future needs. It has identified the following activities as meriting the earliest possible attention:

State the Problem

- Establishment of repositories for clonally propagated plants
- Provision of facilities, staff and funds for increased activities of the national, regional and interregional stations.
- Establishment of a tropical facility.
- Funding selected curators
- Identification and subsequent closing of gaps in major collections
- Computerization of information concerning all plant genetic holdings in the U.S.
- Consolidation of holdings aimed at safer maintenance and reduced costs
- Expansion of preliminary evaluation of plant germplasm.

From this list of priorities it is apparent that although the National Plant Germplasm System has been established and is seeking to rectify the perilous situation that existed at the time of the corn blight in 1970, many of the essential tasks have not been completed.

Maintenance and encouragement of indigenous subsistence agriculture as a program in genetic resource conservation of crop plants in developing countries

This is based on the concept that domestication is an ongoing process of interaction between genetic variation in populations of cultigens and varied selection pressures of subsistent agriculturists. The two part process has led to (and will continue to lead to) useful variations in cultivated plants. An approach based on this concept could complement the present program of storage of germplasm presently in operation as well as that of preservation of the landscape where the putative progenitors of cultivated plants grow. Both of these other approaches ignore the two step process by which our present day genetically variable crop plants have arisen.

The variation seen in cultivated plants is not a static condition. It is the product of continuous interaction between natural variation (in progenitors and cultigens) and continued human selection for maintenance of that variation. The process of maintenance of variation is carried on by subsistent agriculturists who depend on a reliable annual yield of crops. Presumably each type of crop plant produces some yield, no matter how poor the annual weather conditions and soild conditions may be. Our modern agri-business approach tends toward a maximum yield based on genetically uniform and superior cultivars that respond to predictable water and nutrient levels for a longer period of time.

The native cultigens may be nutritionally superior and tolerant of a diversity of environmental fluctuations--this tolerance may be due to greater genetic diversity within the population as well as a broader range of yield response along environmental gradients

in contrast to commercial cultigens with few genotypes and narrower ranges of positive response to enviornmental gradients.

The importance of maintaining present day indigenous agricultural systems and cultigens could be a valuable contribution to the conservation of germ plasm resources if:

1- indigenous agricultural systems are adequately studied from ecological, genetic, and nutritional viewpoints

2- attempts are made to allow subsistent agricultural systems to survive, especially if it is shown that they are nutritionally and ecologically sound for a given region.

3 socio-political efforts to modernize agricultural systems are eliminated in these regions, especially where the soil and climatic conditions are such that the "green revolution" efforts are too costly to governments and native peoples and result in a meager return, if any.

In western Mexico -- a semi-arid region which appears to be the type of area where biological evolution of plants (Stebbins, 1972) and of domesticated plants (e.g., Zea mays, Phaseolus vulgaris) have been important. This concept is based on the observation that the Tarahumara Indians of western Chihuahua maintain a diverse number of cultigens (Zea mays -- 6 races formally recognized and possibly more; Phaseolus vulgaris, 14 native-taxa races). In addition, their activities encourage the interaction of the natural variation of useful plants and of the continual selection for beneficial variations. For example, the most common race of maize is "maíz pinto" which consists of several forms of "Maíz Azul"[1] introgressed with "Maíz Cristalino de Chihuahua" and "Maíz Blandito de Sonora". "Maíz pinto" is recognized as the form which provides the most reliable yield despite the poor soil and unpredictable weather during the growing season (spotty rain distribution, hail, early and late frosts). Also the cultural commitment to the basic selection process is strongly evident in the Tarahumara culture as seen in their attempt to domesticate an introduced, weedy Brassica.

The proposal of incorporating indigenous subsistent agricultural systems into the genetic resources program is based on the principle that genetic diversity is not only a function of the plants (past and present cultigens and progenitors) but also a function of the human element which provides the opportunity for expression of these variations and the selection forces to maintain genetic heterogenity. The success of this two component process is a matter of life or death to the subsistent agriculturists. The necessity of a reliable yield on an annual basis in a region of poor and unpredictable growing conditions should maintain and provide tremendous genetic variation in the food plants that are cultivated.

The following examples may also illustrate the variations of the problems of conservation of genetic resources, particularly of plants of major importance to man's economy. The range of wild species with no known economic significance is great. However, we are never certain that some wild species will not become significant in our culture.

An example of the value of species not in cultivation that provides a very potent argument for conservation of wild materials is the grape. Vitis vinifera, the Asiatic-European wine grape, is highly susceptible to a root-attacking insect, ~~phytophthera~~ Phylloxera. (There are controversies about the proper name for the parasite, not yet fully settled.) The disease, apparently of American origin, has had serious effects on the wine industry here and abroad. The only known resistance to the disease occurs in native American species, found growing as natives in much of the North American continent. Wine grapes are also susceptible to other types of plant diseases, and require use of wild stock for grafting. However, these species are being seriously reduced by the inroads of man and his destructive tendenceis, and unless efforts are made to conserve these species, there will be a serious effect on the largest economic fruit crop in the world. Probably two types of measures are needed for the American species of Vitis: one is an active collection of representatives of the wild species, maintained in viticultural collections, and the other, conservation of regions in which the species are found as natives.

Another example that can be cited involves the requirement for new sources of energy, particularly for coal, and particularly in the regions of the western part of the US, cause some very serious problems of maintenance of environmental quality where strip mining is done. In the west, which is not supplied with large quantities of water, reclamation of strip-mine tailings is a horribly difficult problem. Selection of plant material for reclamation is extremely difficult, because most of the native species that probably are best adapted to the natural conditions in the west are very poor colonizers of disturbed habitats. Those that might be most useful have not been tested. Our best resources for plant materials where conditions similar to those found on the mine tailings exist, are frequently derived from other regions. Most often, the plant materials that fit these requirements are natives (frequently weedy) in other parts of the world. Conservation with plants of this type is naturally, a very risky business, because the chances of introducing plants that produce more problems than they solve is very high. But with respect to conservation of genetic resources, we have an example of the complexity of the environmental problems that man has produced for himself, and an example of the need for much more intensive research into them than has so far been available. Conservation of some of the best known genetic resources are maintained by the USDA Regional Plant Introduction Stations. These are limited, however, and many more such species are required for the various localities that will be mined with more intensity as our supplies of fossil fuel, both foreign and domestic, decline.

Economically important plants in the lesser developed countries

1. Diet (food). With a few notable exceptions (such as white potatoes--species of the tuber-bearing section of the genus Solanum; sugar cane--Saccharum spp.; tomatoes--Lycopersicon spp.;) the food plants of the lesser developed countries have not suffered from the same problems of genetic resources loss as some of those crops used in the temperate zone. For example, it is doubtful that inroads into the genetic diversity of the cultigen Manihot esculenta, cassava, has been nearly so serious as that for Triticum, where the newer cultivars produced in highly advanced crop breeding programs have had the effect of eliminating a large number of so-called land (or primitive) races. Only very recently have efforts been started to conserve genetic resources of any of the major crops and there are still no efforts to conserve those of lesser significance, such as the fruit and nut crops (of which there are very large numbers). Much less there is very little effort towards improvement of these lesser crops. Those vegetables, and many legumes, that are consumed by local peoples in the tropics (which is equivalent to "lesser developed") are practically an unknown quantity when it comes to such matters as intensive cultivation in the same type of endeavor as that done for cultigens in the temperate zone.

The above does not indicate that there is no need for conservation of genetic resources. Clearly the same processes that destroy large land surfaces by road-building, new farming areas, urban construction, et al. are very likely destroying genetic resources of these crops and the related wild relatives. However, the status of these is very poorly known, and only in the case of a very few (such as the common bean, cowpeas, and corn) are any efforts being made to conserve genetic

resources. Zea mays has had the longest record of collection and maintenance of the tropical cultivars, and even in this case, the intensive initial efforts made in about 1943 onward have not ensured the complete collection of genetic resource material and certainly it has not been maintained consistently).

Crops of the lesser developed countries affecting the economies

The variety of crops that provide the cash for many of the underdeveloped countries around the world is very large, ranging from beverages, to fibers, to elastic substances, to medicinals, and to lumber. In the latter category, the largest lumbering areas of the tropics that are being exploited ruthlessly are the so-called dipterocarp (Dipterocarpaceae) forests of the Indomalaysian regions. Large areas are being completely destroyed, and the countries in which this ruthless exploitation occurs are not sufficiently strong (in a political sense) to enforce any kind of forestry methods on the companies who extract the timber with extremely wasteful procedures. In other areas, an equally disturbing process is at work. Herein, large plantings of single genotypes are raised in plantations to the exclusion of all other trees. Where teak (Tectona grandis) is planted, no other plant can live--the dead leaves of the teak act as a sort of herbicide to any other vegetation. Similarly, Eucalyptus forests are (and have been for many years) being planted in pure stands, and the forliage inhibits growth of many native species. Usually, the commercial interests are responsible for the single genotype planting schemes--it is not so profitable to plant

a mixed culture because of variations in growth pattern of the plants, as well as variations in the products.

Rubber, still an important crop in many parts of the world, is seriously attacked in land where this species is native. All of the Hevea brasiliensis (rubber) Latin America plants are subject to the disastrous disease (Dothidella ulei) of the leaves, preventing any large scale plantings in the Western Hemisphere. Since petroleum products are the starting point for production of synthetic rubber, it becomes more than a botanical problem to maintain a pool of diverse genetic resources for this important species.

B. ii. Genetically defined stocks - small mammals

Abundant experimental collections are available for guinea pig, hamster, rabbits, rats, and mice. For most, maintenance and supply is by commercial firms or grant support by governmental agencies with significant assistance from the educational and non-profit organizations with which they are associated. Rather broad and secure support is available for the relatively sophisticated genetic collections of the mouse. The support comes from a variety of sources and collections are maintained in several countries. Stocks available for research include upwards of 50 genetically independent inbred strains and several interbreeding subspecies, some of which have been inbred. Further, there are over 400 loci for which there are mutant or variant alleles and these loci produce a variety of phenotypic effects from immediate gene products to more complex physiological, anatomical and behavioral effects. There are also good collections of translocations, inversions, X-chromosome aneuploids, and a few deficiencies and duplications. Several Robertsonian chromosomes have been found in natural populations in Alpine regions and others have been known to occur de novo in laboratory strains. World-wide research on these stocks fosters their exchange among scientists and relatively good protection against loss.

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FOREST-TREE GERM PLASM RESOURCE - ~~IVS FUTURE~~

~~Stanley P. Krugman~~
~~Forest Service, USDA~~

The forests of the world especially those of the United States represent a substantial genetic pool of considerable diversity and richness. But this unique resource is not limitless and is being subjected to numerous pressures. Genetic variability is the basis for all breeding efforts. Maintaining an adequate diversity of genes in a population permits new combinations that can result in individuals that are better adapted to specific environmental situations. Through breeding new combinations that fit specific criteria such as disease resistance, rapid growth and drought tolerance can be produced. Without genetic diversity there is no basis for breeding programs. Without question diversity must be maintained for the future. Once genes are lost from a species they cannot be readily recovered, if at all. Manmade practices such as uncontrolled and ill-managed large scale forestry operations could lead to impoverishment or even elimination of selected natural gene pools. Indiscriminate mixing of natural forest gene pools could further accelerate genetic impoverishment in the future (Barber and Krugman 1974; Maini 1973; Yeatman 1972).

Such concerns are real since forest practices in most countries are becoming more intensive and shortly few forest stands will remain untouched. In many countries the native forests are being removed often to be replaced by exotics, or non-local reforestation stock. There needs to be a real concern for maintaining ancestral types as well as a broad genetic basis for future selections and breeding. Who knows what genotypes will be needed in the future?

INSERT Something about Teak, Eucalyptus etc from minutes

Strategies for meeting these issues must be based on a clear understanding of the genetic variation associated with forest trees. In addition, it must clearly be understood that forestry problems associated with maintaining a genetic base are not identical to general agriculture and the proposed solutions certainly will vary from those now being attempted in horticulture and agronomy.

Forest trees have evolved inherent adaptations to a series of factors of the environment at the site where they grow. Research in the last 100 years has clearly shown that diverse environments throughout the range of a tree species leads to a genetically variable species. Similarly, widespread species tend to be more variable than restricted species. Often patterns of inherent variation parallel patterns of environmental variation. Likewise, races of a species growing in different climatic regions may differ in inherent adaption to environment factors. Still, limiting factors generally are not always the same for cohabitating species (Callaham 1970).

More recent studies show that for native species local seed sources are best adapted but not always the most productive (Callaham 1970). However, in many areas of the world, there is an inadequate information base on the proper seed source to employ due to a lack of testing and local seed sources must still be employed. In fact, in the absence of better information conservative forestry programs are still dependent solely on a local reliable seed source.

It is apparent then that if forestry is to avoid the difficulties of general agriculture, i.e., the loss of the original genetic base, strategies must be developed to maintain a reliable and varied genetic reservoir for future improvement, to provide standards for progress in improvement, and to ensure

and perpetuate selected large or small populations for future mass seed production (Maini, Yeatman, and Teich 1975; Yeatman 1972). Even if we were only concerned with shortrun situations the maintenance of the integrity of current populations of selected and provened seed collection areas is a serious problem we are now facing. This is not some abstract problem to be confronted at some future date. Virtually all nations with active forestry programs are encountering this problem now.

Forest trees have many features that lend them to easy gene pool protection although the cost may be relatively high by present forestry standards. Normally, the depletion of the gene pool each year when the annual life cycle is complete is not a serious problem because of the longevity of forest trees. Furthermore, foresters, in contrast to agronomists, are still working with essentially wild populations. With a few exceptions, foresters in the United States are not dealing with a crop derived from exotic germ plasm or with material that has been selected over many generations.

There are, of course, some specific problems which must be faced. Diversity in areas of intensive management must be maintained. But even of more importance we must be aware of possible risks of losing germ plasm from species with disjunct distributions, i.e., isolated stands and outliers, through the influence of man or catastrophies such as fire and severe weather. Where these isolated stands may contain genetic diversity not present in the general population, the stands should be protected or some means provided for their continuation.

Currently, there are various strategies now being applied in a serious effort to maintain and protect forest gene resources. Among the more common methods

employed are:

Seed, pollen, and tissue culture storage: Once forest tree material has been selected, for whatever reason, it is feasible to maintain certain biological material as seeds, pollen, and more recently, tissue culture by means of various forms of storage--a method which is common to general agriculture. Obviously, when this method is employed, a decision has been made as to what is unique, superior, or essential for the future. Our needs often change and so will our selection criteria. This method is also limited in scope since at this time not all material can be stored. Storage obviously leads to a static development in genetic composition since normal selection and evolution is halted. At this time, there are still some mechanical problems with long term storage, and the long term value to forestry is uncertain.

Seed stands and plantations: A common practice for future seed collection is to set aside selected stands or develop plantations of material considered essential. Commonly, these represent only relatively small areas and can be employed to guarantee only limited genecological groupings. Unless great care is taken natural selection pressures will modify the genetic composition of the original stands during the long maintenance period.

Seed orchards and arboreta plantings: These provide a means for maintaining genetic selections, especially related to commercial forestry. As with storage these methods are static and assume what will be needed in the future. They do not permit normal evolution. Again, natural selection can be expected to modify the population.

Research and Ecological Natural Areas, National Parks, Primitive and Wilderness Areas: These are set up for a variety of reasons, often for recreational and social purposes. Unfortunately, they are rarely established or managed for maintaining a broad genetic resource which is needed in forestry. They do

provide a form of germ plasm conservation and certainly are needed and useful in the broad development of a germ plasm conservation program. Since the major use of these areas often precludes disturbances and commercial activities they are not always suitable for mass seed collections and related activities needed in production forestry and related activities of restoring damaged forest ecosystems.

Special Gene Pool Centers - Forest Genetic Reserves: Special areas of natural forest ecosystems need to be established in which both static and dynamic management can be applied. To be effective the Genetic Reserve must include forest ecosystems which are representative of forest gene pools commonly found in areas where consumptive forestry is practical and will be practiced, and where there are other pressures on the forest ecosystem which may seriously modify the genetic composition. The Genetic Reserve must be sufficiently large so as to reflect a full range of biological and environmental diversity. It should be possible within the Genetic Reserves to manage and manipulate given forest ecosystems. Finally, it should be possible to make both small and mass seed collections for consumptive forestry purposes, or for restoring damaged ecosystems.

It is rather obvious if Genetic Reserves are to be employed in advancing the science of forestry that they must be representative of forests in which consumptive practices are employed. Their selection criteria should include this element whenever possible. Otherwise, studies and maintenance of Genetic Reserves will become merely an academic exercise in terms of modern forestry.

The gene pool of natural forest population is in adaptive and dynamic balance with a given environment and can only be maintained through successive generations within the environmental context in which it evolved. And, since

patterns of inherent variation of forest trees reflect patterns of environment variations it is essential that as many patterns of environmental variations are included in a Genetic Reserve System. Thus, their size should reflect the extent of the biological and environmental variation encountered. Most often then Genetic Reserves must encompass extensive forested areas. Lest we forget, the area should be sufficiently large to minimize the hazard of foreign pollen contamination. Included should be those stands which are highly unique and exceptional in growth and form as well as the typical representative stands of the area. In addition, the sensitive and often unique transition zones of the various species should also be included. Distinct forest tree populations threatened with destruction should be part of the Genetic Reserve.

Many of the current attempts at gene pool conservation are static systems. They are directed at arresting the present rate of evolution, i.e., permit fire control. Similarly, all too often under undisturbed forest conditions shade intolerant species are at a distinct disadvantage and can be eliminated. Yet many of these same intolerant species are a major source of current and future supply of wood and fiber. It should be possible by proper management, i.e., fire, logging, planting, to maintain repeatedly a segment of a Genetic Reserve in a halted successional sequence (Maini, Yeatman, and Teich 1975; Yeatman 1972).

We noted earlier there is a serious problem of recovering and maintaining proven forest tree seed sources. By permitting mass seed collections the genecological pedigree of a seed source can be guaranteed which in this day of declining intact gene pools is rather important to modern forestry. In

essence, selected portions of a Genetic Reserve would serve as a tested, reliable, and varied genetic reservoir for perpetuating selected populations for forestry and related uses. In fact, certain portions of the Genetic Reserve should be preserved intact after the initial screening has taken place.

To meet the challenges of maintaining an adequate forest gene pool for the future, these various strategies must be applied and they should be initiated in those areas where they are now lacking.

Important Drug Plants in the U.S.

Medicinal plants of importance fall into several categories, i.e. (a) those that yield pure chemical compounds of established value in the treatment of disease; (b) those that are used in a crude or advanced form, and which have established value in the treatment of disease; (c) those that yield chemical compounds of importance as starting materials for the semi-synthetic production of drugs which have an established value in the treatment of disease; (d) those that yield extracts or compounds which in themselves have no well-established medicinal value, but which may be necessary for the preparation of compendial dosage forms; (e) those that yield chemical compounds of known structure that are not in themselves useful as drugs, but which may have a valuable utility as pharmacologic tools in that they contribute to a better understanding of the mechanism of action of other drugs; and (f) those plants that have a widespread use as "herbal remedies", "teas" and the like, in which case the medicinal value may not have been established.

There is a false conception by many as to the importance of higher plants as drugs, or as sources of useful drugs. This is prevalent in the United States, as well as in many countries abroad. Perhaps a few factual aspects of the role of plants as medicines, or as sources of

medicines, will be useful to place into perspective the seemingly large number of species making up this report. In 1974, we obtained data from a National Prescription Audit (R.A. Gosselin and Co.) which detailed the frequency of use of all drugs (synthetic and natural) dispensed from community pharmacies in the United States. The data spanned the period 1959-1973. We analyzed every prescription product in order to determine those in which naturally occurring substances were present as the major drug ingredient. It was found that in each year over the period 1959-1973, prescriptions containing plant-derived drugs accounted for 25.0 ± 1.0% of all new and refilled prescriptions (1.532 billion total prescriptions were dispensed in 1973). Some 38.3 million prescriptions in 1973 contained only powdered plant drugs, or advanced forms of the crude drug. Also, in 1973, a total of 76 different chemical compounds of known structure were represented as therapeutic plant-derived ingredients in the prescriptions. Of these 76 compounds, seven are produced by chemical synthesis (emetine, papaverine, caffeine, theobromine, theophylline, ephedrine and pseudoephedrine), the remaining 69 are still commercially extracted from plants.

An important additional point to be recognized, is that in terms of the total numbers of prescriptions dispensed each year in the United States, there is every indication that those containing plant-derived active constituents will remain at about the 25 per cent level for the forseeable future. In 1974, the cost to the American public for ca. 1.3 billion prescriptions containing plant-derived active ingredients was about $3.0 billion.

If one would look to the future, there is abundant evidence that the "herbal tea" market will expand at a rapid rate. It is my understanding that this is currently a multi-million dollar market in the United States, and we should look forward to a need for expanding quantities of plants

used as teas by the laiety.

With this introduction as a basis for selection, the plants of importance are grouped into three major categories below. The categories are in general, of decreasing importance relative to therapeutic effects of the plant and/or it's active principle(s). Plants grouped in I and II have been selected on the basis of a good data base, and should be reliable. Plants in group III, on the other hand, have been selected on the basis of information made available to me by a number of major botanical suppliers in the United States, regarding their major sales volume items.

The list of plants provided below may well be considered as conservative, but within the constraints of difficulty available and/or factual data, it should include the most important of our medicinal plants.

I. MEDICINAL PLANTS OF MAJOR IMPORTANCE AS PRESCRIPTION DRUGS OR AS YIELDING PRESCRIPTION DRUGS (In order of relative importance).

PLANT NAME	USEFUL CONSTITUENTS
Dioscorea floribunda (and other diosgenin-yielding species)	Diosgenin (steroids)
Papaver somniferum	Codeine, morphine, noscapine
Atropa belladonna	Atropine, hyoscyamine, scopolamine
Duboisia myoporoides	Atropine, scopolamine
Rauvolfia serpentina	Reserpine + whole root
Rauvolfia vomitoria	Reserpine
Digitalis purpurea	Digitoxin ± whole leaf
Digitalis lanata	Digoxin
Pilocarpus jaborandi	Pilocarpine
Cephaelis ipecacuanha	Emetine, cephaeline + whole root
Rhamnus purshiana	Bark

Catharanthus roseus	Leurocristine, vincaleukoblastine
Veratrum viride	Alkaloid extracts
Cinchona species	Quinine, quinidine

II. MEDICINAL PLANTS OF LESSER IMPORTANCE, BUT YIELDING IMPORTANT COMPOUNDS USED AS PRESCRIPTION DRUGS THAT ARE NOT AMENABLE TO SYNTHESIS

PLANT NAME	USEFUL CONSTITUENTS
Colchicum autumnale	Colchicine, demecolcine
Ammi visnaga	Khellin
Ammi majus	8-Methoxypsoralen
Ananas sativus	Bromelain
Carica papaya	Papain
Cassia acutifolia	Sennosides A & B
Cassia angustifolia	Sennosides A & B
Plantago lanceolata	Psyllium hydrocolloid
Ricinus communis	Castor oil
Physostigma venenosum	Physostigmine (Eserine)
Erythroxylum coca	Cocaine
Cytisus scoparius	Sparteine
Carthamus tinctorius	Safflower oil
Veratrum album	Protoveratrines A & B

III. OTHER PLANTS YIELDING MATERIALS USED IN PHARMACEUTICALS AND/OR ARE IMPORTANT TO THE PUBLIC. THESE MAY OR MAY NOT HAVE THERAPEUTIC VALUE, BUT ARE WIDELY USED.

PLANT NAME	USEFUL PRODUCT
Astragalus gummifer (and other species)	Gum Tragacanth
Acacia senegal (and other species)	Gum Acacia
Sterculia urens	Karaya Gum (Sterculia gum)

Sterculia villosa	Karaya gum (Sterculia gum)
Sterculia tragacantha	Karaya gum (Sterculia gum)
Cochlospermum gossypium	Karaya gum (Sterculia gum)
Cyamopsis tetragonolobus	Guar gum (Guaran)
Ceratonia siliqua	Locust bean gum (Carob bean gum)
Aloe barbadensis (or related species)	Aloin
Rheum officinale or R. palmatum	Rhubarb
Glycyrrhiza glabra	Licorice
Panax ginseng	Root
Vanilla planifolia	Vanilla flavor
Anamirta cocculus	Picrotoxin
Mentha piperita	Peppermint oil
Cinnamomum cassia	Cinnamon oil
Mentha spicata	Spearmint oil
Eugenia caryophyllata	Clove oil
Pimpinella anisum	Anise oil
Eucalyptus globulus	Eucalyptus oil
Strophanthus gratus	Ouabain
Acokanthera schimperi	Ouabain
Hydrastis canadensis	Berberine
Chondodendron tomentosum	d-Tubocurarine
Strychnos castelanaei	d-Tubocurarine
Strychnos crevauxii	d-Tubocurarine
Strychnos nux-vomica	Strychnine, brucine
Arctium lappa	Burdock root
Cnicus benedictus	Blessed Thistle herb
Hibiscus rosa-sinensis	Hibiscus flowers
Juniperus communis	Juniper berries
Ulmus rubra	Slippery elm bark

Valeriana officinalis	Valerian root
Cichorium intybus	Chicory
Matricaria chamomilla	Chamomile flowers
Symphytum officinale	Comfrey
Rosa canina	Rose Hips
Leonurus cardiaca	Mugwort
Verbascum thapsus	Mullein

ii. Livestock including poultry and bees.

The problem of preserving germ plasm resources for livestock has received more attention outside of the United States than within. FAO reports of 1967, 1969, 1971 and 1973 have considered many aspects of the problem and have included some recommendations for improvement. Actually, the problem is much greater in older countries where native local breeds have been developed and selected, often over centuries, to meet special needs or to fit particular environments. Many of these breeds might be used to increase efficiency of livestock production in the United States if ways could be found to import them without danger of introducing exotic diseases.

Livestock production is declining in the United States, in spite of considerable unused feed resources and in spite of the growing needs for food by an expanding world population. Grain production is leveling off and appears to be closer to its ceiling than meat production. Then, each year some grain is diverted from livestock to human consumption resulting in an reduction of both meat and total food production.

Wildlife is generally favored over domestic livestock in allocating range resources, in preserving germ plasm (endangered species), and in controlling wildlife pests that reduce meat and food production. Wildlife damage has been greatest on sheep and goats that offer the greatest hope of increasing food and animal fiber production from nongrain resources.

Exotic germ plasm

The importation of livestock germ plasm, free of exotic diseases, presents opportunities to obtain unique genetic characterictics not present in domestic breeds such as high fertility and milk production in sheep, high growth rate in goats, and high productivity from selection in rabbits and other species. Breeds with high production efficiency, but adapted to special environments in other parts of the world could be used in areas in the United States with similar environments. Gains from crossbreeding may be enhanced by using exotic, highly productive breeds, that differ genetically from domestic breeds. Changing conditions such as the shift from species depending largely on grain to those depending largely on nongrain feedstuffs increases the need for exotic germ plasm.

The preservation of exotic germ plasm outside of the United States is important because of its potential usefulness in the United States. Original importations of livestock came from Britain to a considerable extent and importation from other countries was quite limited. Importation of poultry breeds was more complete and many exotic breeds of cattle have been imported recently. Exotic germ plasm is more prevelant for sheep and goats and to a certain extent for swine than for the other domestic species. The special genetic and physiological traits of many exotic breeds that were not imported are still uncertain or unknown. Preservation of germ plasm within the country or origin until the special characteristics are more completely measured and described would be advantageous.

Need for livestock germ plasm preservation

The need to preserve germ plasm of livestock has been thoroughly presented by Jewell, 1971; Bowman, 1974; Mason, 1974, 1975; Lauvergne, 1975; Rendel, 1975; and Bereskin, 1976. A variety of agricultural, scientific and cultural justifications can be cited for preservation of livestock germ plasm but the principal ones involve the potential for increasing the efficiency of food production.

Preservation of rare breeds provides a reservoir of genetic variability, unique genetic and physiological traits, and unknown genetic factors that provide genetic flexibility in meeting new demands and forming new breeds. Rare native breeds are often highly adaptable to special environments and sometimes unfavorable environments where livestock production may not have been needed when food was in surplus. Rare breeds may be useful in crossbreeding to produce maximum hybrid vigor, to overcome lack of adaptability in highly productive breeds, or to meet changing product demands.

Scientific interests in preservation of germ plasm usually are related to agricultural potentials. Unique genes or physiological traits may provide opportunities to study relationships among traits that is not possible in highly developed breeds. There may also be opportunities to trace the history and domestication of livestock through rare breeds.

Cultural interests may be very similar for domestic livestock as for wildlife. Whenever a domesticated species or breed becomes extinct an irreplaceable element in the diversity of life is lost. Maintenance of domestic livestock to which man owes his survival may be more important than the preservation of endangered wild species. Rare breeds are valuable for cultural education and diversion. Country parks with herds of rare or curious livestock may be just as attractive as zoological parks. Mason, 1974 states--"The case for preserving the old breeds is more akin to that for preserving ancient buildings-they form part of our cultural and aesthetic heritage. We can only appreciate the present if we are aware of the achievements of the past and we can only be truly aware of past breeds if we can see their living representatives."

Preservation of germ plasm

Mason, 1974 has listed criteria to determine what breeds should be preserved such as indigenous breeds, local productive breeds, genetically unique breeds, bizarre or beautiful breeds and historically important breeds.

Means of preserving germ plasm are quite limited. Maintaining of pure breeding populations of sufficient size to avoid extreme inbreeding and accidental loss is most desirable. Storing of frozen semen, eggs or embryos is often advanced as a means of preservation but the practicality and permanency of such means are quite uncertain. Using the rare breed or species as a research animal both to completely measure genetic attributes and to develop more reliable methods of freezing and storing semen, eggs and embryos offers a temporary solution if such research can be justified and funded. Gene pools offer a somewhat unsatisfactory

means as the rare breeds cannot be maintained as such.

Research needed for more adequate preservation of livestock germ plasm involves cryopreservation of sperm, ova and embryos including research on media, freezing, handling, storage, disease control and artificial insemination and transplanting of embryos.

Studies to show relationship of blood types and other genetic factors to economically important traits of swine, sheep, goats and other animals are needed.

Research on breed differences with emphasis on traits for which little information exists such as chemical composition of fat, physiological traits, primordial germ cells and anatomical variations is also needed.

The most difficult aspect of germ plasm preservation is to obtain financial support. Farmers or private individuals cannot be expected to support such efforts as they generally benefit the public and are uneconomic or they would not have become rare in the first place. Public funds have sometimes been made available for endangered wild species but generally not for endangered domestic species.

The formation of trust to preserve rare breeds in the United Kingdom has been described by Bowman, 1974. This may be the most desirable solution to the problem particularly if a permanent endowment or some involvement of public agencies is included so that perpetuation is fairly assured.

Breeds and strains in danger of being lost in the United States are listed by species:

CATTLE

There does not seem to be concern about strains being lost. Still, research is needed to better evaluate strains and to preserve frozen germ plasm. Additional importation of germ plasm for either dairy or beef does not seem urgent.

SWINE

Some research efforts with swine in the past and at present have contributed information and technologies that are needed in the area of germ plasm preservation. However, none of these studies have been or are designed with germ plasm preservation as the principal objective. The principal reason for preserving germ plasm of swine is to assure survival of the most efficient breeds or strains that are economically important in providing an adequate supply of meat for the human population in the future. In addition, research with germ plasm from these breeds or strains is needed to: establish current production levels and measure progress in the future; accelerate reproductive rates and reduce the generation interval; and reduce production costs.

At present, there is no coordinated effort in the world to preserve germ plasm from breeds or strains of swine. However, there is a need for such a program to: make decisions on breeds or strains to be preserved; formulate plans and direction of current and future needs and how they would be approached; and identify a means of financing the program. The

present needs call for intensive research efforts in the appropriate disciplines of biology and technology. Current scientific thinking supports the field of cryobiology as the immediate hope to preserve germ plasm. Other fields of science related to the long-range plan for the preservation of germ plasm should include immunology, biochemistry, physiology, and cytogenetics. More specificially, research should be initiated on blood types and genetic polymorphisms, in order to identify and characterize genetic components of existing breeds and strains. Knowledge obtained from these studies is important in order to identify the most important breeds or strains that must be preserved for the future.

SHEEP

Three strains are in danger of being lost including the Karakul, Old Type Navajo and the Southern Native sheep. The Karakul is the only fat-tailed, fur breed of sheep in the United States. The Old Type Navajo is the only truly coarse wool type we have that is adapted to very rigorous conditions. This breed appears to have been lost already. The Southern native is unique in its adaptation to subtropical conditions and particularly for its tolerance to internal parasites. All three breeds are generally uneconomic. The Southern native might not be lost if the invasion of the coyote is halted. Information has just been received of a unique group of sheep on Hogg Island, Virginia, that may have been isolated for as long as 200 years.

POULTRY

The only currently successful method of maintaining germ plasm stocks for birds is by maintaining breeding colonies. For chickens, turkeys and a large number of other domesticated birds this is accomplished by numerous fanciers throughout the country. Some commercial breeders retain up to 100 different lines that they think might be useful to them in the future. Some universities maintain a few lines, usually not more than a half dozen or so, for their own interests. The Department of the Interior is involved in propagating some endangered species. There are no current germ plasm stocks nor are there plans for such stocks in any branch of the Federal government in the foreseeable future.

Poultry producers have a unique and a great need for the technology of artificial insemination. Freezing of semen is incidential to this. Artificial insemination is needed because turkeys and meat-type cheickens have been bred with such large breasts and so heavy that they are unable to consumate coitus. It is estimated that over 90% of commercial turkeys are artificially bred and the technique is currently being applied to broiler-breeders. Natural mating results in extremely low fertility in turkeys and the fertility is ever decreasing with broiler-breeders. Thus, techniques for short-term preservation of avian semen and for artificial insemination are greatly needed in the poultry industry.

OTHER ANIMALS

Important food animals such as the goat and rabbit should receive attention although information is lacking on needs. We assume the horse is adequately provided for by private industry but this may not be true of work horses as energy costs increases. Other farm animals such as domestic dogs, cats, other pets, fur animals and fish have not been included because of lack of information.

BEES

There are probably 20,000 species of bees worldwide. Most of these are classified as solitary bees which collectively contribute a great deal by pollinating many species of flowering plants throughout the world. They all require pollen for their survival, so in collecting their food, they pollinate flowers of wild and cultivated plants.

Only four species are correctly called honey bees. Two of these, Apis dorsata and Apis florea, are bees of South Asia which build open air nests with combs attached to overhangs in cliffs or buildings or sometimes branches of trees. They are not controlled for commercial use. The two other species of honey bee in nature build their nests in hollow trees or caves or dark cavities of some type. These are Apis indica, the Eastern Honey Bee, which is the honey bee of commerce of South Asia, and Apis mellifera, the Western Honey Bee, which evolved in Europe and has behavior adaptations to survive long winters. This bee was introduced to North and South America with the coming of European settlers. Before arrival of the white man, the Indians did obtain some honey from stingless bees of ghe genus Melipona and Trigona which inhabit Central and South America, as well as parts of Africa.

There are several races, or more correctly, subspecies of the Western honey bee that have evolved in different parts of Europe and North Africa. These include the Italians, Caucasians, Carniolans, Northern European and six or eight other types. There is also Apis mellifera adonsonii, a tropical African subspecies of the Western honey bee, which has developed a tropical African subspecies of the Western honey bee, which has developed an unsavory reputation in Brazil in recent years. There is variation in color, stinging behavior and other characteristics of these different strains.

The original settlers from Europe brought the dark colored bees of Northern Europe to this continent. In the late 1800's and early 1900's, many queens of the yellowish colored Italian race were imported. Importations were made mostly by importing mated Italian queens and so the bees, particularly the Southern United States, tended to become an American version of the Italian bee. An extensive queen-rearing business developed in the early 1900's in the Southern United States and queens are now shipped by the hundreds of thousands to commercial beekeepers in Northern States and in Canada. American queen breeders also ship queen bees in fairly substantial numbers to other parts of the world. They sell for $5.00 to $7.00 each.

Breeding Efforts. In 1922, a law was passed in the United States and simultaneously in Canada, which prohibited further importations of adult bees from other parts of the world, except Canada. The law was designed to prevent the introduction of a mite which was particularly harmful to

bees in Europe. Bee breeders of the United States have since been restricted to using material already in this country or Canada. Breeding for stock improvement has been difficult because in nature virgin queens fly into the air to mate and so there has been no control of the drone or male line. Queens mate with six or eight different drones in their initial mating flights. Sperm is stored in a spermatheca and they may lay fertile eggs for three years or more with no further mating. Artificial insemination was first achieved in 1926 and since the 1940's, it has become quite widely used, particularly for research purposes.

ARS researchers, particularly Roberts and Machenson at the Baton Rouge Bee Research Laboratory, developed techniques of inbreeding bees to highlight certain characteristics and then crossing certain inbred lines to achieve desirable hybrids. In the 1950's and 60's, an extensive project was carried out at the Bee Research Laboratory in Madison, Wisconsin, aimed at the development of bees of superior quality. Since that time, Dadant & Sons of Hamilton, Illinois, have developed three different hybrids which have been rather widely used. Southern queen breeders to whom they released their stock had to rely on open-air matings so the production of hybrids was not under full control. During the past year Dadants have set up a bee breeding operation in Florida and all queens sold from this operation are artificially inseminated.

The gene pool. There is wide genetic diversity within the honey bee population worldwide. The races of the Western honey bee mentioned above have distinguishable characteristics. Five or six years ago, a Stock Center was developed at Baton Rouge in conjunction with the Bee Breeding Laboratory. An effort has been made to accumulate representative stocks of bees, but stocks are by no means comprehensive. Inbred lines are maintained and queens are sold from these for breeding or research purposes. There are difficulties in maintaining inbred lines because egg viability decreases rapidly with inbreeding and the lines become quite perishable. Facilities have not been such that we can claim that the Stock Center maintains a wide range of identifiable genetic material.

There has been some success in maintaining and shipping semen and if we can find how to store semen for long periods of time, this will lead to progress in possible expansion of the gene pool. Importations have been very restricted since 1922, but some have been made, both as sperm and as immature stages of the bee. The law was strengthened in 1975 to prohibit importations of any stage of the bee, but it is possible to make exceptions for research purposes under safeguards.

~~B2c.~~ Economically important microorganisms

a. The pathogens in particular

The economic importance of the microorganisms which cause disease in human beings and in the plants and animals upon which humans are dependant needs no emphasis. The germplasm resources essential to the constant effort to control infectious diseases consist of large and comprehensive laboratory collections of pathogenic microorganisms. These organisms cannot be obtained at will from nature and they are required constantly for use in diagnosis of disease, for development of therapy and control measures, for selection or development of resistant host strains, for the study of epidemiology and for the study of fundamental pathogenic mechanisms.

The problems are world-wide and require organization at the national and international levels in order to ensure both adequate coverage and safety back-ups, while avoiding inefficient duplication of effort. The system must be flexible in order to cope with newly arising problems such as can result from changing social conditions, the migration of peoples, the introduction of new species, and the evolution of both hosts and pathogens.

It is essential that the major collections of pathogens be maintained in laboratories with stable support and continuity of expert supervision. The necessity to preserve the agents of past outbreaks of disease, including all significant variants encountered, leads to the steady growth of these collections. Great discretion must be exercised in making decisions to reduce the holdings of pathogens or to transfer their supervision from persons with direct experience in a given field to less knowledgeable individuals.

The World Health Organization (WHO) has assumed responsibility for the organization at the international level of germplasm resources essential for the control of infectious diseases of humans. WHO has designated certain laboratories around the world as International and Regional WHO Reference Centers and has charged them

with the responsibility for maintaining reference collections of specific groups of microorganisms for the entire world. (See Medical Research Programme of the WHO 1964-1968. Report by the Director-General 1969, WHO, Geneva.)

[Approximately one-fifth of these Centers are located within the United States, in government laboratories, universities and private institutions. The Centers have been to some extent supported by the institutions in which they are located and by grants from governmental and private agencies, as the funds available from the WHO are insufficient for their continued operation.]

WHO has also attempted to organize, and to some extent supports, efforts to collect, organize and manipulate data relating to infectious diseases of humans at the international level. The problems are formidable and are by no means solved.

Within the United States the situation is chaotic. The US does not have any public centralized national culture collections charged with the responsibility for maintaining comprehensive collections of pathogenic microorganisms to meet the needs of the nation for the control and study of disease in humans, plants and animals. [This is in marked contrast to the situation in some other developed countries. England, for example, has national collections of bacteria of medical and veterinary importance, of bacteria pathogenic for plants, of fungi and yeast pathogenic for man and animals, as well as other specialized national collections of economic importance.]

The essential collections of pathogenic microorganisms in the US are widely scattered. It is difficult to learn where they are and what their holdings are. They have been built up out of necessity in government laboratories of the US Public Health Service, the Department of the Army, the US Department of Agriculture, and other agencies; in university and other research laboratories; and in commercial laboratories. In these situations collections of great importance are subject to loss due to changes of supervisory personnel, shifts in responsibilities

within agencies, death or retirement of individuals or decrease of funding of research grants. Such losses can leave us in a vulnerable position in certain areas, and are tremendously wasteful. It also appears likely that there is tremendous waste and inefficiency due to overlap among collections.

The microorganisms in general.

The microorganisms of great economic importance include, among others:

a. the agents of infectious diseases of plants, animals and humans

b. the symbionts of the plants and animals which are of economic importance, which includes some of the nitrogen-fixing microorganisms

c. the agents of food processing, as in the dairy and brewing industries

d. organisms which may serve as direct food sources for man or domestic animals, including both conventional agriculture and aquaculture

e. organisms which may be used in the control of insects or other pests

f. the spoilage organisms which attack harvested foods

g. the destructive organisms which attack fabrics, fibers, forest products and other material

h. organisms used to render wastes harmless (as in sewage purification and the treatment of industrial plant effluents) or to convert wastes into useful products

i. organisms used for the production of chemicals (pharmaceutical products such as antibiotics and steroids, and industrial organic chemicals)

j. organisms used in monitoring the environment for the presence of harmful chemicals, such as mutagens

k. microorganisms used in applied and basic research

The major immediate germplasm resources for man's effort to control and utilize these microorganisms consist of collections of cultures of microorganisms maintained in the laboratory. These collections constitute a major resource of the society and represent a major social investment. Many of these cultures consist of strains of microorganisms which have been selected and developed for specific useful properties and they therefore correspond to the crop plants and domestic animals among the higher organisms. They are domestic organisms and are important to the economy. Our ability to control and utilize still other microorganisms depends upon our ability to isolate and manipulate them in the laboratory as well.

Theoretically, the existing culture collections of microorganisms, probably comprising a few hundred thousand specimens, could, if lost, be replaced from nature whereas the same may not be true of the higher organisms of economic importance. ~~The~~ loss of such microorganisms would lead to severe disruption ~~of some essential social functions, however,~~ and their replacement would require years of effort and a tremendous expenditure of (public) funds and resources.

A survey and evaluation of the existing major collections of cultures of microorganisms in the country would be a difficult task, but it is one that should certainly be performed. Previous efforts to compile lists of these resources have failed to achieve complete coverage and have not attempted to assess the value to society of the collections listed. It is impossible to describe the present situation accurately without more information than is available at present. Enough evidence exists to suggest the present situation is extremely wasteful and inefficient, with considerable duplication in some areas and perilously sparse coverage in others.

The one central, widely-recognized, general culture collection of microorganisms in the United States is the American Type Culture Collection (ATCC.) The ATCC is a private, non-profit organization supported largely by government agencies, by fees charged to recipients of stocks, and by fees charged to donors for the maintenance and/or distribution of stocks. The ATCC constitutes a major resource of type strains of a wide variety of microorganisms and is a repository for many strains of economic importance, as well. With its present holdings, it probably could not serve as the sole national resource for any group of microorganisms.

Some of the industrial collections may be among the largest and most stable. A few of these collections are listed as having holdings which numerically exceed those of the ATCC.

The other microbial collections upon which the nation is dependent are widely scattered in laboratories within government agencies, universities and elsewhere. These collections are difficult to locate, which leads to under-utilization and duplication of effort. Their availibility is often restricted for other reasons, as well. In these situations the continuity of collections is often dependent upon the efforts of dedicated individuals who possess the public interest and the expertise to assemble and maintain the collections, and upon the ability of these individuals to obtain financial support. This social responsibility is assumed on the initiative of the caretaker, often at some personal sacrifice. Most often the collections are not supported by funds designated specifically for that purpose. These collections are sometimes subject to dissolution upon the retirement or death of the caretaker, the loss of support for research projects, or the transfer of personnel within agencies. Valuable resources, representing considerable investment of effort, expertise and (often public) funds have been discarded before anyone who might have been able to make arrangements for their preservation was aware of what was happening.

Some of the major factors contributing to this chaotic situation would appear to be the following:

- The lack of organization at the national level and the consequent lack of assignment of clear responsibilities and of support for the long-term maintenance of essential resources. There is no government agency clearly responsible for resources in this area.
- A general mistrust of centralization resulting from the lack of continuity of support and of adequate supervision provided for major essential collections.
- The failure to develop a system of communication adequate for the numbers of laboratories, the types of activities and the masses of data that have evolved.
- An economic situation of some years' duration which provided research funds at a level such that extensive culture collections could be built up and maintained on research grants not specifically designated for that purpose.

The prospect of a more stringent economy and the possibility of a greater dependance on microorganisms in the future for both food and chemicals lends a sense of urgency to the need to assess our resources in this area and to provide for their utilization and preservation in a more efficient manner. The problem is exacerbated by trends in education which have lead to the training of narrow specialists in fashionable fields of research currently enjoying lavish support. This has lead to a greater dependence of research workers on culture collections, a shortage of persons with the expertise required to maintain collections of broad coverage, and of persons willing to assume such unglamorous tasks.

Efforts to introduce some organization at the national and international level have been made.

At the international level an effort has been made to compile a list of collections of cultures of microorganisms. A World Federation for Culture Collections (WFCC; formerly Section on Culture Collections) has been formed within the International Association of Microbiological Societies (IAMS) of the United Nations Educational, Scientific and Cultural Organization (UNESCO.) of the United Nations Organization. This organization sponsored a world-wide survey of culture collections of microorganisms with support from UNESCO, the World Health Organization (WHO) and the Commonwealth Scientific and Industrial Research Organization (CSIRO), Australia. A World Directory of Collections of Cultures of Microorganisms was published as a result of this survey (Martin and Skerman, 1972.) The 349 collections listed in the Directory are acknowledged as representing only a small fraction of the existing collections, but it is a beginning. The coverage for the United States is inadequate, some large important collections being omitted and some trivial ones being included.

Only 32 collections are listed for the United States. Of these, there are no national collections, unless the ATCC be regarded as such, 7 are located in federal government laboratories, 5 are located in universities and 13 are located in industrial laboratories. [Of the 32 collections listed for the United Kingdom, 13 are national collections (some apparently designated as such since the list was published), 10 are in universities, and 5 are in industrial laboratories.]

Marine Fish

Fishes remain amongst the most poorly known groups of vertebrates despite their potential for exploitation for food, and the important roles they play in aquatic ecosystems. New species are described every year at a rate which has not decreased, indicating that a large task lies ahead in completing the inventory of the fishes of the world. Many undescribed fishes are marine, but inadequately known continental areas such as the Amazon Basin may have just as many unknown species.

Fisheries experts have documented numerous cases of local extinctions of races or entire species of commercially important fishes, extinctions which can usually be traced to the activities of man. In most cases overfishing alone is not the cause, but a combination of changes in the physical or biological environment which leads to species decline. The complexity of the problem may be illustrated by the Atlantic salmon. One species is found throughout Europe and North America, but there are estimated to be at least 500 separate stocks, many of which are found in a single tributary of a river system. The strong homing instinct in this species ensures that adults return to their stream of origin, contributing to the formation of distinct breeding stocks. Certain stocks have already been lost (eg. in the Connecticut River), and difficulties have been encountered in restoring such rivers with stocks adapted to other rivers. Genetic research on the Atlantic salmon, and on other important fish species is of relatively recent origin. At the Atlantic Salmon Research Station (New Brunswick) a program has recently been established to examine the fine scale variation in breeding structure and the range of genetic variation in the Atlantic salmon. The life history and migratory patterns of stocks from different streams will be characterized. Four strains of salmon will be crossed in a diallelic crossing system to estimate the heritability of certain desirable characteristics. One aim is to develop a relatively opportunistic strain which can be used to

colonize a variety of rivers from which unique stocks have already been eliminated. Similar programs could be cited for other fish species where scientists are striving to recoup the loss of strains that were well-adapted to given habitats. In all cases it appears that the number of species or stocks that can be maintained in hatchery breeding programs represents only a tiny fraction of the total genetic variability available in nature, and that this variability can only be preserved effectively by preserving the natural habitats of as many species as possible. For certain species it may be found worthwhile to go to heroic lengths to preserve some portion of the germplasm in frozen banks of eggs or sperm, but this will require an intensification of the research effort into criteria for cryopreservation of non-mammalian gametes.

Marine Invertebrates.

The marine environment supplies not only fish, but a wide variety of commercially important species -- lobsters, shrimp, crabs, clams, scallops, oysters, quahogs -- the list is long, and undoubtedly could be extended by the exploitation of hitherto unexplored species. The United States lags behind most other highly developed countries of the world in its use of aquaculture techniques for the improved culture and management of these species, and the Committee on Aquaculture (NAS) is currently preparing a report which will list the reasons why this should be so. A case study of the failure of many aquaculture projects in the State of Florida (Mannheim, personal communication) indicates that the reasons are numerous, ranging from legal barriers, to the effects of vandalism and environmental perturbations, but most basic of all, due to a lack of fundamental knowledge on the biology of the species which have been the object of exploitation. The most successful aquaculture projects have involved oysters and salmon, species whose biology has been comparatively well-understood for more than 50 years. Nevertheless, culture of other marine species is being attempted, often without any fundamental understanding of

the extremely high levels of genetic variability which characterize these species (Section B1, ii) in their natural setting, nor any understanding of the consequences of inbreeding and selection under hatchery conditions.

The Genetics Society of America is currently preparing a national plan for maintaining and supporting genetic stocks and strains which have the potential of becoming important in biological and medical research. An examination of the Society's questionnaire for stock-keepers suggests that few of the stocks of marine species which have been developed and maintained by individual workers would begin to qualify for sustained long-term federal support (for example, from the Biological Research Resources Panel at NSF). There are so few workers in marine genetics that most species have been studied by one or a few investigators. Such stocks, no matter how faithfully maintained, do not receive the intensive use that would qualify them for federal support. Yet, given the extreme paucity of genetic information on marine species, it may be wise to develop a mechanism for ensuring some modest long-term support of genetic studies on marine species. Many of the food organisms used in aquaculture projects (for example, diatoms, Artemia salina - brine shrimp, and Brachionus plicatilis - rotifer) have short generation times, and would lend themselves well to genetic studies. Species of marine bivalves such as oysters have been maintained under hatchery conditions through many generations, but the genetic component in such studies has often been weak, or the stocks have had to be discarded for lack of sustained financial support.

Many highly active chemical compounds have been found to occur in marine species of plants and animals, and have served as model compounds in the development of new drugs. They include antibacterial, antiviral and tumor-inhibiting substances, some anticoagulants, and neurobiologically active materials. Many are uniquely found in a single marine species. A large number of these substances occur in tropical species, and this reflects the fact that highly diverse ecosystems are likely to provide the greatest potential

source of new model compounds. In addition to the fauna and flora of tropical coral reefs, the diverse fauna of the deep sea is an almost totally unexplored source of new pharmacologically active compounds.

2. Economically Important Organisms

v. Pests and Pathogens

Pests and pathogens that afflict humans or those species important to humankind as sources of food, fiber, medicines etc. are among the most prominent groups of economically important organisms. Extensive research and development has been devoted to the eradication or control of numerous pests and pathogens. The understanding of the role that these species play in the balance of nature is of utmost importance. It is clear that much of the effort expended for developing poisons and other control chemicals has been sometimes misdirected. Most poisons adversely affect many different members of natural communities including humans. It is becoming increasingly clear that the control of pests and pathogens must be through the thorough understanding of their biology. This of course includes their life cycles, physiology, reproduction, population dynamics and ecology.

The status of our knowledge about pests and pathogens is still embryonic despite the years of study and the volumes of research papers that have been produced. That even more effort be expended for the study of injurious species is a question that must be addressed by the nation.

B3 Specialised Collections.

i General rationale

ii Genetically defined stocks

iii Medical & assoc. uses

iv. Pollutant monitors

v. Cell lines

vi Others.

B. Status of Germplasm Resources

3. Specialized Collections

i. General Considerations

Because of the roles they have assumed in the welfare of humankind, a large number of different species of organisms have for centuries been collected and maintained as sources of food, clothing, transport, medicine, research materials, etc. Aside from the obvious convenience of providing a reliable source for obtaining the organisms when needed, such collections provide the opportunity to select particular varieties which are better adapted to human needs than those found in natural habitats. As a corollary to the selection process, many collections have been developed through the activities of researchers interested in understanding all aspects of living organisms.

The status of specialized collections varies widely depending on the type of organism and the use to which each collection is put. The size of a collection, the form in which it is maintained, its value in terms of the human endeavor that went into its development, its future usefulness and so on are some of the issues which must be considered for each organism and each type of collection. We must, however, recognize the value of collections in a general sense and the need to ensure their high quality and continuity.

Economically important species that have been under domestication for a long period of time represent valuable resources in terms of the effort that has gone into their

selection and propagation. That many useful strains which have been developed through selection exist only in specialized collections and that such strains are virtually irreplaceable if lost, are facts that command our attention.

Research collections of organisms used as models for exploring the fundamental properties of life also represent an invaluable and irreplaceable resource because of the mutant strains that have been accumulated and the knowledge which has been developed from their characterization. Because of this knowledge these strains can be further utilized in the investigation of yet unsolved problems whose solutions would greatly enhance human welfare.

For the most part collections have been the result of individual effort. However, in the case of many domesticated plants and animals the continuity and to some degree the diversity of types are maintained through widespread use. Useful organisms are passed along through very informal channels to future generations. Usually there is no conscious effort to maintain diversity and in fact there is a danger of the discarding of useful varieties as the popularity of some types temporarily declines.

The selection process itself, one of the great advantages of maintaining collections, can be the source of serious loss of germplasm. Selection for particular traits at the expense of others frequently leads to a narrowing of the genetic diversity in the species. The reduction in genotypes is particularly likely to occur as techniques for rearing an organism

become mechanized and culture conditions become more uniform through artificial control. The discarding of all but the strains best adapted to the conditions of domestication becomes a serious threat to the maintenance of the organism if environmental conditions change, changes in susceptibility to predators, parasites or pests develop or the need arises for selecting for different sets of characteristics. Limiting the available types in a collection can be particularly serious if human or other encroachment on natural habitats has depleted the species diversity or has eliminated it from natural communities completely.

With less widely used and more specialized types of collections particularly those developed by a researcher or fancier, the continuity is even less secure because the value of the collections depends so strongly on the knowledge and skill accumulated by one person. To recognize the true value of such a collection is at times difficult and even when value is perceived, finding someone who is capable and willing to carry on the work is unlikely.

Specialized collections, in addition to requiring specialized knowledge to use them advantageously, present a variety of problems in their maintenance and quality control. Some collections require elaborate conditions for culturing and/or preservation. Examples of this are many but it is possibly best illustrated by mentioning organisms that have formed symbiotic relationships where mutual dependence has developed to a degree that neither organism can be readily cultured in

the absence of the other. Leguminous plants and nodules of nitrogen fixing bacteria, insects and cellulose digesting protozoa are but a few cases. The cost in labor and equipment for maintaining cultures can be large and clearly it must be considered in relation to the benefits derived from maintaining collections. At the same time efforts must be considered for improving culture techniques and cutting costs.

We have reached a period in our history when our encroachment on the natural habitats of some species limit their numbers and their diversity and endanger their continuity. This fact forces us to evaluate carefully those collections already in existence and to consider what steps, if any, should be taken to preserve other species and varieties not yet represented in specialized collections. For the most part collections have been developed by individuals who have had rather specific objectives in mind. We must now consider maintaining a more comprehensive and orderly approach to the preservation of irreplaceable germplasms.

3. Specialized Collections

ii. Genetically defined stocks

Relatively few species have been extensively studied and genetically characterized. For the most part genetically well defined organisms comprise two general categories. First, economically important species that have been studied in efforts improve their usefulness or, in the case of pests, improve methods of their control and second, species serving as model systems for investigating questions about structure and function of living things. Some species such as maize and yeast serve in both groups. In both categories, but particularly in the latter, sizeable numbers of mutant strains have been created and characterized. These strains are collected and maintained in a variety of circumstances that range from specialized varieties of microorganisms or fungi used in industrial synthetic processes which are in some instances closely held and jealously guarded to widely distributed and freely exchanged strains used in genetic or medical research. Clearly the value of such highly specialized collections is the tremendous amount of human effort that has gone into the creation and characterization of such strains. That they are of great value for their role in research and as industrial tools and resources is in many cases beyond question.

Many of the important species are maintained through the efforts of the community of users. Research collections are indeed usually built through the efforts of individual scientists but as research results are published, it is traditional

that special strains of organisms used in that research become available to other workers who can use them. For some species considerable effort has gone into the establishment of stock centers containing a number of genetically studied strains. Such centers function mainly to ensure the maintenance and provide for the dispersal of useful strains among the research community. For the most part, however, there still remains a considerable burden of responsibility on the individual researcher for making his/her strains available and guaranteeing their fidelity.

It is important that the value of special collections as a national resource becomes widely recognized and that a national program for their maintenance be instituted.

Research collections represent a diversity of things, from a few specially constructed stocks or wild collections, useful mainly to an individual investigator, to a large systematic and well catalogued resource, valuable to a considerable community of scholars. The extremes pose no problems. The "personal" collections cannot ordinarily be maintained once the "person" disappears. The obviously useful public collections will surely be preserved by some means.

The problem is the continuum of values and the limited funds for preservation. Some "system" must be established for objectively evaluating collections and for assigning available resources to their continuation. Such a system is perhaps emerging from the Genetics Society's Committee on Genetic Stocks, and the NSF's funding capability. A system of funded "curators," such as that being considered by the Department of Agriculture, is attractive.

A second point to be noted is a distinction between "genetic stocks" and "type organisms." Most genetic investigators seem to think that genetic stocks need to be under continuous surveillance by genetic specialists, preferably adjunct to a research program. Type organisms for comparative studies, on the other hand, are less likely

to vary significantly and undetectedly in relatively unsophisticated surroundings. If an investigator needs only a reliably determined member of a certain species, he/she can probably be satisfied with an ATCC-like facility. Specialized "stock centers" for one or a few related species are more suitable for breeding stocks.

Identifiable formal structures concerned with Germplasm Resources in Eucaryotic Protists.

1. National Science Foundation--Research Resources Program. Provides funding for stock centers on a renewable grant basis.

2. Stock Centers: Current

   Algae: R. Starr, Curator, University of Texas, Austin.

   Fungi: Yeast, Fogel, Mortimer and Bassel, University of California, Berkeley.

   Neurospora, Barratt, Humbolt State U., Arcata, California.

   General, Amer. Type Culture Collection, Rockville, Maryland.

   Ciliates: Paramecium, Amer. Type Culture Collection, Rockville, Maryland.

3. Genetics Society of America, Committee on Genetic Stocks. Not involved in the management or funding of stock centers, but an influential coordinating agency.

The chief source of funds for stock centers is the National Science Foundation; the only comprehensive U.S. organization dedicated to the preservation and distribution of these protists is the ATCC. Specialized stock centers for active research groups--particularly genetic research groups that need large numbers of distinctive strains--perform essential functions for the research community.

These funded programs, however, represent only a small fraction of the effort of stock acquisition and distribution. Nearly every research laboratory, particularly every genetic or comparative laboratory, has some unique materials. In many cases, the only source of suitable strains for a particular study is one of these informal and unfunded adjuncts to a research program. The lack of a dependable reservoir and carelessness in inadequately managed working collections have caused much confusion, inefficiency and irreproducibility.

The problems of the basic researcher fall into two main categories. The first is the problem of maintaining in good order the many different mutant strains used in genetic analysis. Each mutant strain, or combination of mutants, costs time and effort. In each case the same mutant or combination could theoretically be recovered from an undifferentiated wild stock, but the probability of getting

precisely the same variant is small. The preservation of the strains saves subsequent students considerable work--or even makes possible more sophisticated studies which would otherwise be prohibited. I am aware of instances in which mutants with interesting properties were discarded because of the lack of an appropriate storage facility and in which the mutants could not be discovered anew with the time and facilities available.

The major issues to be addressed with respect to this particular problem are the cost-benefit ratio for the maintenance of strains and the mechanism for evaluating that ratio on a broad comparative basis. Perhaps the student of a particular organism would want all interesting strains of that organism preserved forever, so long as the costs of preservation did not prevent his/her continuing to do research. The advantage of the coupled research-laboratory-stock collection is that the cost-benefit question is constantly asked. So long as only one or a few investigators use a collection, the work of those investigators is its chief justification. At some point, however, the research group becomes sufficiently large and the work itself of such consequence that a common stock center is economically feasible and is a necessary assurance of reproducibility.

Research activities on particular organisms wax and wane. A few organisms graduate into permanent research technologies, but many aspire to that condition. The investigators of the organism are not themselves likely to be disinterested evaluators of the status of their pets. Hence, one needs a centralized organization to evaluate priorities and allocate resources for the benefit of the general community.

There is a need for living reference organisms, quite distinct from the need to maintain many slightly different strains of the same species for genetic analysis. These protists have an ancient evolutionary origin and are remarkably conservative in many essential forms and functions. Nevertheless, they have undergone genetic and molecular diversification over enormous spans of time by usual vertebrate standards. Organisms that look alike may in fact have almost no macromolecules in common. When conflicting claims arise about the behavior of some species, one must first suspect that the species are not the same. But one cannot test the suspicion without a living reference strain, and one cannot recover the original strain from nature with any certainty, because of its confusion with many other superficially similar species. Several efforts have been made to recover the ciliate called Euplotes patella--the

subject of important observations by Kimball and Powers in the early 1940's. But these attempts have failed, and our understanding of the molecular basis of cell union has been deferred.

The same kind of cost-benefit analysis is involved in the maintenance of a microbial zoo (garden?) as in a genetic research collection. Obviously all organisms collected from nature cannot be perpetuated in collections. But all organisms on which "significant" observations have been made should be retained for later workers if at all possible. Again one needs a disinterested judge of significance.

Specialized Collections of Microorganisms

Most of the collections of cultures of microorganisms in the country are specialized collections assembled with regard to public health services performed, agricultural and industrial needs, the conduct of scientific research programs or, very often, mixtures of research and immediate practical needs. The status of these collections has been discussed in very general terms above under economically important organisms( ).

In addition, there exist some extremely valuable, broadly

specialized but central collections which have been set up in recent years by individuals responding to national needs. The Algae Collection at the University of Texas at Austin, assembled by R. C. Starr and now supported by the National Science Foundation, is an important resource operating at a very effective level. The International Collection of Phytopathogenic Bacteria established at the University of California at Davis by M. P. Starr is another effort to make widely available a comprehensive collection in a broadly specialized field.

A system of such collections in the hands of competent curators could do much to reduce the inefficiency and redundancy of the present situation. Once the areas of coverage were firmly established and widely known and curatorial expertise and continuity were assured, many small specialized collections could be eliminated or consolidated, and unique, valuable stocks from small collections could be saved when endangered.

[The Algae Collection is currently arranging to acquire the more valuable stocks from the collection of L. Provasoli, of the Haskins Institute and Yale University, who is now retired. These include unique pure cultures of marine phytoflagellates and marine invertebrates which are being used in

efforts to establish mariculture in the United States (Provasoli, 1976). There is no other repository with the expertise to assume the responsibility for maintaining these organisms.]

The system of broadly specialized national culture collections for microorganisms of economic importance has been adopted in the United Kingdom and the Commonwealth nations. It would be worthwhile going to some effort to determine how well this system has worked out in these countries.

Breeding and Reference Stocks of the Ciliated Protozoa

The ciliated protozoa may provide a characteristic case history in germplasm management. They do not constitute a problem of sufficient magnitude for extensive treatment in a report of this sort, but they may represent a class of problems. Approximately 6,000 species have been described, but the more intensively studied species are found to be seriously underclassified (see Sonneborn, 1975; Nanney and McCoy, 1976), and little systematic attention has been given to many major groups. A conservative extrapolation would suggest that the number of isolated gene pools is at least ten times the number of named species, and more probably one hundred times.

The number of species that have been used extensively in biological research is, of course, much smaller. Genetic studies of any magnitude have been restricted to species of the genera Paramecium and Tetrahymena. Important experimental work has also been carried out on species of the genera Euplotes and Blepharisma, even though breeding analyses have been restricted in these groups. Two major collections of strains have provided the basis for many studies in genetics and comparative biology. The first of these is the collection of strains of the species of the Paramecium aurelia complex, assembled by T. M. Sonneborn at Indiana University. The second is composed of strains of the Tetrahymena pyriformis complex, put together by A. M. Elliott at the University of Michigan. Both Sonneborn and Elliott have now retired, and their extensive research collections have posed a problem in continuity.

The two groups of organisms are different in important ways and require different management. None of the organisms regularly used can encyst, and none recovers from desiccation. The P. aurelia species are generally inbreeding organisms that undergo autogamy periodically. They can be maintained easily in monoxenic culture (with Klebsiella aerogenes) and retain their capacity to mate and their other major biological

features when fed monthly or even more rarely. However, after long culture, the strains adapt to the growth conditions. This genetic plasticity causes problems when they are grown for long periods under conditions different from those employed in experimental work.

The T. pyriformis species, in contrast, never undergo autogamy and are much less capable of undergoing genetic adaptation in stock cultures. [Some "somatic" adaptation does occur, but this is lost at a subsequent conjugation when the somatic nuclei are destroyed.] The strains may be grown, like the P. aurelia species, in monoxenic cultures but are equally well retained in axenic medium (e.g., 1% proteose peptone). Unfortunately, most Tetrahymena strains after a few months or years lose the ability to survive conjugation. They retain their physiological properties and are valuable in comparative studies, but their genetic utility is lost (Nanney, 1974). Some success has been achieved in selecting strains which avoid genetic senescence, or at least delay it for several years, but this procedure can be used economically only for widely-used strains. For years, the only general way to maintain breeding stocks was to cross them at least once a year and to progeny-test their offspring. This expensive procedure greatly limits the numbers of strains which can be maintained for genetic studies.

define

The technological solution to the maintenance of breeding stocks of both Paramecium and Tetrahymena is emerging from the storage of strains in liquid nitrogen with dimethyl sulfoxide. The feasibility of such procedures was first established for Tetrahymena by Hwang et al. (1964) at the American Type Culture Collection. Later, Simon and Hwang (1967) showed that freezing and thawing did not impair the breeding performance of Tetrahymena. Moreover, strains stored several years in liquid nitrogen showed none of the signs of senescence manifested by control lines held under ordinary conditions (Simon, 1972). The procedures do not work well for all Tetrahymenas; those for preserving Paramecium in liquid nitrogen have been even more difficult to perfect, but marginal success has been reported (Simon, 1971; Simon and Schneller, 1973). Most cultures can be recovered, but the fraction of viable cells is often very small. Much more systematic effort needs to be directed to the cryogenic procedures necessary for the preservation of a wide range of ciliated protozoa.

Nevertheless, storage in liquid nitrogen, even in its present primitive state, has greatly opened up the genetic potentialities of ciliates as experimental organisms by allowing the accumulation of large numbers of mutants, wild

strains, and complex stocks and assuring the continuity of these collections. The chief hazard to such collections is human error in the maintenance of records, or the levels of liquid nitrogen in the tanks. These are substantial hazards, however, and should be met by the resources needed for fail-safe operations.

At the present time the American Type Culture Collection is specifically funded to house the Sonneborn Paramecium strains, and it also acts as a repository for type cultures of the Tetrahymena species. It does not attempt to provide continuity to special breeding strains or mutant collections.

Amphibian Genetic Stocks

In the United States large numbers of leopard frogs (R. pipiens) are used in teaching laboratories, and large numbers of bullfrogs (R. catesbeiana) are used as food. Virtually all of these animals have been collected from nature. So long as their habitats remain intact the populations of these frogs withstand both extensive collecting and occasional natural decimations. For example, under some circumstances (long Indian summer followed by freezing rains) the leopard frogs in Vermont may fail to migrate to Lake Champlain to hibernate, and may suffer large losses over the winter. The population recovers completely in about 3 years, (Nace, 1976). Also, some experiments have been performed in which populations of lakes were almost

completely removed, and again the populations recovered in about 3 years (Nace, 1976). Thus, amphibian populations show a remarkable capacity to recover from extensive losses so long as their habitats are preserved. This situation appears to have changed during the past several years, with losses and changes in habitat and perhaps from excessive collecting leading to reduction in the supply of the commonly used species. Several measures may be taken to combat this trend.

1. Habitat preservation
2. Establishment of laws governing collecting of amphibians, where such laws do not already exist
3. Preservation of migration routes--between foraging and hibernating areas. Highway underpasses
4. Commercial culture of commonly used species. It appears that no one has yet succeeded in the large scale commercial breeding of amphibians, though efforts are currently underway in this direction. (Personal communication) Nace indicates that Culley (LSU-bullfrogs), Griffen (London, Xenopus), Kawamura (Hiroshima, frogs), and Nace himself (Michigan, frogs) are working on the problem. Nace now has about 15000 frogs in his amphibian facility. He and his associates have developed new population cages, feeding devices, nonliving foods, and better methods of getting the animals through metamorphosis, all of which have led to improved efficiency and productivity.

However, Nace estimates that he is still 3-4 years away from a commercially feasible operation--i.e., an operation that could be scaled up to produce large numbers of animals at reasonable cost. Approximately the same situation seems to hold for R. catesbeiana as for R. pipiens (Culley).

It should be said that of all the anurans, Xenopus appears the easiest to culture, and it is still available from natural populations in large numbers, though import restrictions may affect availability in this country.

In many ways the simpler aspect of the overall problem of maintaining amphibian germplasm concerns genetic stocks. These stocks are important in several types of biological research. They are currently being maintained in a small number of stock centers and in research laboratories. The long-range objective will be to ensure that mutant stocks are preserved, since once lost they might be very difficult or impossible to produce again and would require additional expenditure of human effort. It seems obvious that at

least the more important mutant stocks should be maintained in more than one stock center. At present all stocks are being maintained through normal breeding methods and in some cases through artificial insemination. It seems reasonable to hope that this will be supplemented by the development of methods for preservation of germ cells or early embryos by freezing. Such methods should be successful for sperm cells, but as yet the methods for routine preservation of amphibian sperm by freezing appear not to have been worked out, and sperm freezing is not yet a part of the system of maintaining genetic stocks in any stock center or research laboratory. Amphibian eggs and early embryos are large and have a great deal of yolk and are impermeable to most molecules, both large and small. Thus, the use of glycerol or dimethylsulfoxide (DMSO) for the purpose of protecting eggs against freezing damage may be ineffectual. However, attempts should be made to develop methods for preserving eggs and early embryos, as well as sperm cells, by freezing. Under some circumstances genetic stocks may be propagated by gonad grafting and by nuclear transplantation, but these methods do not lend themselves to routine or large-scale propagation because they require a lot of time and expertise. Some tissue cultures have been established and are available for amphibian cell lines.

Breeding colonies of amphibians are currently being maintained at the following places:

*University of Michigan (Nace)--mainly frogs, but several other species have been maintained in smaller numbers. Some mutant genes, affecting pigmentation, are maintained.

*Hiroshima (Kawamura)--a large colony, mainly of Japanese anurans. Includes mutants affecting pigmentation.

**Louisana State University (Culley)--bullfrogs (R. catesbeiana).

*Geneva (Fischberg)--large colony of Xenopus. Includes several species and several mutants of X. laevis.

Cambridge (England) (Gurdon)--Xenopus. Some mutants.

Cornell, Ithaca (Blackler)--Xenopus. Some mutants.

**London (Griffen)--Xenopus. Griffen has set up a breeding colony which produces animals commercially.

*Indiana University (Brothers & Malacinski)--Axolotls. About 35 mutant genes maintained (Humphry ?).

*Ithaca College (DeLanney)--Axolotls. Some mutant stocks.

Ottawa (Armstrong)--Axolotls. Some mutant stocks.

Utrecht (Nieuwkoop)-- " " " "

Moscow (Lopashov)-- " " " "

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*Indicates major colonies which have been maintained for several years. The largest numbers of mutant stocks have been maintained at Indiana University (Axolotls), Geneva (Xenopus), and Paris (Pleurodeles). Other colonies maintain some mutant stocks.

**These colonies are concerned with development of procedures for producing amphibians on a commercial level as well as for research.

Tulane (Tompkins)--Axolotls. Some mutant stocks.

University of California, San Francisco Medical Branch (Lemanski)--Some mutant stocks.

University of Chicago (Manasek)--Some mutant stocks.

A.S.U. (Justus)--Some mutant stocks.

Toulouse, France (Beetschen)--Pleurodeles, Axolotls. Some mutant stocks.

Caen, France (Signoret)--Pleurodeles, Axolotls. Some mutant stocks.

*Paris, France (?)--Pleurodeles. This colony was built up by L. Gallien. Gallien died in September 1976. It is not yet clear what will happen with his colony, which contains some mutant stocks.

The list may be incomplete, and the information about each of the colonies listed is incomplete. Recommend survey.

The relatively small number of laboratory held stocks, coupled with the observed declining numbers of natural populations, merits serious attention in the light of the multitude of uses of amphibians in various aspects of biomedical research and teaching. The reliability of future supplies of amphibian species needs to be assessed. A short review of the biomedical uses is given by Wake et al. (1975). Research with amphibians has led to fundamental discoveries in molecular biology and genetics (gene reiteration, gene amplification, gene sequence organization), cell biology

*See footnote on preceding page.

(chromosome structure and function), developmental biology (embryonic induction, cell-cell interactions, gene regulation, gene content of differentiated cells), neurophysiology, and pharmacology. Amphibians, mostly from natural populations, have also been extensively used in biomedical training in such subjects as embryology, physiology, anatomy, cytology.

Amphibian colonies are maintained by natural breeding and, more rarely, by artificial insemination. Methods for routine preservation of sperm, eggs, or early embryos by freezing are not yet available. The most commonly used species of amphibians produce large numbers of eggs at each spawning and, in captivity, may be induced to spawn every 2 to 3 months. However, generation time is long, ranging from about 7 months to more than a year. For genetic studies it would obviously be desirable to search for amphibian species with generation times considerably shorter than this.

3. Specialized Collections.

iv. Pollutant monitors

In the natural environment the indicator species concept has often been used to provide the first hint that not all is well with the system. Such species are usually highly opportunistic organisms, with short generation times and wide dispersal capabilities, with the ability to take advantage of disturbance by increasing their population size very rapidly. The appearance of these blooms of pest species is usually an indication of gross changes in the ecosystem. Earlier effects of pollution are usually accompanied by the disappearance of the rare species in the ecosystem -- but most environmental surveys and laboratory tests of pollutants are not equipped to perceive or monitor these effects.

Guidelines for toxicity testing of pollutants (for example, Stephan, 1975) recommend the use of large or relatively robust species, the primary justification for their use being that they should be readily available and/or commercially important. Most of these species are in fact obtained from commercial suppliers, and only in some species of fish are hatchery stocks, whose genetic backgrounds are known, used in such tests. (for example, the fathead minnow) Some pollutant studies on marine invertebrates have used species which have been inbred over several generations, but in most cases little is known about the effects of inbreeding per se.

Marine pollution studies have increasingly recognized that the pelagic larval stages are likely to be highly sensitive stages in the life history of any species, and have used these larvae in studies of the effects of pollutants. However, the adult parents used to produce the larvae, are, in almost all cases, taken directly from wild populations, so that genetic differences in susceptibility to pollutants are not being taken into account.

One broad-scale study recently initiated by the Environmental Protection Agency will use the mussel, Mytilus edulis, as a monitor of pollutants. This species has a widespread distribution along the coasts of Europe and the United States, the postlarval stages are sessile and relatively long-lived, so that

populations provide evidence of trends in the environment, and individuals act as integrators of environmental effects .

Description of the Status of Germplasm Resources for Zoos, Etc.

Zoos and aquaria represent a substantial industry in this country and the world over. This country has 230 zoos and aquaria listed in the International Zoo Yearbook (Olney, IZY 16, 1976), an annual publication devoted to improving

zoo management. It records not only the location and staff of all zoos but also the species of animals bred in zoos and gives a census of rare animals in captivity. The designation of "rare" follows that of the "Red Data Books" (IUCN).

Zoos and aquaria hold a large number of species that have become extinct in nature (best known are Père David's deer, Arabian oryx, Przewalski's horse) or are severely threatened. While in the past zoos have replaced their losses by purchase from dealers, in the last decade very serious conservation programs have been started, and reproduction is foremost on the minds of most modern zookeepers. The listing in the IZY of rare animals provides information that enables animals to be exchanged for breeding purposes. ISIS (International Species Identification System) is a computerized program located at Minnesota State Zoo that attempts to gather similar figures to augment such information and make it more rapidly accessible. Over 18,000 animals are now recorded from 111 participants. Because of specific pressures on certain animals (34 mammals, 2 birds), studbook keepers have an international obligation to provide updated information on number and breeding of these 36 species (Appendix 1). This enhances elective exchange of specimens to avoid inbreeding depression, etc. Two international

Do we want an Appendix?

congresses have been held on "Breeding Endangered Species in Captivity" (Martin, 1975; IZY, 1977). These publications have addressed the major causes of extinction and the results of artificial breeding programs.

These activities and publications give evidence for the concern that exists in the zoological world over the rapidity of extinction of many species and the severely threatened status of many others. Numerous books and other publications have been issued that deal with the problems. There are a number of national/international societies and organizations that address this situation. In addition, many laws have been passed in attempts to stem the loss of many forms of wildlife.

The increasing demand for animals for biomedical research, especially primates, has led to the suggestion that breeding colonies be established. Progressive zoos are doing just that for all species in their care and reach. While it must be recognized that few zoos have the financial privilege to undertake this massive job and most have at least self-preservation in mind, their staffs are usually deeply concerned about and motivated by conservation. Aside from primates, biomedicine is gaining enormously from the spontaneous models that exist in zoos and aquaria. Witness

the comparative genetics of mammals, e.g., the lowest chromosome number (6) in a deer, of which only 5 exist in zoos at present, although not endangered, etc. (Benirschke, 1977). A plea has also been made to seek amongst zoo species future domestic stock (Short, 1976); candidates include eland and beisa oryx, special breeds of wild sheep and pigs with different food preferences from current domestic animals. Zoos and aquaria represent an important reservoir of animals and germplasm resource. Research in these institutions has been minimal compared to other institutions, but there are indications that this is changing (Research in Zoos, 1975). Funds have not been available to zoos for biomedical research because many institutions lacked qualified investigators and facilities, research was unacceptable because of the endangered nature of their specimens, public pressures and other factors. For these reasons, most of the research in the past has been behavioral, taxonomic, or confined to autopsy material. The situation is gradually changing, but a vast amount of material is not properly utilized by investigators. Appropriate research in zoos would make available resources otherwise available only in the field, at the cost of further endangering wildlife.

Cell strains from a wide variety of animals are currently used in biomedical research. A large number of nonhuman primates are killed in order to obtain kidney cell cultures for the propagation of viruses in vaccine production. Most of these animals are imported from the wild with little concern for their decrease. Recently, primate production centers are being established or contemplated in this country and abroad. In part, this is so because of the vanishing nature of this resource, stricter importation laws, and the existence of numerous viruses in imported animals.

There is evidence that primates dying in primate centers, zoos, and other installations are not being used to derive needed cell strains. This should be avoided. Most mammalian cells are readily preserved (with adequate protective agents) in liquid nitrogen. A large number of animals die in zoos and at importation points whose cells are sought after by geneticists, aging-process investigators, and other scientists. The one center that seeks to collect such cells for its own purposes is the San Diego Zoo. These cells should be widely disseminated, as they serve as the only reference point for genetic diversity. They can subservice the needs of many current scientists and, more importantly, for future generations when many animals (species or subspecies) have become

extinct, even before their genetic characterization. Very few cell strains of this irretrievable resource reside in the ATCC; more should be collected and systematic input must be encouraged by other zoos. They must be properly identified and genetically characterized. Only approximately 250 strains of various species are on hand in a single collection with the San Diego Zoological Society serving as perpetual "curator." In a few other laboratories, a few species' strains are collected without the obligation of continuance. Almost all of these cells are mammalian. Ten cell lines of 8 species of fish have been preserved for diagnostic purposes by the Eastern Fish Diseases Laboratory, Virginia, indicating that preservation of fish cells is feasible. No existing lines of bird and amphibian cells are known to the Committee.

The serious deficiency of this potential loss of genome has been recognized by many people. Some, lacking scientific expertise and affiliation with research or scientific organizations, freeze materials indiscriminately so that, with current techniques, they cannot be resuscitated. The deficiencies of amateur efforts point to the necessity that a scientist/curator must have the direction and supervision of such cell banks.

B 3iii - Experimental Mammals

Biomedical research depends heavily upon the availability of genetically controlled animal stocks usually, although not exclusively, experimental mammals for increasing understanding of normal and abnormal growth, development, differentiation, function, and aging deterioration. Analyses which require planned matings or tampering with the life history can only be based on experimental animals. Primates, mice, and rats are, and will continue to be, extensively used in paramedical research both because of their intrinsic suitability and because of the "added value" of accumulated knowledge resulting from previous experimentation with the same species, inbred strains, or mutants. For researchers to gain from the experience of past investigators, and from special stocks which they have created, it is necessary that a considerable variety of genetically controlled animal stocks be permanently maintained.

Primates: Both old-world and new-world primates have become important in medical research. Collection of primates from the wild, followed by importation into the United States, has up to now been the chief source of research primates. Very large numbers of rhesus monkeys have been used to provide kidney cell cultures for testing safely and effectiveness of polio vaccines. For comparison with humans, primates are especially important in studies involving the nervous system. Several different species, and variant populations within the squirrel monkeys, provide excellent material for the study of atherosclerosis. Recent advances in understanding of slow virus diseases such as kuru and multiple sclerosis depends on research using

monkeys as hosts. The reproductive physiology of primates corresponds more closely with that of humans than does that of any other animal group. Monkeys are slow to mature and have few offspring and establishment and maintenance of breeding colonies is necessarily slow and expensive, but nevertheless essential for future supply of primates for research.

Laboratory mice have become very important in biomedical research, largely because many and diverse inbred strains and mutants are currently available.

The genetically homogeneous mice in each inbred strain (result of more than 20 successive brother-sister matings) tend to be much more like each other than like mice from other strains, including having some histocompatibility genes, the same isozymic forms of enzymes, and a tendency for the same types of pathological lesions.

Inbred mice, plus special congenic stocks in which specific alleles characteristically found in one inbred strain have been transferred to a different genetic background, are very widely used in immunogenetic and other immunological research. All mammalian species which have been studied have one major histocompatibility locus, similar to the human HLA, and numerous minor histocompatibility loci. More is known about effects and genetic fine structure of the major H-2 complex locus of the mouse, which corresponds to human HLA, than about any other histocompatibility locus in any mammalian species. The complex has effects on immune responses as well as transplantation. Advances in immunology will be greatly helped by continued maintenance and availability of a number of special genetic stocks carrying recombinations within the H-2 locus.

Such recombinations happen very rarely, but each must be kept as a critical research tool. A great deal of research on T- and B-lymphocytes and their interactions uses special congenic mouse stocks.

A predictable proportion of the mice in any one inbred strain develop lung tumors, or mammary tumors, or lymphatic leukemia, or Hodgkin's-like lesions, or amyloidosis, or plasma cell tumors, and thus provide valuable tools for cancer or aging research. The incidences can often be elevated by carcinogenic agents, thus providing test animals for experimental carcinogenesis or for screening of chemicals. They are also valuable for screening of potential mutagens. The use of appropriately sensitive strains, and of a variety of strains, is important for obtaining valid evidence.

Deleterious mutants which occur in mice, whether spontaneous or induced, provide valuable tools for study of constitutional diseases which correspond to, or are at least similar to, human hereditary diseases. These mutants are most valuable if they occur in otherwise genetically homogeneous inbred mice, since the availability of a single gene difference segregating against a homogeneous genetic background greatly facilitates analysis of the action of the mutant gene. Mutants are known in mice which produce achondraplastic dwarfism, adrenocortical lipid depletion, cerebellar ataxia, autoimmune diseases, cartilage anomalies, two kinds of cataract, cerebral degeneration, Chediak-Higashi syndrome with abnormal lysosomes, copper-transport defects, diabetes mellitus and diabetes insipidus, dystonia musculorum, epileptiform seizure susceptibility, three kinds of homolytic anemia, congenital hydrocephalus, hypophosphatemia (vitamin-D resistant rickets), ichthyosis, immune deficiency diseases, iron-transport defects, four kinds of macrocytic

anemia, megacolon, microcytic anemia, microphthalmia, motor end-plate disease, muscular dystrophy, muscular dysgenesis, myelin synthesis deficiency, obesity, osteopetrosis, three kinds of pituitary dwarfism, prolinemia, retinal degeneration, siderocyte anemia, spina bifida, spherocytosis, and testicular feminization, to name only a partial list. The human counterparts of these mouse diseases are individually rare, but in the aggregate form an increasing proportion of human disease states. Since each mouse mutation also is a rare event, it is important that new mutations be recognized, preserved, characterized, and exploited for bearing on medical problems.

Laboratory rats have been widely used in research, especially in studies of growth, learning and behavior. Much of such research has been based on well-characterized non-inbred stocks, but recent studies frequently are based on inbred rats. It seems probable that genetically homogeneous inbred rats will become increasingly important as research tools. Old rats from different inbred strains develop specific pathologic lesions, including important tumor types. Two contrasting rat inbred strains have been developed with low vs high incidence of dental caries. Special rat stocks have been developed for study of hypertension, one which develops hypertension spontaneously, and one whose hypertension develops only with a salt-containing diet.

Important genetic susceptibilities have also been recognized in a variety of other mammalian species. Mutant rabbits provide chondrodystraphy, adrenal hyperplasia, hemolytic anemia associated with leukemia and scoliosis, among other diseases. Mutant alleles carried by both pigs and dogs cause von Willebrandt's disease. Other dogs have congenital

heart defects, similar to human anomalies, and still others develop rheumatoid arthritis or hemophilia. When armadillos are infected with leprosy they develop lesions corresponding more closely to human diseases than those found in any other experimental mammal.

Each of these diseases has been identified in an experimental animal colony, and documented by an alert investigator. Each can be reproduced and studied further by other investigators, provided that the original mutant stock still exists. The true usefulness of scientific reports describing all of these (and many other) constitutional diseases in animals depends on their future maintenance. Recognition and long-term maintenance of mutants in species not otherwise widely used in biomedical research is a problem which should be studied.

While primate centers concern themselves with the most "biomedically useful" species, large numbers of threatened species "fall between the cracks" (langurs, pigmy chimpanzees, lemurs, orangutans are a few examples). Zoos are establishing priorities, and breeding consortia are either being formed or in the planning stages. There is, however, no overall plan to see that maximum conservation occurs. Research in zoos is beginning very slowly and facing enormous tasks. It is virtually unfunded and in great need of resources and participation by the biomedical research community. New journals (Animal Regulation Studies) aim to correct the lack of awareness among scientists. Only the Fauna Protection Society (Oryx) and IUCN attempt to bring all available data together systematically, However, they possess few funds to change the decline of fauna appreciably. When broadly based biologists are available, which is rare, virtually no available funds exist to support them or their needed research. There has appeared a flurry of new laws and regulations that severely impede captive breeding. While it is recognized that the regulations are produced to be beneficial--and many have been most effective--they are at the same time so restrictive or the processing of forms so laborious or restrictive that interest is thwarted. Large zoos now require special staff dealing only with permits to effect simple breeding loans of

endangered species by loaning opposite sexes, etc., within this country. Without special permit (lengthy and difficult to get) it is not allowed to send a tissue culture of a stranded dead whale to Edinburgh, where the only DNA research of this type goes on, etc., etc. The zoological community is frequently confronted with new regulations. There is very little communication between government officials and those individuals who do the actual breeding or research into reproductive biologic phenomena of endangered species. So complex is the legislation that the Society of Mammalogists saw fit to publish a summary of these laws to its members but warns that the report not be used as primary source of information because of constant revisions and reviews (Genoway and Choate, J. Mammal., 1976; ILAR News, 1976; Appendix 2). Indeed, new proposed legislation on injurious wildlife (50 CFR Part 16) has appeared on March 7, 1977. An "Endangered Species Technical Bulletin" is now produced by the Department of the Interior, U.S. Fish and Wildlife Service, in order to keep the public abreast of the rapidly changing situation.

A special problem may be encountered in attempting to maintain collections of closely related species of plants in botanical gardens over several generations. This arises from the breakdown of normal ecological barriers to hybridization. Although first generation interspecific hybrids are usually obvious, intraspecific crosses may not be, and introgression can produce subtle variations that are not easily recognized.

The problem of labelling and record keeping for field grown plants can become serious. Support for botanical gardens is often inadequate to hire the help needed to keep the specimens labelled and the records properly filed.

In most countries, botanical gardens formerly were the institutions in charge of introduction and spread of all types of plants, including those of commercial or food-producing interest as well as those of floricultural or other horticultural value. These institutions, at least in the United States, are today more likely concerned only with a relatively few of those plants of commercial or food producing importance. However, to make this generalization is to oversimplify the functions of botanical gardens, both public and private. Many of the larger institutions are interested in the distribution and classification of wild plants, both at home and abroad. They form a network of institutions that are concerned with inventories of the world's plant resources. The botanical gardens and allied museums (herbaria) exist either independently, or more frequently, in conjunction with some large university where botany is taught as a major scientific discipline. Through

these institutions, we have the best opportunity to know what needs conservation, where the plants grow (geographic and environmental data), and where one may find may samples of the plant material, if these exist in some conservation program. Larger botanical gardens frequently maintain and propagate plants which they are willing to distribute to other institutions upon request. Lists of these are index semina.

There are other sources of plant material that frequently are not recognized as genetic resource conservation but still provide a broad range of plant material that would otherwise not be generally available. These are the commercial seed companies. Throughout the U.S., these companies provide many vegetables, fruits, flowering plants and curiosities raised for some particular interest, such as the sensitive plant (Mimosa pudica), the telegraph plant (Desmodium gyrans), the Venus fly trap (Dionaea muscipula), and many others. The seed companies are not interested in long-term conservation of genetic resources themselves, because their market demands new cultivars with differing qualities from time to time. They usually do not store viable seeds of those cultivars that are no longer popular. However, these institutions may maintain certain breeding stock which contains much of the

genetic constituency of a particular cultigen and thus does maintain some of the genetic diversity for those species kept. However, this does not address the overall problem of conservation of genetic resources where genetic drift is an important problem, nor do these institutions constitute organizations whose primary objective is conservation.

Another resource for maintenance of genetic resources in cultivated plants with interest in many differing types of plant material is the amateur and professional societies that sponsor various cultigens, such as the African Violet Society, the Iris Growers, etc., etc. By encouraging each of these societies to consider as a part of their mission the conservation of genetic resources in their cultigens, much could be accomplished towards the goal of overall genetic resource conservation.

Add to the above all the agencies for environmental protection, both private and government, and a very large part of the organizational requirements for germplasm conservation can be met at little more than the cost of a central office charged with assuring that the various endeavors for germplasm conservation are consistent in their efforts. Certainly there will be gaps in such conservation, no matter how many organizations are involved. This merely indicates

that one of the most significant requirements for assurance of conservation is a well-designed and managed data system.

C1 Problems & Solutions
Maintainance of Reproducing
colonies.

C. Problems and Solutions

1. Reproducing colonies

The problems surrounding the proper care and maintenance of reproducing colonies of organisms are comprised of three general types. The first of these is essentially a decision of whether any given colony is now or is potentially valuable to the point of expending effort and money to conserve it. Second, the technical problems of culturing and controlling the quality of the collection. The third category includes the administrative problems of providing support and continuity for the collection. Within each of these general areas there are a number of important problems that must be considered some of which depend largely on the organism being cultured.

There are some colonies that are easily identified as important enough to justify the effort to preserve them, whether these be natural stands of plant or animal species or laboratory cultures of important bacteria, viruses, fungi, insects, vertebrates and so on. There are some colonies, however, which are equally valuable but which for a variety of reasons are not recognized as such. There is a need for a central agency to act as a collector of information about these assets and as the administrator for intelligent evaluation of such data. One of the difficulties is that though a problem (such as a researcher retiring and leaving a valuable collection) may be recognized by a few knowledgeable people, there is now no easily identifiable mechanism of solving the problem. Often problems such as these are neglected and the collection is lost.

At best the research workers in the field have formed their own agency such as the Genetics Society of America Committee for the Maintenance of Genetic Stocks which is active in providing a conduit through which efforts for maintaining valuable genetics research collections can be channeled.

With regard to the technical problems involved in maintaining colonies, there should be avenues available for identifying competent and willing curators and exchanging information for improvement of techniques of culturing. The technical problems vary with each type of organism and deal mainly with culture conditions, states of preservation for storage, the control of pests or parasites, changes in strains through mutation, inbreeding, selection etc. Solutions for most of these problems depend on the competence and alertness of the curator and on the free exchange of information between curator and the users of a collection.

Administrative problems must include seeking long term financial support and providing a continuity for the colony through competent curatorial personnel. Agencies through which colony support can be sought do exist but there is need for a broader responsibility at a national level for ensuring these resources against decay or loss.

A program for the preservation of natural ecosystems and their individual components must include the following steps: (1) identification of the systems that must be preserved; (2) establishment of the areas under stable long-range ownership; and (3) stewardship or management of the preserves to maximize the maintenance of their biological diversity and the survival of all of their individual components.

Activities involving each of these steps have been proceeding for quite a few years at the federal, state, and private levels. At this point, however, the most urgent need is for a comprehensive national natural areas inventory (Jenkins 1972). This inventory should be stored in a computerized data bank, the format for which has already been worked out (Note: ask Dr. Robert Jenkins for a reference). This project will reveal the true dimensions of the problem. At the present time progress toward this goal is being made on a state by state basis, funded by state natural heritage programs. About eleven of the larger states are already being inventoried in this way.

The second step, the development of a nationwide system of ecological reserves, is outlined in recommendations already quoted (The Nature Conservancy 1975) and in a report entitled A Natural Heritage Trust (The Nature Conservancy, undated). This system should build upon existing natural areas already established through public and private action, and expanded through the addition of areas in public and private ownership. Areas recognized in the National Registry of Natural Landmarks administered by the National Park Service should be considered for inclusion in the system.

The management of these areas presents the most serious constellation of problems, because of the ecological complications involved, present ignorance of some of the parameters and pressures for other human uses. A long-range plan will have to be drawn up for each preserve that will implement the basic objective of maintaining biological diversity. In some cases this will require procedures such as prescribed burning to maintain a prairie, the restoration of an ecosystem by the introduction of an original component--e.g. buffalo, wolves, beaver--, or the removal or control of an aggressive exotic. The funding of trained personnel to carry out this stewardship function will be difficult. It will be essential that the program be established with assured continuity.

Data on the present status of governmental and private programs and related legislation is reviewed in the Preservation of Natural Diversity (The Nature Conservancy 1975).

Technical Problems and Solutions

The number of higher plants for which we have specific knowledge about the mechanisms and means to conserve them is very small. At most, only about 10,000 species of plants are actually husbanded by man, and of these only a very small percentage are actually raised in gardens, farms, woodlands, etc.; and of this small number, an even smaller number are actively conserved (or indeed have been tested to find out about conservation over long periods).

Various tactics have been employed for genetic resources conservation of plants. Unicellular organisms pose the least problems--lyophilization may be practiced, even on algae, and failing this, pure cultures in the test-tube are very frequently successful. Single-cell culture of multicellular organisms has been successful in a number of cases, where regrowth from meristematic tissues has been the source of cultures. But in a number of woody plants it has yet to be demonstrated that whole plants can be produced from tissue cultures. Perennial fruits are but one example of the need for investigation. Some methods of maintenance of crowded rooted cuttings have been proposed to serve conservation purposes in these plants--apples, pears, peaches, various citrus and nut trees--but these types of culture are still

space consuming and demand considerable attention. Large acreage set aside for the culture of single cultigens is usually unsatisfactory for economic, pathologic and management reasons, among others. Additional technically trained individuals are urgently required.

Facilities for conservation of considerable numbers of accessions of seed-bearing cultigens exist in the U.S. The different types of storage requirements for a variety of cultigens have not been thoroughly investigated in production size quantities, though many have been tested in pilot programs. Roberts (see 1975, for example) has been concerned for many years with means and methods of seed storage and has produced the most significant understanding of seed storage problems. Peter Thompson at Kew Gardens is probably the outstanding authority on long-term maintenance of seeds of wild species, but his experience does not cover a large segment of the plant kingdom. Knowledge of the conservation of ferns and fern allies is minimal, though a number of these are maintained in botanical garden collections. Members of the Cycadaceae are usually well represented in various botanical gardens. Pollen and seed of many gymnosperms are preserved in forestry establishments around the world. Bryophytes have not been identified as requiring conservation

except perhaps in certain Japanese gardens, and there may have been serious losses which have not been reported. The case for bryophyte collection depends upon definition of endimism, and in these circumstances conservation of natural areas would be the method selected.

Administration of conservation procedures is perhaps the most neglected area. There are so few networks of conservation efforts established that they have not had the opportunity to have the same impact as have other, older biological endeavors. An emergency exists, but nothing can be achieved until a responsive administrative framework is developed. No single nation can develop such a scheme in isolation from all others. The program of UNESCO, Man in the Biosphere, could certainly be an important program involving coordinating efforts to accomplish conservation. The United Nations Environmental Program needs to be unified, expanded, and strengthened. The International Biological Program studies were certainly important in public-consciousness raising, but these international endeavors only served as precursors to ongoing organizations that must focus on conservation. As yet no physical organizations exist to accomplish these aims. One may suggest that the Consultative Group for International Agricultural Research, through a newly-formed

structure within it, the International Board of Plant Genetic Resources, is set up for this purpose; but its long-term effectiveness seems to be in doubt, as it has no operating arm. For a limited number of crop species, the United States Department of Agriculture is the most organized functioning body across the country. The largest mission of this body is to conserve genetic resources of species that are not indigenous to the U.S. Therefore, it must rely upon the good will of other nations to supply the needed plant and animal materials for conservation. The conservation of genetic resources will certainly fail unless a central authority is established. This body must organize and co-ordinate individual ongoing activities--private, commercial, and governmental. It should have an obligation to all living organisms without regard for the usual barriers between differing groups--i.e., agricultural versus wild plant interests.

change

Genetic ~~Drift~~--initial sampling

There is no positive way to accumulate all variants that exist by collection, even in those species that are best known. Sampling can be statistically organized to ensure maximum possible representation of genetic resources.

However, because of the enormous number of ecological zones, there is little chance that all the germplasm for any one species can be collected. Also, it must be emphasized that genetic change is due not only to man's selection of some part of the genetic material of a species but to the inevitable processes of natural selection and chance isolation.

The processes of conservation are continuing ones, and there is no end point when we can safely say that conservation has been completed. A long time commitment is required, together with continuous monitoring of the genetic resources. Some efforts by plant breeders for such outbreeding crops as Zea mays include mixing collections of similar accessions, thereby preserving a mixed gene population. It is not at all sure that this tactic may provide a partial answer to the problems of genetic drift.

The reproduction of vanishing species has become a self-imposed duty to most major zoos and aquaria. There are formidable problems. First, only a fraction of species is available or feasible for reproduction. Thus, very little whale breeding is occurring, although the feasibility of it exists (Ridgway and Benirschke, 1977). Virtually no bats exist in zoos. Only some 10% of reptiles breed in captivity;

hummingbirds and penguins are notoriously difficult to breed, to name but a few select species. Individual breeding programs are not feasible for each of the numerous endangered or threatened species. The requirement for caging and care would be astronomical. Consequently, zoos and aquaria have accepted the challenge to propagate a selected number of severely threatened species that are also useful for exhibition. For many of these species, some basic information and techniques are required--for example, the ready ability to sex birds (currently undertaken by sex steroid analysis from feces--a promising method. IZY Volume 17, 1977). In other species (e.g., Przewalski's horse, Wisent) genetic information is needed to prevent inbreeding depression. Others (e.g., Siberian tiger) suffer diseases in captivity that need study and elimination. Many problems in reproductive physiology and communicable disease present technical barriers to the ready reproduction of endangered species in captivity.

From an administrative point of view, there are few barriers in communications, due to the efforts of the technical societies (e.g., A.A.Z.P.A), publications (Oryx, IZY), and specialty journals. There are various laws aimed at limiting extinction, and the IUCN attempts to coordinate such efforts. There is, however, no overall watchdog activity that monitors

those species that might fall "between the cracks." Occasionally interest groups take on severely threatened organisms, but generally only if motivated by special interests or pressures. For instance, although one female California condor (total population approximately 40) has been captive in the Los Angeles Zoo, no captive breeding program has been implemented. Many species are not spoken for and will face extinction.

Legislation and quarantine regulations abound and are summarized elsewhere. Most are intended to be helpful either to the protected species or to domestic species. Nevertheless, well intentioned captive breeding programs suffer from over-regulation, delays, and many poorly-thought-out restrictions. Better avenues are needed for communication between regulatory offices and the major centers for reproduction and research so that captive breeding can be maximized. An important problem that emerged recently is the destruction by the USDA of thousands of quarantined birds, including endangered species, after Newcastle disease was identified in a single specimen. Other ways of dealing with this problem should be explored. Similarly, new ways of assuring freedom from hoof-and-mouth disease and hog cholera should be developed.

There is virtually no broad attempt to identify the

genetic composition of captive wild and endangered species. No assurance can be given that captive breeding will be successful and not lead quickly to inbreeding depression. More research is needed to characterize breeding groups and assure meaningful exchange between gene pools. While these questions have been addressed in two publications (Martin, 1975; Research in Zoos and Aquariums, 1975), much more work is deemed necessary in this area. At present, no specific responsibilities are assigned to any zoos or other institutions to maintain any given species. Breeding consortia are being formed (e.g., gorilla) and the Trustees of the Arabian Oryx have assumed international responsibility for the maintenance of this species, which is believed to be extinct in the wild. While regulation is deemed unwise to promote such responsibilities, their encouragement is essential. Removing or modifying restrictive legislation is one aspect that would be helpful to motivated groups.

C2 Problems and Solutions:

Presentation in the
Dormant State

MATERIAL FOR SECTION C.2. OF THE REPORT OF THE COMMITTEE ON GERMPLASM RESOURCES

C. PROBLEMS AND SOLUTIONS

2. Preservation in the Dormant State

An alternative to the preservation of germplasm by the maintenance of reproducing colonies in the wild or in the laboratory is the preservation of germplasm in a dormant state. The effectiveness of such an approach depends on the extent to which dormancy can be induced reversibly in a variety of cells, and it depends on the economics and reliability of the preservation techniques, and on the institutional arrangements that are utilized. We will discuss each of these topics in turn.

The principal procedures for inducing long-term dormancy in biological systems are (1) lowering the temperature (freezing), (2) removing water (dehydration), or (3) doing both (freeze-drying). Other methods have been described from time to time, but they are mostly applicable to only a restricted number of organisms (e.g., preservation of fungi under mineral oil) and will not be discussed further.

Closely coupled with the problem of preserving germplasm in a dormant state is the problem of its resuscitation when needed. There are of course the scientific and technical problems associated with the thawing of frozen cells and with the rehydration of dehydrated or freeze-dried cells. But beyond those lie questions of the utility of the preserved materials. The use of preserved vivaparous embryos, for example, requires either the existence of suitable foster mothers or of techniques that permit complete embryological development in vitro. The use of preserved plant tissue cultures requires the existence of techniques for inducing the development of the full plant from somatic cells in culture. The use of preserved sperm of species requires the existence of the mature female of that species or the existence of preserved ova and the ability to carry out fertilization and embryogenesis in vitro.

To gain an appreciation for the potentials and limitations of the conservation of germplasm in the dormant state, it is first necessary to review briefly the scientific principles involved and the status of current knowledge.

I. Cryobiological Principles

A. Freezing. Preservation by freezing subjects cells to a number of sequential steps any one of which is potentially lethal. These steps are (1) the collection of the material and its transfer to media that generally must contain molar concentrations of nonphysiological protective solutes (e.g., glycerol or dimethyl sulfoxide), (2) freezing per se to temperatures below -130°C, (3) low temperature storage, (4) warming and thawing, and (5) removal of the protective solute and a return to normal physiological conditions.

Steps (2), (4), and (5) have the greatest potential for inducing injury. The cooling rate in step (2) is especially critical--cells must be cooled at controlled rates, the numerical values of which can differ by as much as a 1000-fold depending chiefly on the size of the cell and its permeability to water, and on the protective additive used. The mechanistic basis of the effects of cooling rate are becoming better understood: Too high a cooling rate results in death from the formation of ice crystals within cells and its growth during warming. Too low a cooling rate can cause death from the chemical consequences of the concentration of solutes during freezing or from osmotic forces operating during cooling and during subsequent warming ( ).

The effects of waming (step 4) depend in a rather complex way on the prior cooling rate. Injury during warming can result from growth of intracellular ice if cooling has been fast enough to induce it. Such potential injury is minimized by rapid thawing. Injury during warming can also result from osmotic forces originating from events that occurred during slow cooling. The osmotic forces seem to be minimized by slow warming. Osmotic forces similarly play a role in step (5), the removal of the protective solute.

Step (3), low temperature storage, is not a problem provided that the storage temperature is sufficiently low. "Sufficiently low" is at least below -130°C (the glass transition temperature of water) or preferably -196°C, the temperature

of liquid nitrogen. At -196°, there are sound reasons for expecting that most of a population of stored cells will remain viable and unchanged for centuries or more likely for a millenium. At -196°C (and probably at -130°C as well), no known, biologically relevant, thermally driven chemical reactions can occur. The only sources of damage are photophysical events--namely, ionizations produced by background radiations and high energy cosmic ray protons. It has been calculated that 200 years would have to elapse to accumulate sufficient radiation to kill 63% of one of the most sensitive known mammalian cells, the early mouse oocyte, and some 3,000-20,000 years would have to elapse to kill 60-90% of a population of "average" mammalian cells ( ). As far as the induction of mutations is concerned, since less than 1% of the spontaneous mutation rate at physiological temperatures is due to background radiation, at least in mouse germ cells (Russell, W. L., 1963), and since background radiation should be the only factor contributing to mutational alterations at -196°C, the mutation rate at -196°C should be considerably lower than the spontaneous rate, even though biological repair is precluded at -196°C ( ).

These theoretical considerations are consistent with experimental evidence. There is no confirmed case of biological decay at -196°C. Experiments in progress are subjecting mouse embryos to 100X background γ radiation at -196°C. As expected, no adverse effects have been detected after two years of irradiation ( ). At higher temperatures, above -80°C there are many well-documented cases of a rapid decline (days to months) in cell viability. Here too there are sound physical reasons for expecting such declines.

An important question regarding the use of low temperatures to preserve germplasm is the extent to which the several steps involved in freezing (which for brevity we will refer to simply as "freezing") either induce mutations or act to select preexisting variants in the population. Although the question has not been studied with sufficient care, all available information indicates that

the extent of mutagenesis or selection is nil. There is direct experimental information on microorganisms (Ashwood-Smith), and there is crude "epidemiological" evidence from the experience of repositories of frozen cell lines and from the large-scale use of frozen bull spermatozoa in the cattle industry. Also several experiments on the freezing of DNA itself have shown no discernible effects except some shearing of high molecular weight froms ( ).

Finally, there is the question of whether the processes in freezing cause nonheritable and nonlethal morphological and physiological changes, and whether the freezing of germ cells and early embryological stages could have teratogenic effects. The question has not been studied in depth, however two statements can be made. In most cases the cells or organisms that survive freezing are indistinguishable morphologically, physiologically, and biochemically from the normal population, and there are no reports of teratogenic effects from freezing of sperm, or early embryos. Either freezing kills them outright, or they survive and develop into normal offspring ( ). The second statement is that there are some well-documented cases, where freezing does induce nonlethal alterations. Frozen-thawed sperm, for example, may have retained motility but have lost their ability to fertilize (see below).

In summary, the chief problem in using freezing to preserve germplasm is not low temperature storage and it is not genetic or physiological defects in the survivors--it is to understand the events in freezing well enough to allow desired cells to survive cooling to -196°C and the later return to normal physiological temperatures.

B. Freeze-Drying and Dehydration. The process of freeze-drying (lyophilizing) consists of first freezing cells and then removing their frozen water at low temperatures by sublimation in vacuo. A major fraction of the cellular dehydration actually occurs during the initial freezing as liquid cell water is converted to ice. The chief difference between freezing and freeze-drying is the ultimate

extent of dehydration. Freezing effectively removes the ~ 90% of cell water that is "unbound" or freezable, but does not remove the residual bound water. Freeze-drying removes most of the latter as well ( ). So also do processes involving dehydration from the liquid state at above-zero temperatures.

This difference between freezing on the one hand and freeze-drying or dehydration from the liquid state on the other hand probably explains why the latter two processes are considerably more deleterious to living systems. Generally the only cells capable of withstanding freeze-drying are bacteria, and the spores and other dormant forms of fungi which are equipped to withstand dehydration in nature. The ability to survive freeze-drying is often critically dependent on the final water content. Drying to too low a water content is immediately lethal; drying to too high a water content causes rapid killing during storage of the dried product ( ).

An original rationale for freeze-drying was that long-term preservation of cells would not need refrigeration, but this is not so. Storage at 4°C to -18°C is required to prevent rather rapid declines in viability ( ).

There are well-documented instances in which freeze-drying and air-drying induce mutations and chromosomal aberrations. Examples have been reported for microorganisms, and dried plant seeds. Roberts (1973), has calculated that the mutations accumulating in dried barley seeds stored under conditions that have led to a 50% loss of viability are equivalent to those induced in fresh seeds by 10,000 rads of X-rays. There is also a potential danger of selection since in many cases only small percentages of cells in a population survive dehydration.

Although mutagenesis and selection are portential problems, various culture repositories routinely freeze-dry and store microorganisms without apparent problems of heritable differences.

It is conceivable that procedures could be developed for successfully freeze-drying a broader spectrum of cells, but in our view the outlook is much less

sanguine than for freezing itself. The fundamental causes of dehydration injury are scarcely understood and they represent difficult problems to attack experimentally. Pragmatically, the only advantage of freeze-drying over freezing is that for long-term storage the former does not require exceedingly low temperatures. While important, this advantage in our view does not compensate for the several disadvantages cited. The expense of long-term cryogenic storage of frozen cells is not prohibitive. Furthermore, it is certainly within our technological capacity to design adequate sensors, alarms, and backup systems to detect and rectify failures in the primary refrigeration systems.

II. Current Status of the Preservation of Germplasm in the Frozen State

Germplasm can be preserved in four ways: (1) By preserving the intact reproductively competent plant or animal, (2) by preserving somatic cells derived from the plant or animal, (3) by preserving individual germ cells or their direct progenitors, e.g., sperm, ova, pollen, spermatocytes, oocytes, and (4) by preserving fertilized embryos. Categories (2), (3), and (4) have in common the requirement that procedures must exist for reconverting the preserved cells into reproductively competent mature plants and animals.

A. Prokaryotes. Most, although not all, bacteria can be frozen with high enough survivals to permit the establishment of subcultures from the thawed progeny. Some blue-green algae survive freezing easily; others do not. The reason for the difference is not known ( ).

B. Plants:

1. Intact Plants. With the exception of some algae, most reproductively competent mature plants do not survive freezing to temperatures $\leq$ -130°C. This is true of fungi, mosses, ferns, and tracheophytes. There are exceptions to this statement. Some higher plants undergo a hardening process in nature which permits them to survive temperatures to $\leq$ -60°C ( ). Some vegetative fungi such as yeast can survive freezing under appropriate conditions ( ).

2. Somatic Vegetative Cells. The above statements on intact higher plants apply equally to the preservation of differentiated plant

tissues such as leaves, stems, and roots. In contrast, there have been recent reports of the successful freezing of plant tissue-culture cells using procedures quite similar to those developed for animal tissue cultures. To date cultures from seven species of plants have been frozen with variable success ( ). The potential for the preservation of germplasm lies in developing procedures for the generation of whole mature plants from relatively undifferentiated somatic cells. Full regeneration can now be achieved in carrot and tobacco ( ), and procedures for other species will soon follow. The problem may not be simple, however, for some forms are exceedingly sensitive to freezing ( ).

3. The Freezing of Plant Reproductive Bodies. Spores, cysts, pollen, and seeds which withstand air-drying in nature also usually withstand freezing provided that they remain aid-dried at the time of freezing. Full hydration or germination prior to freezing, may cause them to lose their resistance ( ). Some spores, pollen, and seeds have not been successfully frozen. This may be correlated with their possessing high water contents in nature and relatively low resistances to desiccation.

C. Animals:

1. Intact Animals with the exception of embryos. Cases of intact animals being frozen are restricted mostly to the protozoans, although there are intermittent instances of the successful freezing of members of metozoan phyla (e.g., rotifers, nematodes, insects) ( ). No vertebrate has ever survived freezing to more than a few degrees below 0°C. Even among the protozoans there are numerous forms which either do not survive freezing (e.g., Amoeba proteus) or in which only small percentages survive even under optimal conditions (e.g., Paramecium) ( ).

The outlook for the successful freezing of the larger more complex metazoans is bleak, partly because they are composed of cells of widely differing characteristics, and partly because increasing isize itself introduces formidable barriers to successful freezing.

2. Animal Somatic Cells.

A number of individual cell types can be frozen with high viabiability (e.g., erythrocytes, lymphocytes, skin, cornea, marrow, myocardial cells) ( ). Exceedingly few mature mammalian tissues and organs have ever retained function fter freezing to below -20°C. Fetal organs (heart and pancreas) have recently been frozen successfully ( ).

Among the more successful examples of freezing in animal cells have been tissue-culture cells. Most lines can be frozen so as to yield high enough survivals after thawing to ensure successful subculture ( ). There exist at least two major repositories of frozen cell lines in this country.

Unfortunately, unlike plants, the ability to preserve animal somatic cells is not a helpful approach to the preservation of animal germplasm. Except for a few instances in lower forms, it is not now possible to generate a complete animal from a collection of its somatic cells, and it is not at all clear when, if ever, it will be possible to do so.

3. Animal Germplasm. In the foreseeable future the preservation of animal germplasm by low temperature storage consist of the preservation of gametes and early zygotes.

(a) The preservation of spermatozoa. The successful preservation of bull spermatozoa ( ) is said by some to have initiated modern cryobiology.

Frozen sperm from highly selected bulls are used in the artificial insemination of a major percentage of cattle. The sperm of a wide variety of mammals has now been successfully preserved by freezing. Those species in which frozen sperm has 50% or more of the fertilizing capacity of normal sperm include: bull, boar, stallion, ram, goat, and probably man. Those in which more than 30% of the sperm remain motile after freezing but in which the fertilizing capacity has not been tested include: camel, moose, deer, bison, llama, yak, monkey, bear, chinchilla, dog, bighorn and other wild sheep (Graham).

Among birds, chicken and turkey sperm is viable after freezing, but both motility and fertilizing capacity are rather low (20-30%) (Graham). Among fish, high fertilizing capacity of frozen sperm has been obtained for several species of trout and salmon, and moderate fertilizing capacity for cod. Carp sperm is motile after freezing but has lost its capacity to fertilize (Horton and Ott, 1976). We have little information on invertebrates' sperm.

This evidence of rather widespread success suggests that there should be no fundamental obstacles to the freezing of sperm from species not yet studied. But this should not be construed to mean the task will be simple and routine. Over 20 years elapsed between the successful freezing of human

and bull sperm and the successful freezing of ram and boar sperm. Empirical techniques developed for the former did not work for the latter and many empirical modifications failed. Part of the problem was that high motility did not correlate well with fertilizing capacity. Part of the problem is that neither the cause of freezing injury nor the basis of protection by various solutes is well understood.

(b) The preservation of ova and early embryos.

(1) Ova and embryos from viviparous animals. The successful freezing of sperm in 1946-1949 initiated attempts to freeze mammalian ova.

Success was finally achieved for mouse embryos in 1972 (Whittingham et al., 1972) and was dependent on fundamental cryobiological and reproductive physiological information that had emerged in the prior few years. Since 1972, embryos of rabbit, cattle, sheep, goat, and rat have been frozen and have yielded normal viable offspring when thawed and transferred to foster mothers ( ). The Jackson Laboratory and others are now beginning to assess the techniques as an approach to the long-term preservation of potentially valuable mutant lines of mice, especially those not used on a day-to-day or month-to-month basis.

Whether early embryos from other mammals will now be frozen easily is not certain. Early stages of cattle embryos (1 to ~ 64 cells) still do not survive freezing, and pig embryos will not even survive chilling to 0°C, much less freezing ( ).

(2) Ova and embryos from oviparous and ovoviviparous animals.

To our knowledge no ovum or embryo from animals with large yolk bearing eggs has ever survived freezing, for at least two reasons:

only in the last few years has the successful freezing of cell aggregates been achieved approaching a cubic millimeter in volume. The permeability of cells to water and to solutes has a major influence on their fate during freezing, and permeability in turn is influenced greatly by cell size through its effect on the surface to volume ratio. Oviparous and ovoviviparous eggs tend to be very large. The second reason is that because oviparous eggs, especially, are exposed to nonhomeostatic conditions, they have evolved protective mechanisms, one of which is an outer layer of exceedingly low permeability to water and solutes. Exceedingly low permeabilities, especially when aggrevated by large size, present formidable obstacles to successful freezing.

III. Scientific Problems and Needed Research in the Low Temperature Preservation of Germplasm

The discovery nearly 30 years ago that bull sperm and human blood could survive freezing to ≤ -75°C if suspended in glycerol or DMSO and frozen at a suitable rate has been both a blessing and a curse to the field of cryobiology. It was a blessing in the sense that it dramatized the potentials of the field and in that the initial success was achieved with two cell types of considerable pragmatic importance. It has been a curse in the sense that it resulted in an emphasis on empirical approaches to the freezing of other cells, and it conveyed the impression that the field of cryobiology was art and technique and not science in the sense of experiments leading to testable concepts with predictive value. In some cases the techniques empirically developed for sperm have worked quite satisfactorily for other cells (e.g., many animal tissue-culture cells) and are in use today. In other cases, the original techniques did not work at all well for other cells, even for sperm of other mammalian species, and the derivation of successful procedures has involved a slow painful process of the empirical testing of various levels of the several variables involved in the process of freezing. In still other cases, both the original empirical techniques and subsequent empirical modifications have produced only failure. In a few of these cases (e.g., mammalian embryos) success finally has come about because a partial understanding of the fundamental aspects of low temperature biology emerged in parallel with empirical studies. But in other cases the level of understanding is still too primitive to permit success.

We will shortly be discussing some of the biological, agricultural, and medical potentials of the ability to preserve living cells for long periods of

time. To an important extent, the realization of the potential will depend on the extent to which the techniques will be applicable to wide varities of species and of cell types by investigators and organizations with wide differences in interests and in cryobiological knowledge.

We will attempt to highlight what seem the more important fundamental problems that need to be answered in order to expand the potential of low-temperature biological techniques for all species.

1. What are the physical-chemical causes of slow freezing injury -- e.g., concentration of electrolytes, osmotic forces, thermal effects?

2. Where do the critical lesions occur and what is their molecular nature? There is increasing evidence that membranes are the most sensitive target of injury; but the nature of the lesions remains unknown.

3. What is the molecular basis by which certain solutes protect against freezing injury?

4. What are the permeability characteristics and temperature dependence of permeability of the cells in question both to water and to solutes? Knowledge of the permeability characteristics has already proved a powerful asset in quantitatively predicting the optimum approaches to the freezing of several biological systems ( ).

5. Can the "scale-up" problem be resolved or ameliorated? As mentioned, the scale-up problem refers to the fact that water and solute permeation depend on the surface to volume ratios in cells or cell aggregates, and these ratios decrease and become increasingly troublesome with increasing cell size: The larger the cell the more slowly it must be cooled to avoid intracellular ice;

IV. Resuscitation

Germplasm that is preserved by dehydration or low temperature storage will, when needed, have to be converted into a form that can reproduce. This conversion, which we refer to as resuscitation, raises a number of intriguing and significant questions.

A. Resuscitation of Animal Germplasm

1. Ova and embryos. There should be little problem in resuscitating frozen embryos of oviparous and ovoviviparous animals provided that ways can ever be found to freeze them successfully.

The problem of the resuscitation of ova and embryos from viviparous animals (some of which can be frozen now) is far more complex. The more direct approach is transfer of embryos to the reproductive tract of foster mothers. Successful transfer requires that the foster mother be in a suitable stage of pseudo-pregnancy. This requires knowledge of the estrus or menstrual cycle of the foster mother. Such information is available for the most laboratory animals and those of major agricultural importance, it is much less available for wild animals in zoos.

Cases arise where a nation needs to obtain nonindigenous germplasm, a desire that may be thwarted by the restrictions in the exportation and importation of animals that are discussed elsewhere in this report. One route to importation of germplasm is to import frozen embryos. However the importation of embryos will be of no value if quarantine restrictions have resulted in there being no females of the species to serve as foster mothers. Similarly, low temperature preservation of embryos has been suggested as a method for preserving the germplasm of certain endangered species. But again the preservation will not be helpful if there are no females of that

species left to act as foster mothers. There are possibilities to solve the problems. Perhaps the least speculative is the possibility of rearing embryos of the desired species in the uterus of a closely related genus or species. But to do so first requires an understanding of the fascinating problem of the nature of the interspecies barrier to in vivo embryological development. More speculative, but still in the realm of possibility, is in vitro embryological and fetal development. In the last few years increasing progress is being made in achieving further and further in vitro development of implantation stages of early embryos ( ). At the same time, the age at which fetuses can be successfully carried to term outside the female reproductive tract is becoming earlier and earlier.

2. Resuscitation of other forms of animal germplasm. We have pointed out two other ways in which animal germplasm could be preserved in theory: (a) through preservation of the intact mature animal, and (b) through preservation of its somatic cells. In both cases the conversion of theory to practice remains highly speculative at best and perhaps even impossible. Although a few small primitive intact metazoans and a few insects can be frozen, the size and complexity of most metazoans precludes for the foreseeable future their surviving freezing and thawing. Many animal somatic cells, on the other hand, can be easily frozen. Here what remains highly speculative is whether ~~drugs~~ ways can or should be found to derive a whole animal from some of its component somatic cells.

B. Resuscitation of Plant Germplasm

In contrast to animals, the problem of deriving a whole plant from samples of its somatic cells seems well on the way to solution in an increasing number of species. Attempts to freeze plant tissue cultures are still too

recent to know with confidence whether the task will be formidable or simple. But, since it has been achieved in several plant systems, it seems reasonable to assume that the difficulties will not be insurmountable. If so, the combination of the preservation of tissue cultures at low temperatures plus the ability to generate whole plants from the thawed material should prove a powerful approach to the preservation of plant germplasm, especially plants which do not form seeds that themselves survive long periods of storage.

V. Potential Benefits from the Long-Term Preservation of Germplasm in the Dormant State

The potentials of the ability to store viable cells at -196°C are those that derive from the ability to prevent nearly all biological activity and change for periods of up to hundreds of years. We divide the discussion into (A) potentials based on knowledge available now, (B) potentials based on knowledge likely to be available in the near future, and (C) potentials based on speculation.

A. Potentials Based on Existing Knowledge and Existing Procedures

1. Genetics, taxonomy, and evolutionary biology. Induced or spontaneous mutations arise in the course of laboratory and agricultural experimentation. In many instances, only small percentages of these mutants can be maintained because of limitations of space, personnel, and money. The maintenance of variants in producing colonies often puts major demands on all three items. Even in cases where the heavy usage of a particular mutant or strain favors its being maintained in the form of a breeding colony, the strain or variant would be lost by disease or catastrophe, and it almost certainly will become slowly altered by genetic drift. Low temperature storage of germplasm would reduce or prevent all three. Equally important, it can provide a powerful research tool for studying genetic drift by providing a nearly immutable standard against which to assess the magnitude of the drift. The high likelihood that germplasm can be stored in nearly unchanged form for perhaps a millenium even provides future evolutionary biologists and taxonomists with a potential tool for assessing evolutionary changes. If present day techniques had existed long ago, today's taxonomists, instead of comparing a contemporary plant against written descriptions or dried

herbarium specimens of that same species, might literally regenerate a fully functioning plant unchanged from when its cells had been frozen, say, a thousand years ago.

2. Reproductive physiology, aging, and immunology. The ability to preserve germplasm or somatic cells opens approaches to separating time and animal age or time and generation -- especially in allogeneic animals. One can, for example, collect cells like lymphocytes from an animal when it is young and transplant them into that same animal when it is older. One thereby could obtain information about such phenomena as the weakening of the immunological systems in aging animals. Or one could collect two-cell embryos, separate the blastomeres, freeze one of them, and allow the other to develop in a foster mother; then, when it is a mature animal, allow it to serve as the foster mother for its identical twin, which had been preserved as a blastomere in liquid nitrogen.

3. Retrospective studies. New techniques, new instruments, and new questions about older observations arise constantly. Long-term preservation of cells permits one to pose the new questions or apply the new techniques to new samples of the very same population of cells that had been subjected to different questions or examined by old techniques.

4. Implications for humans. Human sperm can be preserved by present techniques of low-temperature storage. Males thus can, if they wish, undergo vasectomies and still retain the option to procreate. Whether the existence of such techniques has or should have any role to play in the control of human population is a question that properly falls in the province of social psychology and politics, but as to the current availability of the option, there can be little doubt.

At the opposite pole, the ability to freeze human ova may aid in providing a method for women with blocked fallopian tubes to bear children. The idea would be to collect ova from one cycle, store them in the frozen state until the next cycle, then thaw them, carry out in vitro fertilization with the husband's sperm, and transfer the fertilized embryo back into the woman's oviduct. Attempts have not yet been made to freeze human ova, but the probability is high that success will be achieved shortly.

There are also important applications for frozen human somatic cells. While some of these applications have a relationship to germplasm and genetics (e.g., karyotyping, enzyme function), more have applications in clinical therapy or perhaps in the assessment of environmental pollutants. We have not considered these in detail.

5. Agricultural implications. Changes in climate or in the costs or availability of energy, raw materials, and land may make it desirable to develop new breeds of livestock with altered characteristics. However, the development and testing of new breeds requires up to 15 years with standard techniques, and still more years are required to augment the size of a selected population. The problem is compounded by the fact that many countries (including the United States) have stringent prohibitions or quarantines against the introduction of foreign breeds. These importation restrictions are important because they severely restrict the available gene pool necessary to produce new breeds.

The time required for progeny testing and for expanding the size of the chosen population might be shortened and the restrictions on importation eased by the relatively recent development of procedures for inducing multiple ovulations (up to 40 ova per treatment) in livestock, for achieving births

from ova transferred into foster mothers, and for the nonsurgical collection and transfer of embryos. Furthermore, the utility of the procedure ought to be enhanced by the ability to store embryos in the frozen state. Freezing eliminates restrictions on the time required to transport embryos over large distances. It also is one approach to reducing the size of the herd of potential foster mothers that must be maintained to ensure a reasonable probability that the donor female and the foster mother are synchronized with respect to their estrous cycles. Furthermore, the combination of freezing and ova transfer provides one reliable approach to inducing multiple births in cattle. Finally, freezing permits the storage of strains for possible future use or for reintroducing a breed in cases where it has been modified by genetic drift or has been lost by disease or other catastrophe.

The degree to which quarantine restrictions on importation can be eased or lifted for frozen embryos depends on the answer to one unknown: Does vertical transmission of agriculturally significant viral diseases occur in embryos? The preliminary indications for a few viruses are encouraging (Zeilmaker,).

We have already mentioned the extensive use of frozen spermatozoa in the cattle industry. Presumably the ability to disseminate widely the spermatozoa from highly selected bulls has been efficacious in improving the overall genetic quality of cattle. Possibly similar benefits might accrue from the use of frozen sperm in other mammalian livestock, in poultry, and in fish culture. We reiterate that for many species the freezing techniques are available now.

B. Potentials in the Future

Extrapolating forward in time from present capabilities is always chancy, but it seems realistic to predict that in the next few years the potentials

of the preservation of germplasm will become available for a wider variety of species. In higher plants we expect procedures to be worked out for the freezing of tissue cultures from numerous species and for the regeneration of whole plants from the tissue cultures. In animals, it should become possible to freeze spermatozoa from most mammals and probably from many other vertebrates and invertebrates, and Ova and embryos from a wide variety of mammals. The limitations to progress in the latter may be the accumulation of knowledge about the reproductive physiology of the animals than the freezing itself.

C Potentials Based on Speculation.

Possibly, progress in reproductive physiology will permit the development of embryos of one species in the uterus of a foster mother of another genus or species. Were this possible, it would have major implications for the preservation of the germplasm of endangered species. A more formidable problem is the possibility of achieving the complete in vitro development from ovum to birth of mammalian embryos and fetuses.

It may become possible to freeze ova and embryos from oviparous and ovoviviparous animals. Such an achievement would permit the preservation of germplasm of nonmammalian vertebrates and invertebrates--some of which approach the mammals in their importance to research and agriculture.

We foresee the possibility of limited progress in the freezing of whole animals in certain invertebrate groups. One possibility is insects. And among insects considerable benefit would accrue to the ability to preserve fertilized eggs, embryos, or adults of the important genetic tool, Drosophila.

VI. Recommendations and Trans-Scientific Problems

The impact that low-temperature preservation will have on germplasm conservation depends on two factors. One is the options and potentials that research makes available. The second is the degree of utilization of the techniques that are available now as well as those that surely will become available in the future.

A. The Utilization of Available Techniques

There are three areas where freezing and low temperature storage are currently used widely One is in the preservation of microorganisms and tissue culture lines. The second area is in the clinical use of human red cells. The third area involves the use of frozen bull spermatozoa in artificial insemination of cattle.

There are other classes of cells for which freezing is scarcely used in spite of the fact that knowledge of how to freeze them successfully has existed for 10-25 years, and in spite of the fact that these frozen cells would appear to have considerable biological, agricultural, or medical use. Examples include human and mammalian lymphocytes and bone marrow, human sperm, skin, and cornea.

The difference between the two groups -- those widely and those scarcely used is striking, and understanding the difference may be important in maximizing the contributions of freezing to germplasm preservation.

The key questions are interest, funding, economics, organizations, available technology, experience and knowledge, reproducibility, and safety. We discuss the relevance of some of these to germplasm preservation.

We have made no attempt to assess the proper balance between centralized and decentralized facilities but recommend that it be assessed by appropriate groups. Included in this assessment should be the matter of the continuity of responsible management for the storage collection. Nor have we attempted to assess in detail whether centralized facilities currently have the proper balance of research and curatorial function. Quality research on preservation seems to be neglected by the organizations and underfunded by federal agencies.

3. Safety and Legal Problems. Safety and legal problems arise in connection with the importation of foreign germplasm for agricultural use, for the stocking of botanical and zoological gardens, and perhaps for research purposes. They also arise in connection with the collection, preservation, and use of human germplasm. With respect to importation, the most pressing problem is to determine whether viruses can be transmitted vertically through embryos. Questions of the medical safety of preserved human materials like spermatozoa were not assessed in any detail by the Committee. However, based on the information surveyed, health hazards are likely to be minimal.

B. The Support of Research

Although existing cryobiological techniques provide important approaches to the preservation of germplasm, further progress in research will be essential to realizing the full scope and power of the approach. We have outlined some of the more critical questions. Some of them pertain to cryobiology proper. Some of them fall in the domains of molecular biology, cell physiology, reproductive physiology, and developmental biology.

complex systems such as embryos came about because of collaboration between those knowledgeable in the fundamentals of reproductive physiology and those knowledgeable in the fundamentals of cryobiology. Progress in the freezing of other sensitive cells is likely to require analogous collaboration. But achieving effective collaboration is not simple. There are a mere handful of organizations worldwide that possess the necessary competence in-house. One limiting factor is that there are only a comparative handful of individuals knowledgeable in the fundamentals of cryobiology.

Recent progress in the freezing of complex biological systems has come about to an important extent as the result of increasing understanding of cryobiological fundamentals. It appears that the successful freezing of cell types that to date have been refractory to empirical cryobiological approaches will require a better understanding of the mechanistic basis of freezing injury and its prevention. One feature of the progress in research is that the fundamental principles have often been elucidated in cells other than those to which the fundamentals were eventually applied. Thus, it was knowledge of the response to freezing of yeast, erythrocytes, and tissue culture cells that provided much of the fundamental cryobiological information that led to the successful freezing of mammalian embryos and fetal organs.

Although progress in the field of germplasm preservation clearly requires a holistic approach with a stress on fundamentals, there are indications that neither the holistic approach or the stress on fundamentals is appreciated by the federal funding agencies. While the point needs further analysis, it seems that research support for cryobiology has tended to stress pragmatic approaches and has tended to emphasize those cells of immediate practical interest.

B Section D.

Information Management, Experimental Mammals:

Mice:

[1] Mouse News Letter, ~~is put out~~ mimeographed pamphlet, ~~publication~~, 50-75 pgs. per issue, Subscription 7.50/yr, appears twice per year. ~~Responsibility currently~~ ~~Contains reports from 36~~ Responsib Editor, Dr. Anthony G. Searle, MRC Radiobiology Unit, Harwell, England. ~~Currently~~ Contains lists of all mouse ~~mutants~~ genes, up-to-date linkage map, cytogenetic map, and research reports, ~~from~~ currently from 36 laboratories ~~in~~ all over the world.

~~Supplement To Mous~~

[2] Subject-Strain Bibliography Listing papers ~~list~~ based on ~~all publications of~~ genetically

(2)

controlled mice ~~th~~ which have appeared in the past 6 months. This is a supplement to MNL, and is edited by Joan Staats of the Jackson Laboratory.

[3] ~~Inbred Strains of Mice~~
"Inbred Strains of Mice," with up-to-date information on location, inbred generation numbers, characteristics and specific alleles carried, is presented ~~at please~~ as a Companion issue to every fourth issue of MNL. This listing is prepared by Joan Staats.

[4] "Standardized Nomenclature for Inbred Strains of Mice," with complete listing of all known ~~extant~~ extant inbred mouse strains, is published every four years in Cancer Research.

(3)

[5] Mouse Membrane Alloantigen News has recently been added with Dr. Peter Démant as editor.

[6] ILAR News, published four times per year by The Institute of Laboratory Animal Resources, NAS-NRC, contains ~~excellent~~ useful lists of ~~publications on~~ ~~[illegible]~~ biomedically important publications based on mice. (ILAR ~~also~~ performs invaluable service in establishing guidelines for ~~maintenance on~~ genetics, maintenance and care of mice).

Rats:

[1] A Rat News Letter has recently been established, with Michael A. Festing, ~~at~~ MRC Laboratory Animals Centre, Carshalton, England, as editor.

[2] ~~The Labora~~ Dr. Carl Hansen, ~~[illegible]~~ Division Research Services, National Institutes of Health, ~~puts out a~~ maintains ~~[illegible]~~ an invaluable handbook

characterizing many rat strains which he maintains.

Rabbits:

Richard R. Fox, of the Jackson Laboratory, has recently ~~prepared~~ presented in ILAR News (Spring, 1977) a ~~an~~ valuable article describing Jax Inbred and Mutant Bearing Rabbits.

Primates:

The Pan-American Health Organization ~~through~~ and ILAR have carried out invaluable studies recently on nonhuman primate populations in Bolivia, Guyana, Colombia, and Peru. (Nancy Muckenhirn has been very

(5)

effective in these endeavors.

[2] The Primate Research Centers ~~put out free~~ frequently put out informative publications covering many aspects of primate research and breeding. ~~These are~~ Titles and reviews appear in ILAR News.

[3] I think there is a Primate News Letter.

[4] Non human Primates: Usage and availability ~~of~~ for Biomedical Programs, ~~DHH~~ 1975, 121 pp., DHEW Pub No (NIH) 75-892, 1975.

Different Sheet:

~~Strong R~~

~~suppo~~

Agencies supporting maintenance of genetically controlled mice:

A. Stock Centers:

NSF - Mouse Mutant Stock Center, Jackson Laboratory. ~~[illegible]~~

NIH, DRS - Inbred strains and mutants for local use.

B. Support of specific strains and mutants as part of research grant support:

Many NIH categorical institutes:

NCI, NIA, NICHD, NIAMDD, NINDS

(Present system ~~unsafe~~ uncertain insecure, because it depends on continuation of specific research projects. ~~It is also to some extent wasteful.~~ Also, within the NIH ~~it is to~~ ~~s~~ intramural program, it is to ~~some~~ some extent duplicative; ~~Between and thos~~ a more efficient ~~sub~~ ~~[illegible]~~ cooperative system could be developed,

~~[illegible]~~

~~added stocks.~~ Combining efforts of

~~# diffe~~ different institutes, and of

overlapping

intramural and extramural programs,

could release moneys for ~~the supp~~ ~~additional~~

support of threatened genetic stocks.

still different sheet:

~~C1~~ ~~C3~~ Section C3:

Problems regarding maintenance of genetically controlled mice:

The situation regarding support of mouse colonies is certainly better than that for many species, but ~~more~~ improvements could be made ~~by~~ through greater coordination of efforts. ~~Many resear~~ Some one or two institution ~~should certainly undertake~~ become ~~could~~ ~~fr~~ centers should be established ~~for maint~~ which would undertake the broad responsibility of maintaining genetically important strains and mutants ~~in the fro~~ as frozen embryos. ~~and then~~ These institutions would accept qualified stocks from all interested investigators, ~~[illegible]~~ fee/

responsible for maintaining each stock permanently. ~~S~~ The ~~center should make~~ representatives of these stocks ~~should be~~ made available ~~should be~~ to qualified investigators by resascitation in pseudopregnant foster mothers. ~~It~~ ~~Fo~~ This arrangement has the potential for preserving valuable materials which are now discarded, while at the sonee time releasing space and time now required for regular maintenance in ~~the~~ laboratory colonies. ~~Work on Research on elimination of disease~~ Further research ~~on~~ in cryobiology is needed to make sure the system is fool proof, ~~It must also be demonstrate~~ One ~~Part~~ possible ~~potential~~ ~~problem~~ is transmission of disease

(3)

for ~~the~~ both the storing center and the receiving user is the possibility of disease transmission through the frozen material. Research in this area should be undertaken immediately.

~~E The syste~~
~~The~~ Biomedical research utilization of mouse mutants could be made more effective through the establishment of ~~science~~ consultation ~~advisory~~ groups, combining ~~mous~~ pertinent medical researchers with mouse geneticists, to survey presently existing and newly arising mutants~~ions~~, in order to ~~promote more~~ ~~promote~~ ~~get each patholo~~ get "get the the right mutant to the right investigator".

Data Management for Conservation of Genetic Resources

The conventional wisdom with respect to describing data on conservation of genetic resources, and describing the genetic resources themselves, is that somehow, some way, each individual will take care of the data which he has obtained and uses, and no particular effort need be made centrally to organize, direct, collate, store, and use these data. Such an attitude leads us today to the point where if there were data, we would not know where they are, what is their quality or quantity, what confidence we can have in them, and how they would fit into a larger scheme of knowledge about our organisms.

Today some of the most useful knowledge about genetic resources resides in the holdings of museums and herbaria and similar institutions that have collections of micro-organisms. It is not always easy to retrieve data or information from these institutions. There is usually only one way to find out about the stored resources, and that is through the names of the organisms. If one wishes to retrieve all the names of the plants for a certain region, for example, it would be very difficult to derive such a list from the museum or herbarium because of the single entry organization of the institution. However, these institutions possess the

best possible data banks. There is a movement to place data about holdings into computerized storage and retrieval systems (see, for example, the SELGEM system of the Smithsonian or the TAXIR system used at the USDA Regional Plant Introduction Station at Washington State University, Pullman, Washington). The major problem is understanding the need for data management systems, which is not fully appreciated by many biologists. It would be instructive to know how much time and money goes for data management in the light of the casual attitudes many scientists have toward their principal product, data.

Data management is, or should be, an integral function associated with conservation of genetic resources, because if we don't know what we have and what we are doing now, we cannot possibly know whether conservation is being accomplished or whether we are merely paying lip service to some general concept. We cannot prove, by recorded data, that any particular organism is, or is not, being lost. Aside from large plants or animals whose existence is usually within the range of civilized man's observations, we cannot say with certainty that this or that species of plant is indeed on the verge of extinction. We have witnessed several cases in which some organism was declared extinct, only to find that it showed up alive and well in some other unexpected part of the world.

There are several examples, but among plants perhaps the most famous one is that of the dawn redwood (Metasequoia glyptostroboides). Known as a fossil in the U.S. for a long time and, therefore, thought to be extinct, it was found during World War II in China. Shortly afterwards it was cultivated in many places around the world and is no longer considered an endangered species. We should guard against using these examples as an excuse not to be more scientific in activities such as data recording and storing. The institutions that deal best with information handling are those with the longest record of management--namely, zoos, botanical gardens, museums, herbaria, and live-culture collections. It is important that a critical and powerful organization concerned with data management be developed at the same time as the organisms are actually conserved.

There are a number of outstanding agencies, public and private, that maintain information storage and retrieval systems of the published literature of biology, such as the USDA, Library of Medicine, BIOSIS, and Chemical Abstracts. Many private organizations, which publish a number of different types of information sources, cannot accept the responsibility for management of raw data. Their financial arrangements require that they recover costs, and there is little assurance that there is a sufficiently steady market

for genetic resources raw data to make it profitable. Other institutions of great interest are the National Agricultural Library and the National Medical Library. Both of these organizations are publicly charged to keep up with and disseminate information about the published literature in their respective disciplines. It is likely that very isolated works in pure biology are not incorporated into their information storage and retrieval systems. These institutions would not be able to cope with unpublished data that should accumulate with conservation of genetic resources. Such data are merely descriptions of the actual plants or animals that are maintained or conserved.

The important data to be kept are those describing the what, where, who, when, and why of the conserved species, genotypes and regions. Data about the organizations and individuals carrying on conservation work are also important. Both of these types of data should be dynamic, continuously updated, maintaining new records and checking validity of old ones. There are critical needs for standards of description, as exist in chemistry and physics. The needs for designing and establishing networks for exchange of data are great and unfulfilled. Many institutions need both funding to update their system of recording and storing, retrieving and

transmitting data and materials, and encouragement that their efforts are appreciated and needed. Continuous research into means and methods of data management for all types of biological data is required. The need is not only for information storage and retrieval systems but for programs for determining diversity within species and in areas of growth, multivariate analysis such as clustering methods, computer-aided graphics for mapping and contouring, among others. A first-class training program for students and research workers is needed to ensure the understanding of proper procedures and the significance of accurate observation and recording. There are many new methods for gathering data, from remote sensing to electron microscopy to protein analyzers, but it is often difficult to utilize these in a comprehensive manner for the conservation of genetic resources.

The Role of Agencies, Publications, Etc.

A number of institutions have begun the process of automating their data storage and retrieval systems for systematic collections, and will continue this work under any circumstance. But at the moment, there is no overall coordination among different organizations. Many different

computer programs are being used, on a number of different types of computers. There have been arguments about whether one program or another serves the purpose better. The most important agreement needed is with respect to some minimal set of descriptors for the accessed materials. If the arguments could be directed to establishing the proper descriptor set, there would be more advances. Most sophisticated computer-aided programs require a set of data that has been structured to fit in certain ways and is adaptable to different machines and differently structured data. Standardization of the data structures on magnetic tape would go far to solve many problems.

Expert data management teams are required which would be guided by experienced biologists. This is perhaps the single most important recommendation that can be made: The management of the conservation process and also of the genetic resources data must be designed by biological scientists, using individuals with systems training and preferably some pertinent biological knowledge as consultants.

A monitoring group or standing committee with responsibility for conservation and for management of the data should be established. It will be necessary to maintain some continuity for a long time, because the process of conservation

will not be a short-term function. Various branches of government, in whatever departments the institutions are located, would still have responsibility for various parts of the overall activity--HEW, Interior, Agriculture would all continue their respective functions. To ensure that certain activities or groups of organisms do not get lost and that there is no duplication of effort, these governmental bodies should be in constant communication. These could share a common data bank of genetic resources conservation material and thereby go a long way towards preventing the problems of oversight or duplication.

The type of publication needed for conservation need not be any other than a report series for conservation methods, for groups of organisms conserved, and for information exchange among the individuals and organizations carrying out conservation functions. The governing organization, if one is established, should supply these functions. Types of reporting mechanisms should be examined and established when the objectives of such organizations are known. Provision for long-term maintenance of the data files must be made. Many previous governmental efforts have not included such provisions, and much valuable work has been lost as a result. Some of the most serious of these were the great World War II

efforts to collect quinine, rubber, and other commodities in tropical America. Other efforts with guayule (Parthenium argentatum) during World War II suffered a similar fate.

However one defines endangered species, their listing must be founded on an adequate data base. Thus the need for a world-wide inventory, of which a comprehensive national inventory for the United States would be a highly relevant part, becomes apparent.

In the past "implementation of the Endangered Species Act has unnecessarily conflicted with certain public works projects. Since endangered species occupy only a small fraction of the total landscape, most of this conflict could be avoided if precise information on habitat locations were available early in the planning process. Reconciling endangered species protection with continuing development is far more feasible than is currently recognized because "a careful inventory" can accurately pinpoint the location and extent of endangered species habitat" (The Nature Conservancy, undated report).

A line drawn between threatened and endangered becomes meaningful only when the biology of the organism in question is understood. It is essential to understand the life cycle, the population dynamics, the ecological relationships and habitat requirements of each species. The survival of migratory animals presents some very special problems, often with international dimensions. Adequate habitat is required at all critical points along the migratory pathway.

The status of a species can change swiftly as a result of human activities that may or may not be anticipated. Examples are the introduction or accidental escape of an exotic species that may have drastic effects on the native biota, the magnification effect of certain insecticides on

predators at the top of the food chain, and the impact of over-exploitation of individual species. It might be anticipated, for instance, that highly effective harvesting of antarctic Krill (Shapley 1977) might disrupt the entire ecology of the Antarctic ecosystem, with consequent endangerment of the less common components of the system that are dependent upon the food chain.