STATE OF THE CLOTTING AND ANTICLOTTING SYSTEMS OF THE BLOOD IN EXPERIMENTAL ACUTE INTESTINAL DEHYDRATION

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An important role in the pathology of acute intestinal infections is played by the syndrome of acute intestinal dehydration, which is accompanied by extensive loss of fluid and electrolytes, disturbance of the microcirculation, metabolic disturbances, and hypoxic and dystrophic changes. The syndrome of acute intestinal dehydration associated with intestinal infections is due both to the pathogenic influence of the etiologic factor and to diarrhea.

Disturbances of the hemostasis system discovered in acute intestinal infections are due to the direct action of the etiologic factor, in consequence of which injuries to the vascular walls and the microcirculation and impairment of platelet function are observed. Increased activity of the blood clotting system in acute intestinal infections has been demonstrated in several investigations. Some workers, for instance, have noted thromboses in the organs of rabbits after parenteral injection of salmonella endotoxin, and they explain these phenomena by the action of the toxin itself, which increases the procoagulant activity of the blood in all phases of the clotting process, and also to its action on the blood vessels with consequent slowing of the blood flow [5-7, 9]. In acute intestinal infections signs of a bleeding tendency are found in the intestinal mucosa, and this is linked with the increased fibrinolytic activity of the blood or the high heparin concentration [1, 2].

There is no information in the literature on the effect of acute intestinal dehydration, which is one of the foremost syndromes of intestinal infection, on the hemostasis system. Yet the disturbance of hemostasis which is frequently observed in intestinal infections may be the result of the combined action of a number of factors, including the etiologic factor and the infectious agent, as well as acute intestinal dehydration.

In order to clarify the pathogenesis of the disturbances of blood clotting in acute intestinal infections and to elucidate the influence of acute intestinal dehydration of the blood clotting system, an experimental investigation was conducted with the aim of determining the state of activity of the clotting and anticlotting systems of the blood in acute intestinal dehydration of nonpathogenic etiology (without introduction of the toxic factor).

EXPERIMENTAL METHOD

For the investigation a model of intestinal dehydration was created in which diarrhea was induced by injection of 50 ml castor oil by gastric tube into rabbits (weighing 2-3 kg). The loss of body weight by the animals as a result of this procedure was: 0-0.5, 1-5, 6-10, and 11-15%. Blood for testing the function of the clotting and anticlotting systems was taken from the rabbits by cardiac puncture, into a syringe filled with sodium citrate solution in the ratio of 1:9 to blood. At the same time blood was taken from control animals which, instead of castor oil, received the same volume of drinking water, also injected through a gastric tube.

To determine the activity of the clotting system the R and K indices of the thromboelastogram in citrated blood were recorded on the Tromb-2 thromboelastograph after the addition of 0.1 ml $CaCl_2$ (1.29% solution) to 0.26 ml of plasma. The degree of aggregation of the platelets was determined by Born's method [10] in platelet-enriched plasma, using the sodium salt of ADP as the aggregant. The degree of aggregation was expressed as the index.

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TABLE 1. Total and Nonenzymic Fibrinolytic Activity, Platelet Aggregation, Antiplasmin Activity, R and K Indices of Thromboelastogram in Rabbits with Different Degrees of Dehydration Due to Castor Oil (M ± m)

Group of animals	No. of animals	Aggregation index	Antiplasmin activity, %	Fibrinolytic activity		Thromboelastogram indices, min	
				total	nonenzymic	R	K
Control	13	2,37±0,25	100±0	100±0	100±0	4,1±0,7	4,5±0,7
Dehydration 0-0,5%	5	2,53±0,14	97,0±5,8	95,0±11,7	91,0±9,1	3,8±0,5	4,5±0,5
Dehydration 1-5%	7	2,20±0,08	87,0±2,7	145,0±5,5	149,0±4,8	3,5±0,1	3,3±0,1
Dehydration 6-10%	10	2,00±0,07	86,0±2,5	114,0±5,5	130,0±2,4	2,3±0,1	2,1±0,1
Dehydration 11-15%	15	2,36±0,02	84,0±1,2	112,0±1,7	96,0±1,9	1,8±0,1	2,4±0,1
Chlorpromazine + dehydration 1-8%	4	2,37±0,30	—	145,0±12,1	140,0±9,3	2,2±0,1	2,3±0,1
Chlorpromazine + dehydration 9-15%	4	2,30±0,25	—	117,0±5,7	117,0±2,4	2,9±0,5	3,0±0,4

To investigate the fibrinolytic component of the anticlotting system, the antiplasmin activity [13], the total fibrinolytic activity, and nonenzymic fibrinolysis [4] were determined. Parallel morphological investigations were undertaken of the kidneys of rabbits killed after losing up to 5, 10, and 15% of their body weight.

EXPERIMENTAL RESULTS

In the initial stage of dehydration (loss of 0-0.5% of body weight) 1-2 h after the beginning of diarrhea all the blood clotting indices tested were normal (Table 1).

In stage I of dehydration (loss of 1-5% of body weight) as a result of diarrhea, edema of the renal stroma and venous congestion were accompanied by slight activation of the clotting system (shortening of the thromboelastogram indices: R from 4.1 ± 0.7 to 3.5 ± 0.1 min and K from 4.5 ± 0.7 to 3.3 ± 0.1 min), accompanied by activation of the fibrinolytic component of the anticlotting system, including both total (up to 145 ± 5%) and nonenzymic (149 ± 5%) fibrinolytic activity. The decrease in total blood antiplasmin activity to 87 ± 3% also points to activation of enzymic fibrinolytic activity. Fluctuation of the platelet aggregation index was minimal: from 2.37 ± 0.25 in the control to 2.2 ± 0.1 in the experimental animals.

With an increase in the severity of dehydration of the animals (stage II, loss of 6-10% of body weight) activation of the clotting system was more marked (shortening of the thromboelastogram indices: R to 2.3 ± 0.1 min and K to 2.1 ± 0.1 min). However, activation of total and nonenzymic fibrinolysis was somewhat reduced compared with the previous group of animals (activation of enzymic fibrinolysis reached only 114 ± 5% and nonenzymic 130 ± 2%).

The increase in activity of the clotting and anticlotting systems observed in the animals in stages I and II of dehydration took place against a background of considerable reduction in the volume and slowing of the flow of blood as a result of dehydration, as well as marked edema of the renal stroma, venous congestion, stasis of blood vessels of small caliber, proliferation of their media, and thickening of their elastic membrane. All these changes may lead to liberation of thromboplastic substances into the blood and may create conditions favoring activation of the clotting system [11, 14]. Liberation of thromboplastic substances into the blood ends with thrombin generation in the blood stream and the onset of hypercoagulation. This phenomenon, in turn, lies at the basis of the triggering mechanism of the protective reaction of the anticlotting system which, in the present experiments, was clearly exhibited as activation of its fibrinolytic component (both enzymic and nonenzymic) [3].

Considering the localization of activators of fibrinolysis in the kidneys [12] and also the ability of the kidneys to secrete certain fibrinolytic components and heparin [8], it can be concluded that when edema of the stroma and venous congestion are in their initial stages the kidneys (as the organ of homeostasis) are able to produce compensatory factors of fibrinolysis in response to hypercoagulation.

The observed fall in activation of the fibrinolytic component of the anticlotting system during an increase in activation of the clotting system (stage II of dehydration, loss

of 6-10% of body weight) may also be explained by several causes: First, the progressive damage to the kidneys with aggravation of dehydration may evidently lead to a disturbance and reduction of the secretion of fibrinolytic activators directly from the kidneys; second, the constant excitation of the anticlotting system, superposed on the ever-increasing hypercoagulation and thrombin formation, leads to exhaustion of its function.

This process was clearly demonstrated in animals in the next stage (III, loss of 11-15% of body weight) of dehydration, when, superposed on the morphological changes in the kidneys with evidence of rupture of the blood vessel walls and with necrotic changes, the state of hypercoagulation was not accompanied by activation of the function of the anticlotting system, and there was virtually no activation of nonenzymic fibrinolysis. Platelet aggregation in the animals of all three groups showed no significant changes during dehydration.

In the next experiments dehydration of rabbits with castor oil was carried out in conjunction with administration of chlorpromazine to the animals, so that the influence of stress factors could be eliminated in the course of development of dehydration. As Table 1 shows, in the animals of these groups the results were similar to those in animals in the same stage of dehydration, but not receiving chlorpromazine. Consequently, changes in the indices of both the clotting and the anticlotting systems in the experimental animals were not due to stress, which could have arisen during the experiment. Furthermore, because of the ability of chlorpromazine to inhibit activity of the nervous system controlling the function of the anticlotting system, it can be tentatively suggested that activation of its function observed in the first stages of dehydration was evidently largely due to the direct liberation of agents of the anticlotting system — fibrinolytic agents and heparin from pathologically changed tissues and vessel walls — into the blood stream.

Summarizing briefly the results of the experiments to study the state of the clotting and anticlotting systems of the blood during acute intestinal dehydration produced in animals by a pharmacological agent, a definite increase in activity of the clotting system will be observed at all stages of dehydration, with a tendency for hypercoagulation to increase with an increase in dehydration. Parallel with these changes activation of the function of the anticlotting system was noted in the initial stages of dehydration. With an increase in dehydration of the animal the degree of activation of the function of the anticlotting system was reduced, and in some cases (when a considerable degree of dehydration was present — up to 15%), it disappeared completely.

The reduction in activity of the anticlotting system, as well as the increase in hypercoagulation, took place against a background of aggravation of the morphological changes in the kidneys with an increase in the degree of dehydration, suggesting that renal factors play a direct role in this form of pathology of the regulation of hemostasis. The experiments to study dehydration of animals receiving chlorpromazine confirmed this hypothesis.

Relations discovered between the clotting and anticlotting systems of the blood during the development of dehydration are evidence that the acute intestinal dehydration syndrome may have a definite effect on the clotting and anticlotting systems of the blood in acute intestinal infections, and the character of this influence must be taken into account when the general state of function of the hemostasis system is evaluated in this form of pathology.

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EFFECTS OF SCORPION (*Buthus eupeus*) TOXINS ON CYCLIC AMP AND CYCLIC GMP LEVELS IN BRAIN AND HEART TISSUES

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Toxic polypeptides isolated from venom of the scorpion *Buthus eupeus* [1, 2] interact selectively with the gating mechanism of the sodium channels of excitable membranes, slowing their inactivation [4]. This action of the toxins on the organ or organism leads to massive liberation of neuromediators from nerve endings [5, 6, 12]. Modification of excitable membranes by certain toxins (anemone toxin ATCP, MCD-peptide from bee venom) is known to stimulate cyclic nucleotide formation [8, 9].

It was therefore interesting to study the effect of the toxins of *Buthus eupeus* on cyclic AMP and cyclic GMP in brain and heart tissues.

EXPERIMENTAL METHOD

Native venom of the scorpion *Buthus eupeus*, obtained by electrical stimulation, and also the active polypeptide fraction (APF) isolated from the venom [2] were used. Isolated hearts of 45 guinea pigs were perfused and the cardiac contractions were then recorded [13]. Atropine, propranolol, and hexamethonium (10^{-4} g/ml) were injected into the perfusion fluid 10 min before injection of the venom or APF (10^{-7}-10^{-3} g/ml). The venom and APF were injected intraventricularly [10] into albino mice weighing 23-25 g (120 animals) in a volume of 5 µl in doses of 0.1-10 µg/20 g body weight. The latent period of the convulsive response was analyzed quantitatively. Atropine, tropacin, chlorpromazine, or haloperidol — all in doses of 1 mg/kg — was injected intraperitoneally into the mice 30 min before injection of the venom or APF. Arecoline (25 mg/kg) and apomorphine (10 mg/kg) were injected subcutaneously. Reserpine (5 mg/kg) was injected intraperitoneally into mice and guinea pigs 4 h before the beginning of the experiment. Sections through the forebrain and cerebellum of the mice were prepared by the method in [11]. The sections were preincubated for 40 min. Venom and APF (final concentration 10^{-6}-10^{-5} g/ml) or the incubation solution with an increased KCl concentration (100 mM) were added to the medium for 10 min. Cyclic AMP and cyclic GMP in the myocardium of the guinea pigs and in the mouse brain sections were determined quantitatively with the aid of commercial kits (from Becton and Dickinson, USA). Radioactivity of the samples was counted and the concentrations of cyclic nucleotides determined with an Ultrogamma automatic counter (from LKB, Sweden) coupled to a Data-Box 1222 minicomputer.

EXPERIMENTAL RESULTS

Intraventricular injection of venom or APF into the appearance of dose-dependent myoclonic contractions, reflex movements, and attacks of violent clonico-tonic convulsions. LD_{50} for the venom and APF was 4.7 and 0.66 µg/20 g respectively. Preliminary administration of blockers of catecholaminergic synapses (chlorpromazine, haloperidol, and reserpine) considerably accelerated the development of convulsions. A similar effect was produced by the central muscarinic and nicotinic blockers of acetylcholine synapses atropine and tropacin. Meanwhile specific stimulators of cholinergic and dopaminergic synapses arecoline and apo-

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