PATHWAYS OF PENETRATION OF *Escherichia coli* 055 THROUGH THE INTESTINAL WALL IN GERMFREE AND ORDINARY ANIMALS

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Permeability of the intestinal barrier in germfree animals for bacteria of the enteropathogenic strain *Escherichia coli* 055 was studied. Germfree and ordinary rats and guinea pigs were used. During the first 24 h after peroral infection with *E. coli* 055 an increase in the bacteriemia was observed in the germfree animals, whereas in the ordinary animals the bacteriemia was transient in character. Electron-microscopic investigation of the intestinal mucosa of the germfree animals revealed disturbances of intercellular contacts and the formation of spaces containing numerous microorganisms between the enterocytes. In the germfree animals more marked changes also were found in the microvessels of the mucosa. In ordinary animals ingestion and digestion of the *E. coli* cells by enterocytes and leukocytes was observed. Disturbances of the intestinal barrier discovered in the germfree animals explain the increased bacteriemia in these animals in the absence of a microflora. The results point to the important role of the microbial factor in the formation of the intestinal barrier.

KEY WORDS: germfree animals; monocontamination by E. coli; bacteriemia; intestinal barrier; phagocytosis.

The mechanisms of penetration of enteropathogenic bacteria through the intestinal wall and also the effect of the accompanying microflora on the state of the intestinal barrier have not yet been studied. New opportunities for the study of these problems are provided by the gnotobiological approach [2, 4, 7]. Experiments were carried out on germfree animals to study how the permeability of the intestinal barrier for microorganisms of the enteropathogenic strain *Escherichia coli* 055 depends on the accompanying microbial factor, and also to study the ways of penetration of microorganisms through the intestinal wall.

EXPERIMENTAL METHOD

Germfree and ordinary guinea pigs aged 2 weeks and Wistar rats aged 3 months were used. The germfree guinea pigs were obtained in an operative isolator by hysterotomy [1]. Germfree rats were obtained from the French center for laboratory animals in Lyon (IFFA-Credo). The germfree animals were reared in isolators of Soviet manufacture, made from transparent plastic, and in French isolators made from polyvinyl chloride and equipped with lock of the DPTE type (from Celster, Paris). The guinea pigs were fed with semiliquid diet of type L-477 and the rats on a diet of type L-474E12 [11]. The diet was sterilized in a vacuum autoclave at 121°C for 25 min. In the course of the gnotobiological experiments the ordinary microbiological control was set up [13].

The animals were contaminated with enteropathogenic strain *E. coli* 055 per os with the aid of a plastic catheter. The dose of microorganisms was 10 billion bacterial cells in 1 ml. Blood from the heart was taken by means of a syringe 30 min and 2, 4, and 24 h after contamination and seeded on Endo's agar. The degree of bacteriemia was assessed from growth of colonies of *E. coli* 055 on agar, calculated per milliliter of blood.

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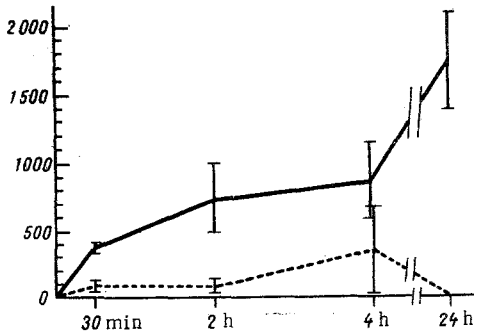

Fig. 1. Dynamics of E. coli 055 bacteriemia in germfree and ordinary guinea pigs. Continuous line, germfree guinea pigs; broken line, ordinary guinea pigs.

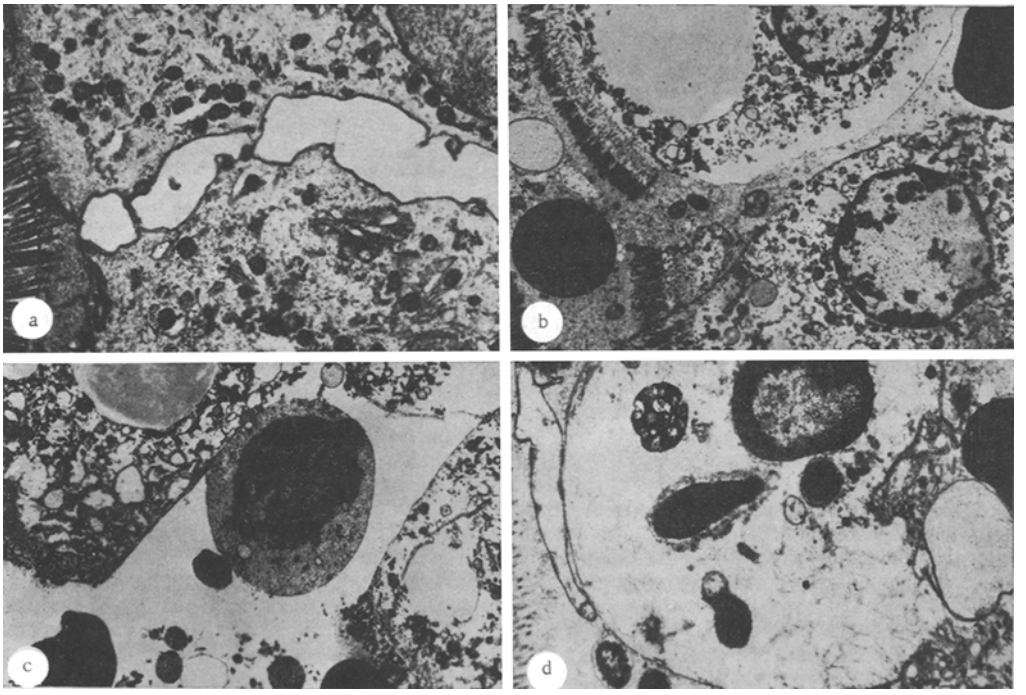

Fig. 2. Small intestine of germfree guinea pig with enteritis caused by E. coli 055: a) complete disturbance of intercellular contacts between adjacent enterocytes without penetration of microorganisms and blood cells into space thus formed (10,000×); b) bodies of E. coli cells and erythrocytes can be seen in space formed as a result of total disturbance of intercellular contacts between adjacent enterocytes. Edema and destruction of organelles present in enterocytes (13,000×); c) lymphocyte and erythrocytes in zone of complete disturbance of intercellular contacts between enterocytes (10,000×); d) E. coli cells visible in digestive vacuoles of neutrophilic granulocyte in lumen of intestine (13,000×).

An electron-microscopic investigation was made of areas of the proximal and middle portions of the small intestine with macroscopic evidence of inflammation 24 h after contamination. Pieces of tissues were fixed in the cold by a modified method of Palade (1962), dehydrated in alcohols and acetone, and then embedded in Araldite. Ultrathin sections were cut on the LKB Ultrotome, stained with uranyl acetate and lead citrate by Reynolds' method, and examined in the JEM-7A electron microscope.

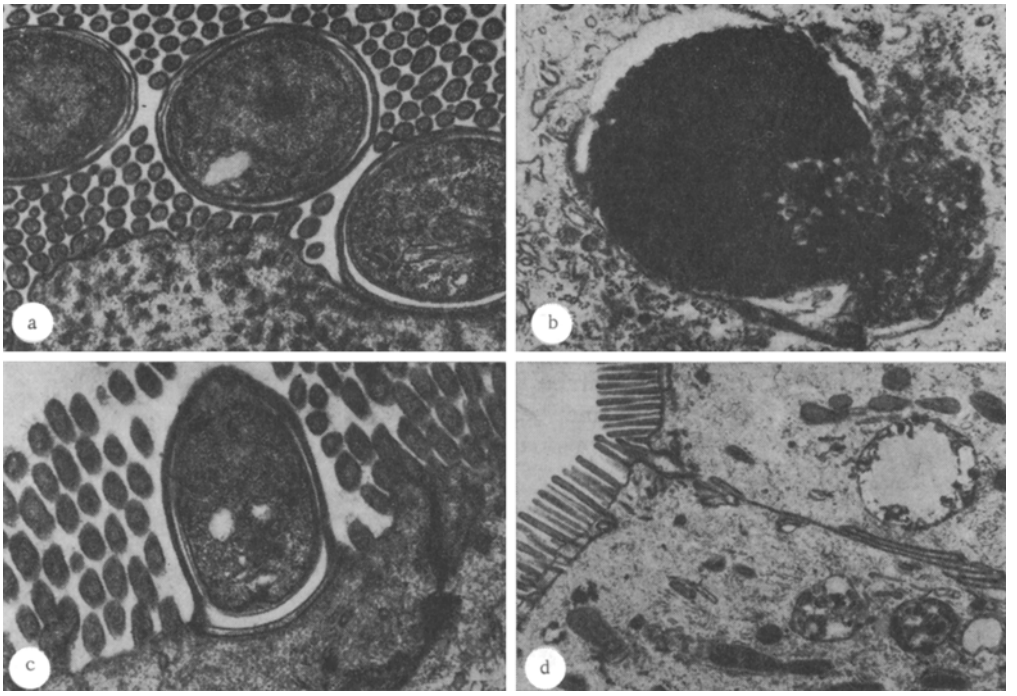

Fig. 3. Small intestine of ordinary guinea pigs infected with E. coli 055: a) E. coli cells on surface of enterocyte among microvilli; one is in close contact with cell surface (30,000×); b) digestive vacuole in cytoplasm of enterocyte containing semi-digested body of microorganism (30,000 ×); c) close contact between E. coli cell and surface of enterocyte (30,000×); d) digestive vacuoles in cytoplasm of adjacent enterocytes, intercellular contacts well preserved (10,000×).

EXPERIMENTAL RESULTS

The bacteriological investigation of the germfree guinea pigs and rats showed a similar picture of increasing bacteriemia, whereas in the ordinary animals the bacteriemia was transient in character (Fig. 1).

The electron-microscopic study of the germfree guinea pigs and rats showed analogous pathological changes in the ultrastructure of the enterocytes. The changes in the germfree animals were more marked than in the ordinary animals (Fig. 2). Swelling, fragmentation, and also complete separation and destruction of the microvilli, intracellular edema, and projection of adjacent areas of cytoplasm of the enterocytes into the lumen of the intestine were observed. Large vacuoles with homogeneous contents were frequently found in the ground substance of the cytoplasm of many enterocytes. Many components of the endoplasmic reticulum were widened and partly destroyed. Migration of chromatin was observed in the nuclei of the enterocytes toward their membranes.

Severe disturbances of intercellular contacts also were found in the germfree animals, with the formation of channels between the enterocytes. Escherichia coli cells, erythrocytes, and leukocytes were present in the spaces of these channels. Signs of phagocytosis of bacteria by leukocytes were observed in these spaces and in the lumen of the intestine. The phagocytic activity of the leukocytes was stronger in ordinary animals. In the germfree animals E. coli cells also were detected in the thickness of the mucous membrane close to dilated microvessels. In the germfree animals marked changes were observed in the microvessels of the mucosa, suggesting that E. coli cells penetrated into them.

Under similar conditions hardly any disturbance of the intercellular contacts was found in the ordinary animals. Bacteria were observed on the surface of the enterocytes. Phagosomes with semidigested E. coli cells were frequently seen in the cytoplasm of these cells (Fig. 3). The same picture of complete phagocytosis also was observed in the leukocytes of ordinary animals. The response of their microvessels was weaker and the ultrastructures were better preserved. No E. coli cells could be seen in the thickness of the mucosa. A comparative investigation thus showed that the ultrastructures of the epithelium and microvessels of the intestine are more vulnerable in animals without preliminary contact with a microflora.

These data showing increased permeability of the intestine of the germfree animals for enteropathogenic bacteria are in agreement with observations by other workers [5, 12]. However, in their investigations the barrier-fixing function of the intestine was not taken into account and the increased permeability was attributed to more intensive proliferation of bacteria in the intestine. Only primary penetration of bacteria into the lymphatic system was described in this case.

The experiments showed that the mechanisms of penetration of enteropathogenic bacteria through the intestinal epithelium differ in principle in germfree and ordinary animals; in the writers' view this difference is connected with the development of physiological inflammation in ordinary animals [10]. As a result of constant contact with the microbial environment a series of morphological and functional adaptations develops in the intestine, including the development of connective tissue, thickening of the mucosa on account of hyperplasia of the cells, the formation of biologically active products (such as histamine, etc.), an increase in the tone of the smooth muscle of the intestine and its blood vessels, activation of various enzymes, increased synthesis of DNA, and the more rapid renewal of the enterocytes. The absence of these changes, which are adaptive and defensive in character, is one of the clearest distinguishing features of germfree animals [2]. The enterocytes and microvessels of the intestine in germfree animals are evidently less resistant to the action of enterotoxins and enzymes of bacterial origin, so that considerable destruction of enterocytes and disturbance of their contacts take place.

With these changes in mind, the most likely way for bacteriemia to arise in germfree animals after contamination is through primary penetration of the bacteria along intercellular spaces and hematogenous spread through the microvessels. In the case of incomplete phagocytosis, transport of bacteria by the leukocytes is also possible. Meanwhile in ordinary animals ingestion of bacteria by the enterocytes as in phagocytosis is predominant. Secretory immunoglobulins and coproantibodies present in the lumen of the intestine of ordinary animals may act as opsonizing factors, facilitating the ingestion of bacteria by the intestinal cells under these circumstances.

The deficiency of opsonizing factors (primarily antibodies) in germfree animals leads to the characteristic depression of the phagocytic activity of their leukocytes and reticuloendothelial system [8, 9]. The lower level of endocytosis observed in germfree animals also agrees with the concept of the inhibitory action of cyclic AMP on phagocytosis, having regard to the fact that in germfree animals the production of this cyclic nucleotide may be increased in response to microbial action [3, 6].

The results, on the whole, are evidence of the important role of the microbial factor in the formation of the intestinal barrier and they shed light on the pathways of generalization of E. coli infections when the barrier is disturbed.

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