research progress report

Germ-Free Amebiasis Studies

This report on progress of germ-free amebiasis research being conducted by the University of Notre Dame and the Public Health Service is based on a paper presented by Bruce P. Phillips at the annual meeting of the American Society of Parasitologists, at Memphis, Tenn., November 4, 1954. Mr. Phillips is a medical protozoologist with the National Microbiological Institute of the Service.

CONVENTIONAL animal experimentation as a key resource in the study of infectious diseases has certain inherent shortcomings. Among them are interference by rival organisms in the animal. An investigator working with a given disease organism in a laboratory animal usually must consider the possible effects of other micro-organisms normally present or accidentally introduced. He must ask if his results are influenced, directly or indirectly, by bacteria normally harbored by the animal, bacteria which in themselves do not ordinarily produce disease.

Germ-Free Animals

To eliminate such uncertain or unpredictable factors is the object of what is known as germ-free medical research. For this work, great pains have been taken to rear and make available for experimental use small laboratory animals—chickens, guinea pigs, or mice—which are free of the bacteria harbored by conventional or "contaminated" animals.

Scientists first attempted to rear germ-free animals—chickens—in 1897. Contamination occurred within the first few days. The investigators concluded that it is practically impossible to free the egg surface from bacteria, and that chickens are therefore unsuitable for germ-free studies. Nevertheless, another scientist, Schottelius, persevered. After 9 years, he reported, he raised about 30 germ-free birds; some remained germ-free for as long as 30 days. But his germ-free birds sickened and died, whereas others which he deliberately contaminated after a short period developed normally. He concluded that intestinal bacteria are indispensable to the nutrition of vertebrates.

Soon after, these conclusions were refuted. Cohendy in 1912 reported rearing 17 healthy germ-free chickens for as long as 40 days.

After a lapse of nearly 25 years, Balzam in 1937 reported he had reared 5 healthy chickens for 59 days, germ-free, for an experiment with nutrition. He concluded that intestinal bacteria had no appreciable influence on the digestibility of food in the chicken.

Chickens have proved particularly appropriate for germ-free breeding. The chick feeds itself from birth. It needs fewer caretakers than the mammals. Advanced knowledge of its genetic and embryonic history is also an advantage to research.

The first long-term program to rear germ-free animals began in 1928 at the University of

Notre Dame. This attempt to resolve the many complex problems of creating and maintaining a sterile environment for germ-free animals drew on resources of mechanics, engineering, physics, chemistry, and biology. The bacteriological triumph gave rise to a specialty known as biological engineering, which designed the instruments and apparatus of the project. Only by these applied skills was it possible to put germ-free animal breeding on a practical basis. Trained technicians were enabled by ingenious equipment to relieve highly trained research personnel of the routine feeding and management of the animals.

The objective at Notre Dame was to introduce an animal, germ-free at birth, into a sterile environment, and to maintain it and its progeny germ-free. The feat required that the animal be isolated from germs in its living quarters, its air, its food, and all its other contacts.

Early attempts to achieve this end depended on a series of steps, each using germ-free apparatus with aseptic technique. Since any one step was a weak link in the chain, Notre Dame devised a system of total control. The entire apparatus for moving the animals, cleaning cages, and handling food is sterilized at one time under steam pressure.

This achievement at Notre Dame, under the leadership of Dr. James A. Reyniers, has been followed with deep interest by scientists in many research institutions. At the National Institutes of Health of the Public Health Service, the availability of germ-free animals led to the initiation of a study 2 years ago by the National Microbiological Institute's Laboratory of Tropical Diseases, in cooperation with the University of Notre Dame.

Amebiasis Study

The problem selected for study at the National Institutes of Health was a widespread intestinal infection known as amebiasis. Many will remember the serious outbreak of this disease in Chicago during the World's Fair 20 years ago. Amebic infection is common in the United States. It is found in an estimated 7 percent of our population.

Amebic dysentery has long been viewed as a clinical anomaly, in that it presents an unusually wide and varied range of manifestations. To explain these variations, studies were devised to investigate the agent of this disease and its capacity to produce infection. Among them have been studies to determine the effects of inoculation of germ-free animals with amebas which are themselves free of bacterial contamination.

Germ-Free Amebas

These studies were proposed because the initial attack of this disease agent usually occurs in the lower intestinal tract, where it lives with a large number of species of bacteria. It had never been possible for the ameba to be grown in the test tube without the presence of bacteria until a scientist in the National Microbiological Institute succeeded in cultivating the ameba in bacteria-free cultures of a South American trypanosome.

Two groups of animals were used for inoculation with the germ-free ameba: germ-free guinea pigs and conventional guinea pigs. Both groups were maintained on identical sterilized rations.

None of the 35 germ-free animals developed amebic lesions before they were sacrificed on the 33d day. Of the 37 conventional animals inoculated as controls, 34 developed ulcerative amebic disease, and the remaining 3 were shown to harbor the infecting agent when sacrificed on the 21st day.

In contrast to these results, 2 series of preexperimental germ-free animals were fed by mouth single species of common intestinal bacteria before being inoculated with bacteria-free ameba. All of these animals developed acute amebic diseases with typical lesions.

Bacterial Effect

These results offered first concrete evidence that bacteria have a role in the experimental production of disease by the ameba. In relation to amebic disease in humans, the evidence suggests a possible explanation for the disparities frequently noted in clinical manifestations and in effects of treatment of amebic dysentery.

This study, made possible by the use of Notre Dame's germ-free animals and facilities, repre-

sented an expenditure by the National Institutes of Health of only $21,000.

The National Microbiological Institute, the National Institute of Arthritis and Metabolic Diseases, and the National Institute of Dental Research have plans for additional germ-free studies.

At the Microbiological Institute, new knowledge is sought about the multitude of infecting agents that assault the tissues of man. Germ-free studies aid in this task, for they supply a method and a freedom from bacterial interference heretofore lacking.

Germ-free animals may be useful in investigating the site of multiplication of the poliomyelitis virus in the intestinal tract and the influence on its multiplication and excretion.

Another project would study resistance of germ-free animals to infection with such agents as staphylococci. This is of interest to clinical medicine because staphylococci, once they become resistant to antibiotic drugs, may cause severe complicating secondary infections.

Other studies may determine whether certain fungi and yeasts become pathogenic, or disease producing, if the effect of bacterial growth in the respiratory and gastrointestinal tracts is eliminated.

The hope is that germ-free techniques have reached the stage where they can be applied to the study of a wide variety of complex biological problems, as evidenced 70 years ago by Pasteur.

New Juvenile Delinquency Division in Children's Bureau

With the establishment of a Division of Juvenile Delinquency, the Children's Bureau of the Department of Health, Education, and Welfare will increase its services to public and private agencies and to organizations concerned with the nationwide problem of juvenile delinquency. The new division will work closely with existing programs of the Children's Bureau to extend and improve its health and welfare services for physically, socially, and emotionally handicapped children.

Philip Gordon Green, former chief juvenile probation officer of the Juvenile Court of San Francisco, will direct the division. Associated with him will be William H. Sheridan as chief of the Technical Aid Branch, Mrs. Elliot Turner Studt as chief of the Training Branch, and Donald George Blackburn as consultant on institutions for delinquent youth. Specialists on juvenile police, courts and probation services, and community services are to be appointed.

The Children's Bureau is also expanding its study program and statistical reporting on children who come to the attention of the courts.