THE PURE-CULTURE CONCEPT AND GNOTOBIOTICS

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Introduction

The first conference on germfree life was held at the University of Notre Dame in 1939.[1] It is encouraging to note that earlier this year The New York Academy of Sciences held a conference on germfree invertebrates,[2] and that another conference was held in Sweden in connection with the Seventh International Congress for Microbiology.[3] Still other meetings are planned in the United States. All of this indicates that the study of germfree life has reached a certain maturity.

When the 1939 conference was held the only active center devoted to the study of germfree life was at the University of Notre Dame, and general scientific interest in the subject had become academic. Indeed, from 1895 to 1950, with the exception of the Lobund group, there had been but eight investigative attempts to use higher animals in such research. These studies were reported by Nuttall and Thierfelder,[4-6] Schottelius,[7-10] Cohendy and Wollman,[11, 12] Küster,[13-18] Glimstedt,[19-23] Balzam,[24] Gustafsson,[25, 26] and Miyakawa *et al.*[27-32]

Today the picture is changing rapidly. There are at present several active centers for research in this field in the United States, Sweden, and Japan.* Many more are being organized or are starting operation in this country and abroad. Interest has shifted from the academic to more applied aspects in which the animals and techniques are used to investigate problems of importance to experimental biology, chemistry, and physics. It is therefore opportune to hold this conference.

The field of gnotobiotics (*gnos*, known; *bios*, life) is new to many investigators. For this reason it may be useful to consider the subject broadly at the beginning of this monograph. The pitfalls of the past have by now been fairly well charted. Those entering the field today can purchase reliable equipment of several designs. The techniques for obtaining and rearing many germfree vertebrates have been reasonably well standardized, such animals can be shipped from production centers to the laboratory, and it is possible to rear several species through generations and in large numbers. While further development may be expected in all these areas, the position of the field is sufficiently sound so that those interested in using germfree animals can devote their efforts to the problem at hand without concern for the technical difficulties that necessarily occupied the attention of earlier investigators.

* In the United States: Lobund Institute; National Institutes of Health, Public Health Service, Bethesda, Md.; and the Walter Reed Army Medical Center, Washington, D. C. In Sweden, the University of Lund; in Japan, Nagoya University.

A publication such as this not only serves to introduce a field to the scientific world, but can also chart the way toward a systematic development of it. At a time when there is an increasing public demand for scientific research and a great expenditure of funds, there is great need of careful planning. The widening interest in controlled environments, which covers the entire field of biology, calls for carefully considered action, since large sums of money are required and large installations are necessary. The choice before us in the United States is between an intelligent, thoughtful approach to the future, or a somewhat frantic opportunism that may generate more heat than light.

Present interest in germfree life stems from the need to standardize laboratory animals and to control their environment. This is merely an indication of the needs of biology as investigation moves from the discovery of a phenomenon to its control.[39] This need has found expression in the pure-culture concept, which is best exemplified by the development of microbiology. This concept demands that a living organism be isolated from the natural complex in which it normally exists. It may then be allowed to multiply as a "pure" animal, that is, in association only with its own kind. As such it may be allowed to live in a "controlled" environment or brought into experimental associations with other life forms. In short, experimental control rests essentially on obtaining a pure animal, or a pure culture of animals, and being able to regulate the environment involved.

The term germfree, when applied to higher animals, means that the animal so specified is free from all other life and that it exists in an uncontaminated environment. The term contaminant refers to other living beings, although it can be extended to nonliving, unwanted elements.

Such a definition may be enlarged to include living organisms that are demonstrable by existing techniques; it also may be given theoretical limits for which it would be necessary to develop new techniques and new definitions. Under any circumstance, the science or art of detecting contamination is always the limiting factor and is at best a temporary situation. However, an inquiry into possible contaminations that might be overlooked with available techniques is useful in suggesting new methods which, in turn, have significance in general biology.

Accidental contamination due to a break in isolation barriers is a technical problem, but contamination of the animal per se means that there has been a break in the surgical technique, in the maternal barriers, or a transfer through the germ cells, and is of a different order. In this area it is possible that we may be eventually forced to concern ourselves with entities that are a part of the cellular economy or close to the genetic apparatus. At any rate, the problem of detection lies within the animal.

There can never be, of course, an unequivocal answer to the question of whether an animal is germfree. For this reason I have long maintained that it is possible to say only that an animal is germfree within the limitations of the tests employed to detect contamination. These tests may very well satisfy the demands of an experiment and may not require extension. If, on the other hand, interest centers on the theoretical limits, this constitutes an investigation of considerable importance in its own right.

At this time it is possible to say that higher animals may be obtained free from contamination by bacteria, yeasts, fungi, protozoa, ectoparasites and endoparasites, and most viruses that cause a recognized disease. It is not possible to say that such an animal is or is not free from inapparent viruses except on the basis of an examination directed to that end. If something is detected that can be regarded as a contaminant, the animal is not germfree, but it may still be very useful. If the contaminant interferes with an experiment the tests for it must be included in the spectrum of tests and the search continued until animals in which the contaminant does not exist are found. It would be absurd to make an unequivocal statement that all animals are contaminated with viruses, no matter how close to the cellular economy this brings us. The important thing to remember is that an animal is germfree within the limits of the tests applied to detect contamination. The theoretical question of whether there is an absolute value to the term germfree involves a search to which there may be no end, but is nevertheless important.

Whatever definition of germfree we may adopt, it is important to consider the design of isolation barriers that make this possible, since it is within these frontiers that all work must be accomplished. In other words, when an animal is placed within a germfree system it is sealed into that system with whatever contaminants it may possess. From this point on it is necessary to be able to eradicate environmental contaminants. If this can be done successfully, then it is further possible continually to narrow the problem with respect to contaminants locked in with the animal by moving it behind new barriers as one or another contaminant is eliminated. For example, in order to press the inquiry about placental barriers, cesarotomy can be performed on germfree animals until the contaminant is eliminated or continually passes. If the contaminant is part of the genetic apparatus, the same might be said by following breeding patterns to eliminate it. The point is that the proper isolation barriers must eliminate with certainty all external contamination and so permit closer examination of internal contaminants.

A reliable apparatus makes it possible to speak of the levels of contamination in terms of the contaminants and to designate an animal as being of the first, second, third, or fourth order; the first refers to parasites, the second to microbes, the third to disease-causing viruses, and the fourth to latent or inapparent viruses.

History

It is difficult to determine the origin of the concept of germfree life, since the concepts of pure air, pure water, and pure food have been present ever since it was recognized that microbes might be involved in the health of man and animals. By the same token, the pure culture concept is buried deep in the historical development of the realization that entities must be isolated from the natural complex in which they normally exist if the demands of the experimental approach are to be satisfied.

The first direct experimentation on germfree life was done in 1885 by Duclaux,[40] who tried to raise peas and beans as pure cultures in a sterile medium.

It is interesting to speculate that in all probability Duclaux's work would have remained unknown had not Pasteur focused attention on it and on the entire problem by his remarks in his introduction to Duclaux's work.[41] Pasteur stated that he thought microbes were necessary to the life of an animal, and that life in the absence of microbes would be impossible. At any rate, it was this statement that was challenged by Nencki[42] on the grounds that microorganisms produced toxic substances, and it was in support of Nencki's veto that Nuttall and Thierfelder attempted to furnish experimental proof with respect to higher animals (guinea pigs).[4-6] This challenge was taken up by Schottelius, who tried to show that microorganisms are needed.[7-10] Again, as in the case of Pasteur and Nencki on theoretical grounds, Metchnikoff supported the thesis that microorganisms are not needed and, indeed, are harmful.[43] Cohendy[11, 44] and Cohendy and Wollman[12] supported Metchnikoff in their experiments with chickens and guinea pigs. By 1916 it was evident that chickens could live germfree much beyond the time limits set by Schottelius.

It was with Cohendy[11, 44] that the original motive for determining whether life without microorganisms would be possible began to evolve into the new concept that the germfree animal might become an experimental tool. While Cohendy did not express his observation, he was certainly not unaware of the possibility, and his observations that germfree chickens were susceptible to infection was brought out in his work with Wollman,[12] using guinea pigs infected with *Vibrio cholerae*.

Küster, working with two goats that he kept germfree for fourteen days, finally turned the tide against the theory of symbiotic helpers and opened up a wider scope to germfree experimentation.[13-18] He emphasized the importance of morphological, physiological, biochemical, and immunological examination of the germfree state and, although he did very little experimentally along these lines, his speculation is interesting.

In the period following World War I the work of Glimstedt[19-22] and Glimstedt *et al.*,[23] done at the Institute of Histology of the University of Lund, Lund, Sweden, should be especially noted. The germfree guinea pigs that he used were intended primarily as tools for investigating the lymph system. His work was followed by that of Balzam,[24] in which germfree chickens were used to investigate nutritional problems. Gustafsson, working also in the Institute of Histology at Lund, reported rearing germfree rats.[25, 26] In Japan the work of Kyoichi[45] and Miyakawa and his associates should be noted.[27-38] Because much of this work with the guinea pig is expounded in Japanese, it is not as well known as it should be.

The first long-range systematic program in the field of germfree life as it concerns higher animals was started at the University of Notre Dame in 1928 and since then has continued uninterrupted.[46] The original program was organized to investigate these five problems: (1) instrumentation, (2) methodology, (3) description of germfree life, (4) exploration of the use of the techniques and animals in problems of importance to experimental biology and medicine, and (5) the establishment of a center for germfree life studies. In realizing these objectives, the Lobund program has served well and stands as

an example of the need for a continued systematic approach to studies of germfree life.

Present and Future Problems

The problem of terminology. As any field of study expands, the need for special terminology arises. The term germfree, when applied to a higher animal, has come to mean something more than is implied by the etymology of the words, and this special meaning now has the sanction of usage. However, it is not adequate to cover the broader area in which the germfree animal is but one level of ecological existence. Substitution of such terms as axenic,[17] bacteria-free,[13] pure,[41] or aseptic,[24] does not suffice.

In an attempt to state the problem, in 1949 my associates and I considered the need for a special terminology based on the pure-culture concept that underlies the conditions in which germfree life is involved.[18] One of these conditions is that an animal may be contaminated with a pure culture of microorganisms. Since the pure-culture concept supposes a knowledge of the kinds of life present in an isolated situation, we suggested the term gnotobiotics to indicate the field of investigation concerned with growing living things by themselves or in association with other known kinds of microorganisms. Using this root, the animal becomes a "gnotobiote," which can mean either that it is free from microorganisms or is in association with other known forms of life. This leaves the problem of a designation for the individuals of a pure culture that would suggest freedom from other life as "germfree" or "axenic" (*xenos*, stranger; *a*, freedom) or, more specifically, by some abbreviation or symbolism. Obviously, the basic term gnotobiotics may well suffice to describe a field of investigation, but it is not sufficient to describe the subjects of experimentation within it.

Whatever terminology is adopted, it should include the poorly defined "disease-free" or "clean" animals that originate from a germfree animal, but are conventionally contaminated except for those specific microorganisms considered undesirable. By the same token, a more satisfactory term than the conventional one is needed to describe the external environment or the normally reared animal. Finally, a proper term is needed to describe those animals in which the microbial flora is reduced or altered by antibiotics, by dietary changes, or simply by housing a conventional animal in an isolated environment.

Trends and needs in apparatus development. The prime requirement of germfree apparatus (isolators) is dependability; a second consideration is adaptability to routines. It follows that the system should be versatile with respect to experimental demands and sufficiently strong to withstand routine operations. When these demands are satisfied, the problem of cost and convenience may be considered.

Ultimately, isolation apparatus must be absolute with respect to maintaining sterility; there can be no compromise with this demand. Such rigid standards are necessarily restrictive and therefore will be reflected in the design, construction, and operation of such apparatus. Probably the closest to the

ultimate design would be a completely sealed chamber, similar to a balanced aquarium, in which an ecological equilibrium could be maintained. With higher animals, however, it is necessary for practical reasons to supply food, water, and air, and to provide devices for manipulating the animals and caring for them. This means also that there must be methods for introducing animals to the isolator, of changing them from one isolator to another as they grow and multiply, and of sterilizing supplies introduced into the isolator. In short, the ultimate apparatus is a goal for design, and incorporated into it must be such things as ultimate air filters or air-sterilizing systems and flexible handling devices.

Also needed, however, is apparatus that will be less expensive initially and that can be used for short-term experimentation with reasonable certainty and at an acceptable level of control. Included here are "benchtype" designs such as hoods, jars, and envelopes that may be sterilized separately in an autoclave or by some chemical means. Such apparatus is useful to those with transient needs, especially if germfree animals can be reared in a central installation and shipped to the point of use.

In between these two extremes is apparatus simplified to the necessary degree for routine operation, but sufficiently dependable to permit long range studies and in which small colonies of germfree animals can be maintained throughout their lives. For routine operations it is important that such apparatus be standardized in construction and design.

Important in this connection is the recent development of an apparatus in which germfree animals may be transported in airplanes, railroads, or motor vehicles.[39, 49] This creates a new dimension for the use of germfree animals, since it permits the installation of one or two isolators at widely separated sites.

It seems almost certain that there will be a demand for large apparatus in which colonies of small animals may be maintained or in which larger animals such as goats, dogs, pigs, and cows can be reared. There have been several starts in this direction, but much remains to be done.[50, 51] The need for such apparatus is further emphasized in experiments in which large numbers of small animals must be used and held in the same environment. This is not easily accomplished if the animals are divided into small groups, each in its own separate isolator and environment.

One of the major problems is the further development of devices such as gloves for handling germfree animals. Inadequately made gloves once accounted for the majority of accidental contaminations, but gloves have been so improved that now they may be used successfully for as long as two years' continuous operation. Nevertheless, gloves still constitute a hazard, and further improvements are needed to strengthen them or to substitute materials other than rubber in their construction.

The dependability of steam versus chemicals for sterilization constitutes an area of investigation that can be solved effectively only by actual use over a sufficiently long period of time in operating germfree equipment. There is, however, no particular reason why the same apparatus should not be used with

both steam and chemical sterilization; this is especially true, if the apparatus is constructed of stainless steel.

The cost of germfree apparatus is properly of concern to the investigator who wishes to enter the field. However, as more apparatus is produced there will be a continuing reduction in costs. At any rate, this problem must be considered in its proper perspective. Dependability and adaptability to routine operation are of primary importance. The real problem is not so much the initial cost of apparatus as the cost in time and money put into an experiment that becomes contaminated because of faulty apparatus. The present cost of dependable stainless steel equipment is about that of an autoclave and, like an autoclave, it can be expected to give many years of service. Thus the initial costs are spread over a long term. Furthermore, in the very near future it will be possible to rent or lease isolators for those with transient interest in germfree life, just as it is possible to rent hospital beds when needed.

Introduction of new species. Thus far at Lobund Institute we have reared the following species of higher animals germfree: the monkey,[52, 53] the rat,[54] the rabbit, the hamster, the mouse, the chicken,[55, 56] the turkey, and the guinea pig. Only the rat,[54] the mouse, and the chicken[57] have been bred through successive generations: the C3H mouse for 8 generations, the Swiss mouse for 11, the rat for 12, and the chicken for 2.

On the basis of information presently available, it is reasonable to believe that other species will be obtained and added to those already reared germfree. One of the neglected areas is that of working with large animals such as dogs, cats, monkeys, goats, sheep, pigs, and cows. The techniques are available and apparatus in which this can be accomplished has been produced. Another area in need of attention is that of working with cold-blooded vertebrates such as fish,[47] amphibia, and reptiles.

Hand-rearing cesarean-born mammals on sterilized diets. In the past, emphasis has been placed on obtaining weaned mammals. This was a matter of expediency. With the establishment of breeding colonies of germfree mice and rats and the weaning of rabbits, guinea pigs, and other such animals, the emphasis should change to one of studying the suckling mammal.

There is an entire area about which little is known concerning the nutritional requirements of the suckling animal, its physiology, transfer of immunity, placental protection, and biochemistry. The most direct approach to the problem is by hand feeding. Complicated as the problem seems, there is need for a systematically planned study involving different species properly selected to provide a spectrum to bear on the problem.

Thus far most attempts at rearing totally dependent suckling mammals by hand have been aimed at simulating the feeding and nursing care of the mother. In view of the difficulties inherent in this approach, especially as regards small mammals such as mice, it seems reasonable to consider nonimitative methods. Since diets must be sterilized and, by and large, approximate the composition of the mother's milk, it might be well to search in another direction. For example, instead of a highly fluid diet it might be possible to use a semisolid diet or even one containing very small pellets. The swallowing process is

present in the young mammal, and devices for feeding such nutriment are available. This approach permits the use of a more highly concentrated diet with less liability of choking the young. The proportioning of ingredients required to achieve sterilization by autoclaving without clotting—a difficult thing to accomplish with concentrated liquid diets—would be eliminated.

Starting with cesarean-born young of one species, it is common practice among those interested in "clean" animals to foster-suckle the young on a lactating mother of another species. Thus, mice have been suckled on rats, rats on hamsters, and hamsters on rats and mice. The same thing can be done with germfree animals, provided the foster mother is acceptable. Foster suckling is, at best, an expedient and not a substitute for hand-rearing, and will not yield the same information.

In my laboratory partially successful attempts have been made to fix a thin-walled, small-bore tube into the stomach of the mouse for continuous and timed feeding—the so-called "pumpkin" technique. In this way automatic devices can be used to supply continuous and known amounts of liquid diet to the animal. In the same category is the self-feeding device or "artificial mother" that has been only partially successful with some species and even less so with others. These advanced techniques need more study, but they are basically workable and sound.

With larger animals such as monkeys, rabbits, dogs and, to some extent, guinea pigs, hand-feeding is less of a problem, but the physiological and nutritional factors assume greater importance. Too little is known to establish hand-feeding techniques on more than an empirical basis at the present time.

The problem of hand-feeding cesarean-born mammals should constitute an investigation in its own right, rather than be simply a means of obtaining germfree animals. Each detail of technique needs systematic study so that standards can be set up, and it is possible to discount the variables that have been inherent in past attempts.

The need for a study and description of germfree animals from birth through old age to death. The germfree animal is relatively unknown with respect to its development, anatomy, physiology, activation of defensive systems, or the reflection of the inactivation of these systems in the general economy of the animal: that is, its physiology and biochemistry and the effects of aging. The study of the germfree animal within the parameters so formed through generations and in individuals through old age to death should constitute a major effort. It is especially necessary to do this if for no other reason than that of establishing a base line relative to its use in experimental research. For example, it is especially important to know that the teeth of germfree animals develop within recognizable limits before these animals can be used in dental caries studies,[58] or to know that in radiation studies we must work with an animal whose intestinal tract is different from that of a conventional animal.[59] Moreover, a study of the germfree animal can well be considered an exploration in which new areas of research are uncovered. Finally, if the germfree animal is to find its widest use experimentally, it must be brought to certain dependable standards, and this objective cannot be achieved until a great deal

more is known about the germfree animal and the state in which it lives. It is not too much to suggest that it will be necessary to study the germfree animal species by species, variety by variety, and generation by generation, lest the literature be strewn with mistakes when the technique is used experimentally and thus become discredited. The history of germfree-life studies over the past seventy years is mute testimony to the need for systematic long-range planning. During this time interest has ebbed and flowed between active efforts, and at times it has receded to a position where the animals were considered merely as laboratory curiosities.

The tendency still exists to compare the germfree directly with the conventional animal. It is natural to do this unless we consider the fact that the germfree animal has never had experience with microbial contamination, while from the moment of birth the conventional animal is host to a wide variety of contaminants over which very little experimental control can be exercised. It is true that eventually the conventional is really the state that must be investigated, and in many ways this furnishes a reason for studying germfree life. However, the conventional state can never be attained simply by contaminating an animal indiscriminately. The animal is not a test tube but a living thing that reacts to each assault upon it, and each assault leaves a mark on the complex whole of its life. Unless the tenets of the pure-culture concept are adhered to and the natural complex built up systematically in proper order, the entire value of the germfree animal can be nullified.

The problem of detecting contamination. The value of a technique lies in the number of questions it poses. In the early period of germfree life studies, interest was centered around the question of whether animals could live without bacteria. While the question was only partially answered, it furnished a stimulus for an experimental approach that has resulted in an extension of the pure-culture concept. The question of whether a germfree animal is free from viruses may very well serve the same purpose today. This is an especially opportune time to raise this question, for it can well serve to tighten up techniques beyond the practicable and toward a theoretical limit.

As matters now stand, there can be little question, that animals may be obtained free from bacteria, yeasts, fungi, protozoa, ectoparastites, and endoparasites; available techniques are adequate to demonstrate these forms of life. Moreover, the animal itself is an excellent culture medium, furnishing a variety of conditions for those forms that can be seen microscopically or as recognized disease processes. Germfree animals reared in colonies can be studied from birth, through old age to death, and through generations, so that if a contaminant manifests itself, it will not be obscured by questions of age, numbers, or generations of animals.

It is time to consider the extension of the tests presently used to detect contamination. Emphasis should be laid on the fact that new tests should be applicable to the living animal. Finding a contaminant in an animal that must be sacrificed before it can be examined will not answer the question for all animals in the group unless the contaminant can be transmitted by means other than through the genetic apparatus. As previously pointed out, once a

contaminant is demonstrated the animal is obviously not germfree. It can be expected that such contaminants will be demonstrated from time to time as virus testing is incorporated into the testing routines. However, this does not mean that such a contaminant cannot be eliminated or that animals of the same species cannot be found that do not harbor the contaminant. This is a challenge for the future.

As we move from a consideration of microscopically visible contaminants to viruses that cause diseases, still another vista is opened by a consideration of those viruses ordinarily considered nonsymptomatic. This is a modern wonderland in which even the terminology is in a state of flux. Whether such viruses will manifest themselves in time as a disease in the germfree animal is a question that is now unanswered, but for which there is now some evidence. Perhaps these viruses must be brought out by forcing techniques or by passing them to another species of animal. It is possible that this path will eventually lead us to still another horizon where a new look at genetics will be required, as seems to be the case where intensive inbreeding has been practiced. The point is that such paths may be followed more certainly if the germfree animal is used. With germfree techniques and pure-culture concepts each level or step is isolated from the other.

Pathogen-free or disease-free animals. It is proper in this monograph to consider the disease-free animal, because today there is a recognized place for it. Such an animal lies in a state between that of the germfree and of the conventional animal. The increasing use of the germfree animal in experimental biology is recognized and is an indication of the trend toward standardization. Moreover, the fact that such animals are commercially available sets the pattern for wider use of them.

From the disease-free animal it is but a step toward the monocontaminated animal in which only a single kind of microbe exists. Because of a broader isolation technique the disease-free animal finds wider usage than either the monocontaminated or the germfree animal. The disease-free animal is accordingly no substitute for the closely controlled animal, which can be and is being used in studies in which such an animal is unnecessary. By the same token it can be used to investigate many problems uncovered by the study of the germfree animal.

Finally, since the disease-free animal is best obtained by contaminating cesarean-born germfree animals and must be held under controlled conditions, it is obvious that the two states have much in common.

The need for a plan to increase the availability and use of germfree animals. The problem for the future is how best to bring the germfree animal and the techniques into the laboratory so that more investigators can enter the field. Because the very nature of the technique requires specialized apparatus, certain steps should be taken to set up centers for producing germfree animals so that they may be shipped alive, or so that the products of germfree animals, such as tissues, sera, and organs may be supplied to investigators who have an interest in using them.

The key to wider use of the germfree animal lies in the fact that they may be produced at a center and shipped by public transportation or private vehicle.

This makes it possible to establish commercial centers for producing germfree animals of different species and strains. At such a center the animals may be brought to the necessary degree of genetic control and standardization. In this way small installations of one or more isolators may be located in any laboratory, since it would be unnecessary to breed animals.

The producing center can also provide isolators on a rental or lease basis. In addition, the animals may be held at the center on special diets and under conditions designated by the investigator before they are shipped for final examination. This is simply a matter of planning and coordination and can be placed on a cost basis.

Commercial centers are made possible by the advances in instrumentation, which make it possible not only to secure an operation, but also permit larger animals to be maintained. With the advance in instrumentation has come simplification and the establishment of routines. These developments have reduced the cost of germfree animals to a point close to that of disease-free animals, which are presently commercially supplied.

It is reasonable to suggest that the United States government, through its agencies, subsidize the formation of regional centers for the production of germfree animals. The objective of such centers would be limited to the production of such animals; research is best done by the individual investigator in his own laboratory. For this reason it would be best to create these centers as subsidized commercial enterprises and not to set them up within the structure of existing governmental research centers or at universities, where the stress would be on research rather than on production. Every such center, in addition to producing disease-free animals, could include a pool of germfree isolators that could be rented or leased. As suggested, such a center could provide germfree animal products for those interested in such matters as tissue culture or biochemistry, and it would have facilities for maintaining animals for given periods of time on special diets or under special environmental conditions; this would be rendered entirely feasible by the use of small isolators.

It is obvious that we are at the beginning of a new period in biology in which larger installations will be necessary. This period is marked by the biologist's awareness of the need for environmental control in the phytotrons, zootrons, and biotrons* that are presently being considered. In the past such demands for large installations have come almost exclusively from the physical sciences, for example, the atomic energy projects. With the needs presently looming on the horizon, it would be well to plan carefully so that biology may advance and realize its promise.

The present tendency toward subsidizing small individual colonies in each institution is probably both wasteful and unsatisfactory. Maintaining colonies of animals properly is a full-time operation involving personnel trained in veterinary medicine, genetics, microbiology, and biochemistry; consequently

* The terms "phytotron," "zootron," and "biotron" have come into use to designate apparatus or systems in which control of the entire gamut of environment of an enclosed plant or animal is possible. The term "phytotron" refers especially to plants, and involves such matters as light, temperature, and humidity. Similarly, "zootron" refers to the rearing of animals and includes, in addition, the control of noise. "Biotron," then, involves a relationship between plants and animals in a controlled environment.

it involves a prohibitive cost in any but very large installations or research centers. Such centers are no substitute for research by visting scientists, who can use their time better in their own locations. The average research institution using tissue culture routinely can buy the cell lines, the media, and the containers, and thus need not maintain these lines, some of which it may use infrequently. This is also true for strains of bacteria and for culture media. There is little reason why animals should not be reared at regional centers. As matters stand at present we are years behind the needs of the times.

Summary

Isolation techniques and apparatus for rearing and experimenting on germfree animals and for controlling the environment are available. One system (Reyniers Germfree System II), which I have described elsewhere in this monograph, has been developed and tested over a twenty-year period. The same apparatus can be used for confining dangerous pathogens in aerobiological or epidemiological experiments. While it is always possible to improve apparatus and techniques, this need no longer be emphasized. It is time to move into the study and use of germfree animals. Germfree apparatus has been standardized and is commercially available.

Some problems presently before us are concerned with terminology, improvement of apparatus, rearing new species of animals, hand-rearing cesarean-born young mammals, description of germfree animals, detection of contamination, nutrition, and the need for federal support of regional centers for producing germfree animals.

The basis of germfree life studies lies in an extension of the pure-culture concept. On this basis the term gnotobiotics has been suggested to designate the field of growing living things in pure culture or in association with other pure cultures. The field can rightly be extended to cover the presently poorly named disease-free or clean animals and toward standardization of environment and animals.

The need for germfree animals has been well demonstrated by their wide use in many different fields of research and in the growing need for better control of the environment. The intent of this monograph is to bring together information on the above-mentioned problems and to outline the present status of the field.

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