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GNOTOBIOTIC ANIMALS IN LIFE SCIENCE RESEARCH

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ABSTRACT- Gnotobiotics is the scientific study of animals or other organisms that are raised in germ free environments or ones that contain only specifically known germs. The gnotobiotic laboratory animal is potentially a very valuable tool for investigating any suspected interaction between the host and its associated microflora or between different components of that flora. However, like many other good ideas, the production of gnotobiotes is simple in concept but complicated in execution. In the early stages the greatest obstacles to the general use of germ free animals were the expense and the restricted amount of space that could be maintained free from contaminants. Nowadays, with modern isolators and facilities it is easier to produce gnotobiotic animals at relatively modest price.

Keywords: gnotobiotes, microorganisms, laboratory animal

I. INTRODUCTION

Gnotobiotic animals or Gnotobiote are an animal stock or strain in which only certain known strains of bacteria and other microorganisms are present. Technically the term also includes germ free animals as the status of their microbial communities is also known (Reyniers, 1959). Gnotobiotic animals are derived by aseptic hysterotomy or hysterectomy, embryo transfer or sterile hatching of eggs and are continuously maintained using aseptic technique where the microbial status of the animal is fully defined; includes both germ free and defined flora animals. Animals reared in a gnotobiotic colony are devoid of normal flora, has poorly developed immune systems, lower cardiac output, thin intestinal walls, low antibody titers low metabolism rate and high susceptibility to infectious pathogens (Wostmann et al., 1996). Lower amounts of serum gamma globulins have been observed in germ free animals of several species and the quantity increases on association with microorganisms. Nuttall and Thierfelder are considered pioneers of gnotobiotics and germ free research. Germ free mice have adapted anatomically and physiologically to life without microbes.

Derivation of Gnotobiotic Animals

The production of germ free animals or birds depends upon the fact that embryos developing inside an egg or the mammalian uterus are microbiologically sterile, provided that they come from healthy parent stock. The uterus is removed and passed into the isolator through germicidal dip tank. Once inside the isolator, the uterus is opened and young ones are removed, cleaned and then placed with foster germ free females. Hysterectomy does not eliminate pathogens that may contaminate fetus after uterine implantation or that is vertically transmitted. Vertical transmission of pathogens can be avoided by using embryo transfer (Foster and Slonczewski, 2010).

Birds are relatively easy to be produced germ free. Germ free chicks, turkeys and Japanese quail can be obtained by passing surface sterilised eggs through a germicidal trap into a sterile isolator, where they are allowed to hatch. The fertilized eggs must be obtained from flocks free from microorganisms that invade the egg in the oviduct.

Rats and mice have been bred germ free through many generations; other species have been bred but are not yet available commercially. The guinea pig is well developed at birth and readily takes solid food within a day or two, but the operator must work fast during delivery into the isolator as the fetuses do not survive more than few minutes after removal from the mother.

Isolator Technology in Gnotobiotics

Isolators are enclosures used to create the sterile environment. They must be made of material with an impervious physical barrier; main components are the chamber, air supply, air inlet and outlet, transfer port and gloves; they come as rigid, semi-rigid and flexible film isolators made of plastic or stainless steel. All manipulation of animals and supplies occurs within the chamber via the use of gloves and sleeves that are attached to the isolator walls. The glove is the most vulnerable part of the isolator in terms of contamination potential. The transfer port is the enclosure that provides a transition between the isolator chamber and room environment, used for loading and removing items from the chamber it is

the physical barrier to prevent contamination of the chamber. Isolators have HEPA filtration on air entry and exhaust. Positive pressure is used to prevent introduction of airborne contaminants through any punctures and is maintained when rearing germ free or gnotobiotic animals. If biohazardous agents are used in the incubator, negative pressure should be used. Air exchange rates are usually higher than the animal room some are 30 or more air exchanges per hour inside the chamber. Test the chamber for leakage using the gas leak detection test. The long term success of any gnotobiotic operation depends on the sterilization of the isolator chamber and the equipment/supplies that enter it (Fox et al., 2006).

Care and Maintenance of Gnotobiotic Animals

Mammals must be aseptically derived by hysterectomy or hysterotomy as late as possible before term. Inside the isolator the young are hand fed on a sterilized liquid diet similar in composition to the mother's milk. If the animals are successfully brought to sexual maturity natural breeding can continue in the germ free environment and other strains or even other species may be fostered onto lactating females. Rats and mice have been bred germ free through many generations, other species have bred but are not yet available commercially. Among the larger animals the difficulties of sterile delivery increase with their size. Beagle dogs have been satisfactorily reared and used for physiological experiments. Germ free pigs have been produced for many years, although the problems of handling and caging limit their usefulness beyond the first few weeks of life. Lambs, calves and goats have all been maintained germ free throughout the life.

Terminologies Related To Gnotobiology

1. Germ free (Axenic) animal: It is free of all foreign life forms (e.g., bacteria, viruses etc.) apart from it thought to be hypothetical state because indigenous or heretofore uncharacterized viruses may be integrated into host genome.
2. Defined flora animals: These are maintained in isolated environment and are intentionally associated with one or more known life forms, usually microorganisms.
3. Specific Pathogen Free (SPF): These are animals free from specific pathogens but otherwise have an undefined flora.
4. Restricted flora: Gnotobiote associated with altered Schaedler flora from isolator but is then moved into a maximum barrier room where it can become colonized with additional organisms (but remains free of adventitial pathogens);higher level of SPF.
5. Conventional animal: Animal reared in a room with an unknown microflora and unknown disease status.

Importance of Gnotobiotic Animal

The gnotobiotic principles used in the production of infection free laboratory animals evolved from the efforts to rear and study animals in the absence of microbes or in association with one or more pure cultures of microbes (Wostmann, 1996). The gnotobiotic animal is potentially a very valuable tool for investigating any suspected interaction between the host and its associated microflora or between different components of that flora. The study of nutrition and metabolism is one to which the gnotobiote has made a significant contribution (Coates, 1970). Germfree animals are used in research involving various fields like toxicology, pollution control, autoimmune disorders, drug metabolism, genetic expression and vaccine tests (Van Bekkum, 1968).

Immunology is another obvious field in which gnotobiotes can prove valuable. The recent establishment of nude (athymic) mice in the germ-free state offers an even more specialized research tool. However, absence of microbial associates does not ensure freedom from all exogenous antigenic stimuli because most diets contain dead organisms or other materials likely to provoke an immune response. The ideal experimental medium is the germ-free animal maintained on a chemically defined antigen-free diet. It is important that the diet should satisfy all criteria of nutritional adequacy since deficiency of some essential nutrient could interfere with the normal defence mechanisms or cause tissue damage that might alter the autoantigen pattern. This object appears to have been almost achieved in mice. A water soluble ultrafiltered diet described by Pleasants et al., 1973, supported satisfactory reproduction through several generations of C3H mice, although occasional abnormalities in the quality of their fur were observed. The authors proposed to investigate the effects of addition of further trace elements to the diet.The microbiological status of an experimental animal may be extremely relevant in toxicological studies. Many drugs and non-nutrient food additives are subject to modification by intestinal bacteria (Walker, 1973).

The study of nutrition and metabolism is one to which the gnotobiote has made a significant contribution in recent years (Coates, 1973). It has been shown that components of the conventional microflora synthesize vitamins K and the B complex, degrade amino acids, modify bile acids and sterols and hydrogenate polyunsaturated fatty acids. Whether or not these activities are important to the host's nutrition depends upon factors such as the site of microbial action, the recycling of microbial products

through coprophagy and the extent to which metabolites are bound within bacterial cells. Interpretation of the findings may also be complicated by the modifications in gut structure described above. The enlarged caecum of the germ-free rodent makes it a poor subject for balance studies, particularly with respect to nitrogen, where the accumulation of nitrogenous materials in the caecum could vitiate any measure of nitrogen retention. Indirect effects of the microflora on the host's nutrition, hitherto unsuspected, are becoming apparent. Several workers have found that in the absence of a microflora the requirement for some vitamins is lower. Germ-free guinea-pigs given a diet devoid of ascorbic acid live longer and show less severe scorbutic lesions than do their conventional controls (Levenson et al., 1962). Analogous findings have been reported in chicks deprived of pantothenic acid (Coates et al., 1968) and rats deprived of vitamin A (Bieri et al., 1969). They probably reflect the imposition of a greater 'work load' in the form of detoxification of bacterial metabolites and mobilization of defence mechanisms, with a consequently greater demand on the metabolic pathways in which the vitamins are concerned.

II. CONCLUSION

The gnotobiotic laboratory animal is potentially a very valuable tool for investigating any suspected interaction between the host and its associated microflora or between different components of that flora. However, like many other good ideas, the production of gnotobiotes is simple in concept but complicated in execution. In the early stages the greatest obstacles to the general use of germ-free animals were the expense and the restricted amount of space that could be maintained free from contaminants. Nowadays, with isolators made of plastic film and improved methods of chemical sterilization, gnotobiotic equipment has become available, even for large animals, at a relatively modest price. Further, colonies of germ-free rats and mice have been established in a number of breeding centres and their offspring can be easily transported to the research laboratory. Already the use of germ-free animals has contributed much to our knowledge of the host-microbe relationship but it has also focused attention on their limitations and on some characteristics that must be taken into account when interpreting the results of experiments with gnotobiotes.

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