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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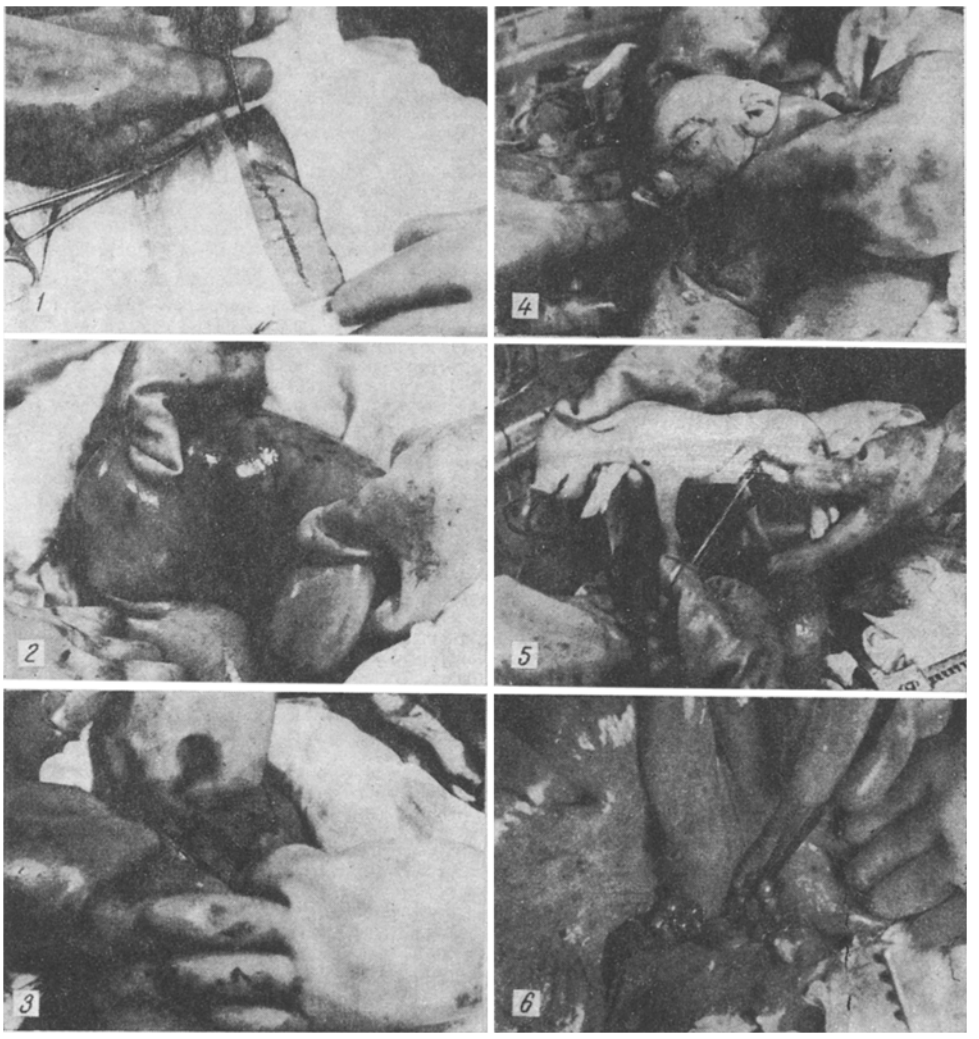

Fig. 1. Hysterotomy on a pig (stages of the operation). 1) Incision of operating film and skin by thermocautery; 2) exteriorization of the gravid uterus; 3) digital widening of the incision in the uterine wall; 4) extraction of a fetus; 5) division of umbilical cord between two clips; 6) inspection of ovaries to confirm complete removal of fetuses.

the risk of asphyxia. For this purpose an operating isolator was devised on the basis of the 10 BP2-OS box. A feature of the design of the isolator is the operating window in the floor of the chamber, 25 cm in diameter, hermetically closed with elastic film of the Saran type. The manipulation isolator is made from a 2 BP-2OS isotope box. The two types of isolators can be joined together by a locking system and they are equipped with a system of sterile air exchange with inlet and outlet filters. The manipulation isolator is equipped with a semiautomatic heating system and a bactericidal drainage system to enable the isolator to be washed and cleaned during use.

Before work the isolators are sterilized by spraying with an aerosol of 2% peracetic acid. After the isolator has aired for a few days tubes containing swabs and nutrient media are introduced. Microbiological control of sterility of the isolator consists of seeding the washings on nutrient media followed by incubation at 37°C and exposure of open solid and liquid nutrient media in open vessels within the isolator chamber. If the results of the seedings are negative, the isolator is considered to be ready for use.

SELECTION AND PREPARATION OF ANIMALS FOR OPERATION

Miniature pigs of the Gottingen and Novosibirsk strains, reared at the Miniature Pig Farm of the Research Laboratory of Experimental Biological Models, Academy of Medical Sciences of the USSR, were used. Sows at the 113th day of pregnancy were chosen for the operation. Their abdominal surface was painted with

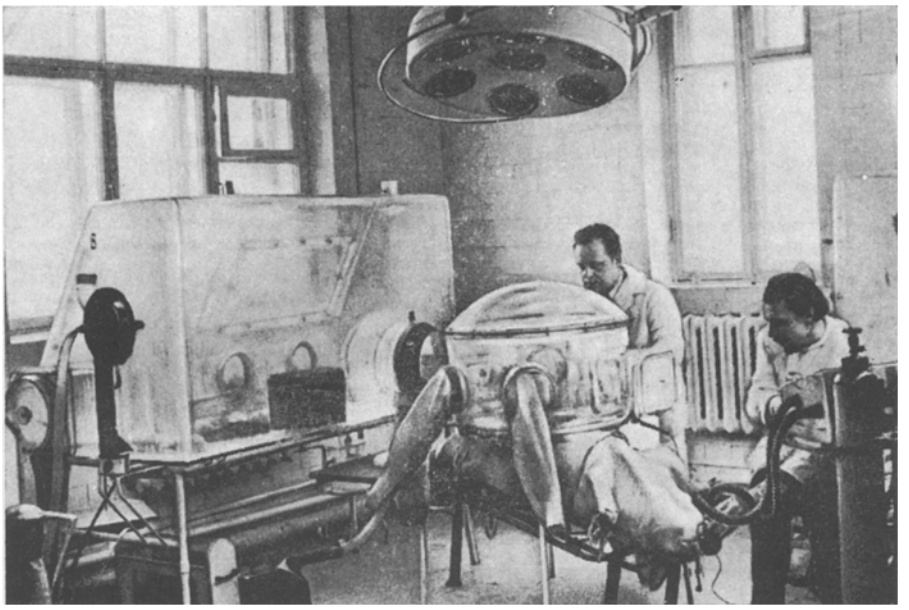

Fig. 2. Hysterotomy on a pig (general view of the operation).

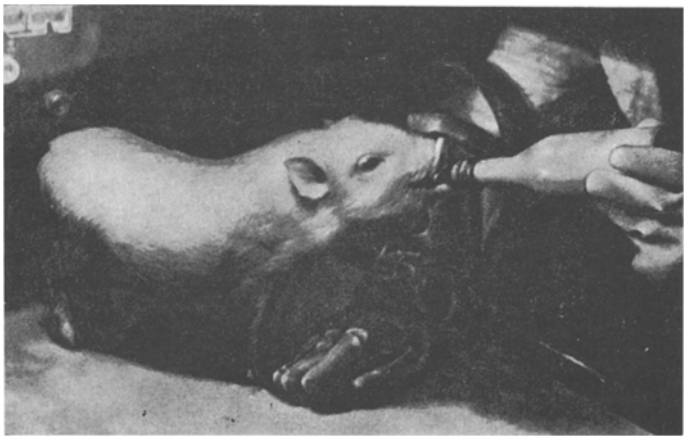

Fig. 3. Germfree piglet in isolator.

0.1% detergent solution (sterile Novost powder), the hair was removed by means of a depilator, and the animal's skin was then washed with a warm 0.25% solution of ammonia water. The skin was then painted three times with 5% tincture of iodine and then dried with a swab soaked in alcohol and ether.

Two types of general anesthesia were used. For intravenous anesthesia the method of neuroleptanalgesia [2] was used. For this purpose, 2-5 ml droperidol was injected into the marginal vein of the sow's ear, after which fentanyl was injected intravenously, slowly, in a dose of 3-5 ml, after an interval of 5-10 min. Meanwhile 1 ml of 0.1% atropine was injected intramuscularly. Gas anesthesia by the NAPP-1A apparatus also was used. For this purpose, after intramuscular injection of atropine and intravenous injection of droperidol (the doses as before), a gas mixture consisting of oxygen and nitrous oxide, with the addition of Narcothane* (Czechoslovakia) vapor was administered to the animal through a mask. In the initial stage of anesthesia the gas mixture consisted of 25% oxygen and 75% nitrous oxide, but later the quantity of oxygen in the mixture was increased to 60%.

COURSE OF THE OPERATION

The anesthetized animal was fixed to the operating table in the supine position beneath the operating isolator. The skin surface and film of the isolator facing it were treated with 88N glue, and after it had dried a little the operating table was raised, so that the film adhered to the skin.

*Equivalent to halothane.

The film and adherent skin were incised by means of a thermocautery in the midline of the abdomen from within the chamber for a length of about 15 cm. The subcutaneous areolar tissue was separated with a scalpel. Along the course of the incision the two large branches of the mammary vein were divided between hemostats. A grooved probe was inserted beneath the aponeurosis of the abdominal wall and the peritoneal cavity opened with a scalpel. A retractor was introduced into the wound and the loops of intestine were pressed aside by means of a liver speculum. Both cornua of the uterus were brought out of the wound separately and the fetuses extracted through an incision in the uterine wall. The membranes were quickly removed from the fetuses, the umbilical cord clamped with two obstetric clips, and divided with scissors between them. Inspection of the uterus and ovaries confirmed the complete removal of the fetuses. The largest fetuses were found in the proximal part of the cornu (Figs. 1 and 2).

Through the locking system the fetuses were transferred to the manipulation isolator. They were carefully dried with gauze towels in the isolator. By means of a rubber suction bulb mucus was removed from the upper respiratory passages. After treatment, the fetuses were placed on warm gauze bedding. The canine teeth were extracted from the newborn piglets, and 1 h later they began to feed.

REARING THE GERMFREE PIGLETS

The piglets were kept in the isolator until the age of 3 months. During the first few days the temperature in the isolator was kept at 30-33°C, after which it was gradually lowered in the course of 2-3 weeks to room temperature (20-25°C).

Preserved sterile condensed milk without sugar (fat content 7.8%) was used to feed the germfree piglets for the first 3-4 weeks. The cans of milk were kept for 3 days beforehand in an incubator at 37°C. If any sign of blowing developed, the cans were rejected. The milk tested in this way was sterilized in an autoclave at 121°C for 10 min. The cooled cans were sterilized in the lock of the isolator with 2% peracetic acid and passed into the chamber. The mixture of vitamins and salt was autoclaved at 121°C for 25 min. To 1 liter milk, 1 ml of the mixture of vitamins and 5 ml of salt were added. The composition of the salt solution per liter distilled water was (in grams): $FeSO_4 \cdot 7H_2O$ 5, $MnCl_2 \cdot 4H_2O$ 3.5, $CuSO_4 \cdot 5H_2O$ 4, KI 0.25. The mixture of vitamins contained (in mg): thiamine 12.5, riboflavin 3, pyridoxine 1.5, pantothenic acid 15, and vitamin B_{12} 0.25. Starting from the age of 1 month, the piglets were given a semiliquid oatmeal porridge with milk, with the addition of vitamins and salts according to the formula given above. Until the age of 2 weeks the piglets were fed by hand from a bottle 8 times a day. After the age of 2 to 3 weeks, the piglets learned to feed themselves. The supply of milk per piglet per day averaged: in the 1st week 110 ml, the 2nd week 160 ml, the 3rd week 353 ml, 4th week 533 ml, and 5th week 550 ml.

Throughout the period of observation the piglets were active and put on weight well (Fig. 3). For instance, on removal from the uterus the mean weight of the piglets was 250 g, at the age of 1 month it was 2 kg, at 2 months 4-5 kg, and at 3 months 6-7 kg.

Microbiological control tests were carried out daily. The recent excreta of the animals, samples of food, water, and bedding, and washings from the body surface of the animals and the walls of the chamber were tested. The material was removed from the isolator and seeded on liquid and solid nutrient media (nutrient broth, blood agar, universal medium for aerobic bacteria and fungi, Sabouraud's medium, etc.). The material was incubated under aerobic and anaerobic conditions. The final conclusion regarding sterility was given at the end of 3 weeks.

By means of the method described above we obtained germfree miniature piglets for the first time in the Soviet Union [1]. Altogether eight hysterotomy operations had been performed and 52 germfree piglets reared. The germfree animals have been used in combined investigations for the study of permeability of the intestinal barrier to Escherichia coli and reactions of nonspecific resistance, as well as special features of the wound process. Germfree miniature piglets have been obtained by our recommended method at the Moscow Veterinary Academy for research in the field of veterinary medicine [5]. Experience so far gained indicates that the methods described above, using materials and apparatus of Soviet origin, are suitable for the production and use of germfree piglets in medical and biological research.

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