Meetings

The Gnotobiotic Animal as a Tool in the Study of Inflammation

The gnotobiotic animal model and its application in medical research, especially in the study of inflammation, was the focal point of a symposium held in Lexington, Kentucky, in June 1970. An earlier symposium, held in Nagoya, Japan, emphasized the technological aspects of gnotobiology (*1*).

In recent years it has become obvious that control of the microbial variable with gnotobiotic techniques is, in itself, insufficient for obtaining a well-defined animal model. Definition of nutritional intake and control of dietary and environmental antigenicity are prerequisites for obtaining reproducible and significant results in studies pertaining to nutrition, metabolism, and the many facets of host defense mechanisms. In addition, a thorough knowledge of the function and metabolism of the gnotobiote, especially in the total absence of a viable intestinal microflora, appears to be necessary. The fact that in the germfree rat the typical enlargement of the cecum leads to a 25 percent reduction in metabolic rate, with comparable reductions in cardiac output and in the size of the circulatory system, indicates that the germfree state has its own phenotypic characteristics (*2*). This finding may now be related to the recent observation that the mesenteric arterioles of the germfree rat show a specific unresponsiveness to catecholamines.

Efforts to further define the germfree animal model have led to the formulation of chemically defined, low molecular weight, water-soluble diets that can be sterilized by membrane filtration to remove all impurities having a molecular weight over 10,000. In germfree, inbred C3H mice that had been fed these diets since they were weaned, gamma globulin levels could not be detected by direct immunoelectrophoresis, even at an advanced age. In similar mice, maintained for 8 months on a practical diet and only subsequently fed the defined, nonantigenic formulation for 3 to 4 months, the levels of immunoglobulin G (IgG) and immunoglobulin M (IgM) were similarly undetectable. Stimulation of germfree mice—reared on membrane-filtered, chemically defined diets—with sheep erythrocytes demonstrated a stronger than average IgM- and normal IgG-producing cell response. Data from germfree, colostrum-deprived piglets, obtained 3 to 5 days before term by means of a hysterectomy, showed that the immune response was also unimpaired in this animal system, which was found to be totally devoid of immune globulins and free of "background" antibody-forming cells. The true primary response that could be elicited in these piglets demonstrated that the occurrence of germinal centers in lymphoid tissues is not a requirement for antibody response in this animal system.

It is becoming generally accepted that the processing of antigenic information by phagocytic cells is a prerequisite for antibody formation; however, the absence of a viable microflora appears to have little influence on this process. The microbial flora may prime the digestive and migratory efficiency of leukocytes, but no major disadvantage exists for the germfree animal. It thus appears that the immune potential of the nutritionally defined gnotobiote is totally unimpaired.

With continued study of the function and metabolism of the germfree animal, and especially of the germfree gut, a more comprehensive picture starts to appear. In the absence of a microflora, the rate of renewal of mucosal cells decreases; consequently, the activity of brush border hydrolases in the typically "older" mucosal cell is higher. In addition, the digestive enzymes in the lumen of the gut are higher in concentration in the absence of microbial degradation. The absorption of calcium and magnesium appears enhanced, presumably in relation to a higher concentration of intestinal bile acids that occur exclusively in the conjugated form. The fiber content of the diet, especially in the absence of a viable microflora, appears as a major factor in determining intestinal processes and intestinal absorption. In general, the germfree rat, as compared to the conventional animal, shows unchanged rates of absorption of actively transported carbohydrates and carrier-transported amino acids but a higher absorption rate of various B vitamins, most of which appear to be passively absorbed. Also absent in the germfree rat is the bacterial deamination of amino acids and endogenous urea, which, in the conventional rat, leads to a constant recycling of ammonia by way of the portal system. This not only relieves the germfree animal's liver of a continuous process of detoxification, but also reduces the loss of amino nitrogen of protein origin, by way of ammonia or urea, or both, in the urine.

The absence of an intestinal microflora is noticeable far beyond the intestinal tract. In the liver, nicotinamide adenine dinucleotide phosphate–dependent, hexose monophosphate–shunt dehydrogenases, as well as succinic dehydrogenase and xanthine oxidase activities, showed 70 percent of the values found in comparable, conventional rats. Cytochrome oxidase activity, on the other hand, was increased in germfree rats, as were fatty acid synthetase and citrate-cleaving enzymes.

Within the context of these quantitative differences in function and metabolism, the germfree animal is a very useful tool for studying a variety of biomedical problems involved in inflammation. Germfree dogs appear to tolerate both intestinal strangulation and bile peritonitis much better than conventional dogs do, thereby indicating the major importance of the microbial factor in these syndromes. Hemorrhagic (Pfeffer loop) pancreatitis, on the other hand, appears to be as lethal in the germfree as in the conventional dog. Hemorrhagic shock has always been considered equally lethal in germfree and conventional rodents. However, after surgical removal of the enlarged cecum, germfree rats tolerated prolonged hypovolemia much better than their conventional counterparts did. Under such conditions, it appears that the germfree animal has an advantage because of the absence of microbes or endotoxins, or both, which were shown to appear in the circulation of hypovolemic, gnotobiotic rats that were monoassociated with selected orga-

nisms (*3*). In the noncecectomized, germfree rat, this advantage appears to be negated by the enlargement of the cecum.

Because they provide a picture of functional and cellular decline that is unadulterated by invading bacteria, germfree rodents are eminent models in the study of healing wounds and kidney pathology. On the other hand, they make it possible to obtain an even clearer picture of the effect of associated microbes on such functions. The posttraumatic, ischemic kidney appears to have retained its function much better in germfree rats than in conventional rats. It appeared possible, however, to obtain a comparable improvement in kidney function by orally treating conventional rats with a mixture of nonabsorbable antibiotics. These rats, although harboring an adjusted intestinal microflora, also showed other characteristics of germfree rats—such as cecal enlargement and more liquid cecal contents, with elevated colloid osmotic value—indicating reduction or absence of at least some functional aspects of the conventional microflora.

Association of the originally germfree animal with selected pathogens has done much to clarify the etiology of infectious disease. The oral administration of virulent cultures of *Pasteurella haemolytica* to gnotobiotic lambs has resulted in the production of fibrinous pleuritis and pneumonia. This syndrome is similar to cases of neonatal lamb pneumonia that occur in nature. Establishing a tracheostomy and occluding the anterior portion of the trachea prior to the oral inoculation demonstrated that the infection of the lung developed after intestinal invasion and subsequent bacteremia. Monoassociation with *Bacillus cereus* prior to administration of *Pasteurella haemolytica* prevented pneumonia.

It was also shown, however, that germfree mice could harbor large populations of *Shigella* and *Salmonella* species as monoassociates without any apparent ill effect. A similar observation had been made earlier in the case of monoassociation of rats with *Salmonella typhimurium*. It thus appears that a symbiosis between the host and its associated pathogen is possible without loss of pathogenicity when the reisolated bacteria are retested in a previously unchallenged susceptible host.

Germfree mice have been shown to harbor leukemia and mammary tumor viruses that are probably responsible

for specific forms of neoplasms but that do not induce inflammatory disease. However, congenital lymphocytic choriomeningitis can produce a viral carrier state in gnotobiotic offspring and appears to lead to overt degeneration, necrosis, and exfoliation of the epithelial cells of the intestinal villi. Germfree mice also proved more sensitive to Friend virus and responded with earlier development of pathological features such as hepatosplenomegaly. A similar reduced resistance was observed in studies of the intestinal pathology caused by Kilham virus in germfree rats.

The gnotobiote is obviously suitable whenever the study of inflammatory processes requires separation of the microbial variable from the systemic response. The meeting provided an opportunity not only to further define and describe the function and metabolism of the gnotobiotic animal, but also to indicate many of the fields in which this tool can be used to elucidate the numerous facets of the inflammatory response.

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References

1. *Technology in Germfree and Gnotobiotic Life Research*, M. Miyakawa and B. S. Wostmann, Eds. (Academic Press of Japan, Tokyo, 1969).
2. B. S. Wostmann, "The germfree animal as a research tool," in *The Use of Drugs in Animal Feeds* (National Academy of Sciences, Washington, D.C., 1969), pp. 123–134.
3. Monoassociation describes the intentional association with a defined microbial species. See, "Gnotobiotes: Standards and Guidelines for the Breedings, Care, and Management of Laboratory Animals," *Nat. Acad. Sci.–Nat. Res. Counc. Publ. No. ISBNO-309-01858-7* (1970).

Forthcoming Events

August

11–13. **Automatic Control** Joint Conf., St. Louis, Mo. (J. Lewis, Dept. of Electrical Engineering, Pennsylvania State Univ., University Park 16802)

12–17. American **Podiatry** Assoc., Denver, Colo. (Secretary, APA, 20 Chevy Chase Circle, NW, Washington, D.C. 20015)

12–3. Pacific **Science** Congr., 12th annual, Canberra, Australia. (A. Harvey, Australian Acad. of Science, Gordon St., Canberra City 2601)