History of Germfree Animal Research at Lobund Laboratory, Biology Department, University of Notre Dame, 1928-1965

JULIAN R. PLEASANTS, University of Notre Dame

The first published report (7) of germfree animal rearing at Lobund Laboratory was presented to the Indiana Academy of Science in 1932, by James A. Reyniers, who had inaugurated the program of germfree animal research at Notre Dame in 1928, and directed it until 1958. In 1939, a colloquium on micrurgical and germfree methods, the first of its type, was convened at the University of Notre Dame, and the ambitious range of the Lobund program was there revealed. When the proceedings were published in book form in 1943 (8), they included a summary of the Lobund program as it had developed up to 1942.

In 1944, another historical summary of the Lobund research program was included in a history of bacteriology research and teaching (3) presented to the Indiana Academy of Science by Robert F. Ervin, who had been associated with Lobund activities since 1936. The word "Lobund" is derived from the initial letters of the term: Laboratories of Bacteriology, University of Notre Dame. The teaching of bacteriology and strictly bacteriological research have been continuing activities of Lobund. Prominent also in the early years of the Lobund program was research in micrurgy, or micro-manipulation, and most of the early publications (8) dealt with this approach to bacteriology. The name "Lobund," however, has come to be associated primarily with the program of germfree animal research, and it is this program which will be summarized below.

A laboratory which is pursuing an ambitious, long range, and stepwise program provides an inviting topic for historical summation. Ervin provided other summaries in 1946 (1) and 1949 (2). The major publications on the rearing of germfree rats and chickens were presented in somewhat chronological form in Lobund Reports Numbers 1, 2, and 3 (1946, 1949, 1960) published by the University of Notre Dame Press. Summary articles were published by Reyniers in 1957 (9) and 1958 (10). A chronology fitting the Lobund effort into the total picture of germfree animal research was included in a 1963 book by T. D. Luckey (4) who was associated with the Lobund program from 1946 to 1954. Lobund history was further brought up to date in 1964 (6) by Morris Pollard, director of Lobund since 1961. The sources for a history of germfree animal research may be conveniently located in a bibliography of germfree research (11) published in 1963 by B. A. Teah, a member of the Lobund staff since 1943.

In order to simplify the presentation of Lobund history, I have divided it into periods which correspond to the four phases of the Lobund program decided upon by its founder in the years 1928-1930. The events, of course, cannot be so neatly divided. Apparatus development did not stop when germfree colonies were obtained. Also, valuable scientific observations were made on germfree animals even during experiments set up to test apparatus. Nevertheless, there is a certain

parallelism between the events and the original plan as published in (8), and this plan provides the basis for the following chronological division:

1928—1944 a. The design of appropriate apparatus.
1945—1954 b. The successful rearing of a variety of germfree species.
1954—1958 c. The testing of the animals for uniformity and general acceptability in experimental research.
1959—1965 d. The use of germfree animals in various aspects of bio-medical research.

First Period. 1928—1944. Reyniers' original concern was to apply engineering principles to assure the isolation of individual microorganisms from all other microorganisms. In 1928, bacteriology had been carried on for 50 years with techniques little different from those of Koch and his contemporaries. These did not, however, provide absolute security from contamination. In the course of developing equipment which would provide secure isolation of microorganisms, Reyniers soon realized that it would have a much broader range of usefulness, permitting also the isolation of higher organisms from microorganisms. Furthermore, a germfree animal, as a living culture medium, would provide a very critical test of any isolating system.

Germfree animal research was already 33 years old (5) when the Lobund program started. However, up until then germfree animals had been reared primarily to test the prediction of Pasteur (4) that an animal would not be able to live without germs. What Lobund brought to the field of germfree research was a determination to turn the germfree animal from a scientific curiosity into a useful experimental tool. This demanded a continuity of effort which was provided in this period by the research team of J. A. Reyniers, P. C. Trexler, R. F. Ervin, A. W. Phillips, J. F. Reback, M. Wagner and B. A. Teah with the support of Dean of Science Francis J. Wenninger, C.S.C. and President of the University John F. O'Hara, C.S.C.

During this period, air-tight containers had to be developed which could be sterilized on the inside and then used to maintain and manipulate the germfree animals. Air filtration methods were devised and tested. Food sterilization techniques had to be developed which did not destroy the nutritional value of the diet. Procedures for detecting leaks and contaminations were worked out, as well as the means for preventing them. A practical technique for caesarian delivery of young mammals into the germfree system, and a method of surface sterilization of chicken and turkey eggs were developed.

Although development of apparatus in this period was basic to germfree animal research, the broader usefulness of this development may be seen in its application to the control of airborne cross-infection in hospital nurseries and in army barracks, and to the protection of laboratory personnel from highly infectious bacteria. In 1937, Lobund had moved from a few rooms in the old Science Hall to 22 rooms in the ground floor of the new Biology Building. During World War II some of these facilities were diverted from the rearing of germfree animals to the propagation, processing and study of infectious bacteria, with the objec-

tive of developing defenses against microbiological warfare. Here the germfree system was successfully used in reverse to keep the germs in—away from laboratory personnel.

This first period had seen the rearing of germfree guinea pigs, monkeys, and chickens through the early portions of their life span. It was now evident that the apparatus was adequate for maintaining animals through long periods, and the way was clear for development of colonies of reproducing germfree laboratory animals, particularly the much-used species: rats and mice.

Second Period. 1945—1954. With the close of World War II freeing some of the staff and facilities, Lobund could now concentrate on the germfree animal itself. It received new support for this effort from the Dean of the Notre Dame Graduate School, Rev. Philip S. Moore, C.S.C., and from the Office of Naval Research. It will already have become evident that the original 4 point Lobund program cuts across all lines in the areas of biology, chemistry, and engineering. The first period's emphasis on microbiology and engineering now gave way to more emphasis on the germfree animal, its physiology, nutrition, anatomy and even its psychology. New staff members active in these fields were added: J. R. Pleasants, H. A. Gordon, T. D. Luckey. In the case of rats and mice, which are very immature at birth, it became necessary for Lobund to pioneer in the development of artificial milk formulas and hand-feeding methods which could be used to rear these germfree mammals from caesarian birth to weaning, since this was the only means by which such species could be obtained germfree.

Concentrated efforts in 1945 resulted in the first successful weaning of germfree rats and the first successful reproduction by hand-reared (but accidentally contaminated) rats. Initial dental and nutritional studies were carried out with hand-reared rats, but the whole operation was put on a much more secure basis in 1950, when full-scale colony production of germfree rats expanded into a new building designed specifically for germfree life research. With the dedication of this building, Lobund was also organized as an institute within the University of Notre Dame.

During this period, germfree chickens were obtained in quantity and subjected to an extensive survey to determine their physiological, nutritional and morphological parameters of normalcy, a survey which indicated their potential value as an experimental tool. They were applied to experiments designed to explain the then recent discovery that antibiotics could increase the growth of farm animals. Repression of certain elements of the intestinal bacterial flora was implicated as the main cause of the increased growth rate. Early work in germfree rat nutrition was also able to prove decisively for the first time that the rat itself could synthesize an important metabolic intermediate (the labile methyl group) and did not have to obtain it from its intestinal bacteria. Germfree guinea pigs were reared during this period by B. P. Phillips. They were used to show that the dysentery-causing amoeba, *Entamoeba histolytica*, was very much dependent, for its survival in the intestine, upon conditions created by intestinal bacteria. By the end of

this period, in 1954, the first pair of germfree mice were weaned, and they had produced 300 descendants in the following year. Thus, by the end of the second period, the problem of producing healthy germfree animals in the quantities required for scientific experimentation had been essentially solved, even though a number of problems, such as the enlargement of the cecum in germfree rodents, demanded further study to improve the condition of the experimental animal itself.

Third Period. 1954—1958. This third and briefer period may be considered an explosive one, in which germfree animals underwent a population explosion, and their production and use expanded out of the Lobund Laboratory into other laboratories in the United States and abroad. While effective means for transporting germfree animals were being developed, a new type of isolator was also developed by P. C. Trexler, who had joined the Lobund staff in 1933 and had done much of the developmental work on equipment throughout Lobund's history. His innovation was an inexpensive and versatile plastic isolator which could be sterilized by a spray of germicide. This made it possible for many laboratories to venture into germfree research without a large capital investment. Lobund rats and mice became the progenitors of germfree rat and mouse colonies in various other centers, and also provided the start of a number of disease-free colonies, which were not maintained under germfree conditions but were maintained free of certain pathogens which had long been known to limit the usefulness of the usual laboratory rats and mice.

With germfree rats, mice, chickens, and guinea pigs now regularly available at Lobund, a period of increasing experimental use began, aided by new staff members, B. S. Wostmann, and the Rev. J. P. Doll, C.S.C. The population explosion was thus followed by the beginnings of a publication explosion, some of the high lights of which are summarized below:

Highlights of Lobund Publications 1954—1958

1954 **Dental studies.** Bacteria are essential for tooth decay. Certain species can produce caries when present as monocontaminants.

Liver necrosis. Bacterially produced toxins are not needed for production of this disease.

1955 **Serology.** Germfree animals show few specific antibodies against common bacteria, but show some so-called "natural antibodies."

Amoebiasis. Pathogenic amoebae do not survive in the intestinal tract of the germfree guinea pig unless certain bacterial associates are added.

1956 **Radiobiology.** Germfree rats show better survival after irradiation than conventional rats.

1957 **Immunology.** Germfree animals show reduced lymphoid tissue and serum gamma globulin.

1958 **Shock.** Bacterial toxins are not needed for production of irreversible hemorrhagic shock.

Nutrition. Penicillin does not improve the growth of germfree rats fed a thiamin deficient diet.

Immunology. Penicillin treated conventional chickens show anatomical characteristics approaching those of germfree chickens.

In 1958, James A. Reyniers ended his 30 years of association with the Lobund program as founder and director. The research administrator for the University, Francis X. Bradley, assumed the role of acting administrative director until 1961, when the post of director was taken over by Morris Pollard, till then professor of preventive medicine and public health at the University of Texas Medical Branch.

Fourth Period. 1959—1965. New staff members were added for increased experimental use of the germfree animal: Brother Raphael Wilson, C.S.C., T. J. Starr and R. J. Downey. In addition to the continued use of the germfree animal as a scientific tool, which will be summarized below, two new species, the sheep and the goat, were added to the germfree list, and the first colony production of germfree guinea pigs and rabbits was obtained. Study of the germfree animal itself, with the goal of increasing its experimental value, was further intensified. A virological survey of the germfree rat and mouse colonies proved generally negative. Subsequently, in 1964 all of the germfree mouse strains were found susceptible to a leukemia induced by four sub-lethal doses of radiation. Thymus glands of the affected animals showed virus-like particles, suggesting the possibility that a virus had been passed into the zygote or through the placenta of the original caesarian-derived animals. This phenomenon is under further study, and efforts are under way to obtain new and virus-free stock for those experiments which require freedom from viruses, including latent ones.

Other efforts to improve the quality of the germfree animal, particularly for nutritional and immunological studies, have involved the development of chemically defined, antigen free diets, in which the exact nutritional intake is known, as well as the degree of exposure to foreign antigens.

Recent uses of the germfree animal at Lobund to answer specific scientific questions are summarized below:

Highlights of Lobund Publications 1959—1965

1959 **Immunology.** Different bacterial monocontaminants may elicit markedly different kinds of immune response.

1960 **Serology and Immunology.** Germfree animals show a complex sequence of responses to added bacterial species and to the action of antibiotics on these species.

1961 **Morphology.** Characteristics of the germfree intestinal tract lead to the suggestion that conventional animals show a chronic intestinal inflammation.

Metabolism. Cholesterol levels in the liver are higher in germfree than in conventional rats. Differences in serum cholesterol depend on age.

1962 **Nutrition.** Thiamin produced by intestinal bacteria is not immediately available to the host.

Immunology. Leucocytes of germfree animals are generally as effective in carbon clearance as those of conventional animals.

1963 **Parasitology.** Germfree turkeys do not succumb to experimental "blackhead" disease.

Oncology. In general, germfree rodents respond to chemical carcinogens as do conventionals, with no association of virus-like particles. A limited variety of spontaneous tumors have occurred in germfree rats and mice.

Radiobiology. Endotoxin is radioprotective for germfree mice. Some bacterial associates may be radioprotective.

Morphology. Germfree rats show reduced cardiac output and lower blood flow to some regions of the body.

Nutrition. Germfree rats succumb rapidly to the effect of a vitamin K deficiency. Natural vitamin K is more effective than the synthetic form for preventing hemorrhage.

1964 **Gerontology.** Germfree rats and mice outlive conventionals. The usual sex pattern of mortality is reversed, germfree males outliving females.

Virology and Oncology. Injected oncogenic viruses produce the same effects in germfree as in conventional mice. Radiation induces leukemia in both germfree and conventional mice, but not in rats.

Immunology. Germfree mice thymectomized at birth show no runting disease or early mortality.

Metabolism. Bacterial action is not necessary to potentiate the hypocholesterolemic effect of certain dietary lipids.

1965 **Dental studies.** The possibility of immunizing against tooth decay is demonstrable in gnotobiotic rats.

Nutrition. When certain diets are fed, intestinal bacteria can play an important role in promoting iron absorption by rabbits.

Immunology. Cortisone increases the mortality of germfree mice exposed to hepatitis virus, but not as much as it increases the mortality of conventional mice.

Radiobiology. Bone marrow transplants permit germfree mice to survive large radiation doses. General radiation resistance in germfree mice is associated with slower mucosal turnover in the germfree intestine.

In this fourth period, as in the preceding ones, the Lobund Laboratory has continued to show its concern not only for short-range experimental use of the germfree animal, but also for the long-range improvement of the germfree animal as a precise experimental tool, through survey of its characteristics, improvement of its nutrition and environment, and exact evaluation of its germfree status. It is these latter concerns, as well as its extensive use of the germfree animal, which has

enabled Lobund to play a decisive role in the over-all development of germfree animal research.

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