FACTORS CONTRIBUTING TO THE DEVELOPMENT OF EXPERIMENTAL CHOLERA IN PUPPIES

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UDC 616.932-092.9

The possibility of infection with experimental cholera by injection of various strains of cholera vibrio of the El Tor biotype was studied in experiments on puppies aged 2-3 months. A high morbidity with the typical syndrome was observed after the use of strains recently isolated from patients. The cholera syndrome developed with a high degree of probability after a decrease in the general resistance of the body or blocking of gastric secretion through starvation or administration of alcohol. It is concluded from the results of the clinical and biochemical investigations that experimental cholera in puppies is an adequate model for the study of many pathophysiological problems.

An experimental model in dogs is the most convenient for pathophysiological and clinical investigations of cholera. This is because of the similar evolution of the physiology of digestion, the fact that digestive processes in dogs have received detailed study, and finally, the identity of the clinical manifestations of cholera in dogs and man (vomiting, profuse diarrhea, dehydration and collapse [2, 3, 6, 10]). A disadvantage of experimental cholera in dogs is that only a relatively small percentage of animals develop a disease with the typical clinical syndrome (about 40% [8]). As Sack and Carpenter [8] state in their survey, attempts to infect puppies with cholera have proved unsuccessful.

In the investigation described below, conducted on puppies aged 2-3 months, the conditions facilitating reproduction of an adequate model of cholera with a high incidence of the disease were studied.

EXPERIMENTAL METHOD

Dogs were infected with cholera vibrios of the El Tor biotype, two of which were recently isolated from patients with cholera and two were museum strains (nos. 569b and 3062). Before and 48 h after infection the animals were weighed, their rectal temperature measured, and their water balance (specific gravity of the blood and plasma, hematocrit, red cell count) and electrolytes (potassium, sodium, chlorides, bicarbonates) were studied. Bacteriological examination of the stools was carried out daily by the usual method [5]. If the animal died, the contents of the small and large intestine, bile and blood were investigated bacteriologically.

The results of infection were taken as positive if the dogs developed diarrhea and hemoconcentration, lost 10% of their body weight, and the strain of vibrio was isolated from the stools 24-48 h after infection. The last factor was particularly important, for the other symptoms could develop as a result of the cholerogenic effect of the vibrio suspension without actual development of the infectious disease [9].

Several series of experiments were carried out in the period 1965-1971: series I (control) – infection by the method of Sack and Carpenter [10] after preliminary starvation for 16-20 h and injection of 6% soda solution into the stomach before infection; series II – the puppies were infected as in the control series,

Laboratory of Pathophysiology and Laboratory of Emergency Prevention and Treatment of Cholera, Rostov-on-Don, Plague Research Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 6, pp. 18-20, June, 1972. Original article submited September 20, 1971.

TABLE 1. Frequency of Development of a Cholera Syndrome in Puppies With Modified Resistance

Series of experiments	Experimental conditions	No. of strain used for infection	Dose (number of vibrios)	Number of puppies	
				used in experiment	developint the disease
I	Control	569 B	$2,5—10\times10^{10}$	9	1
		3062	$3—4\times10^{11}$	2	0
		Recently isolated	$10^{4}—10^{11}$	5	5
II	Treatment with actinomycin	569 B	$1—8\times10^{10}$	5	1
		3062	$7—30\times10^{10}$	7	6
III	Treatment with alcohol	3062	$3—8\times10^{11}$	4	2
IV	Starvation for 3 days	3062	$6—7\times10^{11}$	2	2

but a subcutaneous injection of actinomycin D was given in a dose of 0.15 mg/kg body weight 15 min before introduction of the gastric tube; series III—before infection the dogs received an injection of 60% alcohol in a dose of 10 ml/kg body weight by gastric tube at intervals of 15 h, and the animals were infected 1 h after the second injection without neutralization of the gastric contents with soda; series IV—the puppies received no food for $3^{1}/_{2}$ days before injection, but were given water ad lib.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that infection of the puppies in the control series was successful only in the case of infection of a bacterial suspension of strains recently isolated from patients with cholera. The attempt to obtain a cholera syndrome by infection with museum strains was less successful: a distinct clinical picture was observed in only one puppy.

Administration of actinomycin D, which sharply reduced the resistance of the animals to the action of toxins and to infection, greatly facilitated infection of the puppies with cholera. Most animals developed the typical cholera syndrome, the strain of vibrio was isolated from the stools, and in animals which died it was found in the contents of the small and large intestine and twice in the bile. The experiments with alcohol were particularly interesting, for as was pointed out above, the puppies were infected without neutralization of the gastric juice with soda, and nevertheless two of the four puppies developed the clinical picture and one of them died. Success of infection in this case can be explained by the development of acute hypochlorhydria as the result of "fixing" of the gastric mucosa through the action of alcohol in high concentration [2]. Starvation, by lowering the general resistance of the body and disturbing the normal activity of the digestive glands and, in particular, of the stomach, evidently may also play an important role.

There are grounds for concluding from the results of investigations of gastric secretion in persons excreting NAG-vibrios* or cholera convalescents and carriers that a special role in the mechanism of human infection may also be played by depression of the barrier function of the stomach and lowering of the general resistance of the body [1]. Hematological and biochemical investigations revealed an increase in the specific gravity of the blood and plasma and in the hematocrit index (in individual cases up to 60-80%), a decrease in the levels of bicarbonates and potassium in the plasma and, what is particularly interesting, in the erythrocytes of the puppies developing the disease, i.e., the animals developed dehydration, acidosis, and a disturbance of the electrolyte balance. These symptoms are characteristic of the clinical picture of cholera in man [4, 7].

Injection of massive doses of cholera vibrios of recently isolated strains thus leads to successful experimental infection of puppies with cholera; experiments using museum strains were less successful, even though these strains still retained a definite cholerogenic effect: most animals developed diarrhea, sometimes accompanied by vomiting, during the first days after infection although bacteriological examination of the stools was negative. A decrease in the general resistance of the body and in the barrier function of the stomach sharply increased the chances of success in obtaining an adequate model of cholera in puppies and one which, in the writer's opinion, is perfectly suitable for pathophysiological and immunological investigations.

*NAG = nonagglutinating — Translator's note.

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