THE EFFECT OF HYPOVITAMINOSIS C ON THE COURSE OF EXPERIMENTAL INTESTINAL INFECTIONS IN MONKEYS

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Experimental investigations have shown that with a deficit of vitamin C the permeability of the connective tissue ground substance to microbes decreases [9]. Hypovitaminosis C leads to weakening of the defensive humoral and cellular functions of the organism, diminishing the processes of antibody formation and the phagocytic activity of leukocytes [3]. According to the data of a number of authors, in a whole series of infectious diseases that have clinically mild courses in normal animals, under conditions of hypovitaminosis C a servere infectious process develops, with a lethal outcome [4—8].

There are also reports that, in association with vitamin C insufficiency, the natural resistance of the animal organism to infection decreases. Thus, according to the data of V. P. Davydov [2], in guinea pigs with hypovitaminosis C, inoculated with enteropathogenic serotypes of intestinal bacilli, there develops a manifest infectious process—colienteritis, while in the animals receiving a normal diet the disease is absent.

It was shown [1] that in monkeys—carriers of Flexner's dysentery bacteria, experimental hypovitaminosis C is accompanied by the development of a severe form of dysentery.

In previous investigations, we established that inoculation of monkeys with Schottmüller's paratyphoid B bacteria and enteropathogenic intestinal bacilli did not always give rise to clinically manifest illness. In those cases where it was successful, the infection took a mild form, terminating in complete recovery of the animal.

The purpose of this work was to elucidate the degree of susceptibility to infection, and the course of the infectious processes, in monkeys inoculated with paratyphoid B bacteria and enteropathogenic strains of intestinal bacilli under conditions of hypovitaminosis C.

EXPERIMENTAL METHOD

The investigation was carried out on 24 Macacus rhesus monkeys, 2—4 years of age. Hypovitaminosis C was obtained by maintaining the monkeys for a long time on a diet devoid of vitamin C. For 11 of the monkeys, over a course of 3 months, the following diet was set: bread, cereal, boiled egg, boiled milk, sugar, and A and group B vitamins. The total calorie content of the products fed to the monkeys deprived of vitamin C was the same as for the monkeys maintained on the normal diet.

Twice before initiation of the feeding we determined the concentration of vitamin C in the serum. Subsequently, the serum was studied once every 2-3 weeks. The concentration of vitamin C was determined by the macromethod of Bassi.

Daily observations were performed on all the experimental monkeys for their general condition, and we also daily inspected the skin and mucous membranes measured the temperature, weighed the animals, performed clinical investigations on the blood and carried out bacteriological investigations of the feces.

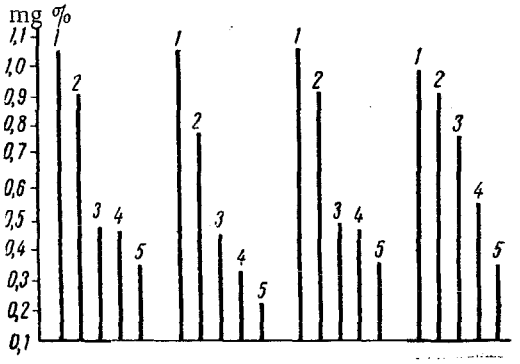

Fig. 1. Concentration of vitamin C in the sera of 4 monkeys inoculated with paratyphoid B bacteria and a strain of intestinal bacillus.

EXPERIMENTAL RESULTS

According to the biochemical investigations of the blood in the monkeys, the concentration of vitamin C gradually decreased. Fig. 1 shows the results of vitamin C determinations in the sera of 4 monkeys. From the data presented, it is apparent that after $2\,^1/_2$–3 months the level of vitamin C decreased significantly—from 1.05–0.35 mg%.

After 2 months, in 6 monkeys maintained on the scorbutogenic diet, we noted that the gums were friable and bled on light touch. At the end of the third month, these symptoms were observed in 10 monkeys. In 3 of the animals, in this period, there were skin and subcutaneous hemorrhages. Almost all the monkeys lost 600–800 grams of weight. In 5 animals, the blood showed a manifest erythropenia (down to 1,500,000–2,000,000 erythrocytes per mm^3). Externally, the monkeys appeared considerably weakened: after 2 or $2\,^1/_2$ months, out of 14 animals with hypovitaminosis C, 3 contracted an acute form of dysentery and died. On autopsy, in addition to changes characteristic for dysentery (colitis), we also noted signs inherent to hypovitaminosis C: gingivitis, diapedetic hemorrhage in the stomach, large and small intestine.

After 3 months, 11 of the hypovitaminotic monkeys and 10 of the monkeys maintained on normal diet (control group) were inoculated with paratyphoid B bacteria and enteropathogenic intestinal bacilli of the serotype 0111:B4. In this case, the animals were divided into 2 groups. Monkeys of the first group (6 hypovitaminotic and 5 control) were inoculated orally, after preliminary administration of ox bile, with Schottmüller paratyphoid B bacteria (50 million microbial bodies). Monkeys of the second group (5 hypovitaminotic and 5 control) were inoculated orally, after administration of 4 ml of ox bile, with intestinal bacilli of the serotype 0111:B4 (30 million microbial bodies). The monkeys were placed in four separate cages, isolated from one another, and located in two different divisions.

Observations on the hypovitaminotic animals, inoculated with paratyphoid bacteria, showed that clinically manifest illness developed in all the monkeys, and showed the following symptoms. Two or three days after the inoculation, the animals maintained a high temperature of 4–5 days (up to 41.5°). We observed a depressed condition, absence of appetite, and frequent fluid stool. In the peripheral blood we found a leukopenia (4500 leukocytes per mm^3), erythropenia (down to 2,000,000 per mm^3), decrease in the percent of hemoglobin, and accelerated sedimentation rate (up to 20–25 mm per h). Three monkeys showed especially severe conditions. In these animals we

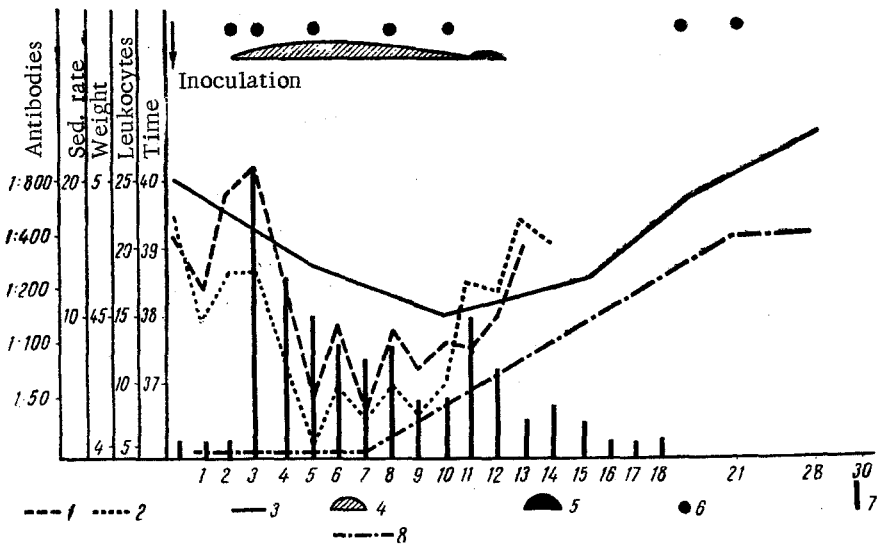

Fig. 2. Condition of the hypovitaminotic animals subsequent to their inoculation with paratyphoid bacteria. 1—temperature; 2—leukocytes; 3—weight; 4—fluid stool; 5—paste-like stool; 6—excretion of bacteria; 7—sedimentation rate; 8—antibodies.

observed signs of toxicosis and exicosis, manifested by dryness and cyanotic appearance of the skin and mucous membranes, and a decrease in skin turgor. Over the total period of illness, the monkeys lost up to 800–900 grams of weight. The duration of the illness was 10–12 days, and bacterially contaminated excretion was observed over the course of 2 months. Antibodies were determined in titers of 1:400–1: 1600 (Fig. 2).

In the control group, out of 5 monkeys, 3 contracted paratyphoid. They showed a minimal elevation in temperature, decrease in appetite, fluid or paste-like stool, loss in weight (by 200–300 grams), and acceleration of the sedimentation rate (10–15 mm per h). The illness took a mild course, and was of short duration. Excretion of bacteria was observed over a period of 6–9 days; specific antibodies were determined in titers of 1:400 –1: 1600.

Comparison of the monkeys in the control and experimental groups showed a significant difference both in the degree of severity of the disease and in the duration of the period of clinical symptoms. Thus, while in the control group clinical manifestation of paratyphoid B was recorded in 3 out of 5 monkeys, in the group of C-hypovitaminotic animals all individuals came down with the disease. In the control monkeys the disease took a mild course, and lasted 5–7 days. In the hypovitaminotic animals, we observed a severe form of the illness, and the clinical symptoms of the disease lasted 10–12 days. In the control animals, the organism rid itself of the paratyphoid bacteria after 6–8 days, while in the hypovitaminotic animals this occurred only after 2 months.

Observations on the hypovitaminotic monkeys inoculated with enteropathogenic strains of intestinal bacilli showed that out of 5 animals, 4 contracted colienteritis. The disease began after 2–3 days of incubation, and was accompanied by a decrease in appetite, frequent watery stools over a period of 8– 10 days, intoxication, and a loss in weight of up to 600 grams. On the third week after inoculation, antibodies were determined in titers of 1:50–1:100. Excretion of bacteria lasted over a period of 1 month. Clinical symptoms of colienteritis were observed in the diseased monkeys for 8–10 days. In one of the 5 monkeys, the colienteritis followed a serious course, and terminated in the death of the animal on the 25th day after inoculation. On autopsy, we noted catarrhal gastroenterocolitis, with predominant damage of the ileum, and diapedetic hemorrhages in the stomach, large and small intestine. On bacteriological investigation of the organs, intestinal rods of the serotype 0111 were cultured from the ileum.

Parallel observations on the control animals, maintained on normal diet, showed that inoculating them with enteropathogenic strains of intestinal bacilli of the same serotype was not accompanied by development of clinical symptoms of illness Throughout the entire period of observation the animals were noted to be in healthy and lively condition. Excretion of the pathogenic serotypes of intestinal bacilli ceased 4–5 days after the inoculation. By the end of the third weed antibodies were determined in titers of 1:50–1:100.

Thus, in monkeys maintained on a scorbutogenic diet for 2 $^{1}/_{2}$–3 months a vitamin C deficiency develops. In the setting of hypovitaminosis C, there is a decrease in the natural resistance of the animal organism to paratyphoid B and colienteritis. The infectious process that develops in the hypovitaminotic animals differs from that seen in the control animals in its severity, and in the duration of its clinical course. The obtained data suggests that in monkeys, under the influence of a vitamin C deficiency, there is a change in the immunobiological state of the organism.

SUMMARY

The present paper deals with studies of the effect produced by vitamin C deficiency on the susceptibility to infection and the course of infectious processes in paratyphoid B (Schottmuller) and colienteritis in monkeys. Vitamin C deficiency was induced by keeping the animals on scorbutogenic diet for a prolonged time. The results show that normal adult animals exhibited a considerable resistance to artificial infection with paratyphoid B bacilli and enteropathogenic E. coli. Infection of monkeys with hypovitaminosis with the mentioned bacteria was accompanied by the development of a clinically pronounced severe infectious process.

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