EXPERIMENTAL DIARRHEA INDUCED IN MICE BY PERORAL INFECTION WITH AN ENTEROTOXIGENIC STRAIN OF *Escherichia coli*

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Attempts to reproduce colibacillosis by peroral infection of mice with classical enteropathogenic strains have often yielded contradictory results. Some workers consider that adult mice are unsuitable for the study of enteropathogenic properties of *Escherichia coli* [6], whereas others have successfully reproduced the characteristic pathological changes in the intestine of these animals by experimental infection with *E. coli* [2]. Another cause of this contradiction is that the same strain of *E. coli* (and its toxin) can give both negative and positive results, depending on the species of experimental animal [4, 5]. There is as yet virtually no information in the accessible literature on the use of adult mice as models for the study of the agents of enterotoxigenic human diarrheas. The possibility of reproducing escherichioses in random-bred mice by peroral infection is mentioned only in a few publications [3]. However, the authors cited did not indicate what serotypes of escherichias were studied, which makes interpretation and comparative analysis of their results difficult. Some investigations have been devoted mainly to the choice of tests for detecting enterotoxins of different strains of *E. coli* isolated from patients for diagnostic purposes, but not tests for studying mechanisms of pathogenesis of the disease itself [8, 10].

The aim of this investigation was to study colibacillary diarrhea caused by enterotoxigenic strain H-10407, isolated from a patient, in conventional adult noninbred and inbred mice belonging to a Soviet collection, by peroral infection. The aim of the investigation was to discover how the character and degree of manifestation of the disease depend on the infecting dose and the times of investigation.

EXPERIMENTAL METHOD

A culture of international strain H-10407 of *E. coli*, serotype 078:HII, possessing CFA/I factor, nonspecific for mice, and forming thermolabile and thermostable enterotoxins [9], was used. Two series of experiments were carried out on male mice aged 1.5-2 months. In series I 96 random-bred mice were used, in series II 30 BALB/c Sto mice.

The animals were infected perorally with different doses of a 24-h culture of *E. coli* H-10407, suspended in 0.62 ml of physiological saline. Since the clinical picture of the disease in the experiments of series I was reproduced with doses starting with $12 \cdot 10^9$ bacterial cells, in the experiments of series II the minimal infecting dose was this same dose, whereas the maximal dose was limited to $60 \cdot 10^9$ bacterial cells, for BALB/c Sto mice are known to be highly prone to diarrhea [1]. The mice were killed under ether anesthesia 1 and 2h and 1 and 2 weeks after infection. To reveal pathological changes in the intestinal mucosa, native preparations of the mucosa were examined under a binocular loupe. To assess the course of the macroscopic changes in the intestine and of the clinical symptoms of the disease, a semiquantitative method (a system of crosses) was used. To monitor the bacteriemia, blood from the heart was seeded on nutrient broth.

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EXPERIMENTAL RESULTS

In the experiments of series I in which random-bred mice were infected with a dose of $1 \cdot 10^8$ bacterial cells, no clinical signs of the disease could be detected during the next 2h. At autopsy on these same animals after 1 and 2h, generally similar pathological changes could already be detected macroscopically: the mucosa was edematous and hyperemic. These manifestations decreased in intensity in the direction toward the large intestine. No formed stools were present, and a large quantity of yellow fluid was found in the lumen of the intestine.

The clinical picture of the disease was observed after 1 week, but only after infection with a dose of $12 \cdot 10^9$ bacterial cells or more (Table 1). Definite signs of diarrhea were observed only when the infecting dose was increased to $36 \cdot 10^9$ bacterial cells or more. At autopsy on these animals pathological changes could also be observed in the large intestine (Table 2).

With a further increase in the dose of the bacteria to 72, 84, and $96 \cdot 10^9$ bacterial cells there was little change in the clincal picture. Nevertheless, on macroscopic examination an increase in pallor and edema of the intestinal mucosa was noted, and the latter became glass-like in appearance.

Two weeks after infection the general symptoms of the disease in the random-bred mice were greatly decreased (Table 1). Clinically the mice appeared healthy, active and ate well. However, on macroscopic examination the changes in the intestine described in the earlier stages were still present as before (Table 2).

In the experiments of series II, the characteristic disease developed in response to infection in all inbred mice; its severity was independent of the dose of infecting material administered (Table 1). Marked diarrhea was observed in all the mice. Pathological changes were discovered in the intestinal mucosa very early in the disease. After only 1-2h after infection severe hyperemia and accumulation of much mucus were observed in all parts of the intestine. These changes increased in severity and by the end of the first week of the disease they were complicated by the development of microerosion (Table 2).

Control blood cultures in all the cases studied were negative, evidence of the absence of both very early and late bacteriemia in the pathogenesis of this disease.

The results thus indicate that experimental colibacillary diarrhea can be produced in mice with enterotoxigenic strain *E. coli* H-10407. Comparative analysis showed that the more severe clinical picture of the disease, with destructive processes (including the formation of erosions) in the mucosa of the small intestine, is characteristic of BALB/c Sto mice, evidence of their greater sensitivity to infection by the particular strain tested than that of random-bred mice. This strain was isolated from man, but nevertheless it causes enterocolitis in mice with a definite diarrhea syndrome, but does not end fatally, unlike enterocolitis caused by murine toxigenic strains, whcih cause death of these animals after peroral infection [11]. The severity of the disease in these cases can be explained by the species-specificity of those strains that are able to colonize the mucosa of the mouse intestine. Reproduction of colibacillary diarrhea by strain *E. coli* H-10407, which is not characteristic of mice and, consequently, may not be invasive and may not colonize the intestinal mucusa of these animals when introduced into them in physiological saline, and not in culture fluid containing their metabolic products, may be linked with the appearance of toxic substances, primarily as a result of gradual destruction of the inoculated bacteria, for we know that 90% of enterotoxins are obtained by modern methods from cell lysates [7].

It must be pointed out that the times of development of the clinical picture did not correlate with the times of development of pathological changes in the intestinal mucosa: the latter were found during the first 2h after infection, before the appearance of the first clinical symptoms of the disease, and they were still present 2 weeks after disappearance of the symptoms. The contradictory or even negative results obtained by some workers attempting to product colibacillary infection in adult mice can perhaps be explained on the grounds that they used strains of *E. coli* which were nontoxigenic and unable to colonize the mouse intestine [6].

Our results are evidence that adult mice can serve as a convenient model (availability, ease of breeding, existence of pure lines) with which to study the pathogenesis of colibacil-

TABLE 1. Trend of Clinical Manifestations of Experimental Colibacillary Diarrhea in Mice Depending on Dose and Time after Peroral Infection with Enterotoxigenic Strain *E. coli* H-10407

Infecting dose, x 10^9 bacterial cells	Random-bred mice		BALB/c Sto mice
	1 week	2 weeks	1 week
12	++	—	++++
24	++	—	++++
36	+++	—	+++ +
48	+++	—	++++
60	+++	—	++++
72	++++	—	n.s.
84	++++	—	»
96	++++	—	»

Legend. —) Symptoms of disease absent; +) animals hunched, curled into a ball; ++) the same, rapid breathing; +++) the same, animals apathetic, move with difficulty, eyes half closed, cyanosis in some cases, fur untidy, tail soiled with pale yellow feces; ++++) the same, eyes closed, some mice lying down, tail soiled with white feces. Here and in Table 2: n.s.) not studied; for each dose and time of infection six mice were used.

TABLE 2. Trend of Macroscopic Changes in Intestine of Mice with Experimental Colibacillary Diarrhea Depending on Dose and Time after Peroral Infection with Enterotoxigenic Strain *E. coli* H-10407

Infecting dose, 10^9 bacterial cells	Random-bred mice		BALB/c Sto mice
	1 week	2 weeks	1 week
12	+	+++	++++
24	+	+++	++++
36	++	+++	++++
48	++	+++	++++
60	++	+++	++++
72	+++	+++	n.s.
84	+++	+++	»
96	+++	+++	»

Legend. —) Macroscopic changes absent; +) intestine abundantly filled with mucous contents, changes present in mucosa; ++) the same, foci of hemorrhages seen occasionally in mucosa, villi only slightly changed (slightly edematous, their pattern undisturbed), mucosa of jejunum pale, of ileum hyperemic, edema of mucosa of large intestine; +++) intestine abundantly filled with unformed whitish contents, intestinal wall flaccid, friable, and highly edematous, villi wide and flattened, peritoneum hyperemic, Peyer's patches enlarged; ++++) the same, microerosions visible in small intestine.

lary diarrhea induced by enterotoxigenic strains of *E. coli*, and also for the elaboration of methods of prevention and treatment of colibacillosis. Mice of the BALB/c Sto genotype are the most sensitive model in this case.

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HEMOBACTERIAL AGGLUTINATION: A METHOD OF DETERMINING ANTIERYTHROCYTIC ANTIBODIES

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The development of a method of detecting antierythrocytic autoantibodies which is simple in use and more sensitive than the Coombs' test, now widely used in immunohematology, is important as a means of making the diagnosis of autoimmune hemolytic anemia (AIHA) more effective, and for use in isoserologic practice and related fields. A positive direct Coombs' test is observed in most patients with AIHA [10]. However, this test in some such patients is negative at the height of the disease because of destruction at this period of the erythrocytes on which most of the antibodies are fixed, whereas the quantity of antibodies on cells remaining undestroyed during the period of crisis is insufficient for detection by the Coombs' test [5]. The test may be negative in chronic cases of the disease, pursuing a sluggish course, when too few autoantibodies likewise are present on the surface of the erythrocytes [4]. A similar situation may arise in the case of incompatible blood transfusion, and also of pregnancy incompatible with respect to erythrocytic antigens.

Attempts to increase the sensitivity of determination of antierythrocytic antibodies has frequently been made [8, 9]. Among the suggested methods, one of the most sensitive is the aggregate-hemagglutination test [1]. However, it is relatively laborious and the agglutination obtained is fine-grained.

A test for antierythrocytic antibodies is suggested in this paper and its sensitivity is compared with that of the Coombs' test. Coagglutination of erythrocytes sensitized *in vivo*

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