NASA TECHNICAL MEMORANDUM NASA TM-76362

GNOTOBIOLOGY IN MODERN MEDICINE

G. I. Podoprigora

(NASA-TM-76362) GNOTOBIOLOGY IN MODERN MEDICINE (National Aeronautics and Space Administration) 20 p HC A02/MF A01 CSCL 06E

N81-18636

Unclas

G5/52 16559

Translation of "Gnotobiologiya v sovremennoy meditsine," Priroda, No. 6, June 1978, pp. 3-15.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D. C. 20546 SEPTEMBER 1980

STANDARD TITLE PAGE

1. Report No. NASA TM-76362	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle GNOTOBIOLOGY IN MODERN MEDICINE		5. Report Date
		6. Performing Organization Code
7. Author(s) G. I. Podoprigora USSR Academy of Medical Sciences Laboratory of Experimental Simulations		8. Performing Organization Report No.
		10. Work Unit No.
9. Performing Organization Name and Address Leo Kanner Associates Redwood City, California 94063		11. Contract or Grant No. NASW - 3199
		13. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address National Aeronautics and Space Administration, Washington, D.C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes
Translation of "Gnotobiologiya v sovremennoy meditsine," Priroda, No. 6, June 1978, pp. 3-15.

16. Abstract
A review is given of currently accepted theories and applications of gnotobiology. A brief history of gnotobiology is supplied. Problems involved in creating germ-free gnotobiota and the use of these animals in experimental biology are cited. Examples of how gnotobiology is used in modern medical practice illustrate the future prospects for this area of science.

17. Key Words (Selected by Author(s))	18. Distribution Statement This copyrighted Soviet work is reproduced and sold by NTIS under license from VAAP, the Soviet copyright agency. No further copying is permitted without permission from VAAP.

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22.
Unclassified	Unclassified		

NASA-HQ

GNOTOBIOLOGY IN MODERN MEDICINE

G. I. Podoprigora

Experimental Biological Simulation Research Laboratory,
USSR Academy of Medical Sciences

Gennadiy Ignat'yevich Podoprigora is a Medical Science Kandidat, senior science associate, and leader of a group of gnotobiologists at the Experimental Biological Simulation Research Laboratory, USSR Academy of Medical Sciences. He is developing Soviet designs of gnotobiological insulators and working on obtaining and studying various kinds of amicrobic animals and implementing the introduction of gnotobiology into clinical medicine. The basic purpose of his research is to study the role of microbes in body defense reaction mechanisms.

The study of regularities in the interaction of the macroorganism and its microbial environment is one of the most vital tasks in medicine and biology. /3*

Although the successes of microbiology have provided extensive information about the properties of various microorganisms, their roles in the vital processes of the human and animal bodies are still far from understood. Our inadequate knowledge in this area is a consequence of less-than-perfect experimental approaches to the problem and, primarily, unsatisfactory requirements for quality in the subjects of medical and biological research -- the laboratory animals. The presence of unregulated and uncontrolled microflora in common laboratory animals has made it impossible correctly to evaluate the influence of the microbial factor on the body.

These difficulties were overcome through the introduction of animals with controlled microflora (gnotobiota) into experimental practice. The term "gnotobiota" (from the Greek words "γνωτο" -- known and "βιότα" -- flora and fauna), proposed by Lucky [1] (1963), designates the microflora in a given organism which are known to the

---

*Numbers in the margin indicate pagination in the foreign text.

researcher, and that realm of science dealing with the study of gnotobiota has been given the name gnotobiology.

The possibility of life without microbes was first advanced by Pasteur in 1885. Ten years later, at a Berlin University laboratory, Nuttel and Tierfelder obtained the first germ-free guinea pigs.

Further development of germ-free research was given a tremendous impetus by Mechnikov. His studies on normal body microflora were so intimately associated with the development of gnotobiology that he is rightfully considered one of its founders. In 1912-1913, Gueno and Volman first bred germ-free chicks, flies, and tadpoles. In the earliest stages of gnotobiology the possibility of obtaining germ-free animals was only an interesting laboratory phenomenon. Further study of the biology of germ-free animals demanded substantial improvement and the development of methodological principles and technical foundations for obtaining and maintaining them.

In the initial experiments, isolation apparati for the animals were borrowed from available technology. Nuttel and Tierfelder used the principle of the diving bell. The prototype of the modern isolator/4 was Kuster's apparatus (1913-1914), with which he obtained germ-free goats. The first production model germ-free isolators (apparati by Reiniers, 1946, and Gustafson, 1948) were made of stainless steel.

The real blossoming of gnotobiological technology occurred with the introduction of polyvinyl chloride films by Trexler in 1957. Various modifications of isolators made of sheeting are most widely used at present.

Methods of obtaining and breeding various germ-free animals and birds (guinea pigs, mice, rats, rabbits, hamsters, dogs, hares, pigs, sheep, calves, monkeys, chickens, quails, etc.) were developed simultaneously with the apparati. Many of them breed quite well in germ-free conditions. Because of the successes of gnotobiological technology, germ-free animals have been converted from a laboratory phenomenon into a precise instrument of medico-biological research.

Gnotobiology in this country began developing in the mid 1960's within the USSR Academy of Medical Sciences, at the Gamaleya Institute of Microbiology and Epidemiology ("IME") and the Experimental Biological Simulation Research Laboratory ("NIL EBM"). Basic research by Chakhava (IME), who was first in the Soviet Union to obtain germ-free animals, contributed greatly to the development of gnotobiology here. Experiments on gnotobiological animals of various kinds (guinea pigs, mice, rats, and pigs) are now being conducted at the NIL EBM. In addition, methods for obtaining germ-free chicks, rabbits, and monkeys are under development, and plexiglass isolators for obtaining large and small gnotobiota are being built.

The Essence of the Gnotobiological Experiment

Gnotobiota are obtained by caesarian section in a special surgical isolator. The newborn are then transferred to an isolator for growth and artificial feeding. The interior of the isolator is sterilized before hand; the air going into the isolator passes through bacterial filters. All material, including food, enters the isolator through a special airlock. Preparation of the diet for gnotobiota involves consideration of the disruptive effect of sterilization on the nutritive components of the food, so it is enriched with vitamins, amino acids, and other nourishment.

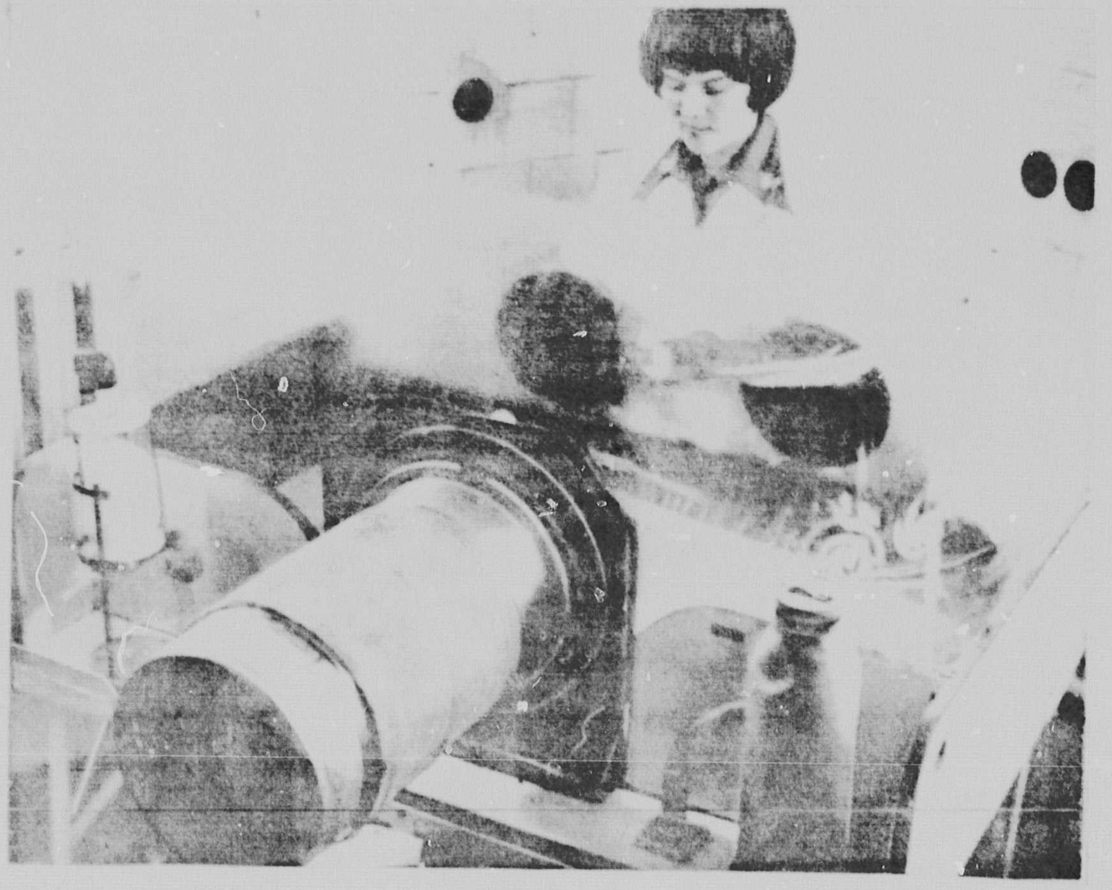
Careful microbiological control is exercised over sterilization by using different nutriments. Modern microbiological methods can accurately determine the extent of bacteriological sterility in gnotobiota. Whether they are carriers of viruses is a more complicated problem. The presence in gnotobiota of viruses transmitted from the mother cannot be ruled out. Virus-free gnotobiota do, however, exist. Colonies of virus-free animals can also be obtained from them. The gnotobiological isolator offers reliable protection against external viruses.

The gnotobiota thus obtained are used to study features of germ-free life and to serve as the foundation for developing various categories of gnotobiota. Four basic categories may be singled out, depending upon the status of microbe content. A few or several types of known microorganisms in an artificial population of gnotobiota indicate gnotophoric animals ("gnotophor" means carrier of a known item). If gnotobiota are inoculated with known microflora not containing specific pathogens, so-called pathogen-free animals are obtained. The term SPF-animals (specific pathogen free) is most often used in foreign publications to designate this category.

New categories of gnotobiota having both controlled microbes and controlled antigen influences are of some interest. Under normal conditions the body comes in contact with antigens from microbes and food. Although not to the same extent as microbes, protein foods nonetheless have a certain antigenic effect and are capable of causing immunobiological alterations in the body. The difficulty of obtaining antigen-free animals lies in the necessity for creating a chemically defined diet with a low molecular weight containing the needed composition of amino acids and other ingredients free of antigenic properties, without protein. Work on obtaining such animals has begun in /5
the USA, Czechoslovakia, and Japan.

The Importance of Gnotobiology

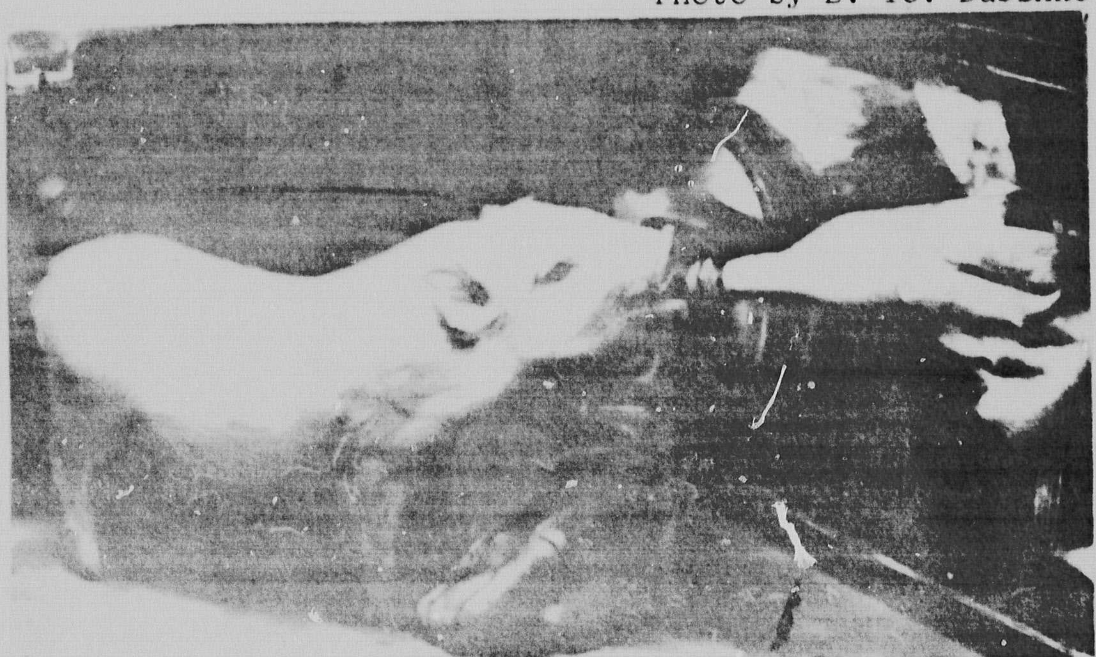
The overall biological importance of gnotobiology stems from the diversity of the world of microorganisms and their vital role in in nature and various spheres of human activity. Principles of subdividing microbiology into general, medical, veterinary, agricultural, and industrial areas are applicable to gnotobiology as well. This differentiation is evidenced in a practical way by the ever-strengthening ties between gnotobiology and the areas of science and practice indicated above. Methods of gnotobiological isolation have extended into clinical medicine in recent years, and clinical gnotobiology is under development. Germ-free research is of vital importance for space biology and medicine, as well as for some branches of industry (production of antibiotics, vaccines and sera, microbiological control in food production, etc.).



A modern germ-free isolator made of polyvinyl chloride sheeting produced by "Celster-Isotechnie" company (France).
Photo by B. E. Dubinko.

The possibility of artificially colonizing an organism with useful microflora has long attracted the interest of researchers. The introduction of pathogen-free or SPF animals is radically altering the concept of the laboratory animal as a subject for research. While the role of microflora in laboratory animals was previously simply ignored, the need for taking it into consideration has now become obvious. Increased demands for microbiological control of laboratory animals have changed attitudes towards the conditions of their /7 confinement as well. The modern experimental biology clinic working on gnotobiota is well supplied with a complex of technical equipment with which, in addition to maintaining optimal zoological conditions, a constant microbial environment is provided (standard microflora). Biological standardization of laboratory animals ensures not only their genetic homogeneity, but also the high quality of biological simulation,

Operation for obtaining germ-free pigs using a surgical isolator. Extracted fetuses are transferred through adjoining airlocks into an isolator for gnotobiotic growth.
Photo by B. Ye. Dubinko.



Feeding a germ-free piglet in an isolator.
Photo by B. Ye. Dubinko.

which, in turn, considerably increases the accuracy of medical and biological experiments. It was not by accident that gnotobiota were used for space research on the "Kosmos-936" biosatellite.

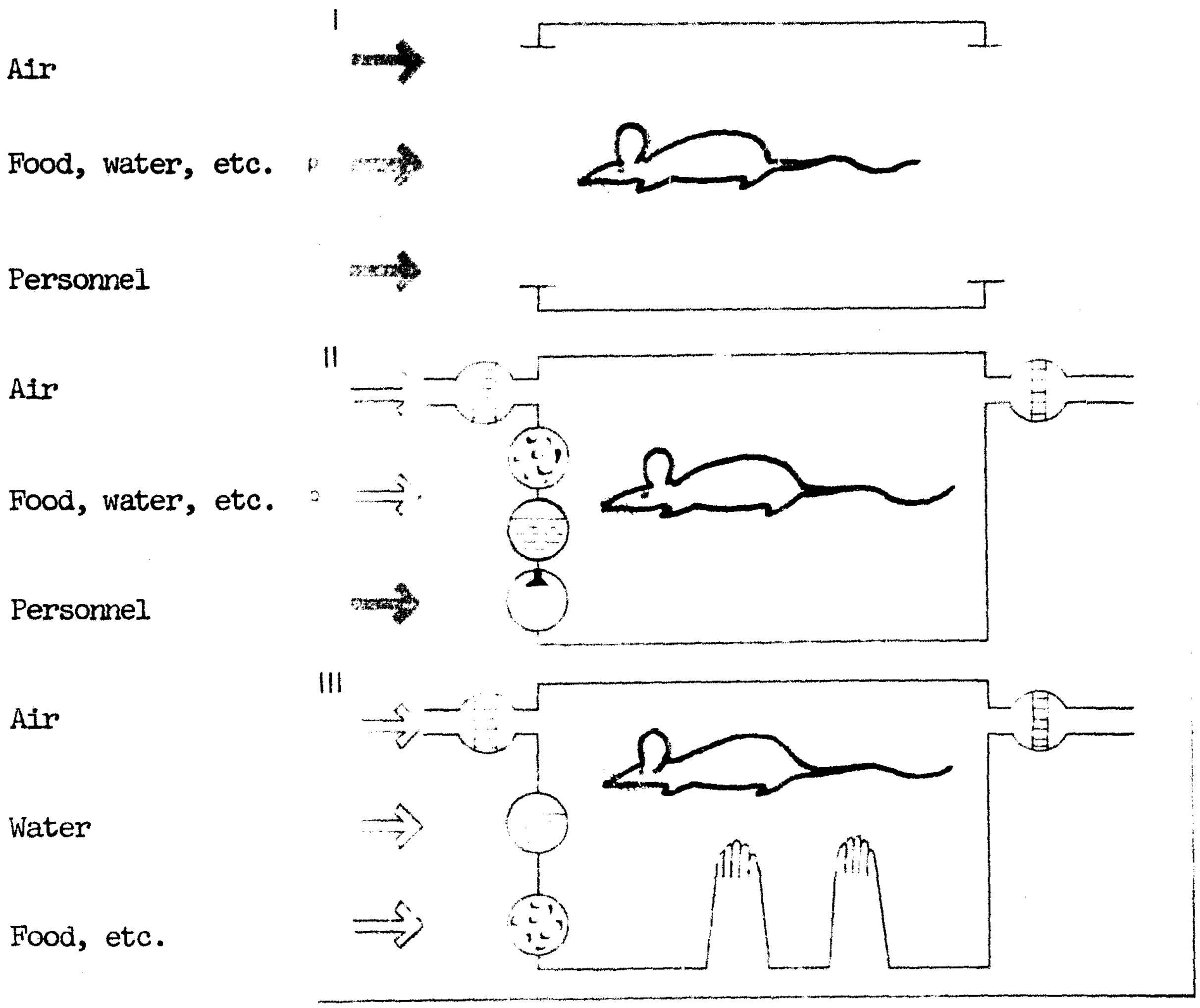
The growing of germ-free animals is one of the most effective measures for preventing infection in agricultural animals. This principle is already being used abroad for breeding germ-free pigs, chickens, etc.

The problem of biological isolation links gnotobiology with space research. Germ-free animals are needed for the creation of inhabited space systems, the study of the effects of space flight factors in the absence of microflora, and for finding microorganisms or other forms of life on celestial bodies (as was done when testing lunar soil).

Gnotobiological experiments in the most diverse realms of medicine make it possible to separate our true "friends" and "enemies" from the microworld that surrounds us. We have as yet learned much too little about the normal microflora chiefly responsible for the health or illness of the macroorganism. It is entirely possible that the development of useful physiological microflora and means of adapting them to the body will solve a whole series of problems related to the defense of the body against infection, increasing resistance to various pathogenic factors, and, in the final analysis, increasing the length of the human lifespan.

Properties of Gnotobiota and Their Use in Medicine

By artificially isolating a macroorganism from its microbial environment we can reveal those structures and functions of the body in the formation of which microbes directly participate. This refers first of all to those organs and tissues which come in direct contact with microflora, primarily to the intestine.



Three types of containment for differing categories of laboratory animals: I -- open system (normal animals), II -- barrier system (SPF-animals), III -- gnotobiological system (germ-free and other categories of gnotobiota).

Under normal conditions, microflora constantly irritate the wall of the intestine and stimulate local blood flow and metabolic processes. This complex of body responses to microbial influences is called physiologic inflammation. Physiologic inflammation plays an important part in nonspecific stimulation of body defense mechanisms, maintaining them in a constant state of preparedness for warding off pathogenic microbes. This stimulates the secretory activity of cells manufacturing various bacteriocidal factors; for example, an acid environment is created which is unfavorable to the growth of most pathogenic microbes.

Blood protein factors and leukocytes which destroy pathogenic bacteria enter the intestine simultaneously.

Gnotobiota have no physiologic inflammation, and thus the barrier-like properties of the intestine are reduced. This is particularly noticeable when gnotobiota are infected with pathogenic intestinal bacteria. In our experiments, one hour after infecting germ-free guinea pigs with intestinal bacilli the microbes had passed through the intestine into the bloodstream and caused the death of the animal. Normal guinea pigs would, as a rule, survive in such a situation [2].

Weakened immunity is a characteristic of germ-free animals, and is manifested most of all by decreased quantities of lymphocytes and /8
blood immunoproteins (immunoglobulins, antibodies, etc.). We have shown that the ability of leukocytes and other cells in gnotobiota to engulf and destroy microorganisms (a phenomenon called phagocytosis) is reduced. This appeared to be associated with a lack of certain blood protein factors (opsonins) in germ-free animals. These protein factors are formed during the interaction of the body with microbes, and they play an important part in defense responses.

Diverse biologically-active substances normally broken down by bacterial enzymes are accumulated in the macroorganism living a germ--free existence. These substances in turn cause a number of alterations in the bodies of gnotobiota. For example, the ceca of germ-free rodents are considerably larger than those of normal animals. This is, of course, linked to the accumulation of substances which weaken the /9
tone of the smooth muscles of the intestine and overstretching of this area by food residues. The cause of this phenomenon has yet to be fully explained, however.

Reduced cardiovascular functional activity is also a characteristic of germ-free animals. Smooth muscle tone is decreased, leading to slowing of the bloodstream and reduced heart stroke volume. Overall blood volume and number of blood leukocytes is diminished in gnotobiota. Metabolism in gnotobiota is reduced 20-25%. The microbial factor is also involved in the assimilation of proteins, carbohydrates, vitamins, and mineral elements, and affects the activity of endocrine glands, particularly the adrenals, which secrete vital body hormones.

The aging process in gnotobiota is quite interesting. The lifespans of some types of gnotobiota are greater than those of normal animals. Gnotobiotic rats live more than three years, whereas normal animals generally only live for two years.

By studying the resistance of the body to microbes we ascertained that phagocytic activity is higher in older gnotobiotic rats than in normal rats of the same age, while it is considerably higher in young normal rats than in young gnotobiotic animals [3]. Peak resistance in normal animals apparently occurs at a younger age than in animals with limited non-pathogenic microflora, after which resistance to infection falls rapidly. Obviously, as a result of constant contact with an uncontrolled microbial environment, the energy reserves of normal animals are depleted more quickly, which is undoubtedly a consequence of body aging processes.

Age-related changes in normal animals are characterized chiefly by inflammatory alterations in organs, but in germ-free animals by reduced muscle tone. The role of microbes in the length of life has been sparsely studied. We may say with certainty, however, that the prospects for using gnotobiotic animals in this field are extremely good.

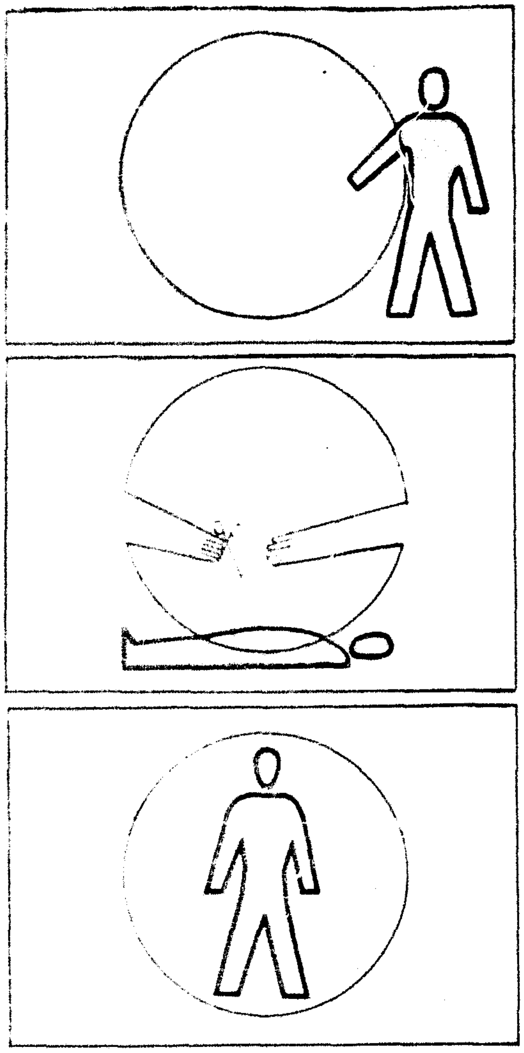
Much new data have been supplied by the use of gnotobiota to study the causes and developmental mechanisms of infectious diseases. Two principly different types of interrelationships between microbial agent and attendant microflora have been established: intensification of microbe-agent pathogenic action (a synergistic phenomenon) and suppression of the pathogenic effect of the agent (an antagonistic phenomenon). An example of synergism is the absence of the typical infectious disease picture when germ-free pigs are infected with amebic dysentery, helminthes, etc. The antagonistic role of normal microflora is well illustrated by the increased sensitivity of germ--free animals to tuberculosis and cholera. In gnotobiotic experiments it is possible to select microbial associations least favorable to the development of infections. The development of sensible methods of treatment using antibiotics must be based on data from gnotobiological research.

Germ-free animals aid in explaining the role of microflora in non-infectious diseases, such as burns and malignant tumors. It was shown that poisoning of burned gnotobiotic rats and guinea pigs was to a considerable extent caused by the products of tissue decay [4]. Moreover, the healing of burns is greatly inhibited by the presence of microorganisms. Regeneration of the burned skin of germ-free animals occurred somewhat more quickly and without wound suppuration and scarring. Based upon these observations, we proposed the use of gnotobiotic isolation for treatment of burns.

Gnotobiology has an important place in the study of the causes and mechanisms of tumor formation, particularly the role of microbes in spontaneous and induced carcinogenesis. It turned out that tumors occur less frequently, develop more slowly, and are more benign in gnotobiota. As an example, the frequency of mammary gland tumors is 30% in normal mice, but only 1.5% in germ-free mice. According to Pollard's data, spontaneous tumors occur considerably less often in gnotobiota than in normal animals, and then only in old age. These were predominantly adenomas of the mammaries, hypophysis, and other endocrine glands [5].

The presence of spontaneous tumors in gnotobiota cannot, however, be viewed as an argument against the viral theory of carcinogenesis, first of all since in a number of cases germ-free animals are not free of viruses. Precise definition of the link between tumor formation in germ-free animals and oncogenous viruses transmitted through the placenta still lies ahead.

Besides the virus factor, microflora as a whole may play an important role in carcinogenesis by facilitating the formation of carcinogenic compounds from initially harmless products. In research done by associates at the Petrov Institute of Oncology and our laboratories



Principles of gnotobiological isolation in the clinic (schematic): above -- local isolation (treatment of long-term non-healing injuries); middle -- abacterial surgery (performance of surgical operations in an absolutely sterile environment); below -- general isolation (treatment of patients with reduced resistance to infection). Dark area indicates non-sterile surroundings.

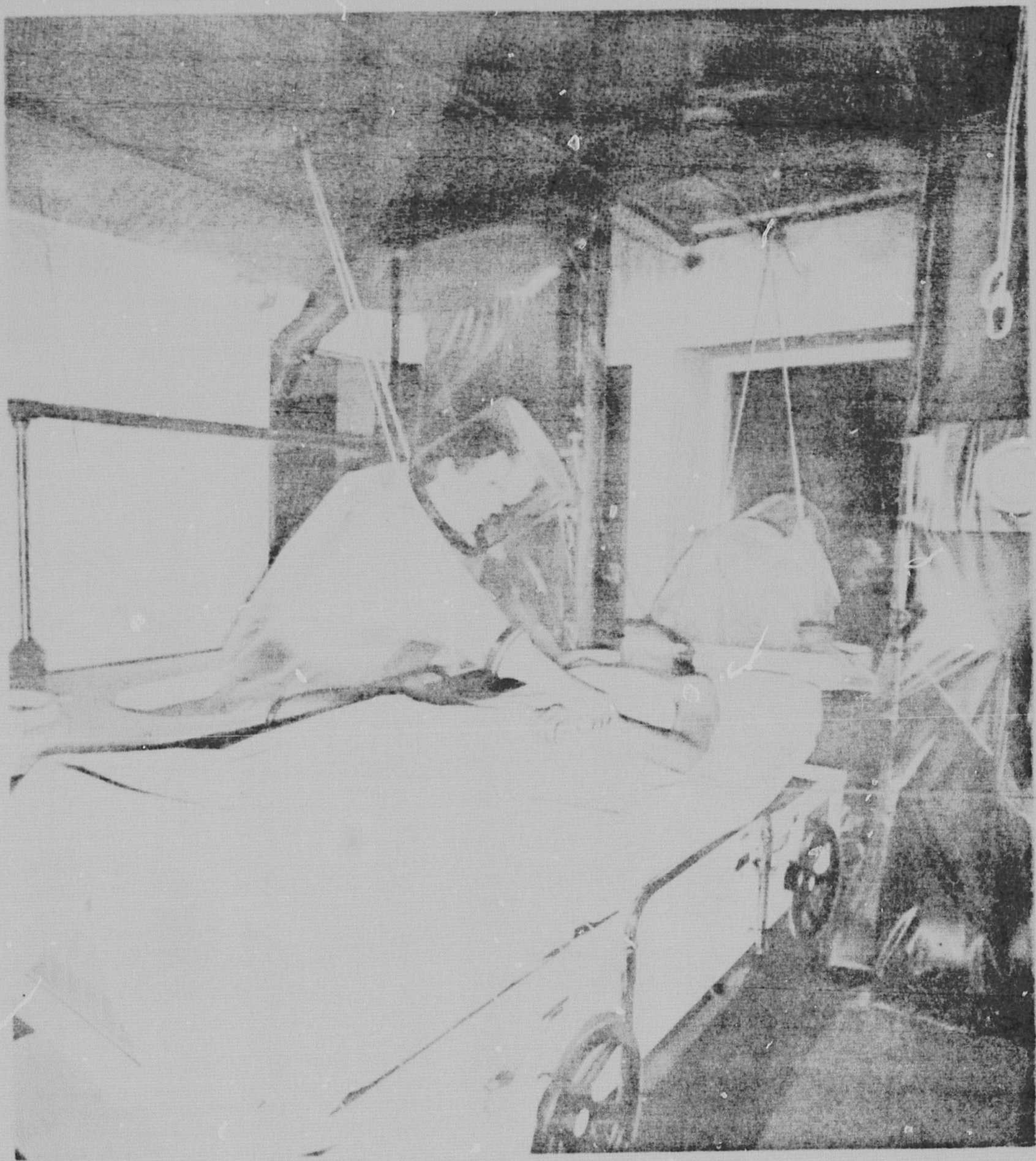
it was shown that a substance like 1,2-dimethyl hydrazine, which normally has carcinogenic properties, does not evoke tumors in gnotobiotic rats. The carcinogenic action of this substance appears only where there is interaction with the enzyme systems of microbes, i.e., where a new compound is formed [6]. The same outcome was found with another substance -- cycasin -- in works by foreign authors [7]. Further studies using gnotbiological models will make it possible on one hand to isolate those chemical compounds whose carcinogenic effect is actuated by microbes and, on the other hand, to ascertain how certain microorganisms participate in the carcinogenic transformation of chemical compounds. These data will make possible the elaboration of oncologic criteria for normal microflora.

Gnotobiology in Clinical Medicine

Development of clinical gnotobiology in this country began in 1970 through the joint efforts of associates at the USSR Academy of Medical Sciences Experimental Biological Simulation Research Laboratory and the Department of Pediatric Surgery at Pirogov 2nd Moscow Medical Institute.

A surgical operation in a germ-free environment using a gnotobiological isolator (abacterial surgery).
Photo by "Celster-Isotechnie" (France).

ORIGINAL PAGE IS
OF POOR QUALITY



Isolation room for general gnotobiological isolation.
Photo by "Celster-Isotechnie" (France).

The clinical use of gnotobiological technology is opening up new prophylactic pathways for combatting intrahospital infections in surgery. Three basic trends in clinical gnotobiology may be cited.

First of all, the use of the germ-free isolator principle has made it possible to conduct surgical operations in totally sterile conditions (the principle of abacterial surgery). A gnotobiological isolator made of sheeting and the prepared instrument set-up are assembled on the patient's operative field and attached to the skin. The operation is performed in total isolation, permitting a sharp reduction in the use of antibiotics. Operations are now done on kidneys, appendices and gall bladders are removed, and traumatologic dermoplasty is conducted under such conditions. Postoperative periods usually pass without infectious complications. The reliability of gnotobiological isolation for antimicrobial protection of surgical wounds has engendered a review of the status of modern operative technique and has broadened the horizons of operational intervention, as for the most unprepared conditions -- in military surgery, flying ambulances, and first aid.

Use of general gnotobiological isolation in the process of treating a newborn with an immunodeficit.
Photo by "Celster-Isotechnie" (France).

Partial (or local) isolation is used in the treatment of injuries, chiefly those to the extremities. A gnotobiological local isolator is placed over the wound or burn site. Constant microbe control demonstrates that wound microflora are decreased, both in quantity and type, during the isolation process. The wound becomes sterile after 7-9 days. It also helps promote wound self-healing and prepares the surface of the wound for autoplasty in extensive processes.

Specialists at the American space research center developed a mobile gnotobiological isolation system consisting of a suit in which the patient may leave the isolation room and go out of the hospital area. This system was used for isolation of a child born with a severe immunodeficit.

Photo by NASA.

In general gnotobiological isolation the patient is totally removed from the surrounding microbial environment. Life-support conditions are created within the isolator, including sterile air circulation, maintenance of a constant temperature and humidity, etc. In total isolation cases it is necessary to make allowances for the patient's own native microflora. The composition of these microbes is quickly brought down during isolation -- to 1-2 different types by the end of 2-3 weeks of isolation [9]. Under these conditions the danger arises of pathogenic microorganisms developing from those present in the body. Hence the fundamental principle of general gnotobiological isolation is reliable decontamination from the body's own microbes using antibiotics or other antibacterial preparations.

Material related to the first use in this country of general gnotobiological isolation at the 2nd Moscow Medical Institute Pediatric Surgery Clinic was reported by us in early 1977 at a meeting of the Moscow Surgical Society. The successful use of this kind of method showed the reliability of gnotobiological protection of a patient's body from infection.

General gnotobiological isolation is required for treatment of patients with general (or, in the accepted terminology, systemic) immunity disorders. There are diseases (so-called immunodeficits) in which the body is totally defenseless against the most benign sorts of microorganisms. The gnotobiological isolator may also provide a reliable antimicrobial barrier for such patients during the process of treatment. Therapeutic measures for reestablishing body immunity may be applied in antimicrobial isolation.

The substantial immunobiological alterations which the body goes through during the use of general isolation must be taken into account. For this reason, before releasing the patient from the isolator int the external environment a complex of measures are applied, particularly repopulation of the body with normal microflora (recontamination) so as to increase its resistance to microbes.

Despite the tremendous successes of gnotobiology, this realm of science has far from exhausted its possibilities. Further development and elaboration of its technological, experimental, theoretical, and applied aspects remains to be accomplished.

REFERENCES

1. Lucky, T. D., Germfree Life and Gnotobiology, Academic Press, New York and London, 1963.

2. Podoprigora, G. I., Byull. eksperimental'noy biologii i meditsiny, 6 (1977).

3. Podoprigora, G. I., and V. A. Dushkin, "A Comparative Study of Phagocytic Activity in Adult Gnotobiological and Normal Mice," Report at the 9th International Conference of Gerontologists, Kiev, 1972.

4. Fedorov, N. A., I. K. Koryakina, G. I. Podoprigora, Byull. eksperimental'noy biologii i meditsiny, 8 (1973).

5. Pollard, M., Gerontologiya 17, 333 (1971).

6. Pozharisskiy, K. M., V. A. Dushkin, G. I. Podoprigora, Byull. eksp. biol. i med., 10 (1974).

7. Lagueur, G. L., Fed. Proc. 23, 1386 (1964).

8. Isakov, Yu. F., E. A. Stepanov, G. I. Podoprigora, G. A. Ginodman, Vestnik khirurgii, 5 (1976).

9. Bengson, M., and F. Tome, "The Importance of Gnotobiology in Space Flight Research," Report at the 9th International Congress on Microbiology, Moscow, 1966.