The following conclusions can thus be drawn from the results. Diarrhea fluid contains many endogenous metabolites, some of them in considerable concentrations. As a result of the action of cholera and salmonella toxins metabolic disturbances arise, and depending on the dynamics of their development, these can be divided into four types (groups I, III, IV, and V). The GLC method likewise reveals disturbances arising actually during the latent period (group IV).

Determination of the structure of metabolites secreted into the lumen of the small intestine by chromatography and mass spectrometry is an essential basis for the interpretation of the changes observed and of differences in the action of toxins.

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INTERACTION BETWEEN HEPARIN IMMOBILIZED ON SYNTHETIC POLYMERS AND BLOOD

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An effective and widely used method of increasing the hemocompatibility of polymer materials intended for contact with blood is by modifying their surface with heparin, through the ionic or covalent addition of this anticoagulant [1, 2, 8]. The reason for the increased hemocompatibility of heparin-containing polymers (HCP), according to some workers, is the permanent elution of heparin into the blood stream [4], whereas according to others it is that heparin can prevent blood from clotting even while bound with a polymer [3]. Evidently it is only through knowledge of the true mechanism of this phenomenon that properly oriented synthesis of HCP can be undertaken.

The object of the present investigation was to study the effect of covalent immobilization of heparin on its biological properties.

EXPERIMENTAL METHOD

The test samples were hydrogels, swelling in water, and containing covalently immobilized heparin, or fabricated articles (films, catheters, artificial blood vessels) made from synthetic polymers (polysilane, polyurethane, polyethylene-terephthalate — lavsan), the surface of which was covered with a layer of hydrogel with covalently immobilized heparin, chemically bound with the polymer.

To investigate the hemocompatibility of HCP in experiments *in vivo*, plastic operations were performed on the carotid arteries (two experiments) and an artificial graft was inserted into the abdominal aorta below the renal vessels (two experiments) using standard lavsan grafts (4 mm diameter, 30-50 mm long), modified with heparin. During and after the

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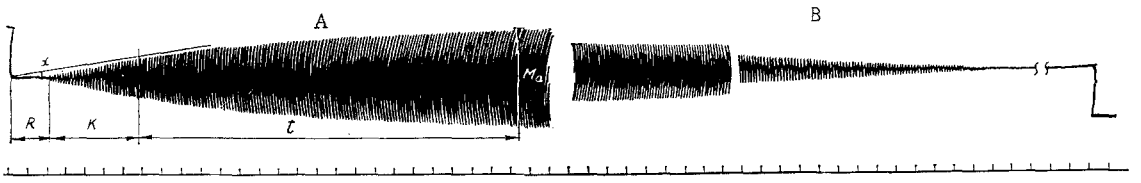

Fig. 1. Thromboelastograms of dog's blood before (A) and after (B) contact with HCP. 2 ml blood incubated with 1 g HCP (concentration of immobilized heparin 11.8 mg/g).

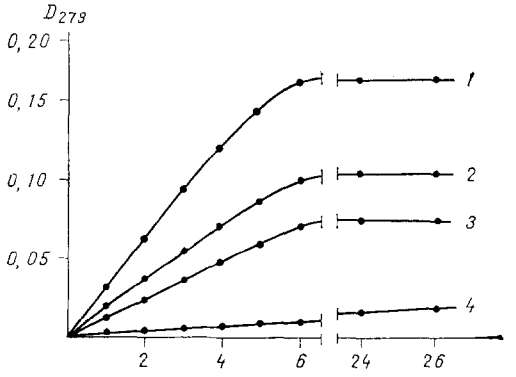

Fig. 2. Kinetics of lysis of unstabilized fibrin by products of interaction between immobilized heparin and fibrinogen (1), thrombin (2), fibrinolysin (3), and serum albumin (4). Abscissa, time (in h); ordinate, optical density of physiological saline above mixture of unstabilized fibrin and HCP-treated protein.

operation no anticoagulant therapy was given. The periods of observation on the animals (dogs) were 14 days and 1, 3, and 6 months. Before these times the animals were killed and the state of the prostheses examined. In all cases the prostheses remained completely patent and no thrombi were present in their lumen. The inner wall of the prostheses until the 3rd month of observation was covered by an isolating capsule, arising from the sites of the anastomoses. Under similar conditions, nonheparinized prostheses were completely occluded by a thrombus in the course of 3 days.

Changes in the clotting system of the blood on contact with HCP were assessed by thromboelastography and by means of coagulograms [6].

EXPERIMENTAL RESULTS

The most typical thromboelastograms are illustrated in Fig. 1. They show that on interaction of blood with HCP a marked decrease in the total coagulant activity of the blood and a substantial change in the parameters of its clotting system were observed. This applies in particular to an increase in the invisible phase of clotting (R) and the time of onset of clot formation (K), a decrease in the fibrinogen concentration, in the number and functional activity of the platelets, in the activity of fibrin-stabilizing factor (M_a), to a decrease in the rate of clot formation (tan α), and so on.

More detailed biochemical monitoring was carried out by means of coagulograms. On contact of the blood with HCP, changes were found in all components of hemocoagulation: procoagulant, anticoagulant, and fibrinolytic. For instance, after contact between 10 ml blood and 10 g HCP (immobilized heparin concentration 11.8 mg/g) the plasma heparin tolerance and recalcification time increased from 55 ± 3 and 57 ± 3 to 15,000 ± 60 and 115 ± 8 sec respectively. Parameters characterizing the second phase of blood clotting also changed very considerably, with the exception of free heparin, the concentration of which remained

constant. The prothrombin index, for instance, fell from 100 to 80 ± 5% and the heparin time rose from 26 ± 2 to 69 ± 4 sec. The study of the third phase of clotting revealed considerable changes. For instance, the thrombin time was increased from 15 ± 1 to 20 ± 1 sec, the fibrinogen concentration fell from 365 ± 10 to 220 ± 10 mg %, and activity of fibrin-stabilizing factor fell from 63 ± 4 to 34 ± 3%. These data are evidence that HCP are active for the blood clotting system.

One of the biological functions of heparin bound with certain plasma proteins, as we know, is to participate in the lysis of unstabilized fibrin [5, 7, 9]. To study whether the same function can be performed by immobilized heparin, interaction between HCP and fibrinogen, thrombin, fibrinolysin, and serum albumin was studied. The experiments showed that all these proteins interact with immobilized heparin, and in order of diminishing strength of binding they can be arranged in the following series: fibrinogen ≈ thrombin ≫ fibrinolysin > serum albumin. The results of investigation of lysis of unstabilized fibrin by products of interaction between immobilized heparin and the proteins mentioned above are shown in Fig. 2. Clearly these proteins (except albumin), like complexes of the original heparin, had a lytic action on unstabilized fibrin.

The results are thus evidence that the immobilization process causes no significant change in the biological properties of heparin and, in particular, in its anticoagulant and fibrinolytic activity, and the enhanced hemocompatibility is an inseparable property of the HCP themselves.

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