FISTULATE DIFFUSION CHAMBER AND ITS USE TO STUDY MICROBIAL ASSOCIATIONS in vivo

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A fistulate diffusion chamber intended for the in vivo culture of microbial associations has been constructed. Relations between yeast-like fungi (Candida albicans), staphylococci, and Shigella sonnei and their effect on the host and also the response of the host to their combined action were studied.

KEY WORDS: microbial associations; fistulate diffusion chamber.

The problem of microbial associations is attracting continually increasing attention nowadays. This is because pathogenic and conventionally pathogenic microorganisms exhibit their action on the host organism during infectious processes not alone, but under conditions of complex and diverse relations with various microorganisms living in the unsterile cavities of the human body. The complexity of the problem is evident from the methodological aspect also. As yet there are no techniques or methods whereby the character of relations between the various members of microbial associations of many different species and the mechanisms of their combined action on the host during the development of an infectious process induced by pathogenic and conventionally pathogenic agents can be comprehensively studied [2]. The development of approaches and methods for the study of microbial associations under conditions as close to natural as possible is therefore of the utmost importance.

The fistulate diffusion chamber (Fig. 1) developed by the writers differs from an earlier model [1] in its structure and purpose. It is made from transparent plastic, and its side walls are Millipore membranes. The chamber has two compartments separated by a similar membrane. The outer diameter of the chamber is 34 mm and the inner diameter 30 mm, the length of the outlet tube is 40 mm, the bore of the tube 2 mm, and the thickness of its wall 1 mm. The outlet of the fistula is closed with a rubber stopper secured with aluminum foil in the manner of antibiotic flasks. The chambers are sterilized by boiling in distilled water for 30 min.

A special operation has been devised for suturing the chamber into the peritoneal cavity of rabbits (Fig. 2). Monocultures were grown in a chamber with one compartment and microbial associations in a chamber with two compartments; each member of the association was kept in a separate compartment of the chamber. The pore size (0.22 μ) of the Millipore membrane prevented the microorganisms from entering the peritoneal cavity; only metabolic products of the microorganism could penetrate from one compartment of the chamber into the other and also into the rabbit's body. The presence of the fistula in the chamber enabled material to be collected and growth, reproduction, and biological properties of the microorganisms to be studied periodically.

Experiments were carried out on 24 noninbred rabbits weighing 2-3 kg. The animals were divided into 8 groups (3 rabbits in each group) depending on the strains kept in the diffusion chambers: Groups 1 and 2 contained pathogenic and nonpathogenic staphylococci; group 3) Candida albicans; group 4) Shigella sonnei; group 5) C. albicans and a pathogenic staphylococcus; group 6) C. albicans and a nonpathogenic staphylococcus; group 7) C. albicans and Sh. sonnei; group 8) sugar broth.

The level of staphylococcal antitoxin in the rabbits' blood serum was determined by Vygodchikov's method [3], using staphylococcal toxin obtained from the Gamaleya Institute of Epidemiology and Microbiology. Toxin of batch No. 853, Lh-0.18, was used. Specific antibodies were determined by the agglutination test in dilutions with living cultures of C. albicans, staphylococci, and Sh. sonnei. The degree of sensitization of the animals

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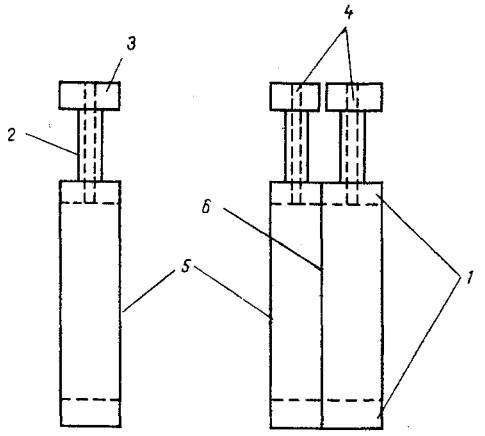

Fig. 1. Fistulate diffusion chamber: 1) body of chamber; 2) fistula of chamber; 3) expanded end of fistula; 4) outlet of fistula; 5) membrane filters forming side wall of chamber; 6) membrane filter between two chambers.

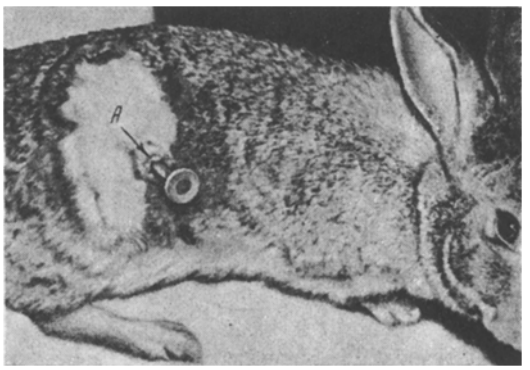

Fig. 2. Rabbit with fistulate diffusion chamber implanted intraperitoneally. A) Fistula of chamber.

to these microorganisms was determined by the blast transformation reaction (BTR) with specific and nonspecific stimulators [4]. A capsule formed around the implanted chamber and the cellular response of the host was studied in it [5]; the behavior of the cell systems reflected the intimate mechanisms of their interaction with the microorganism. The capsules as a whole were fixed in 15% neutral formalin solution. Ordinary histological sections were prepared from pieces of the capsule wall and stained with hematoxylin–eosin and with picrofuchsin by Van Gieson's method for connective tissue. The results were subjected to statistical analysis.

Mutual stimulation of growth and reproduction was observed in vivo between C. albicans and the pathogenic staphylococci or Sh. sonnei. In monoculture, each of these microorganisms grew much less successfully than in association. No mutual stimulation of growth and reproduction was observed in the association of C. albicans and the nonpathogenic staphylococcus. In associations of pathogenic staphylococci and Sh. sonnei the plasma-coagulating and hemolytic activity was increased. The nonpathogenic staphylococcus under these circumstances acquired the ability to produce hemotoxin.

The antibody level in the animals' blood serum was reduced by half in those animals whose fistulate chambers contained microbial associations compared with the control in which monocultures were used. No difference in the degree of sensitization of the host to the microorganisms could be detected in the experimental and control animals. The highest percentage of blast transformation was observed on the 5th-6th day after implantation of chambers containing microbial associations or monocultures into the rabbits.

Three layers could be clearly distinguished in the capsule formed around the implanted chamber. The composition of the cells in them depended on the species of microorganism in the chamber. In the case of a mixture of cultures, namely C. albicans in association with staphylococci and with Sh. sonnei, the particular features characteristic of each species of microorganisms were to some extent cancelled out, but they nevertheless remained perceptible. In all experiments with microbial associations in the chamber the changes in the wall of the capsule were more marked than when the chamber contained a monoculture. These results are described for the first time.

The fistulate diffusion chamber as devised by the writers, when implanted into the peritoneal cavity of animals, can thus be used to study not only interaction between microorganisms under conditions as close as possible to natural, but also the response of the host to the combined action of microbial associations.

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EXPERIMENTAL PRODUCTION AND REARING OF GERMFREE MINIATURE PIGLETS FOR MEDICAL AND BIOLOGICAL RESEARCH

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A method of obtaining and rearing germfree miniature piglets for medical and biological research is described. The germfree animals were reared in strict isolators made from transparent plastic until the age of 3 months. They were fed on a milk diet with the addition of vitamins and salt. They gained in weight satisfactorily throughout the period of observation.

KEY WORDS: germfree technology; germfree miniature piglets.

With the development of methods of obtaining germfree animals and of gnotobiology, the research worker can now control the microbial factor in his experiments [3, 4, 9]. Germfree piglets in which, because of the chorion-epithelial type of placenta in pigs, immunoglobulins and antibodies of maternal origin are absent [7, 10-12], are particularly valuable for immunobiology and infectious pathology. From the morphological and functional points of view pigs also have several common features with man, so that they are being used on an increasing scale in the simulation of human diseases [6, 8, 13].

Considering the great importance of this biological object, it was decided to attempt to develop methods of obtaining and rearing germfree miniature piglets.

APPARATUS

To provide germfree conditions for obtaining and rearing piglets gnotobiological isolators were used; these were made by the authors' own design from isotope boxes of Soviet manufacture.

To obtain germfree piglets the hysterotomy method was used. The advantages of this method are the complete exclusion of contact between the fetuses and the surrounding unsterile environment and reduction of

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