EXPERIMENTAL GAS–LIQUID CHROMATOGRAPHIC STUDY OF THE DYNAMICS OF DEVELOPMENT OF THE DIARRHEA SYNDROME

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KEY WORDS: bacterial toxins; small intestine; diarrhea syndrome.

Attempts to create an integral picture of the action of bacterial toxins are hampered by the absence of data on some of the intermediate stages of the single chain of biochemical processes that lead to the development of diarrhea. The process of secretion of electrolytes and water, which arises after even the brief action of cholera toxin, for example, does not begin at once, but after a delay of 1-1.5 h [2, 3, 6], and as yet no fundamental changes in the biochemical parameters of the mucosa of the small intestine have been discovered during this period.

The composition of diarrhea fluid is known to reflect processes taking place in the mucosa under the influence of bacterial toxins, as has been shown by a study of the dynamics of secretion of inorganic ions and proteins, and this has substantially widened our knowledge of the mechanism of diarrhea [1, 8, 9]. Secretion of organic components also must characterize the functional state of the mucosa, but no research aimed at studying this problem has yet been undertaken.

In the investigation described below disturbances of the turnover of metabolites in the mucosa of the small intestine arising under the influence of bacterial toxins were examined. A perfused rabbit intestinal loop, from which samples could be taken continuously, was used as the experimental model. The composition of the diarrhea fluid was analyzed by gas–liquid chromatography (GLC), the wide possibilities of which for the study of complex mixtures of biological origin have been demonstrated by many workers [4, 5, 7].

EXPERIMENTAL METHOD

Chinchilla rabbits weighing not more than 1 kg were used. Before the experiment the animals were deprived of food overnight. A 2% solution of thiopental sodium (intravenously) was used for anesthesia. Laparotomy was performed on the anesthetized animals, the small intestine brought out, and two ligatures applied to the jejunum. The length of the loop was 25-30 cm. The loop was irrigated with 200 ml isotonic salt solution (150 mM Na^+, 75 mM Cl^-, 75 mM HCO_3^- pH 7.4), after which it was perfused with the same solution in a closed cycle with the aid of a peristaltic pump. After equilibrium had been established the volume of fluid was recorded at 15-min intervals on the scale of a cylinder which was connected successively with the pump and the intestinal loop. Samples of perfusion fluid, 5 ml in volume, were taken every 30 min. After removal of the first sample, the control animals were given an intravenous injection of 2 ml of salt solution, one group of experimental rabbits received 5 mg of salmonella lipopolysaccharide complex, and those of the other experimental group received an injection of 250 mg cholera enterotoxin in 2 ml of salt solution by enteral injection. Each group contained five animals. The period of observation was 3.5 h. Active secretion of fluid was observed about 90 min after injection of the toxin.

Each sample of perfusion fluid was treated with 25 μg of methyl ester of 9,10-dihydroxystearate (internal standard), after which it was frozen and lyophilized and the residue extracted twice with 1.5 ml of a chloroform–methanol system (2:1 by volume) each time. After each extraction the material was centrifuged at 1000g for 7 min.

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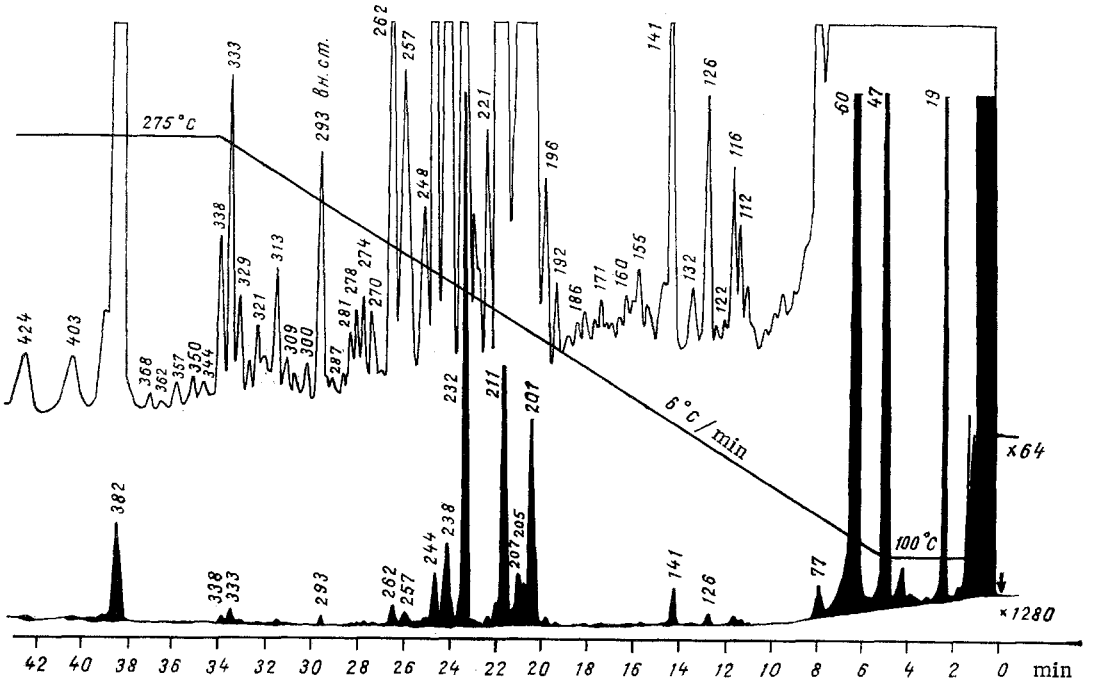

Fig. 1. Chromatogram of trimethylsilyl derivatives of metabolites of intrajejunal perfusion fluid. Column: 6 × 4 mm, OV-1 (3%).

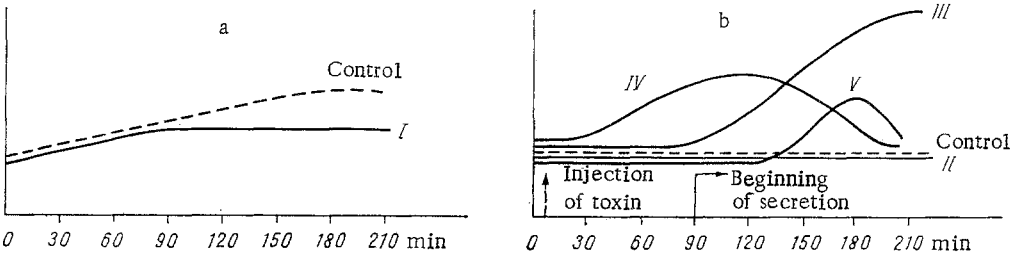

Fig. 2. Dynamics of changes in concentration of metabolites in intrajejunal perfusion fluid: a) group I (metabolites 186, 196, 205, 403); b) group II (metabolites 122, 171, 192, 211, 232, 270, 274, 287), group III (201, 382), group IV (116, 126, 238, 257, 338), group V (132, 244, 300, 313). Abscissa, time (in min), ordinate, relative concentration.

The pooled supernatants were concentrated *in vacuo*, transferred to 0.3-ml conical flasks, dried in a current of nitrogen, and kept at below −20°C. Before analysis the organic components of the samples were treated with 50 µl of N-methyl-N-trimethylsilylheptafluorobutylamide. A gas chromatograph with flame-ionization detector (Tracor-560), working under integral conditions, was used for analysis (glass column, 6 × 4 mm, 3% OV-1 on Chromosorb W-HP, 80-100 mesh). The carrier gas was nitrogen (60 ml/min). The temperature of the injector and detector was 285°C. The thermostat temperature program was: initial 100°C (5 min), final 275°C (12 min), rate 6°C/min. The content of the substances was calculated relative to the internal standard.

EXPERIMENTAL RESULTS

It will be clear from the chromatogram of the perfusion fluid given in Fig. 1 that the fluid contained about 30 clearly separated components, characterized by retention times shown in tenths of a minute. Comparison of the chromatograms of the control and two experimental groups of animals showed no qualitative differences between the control and the experimental series or between the two experimental series of animals. However, the quantitative differences were highly significant, especially between the control and experiment.

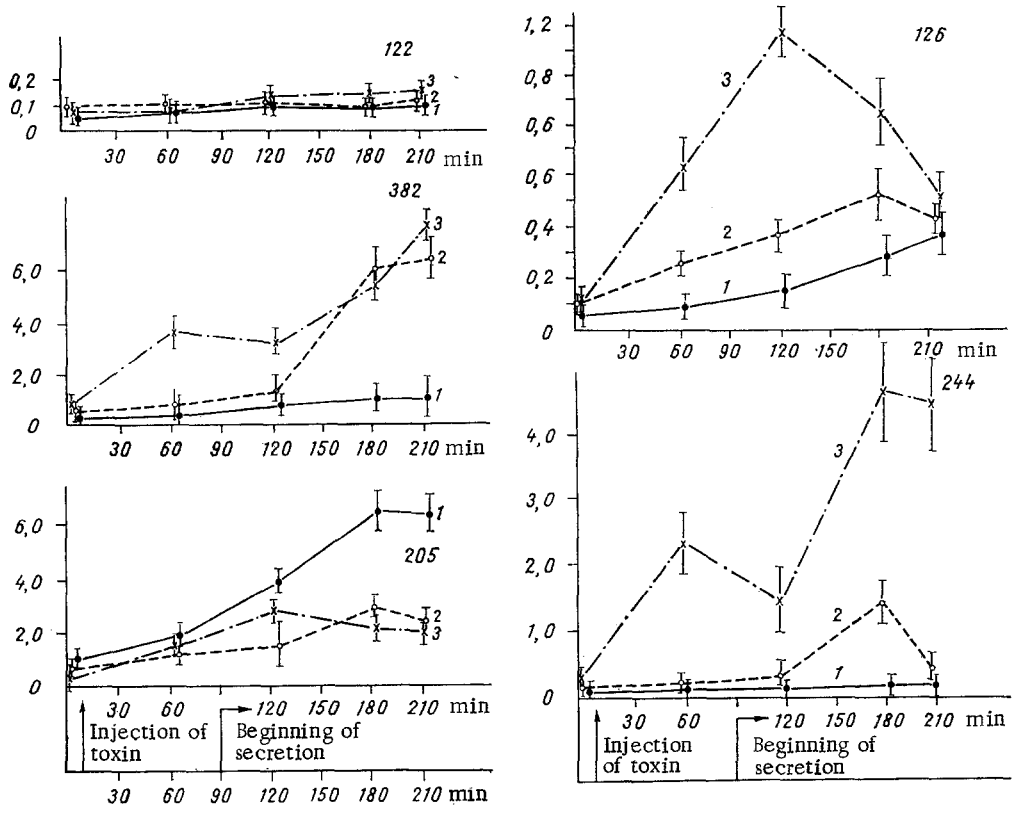

Fig. 3. Changes in concentrations of metabolites 122, 382, 205, 126, 244 in perfusion fluid of control animals (1), of experimental animals receiving salmonella toxin (2), and receiving cholera toxin (3).

When the experiment was set up it was assumed that in the initial period rapid isolation of various metabolites would take place, and that within a comparatively short time a state of dynamic equilibrium would arise. In fact, for most substances a state of equilibrium occurred as early as 30 min after the beginning of perfusion. However, the concentration of some metabolites did not flatten out on a plateau until near the end of observation. The concentration of these substances in the experimental series, considered together in the first group, was not increased (Fig. 2a).

All the other components of the diarrhea perfusion fluid could be combined into four main groups on the basis of the character of the change in their concentrations under the influence of toxins. Substances whose concentration in the experimental series was identical with the control were included in group II; group III consisted of metabolites whose content rose steadily from the time of appearance of diarrhea but did not flatten out on a plateau until the end of observation. Components whose concentration varied during the development of the diarrhea syndrome, passing through a maximum and returning to the normal level (Fig. 2b), also were combined into two groups (IV and V). As Fig. 2 shows, the beginning of accumulation of the group IV metabolites corresponded to the latent period, whereas that of the metabolites in group V corresponded to intensive secretion of fluid.

Disturbances of the turnover of the substances in group V were evidently secondary to the diarrhea, for they were considerably delayed after the beginning of secretion. Groups I and III reflect processes taking place in the mucosa synchronously with secretion. Meanwhile group IV characterizes processes preceding secretion.

Examples of changes in the concentrations of substances illustrating each of the above groups are given in Fig. 3. However, as was noted previously, in some cases differences in the dynamics of outflow of metabolites due to characteristic features in the mechanisms of action of the different toxins tested, were found in the experimental animals. Disturbances after cholera toxin as a rule were more severe in character, possibly because of its action directly on the mucosa.

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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