tration necessary for radical formation with generation of luminescence is two orders of magnitude less than the required concentration of ionic iron (Table 1).

Extraerythrocytic hemoglobin and its destruction products as far as hemin are thus evidently among the most effective pro-oxidants and are potential centers for radical formation in the blood.

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CHROMATO-MASS-SPECTRAL INVESTIGATION OF SECRETION OF THE RABBIT SMALL INTESTINE INDUCED BY CHOLERA AND SALMONELLA TOXINS

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The study of the dynamics of the diarrhea syndrome by gas–liquid chromatography [1] showed that development of the secretory process under the influence of bacterial toxins (cholera enterotoxin and the salmonella lipopolysaccharide complex) in experimental animals is accompanied by marked changes in the time course of entry of low-molecular-weight organic compounds into the intestinal lumen. However, the results were made difficult to interpret because of the absence of data on chemical structure of the metabolites. To solve this problem, a special chromato-mass-spectral study was required, and its results were described below.

EXPERIMENTAL METHOD

Silylated samples of secretion, treated as described in [1], were analyzed on the Finnigan-4021 electron impact mass-spectrometer, connected to a Nara-3 computer. The 6 × 2 mm glass column was packed with 3% methylsilicone OV-1 on Chromosorb-HP (80–100 mesh). The carrier gas was helium (20 ml/min). The temperature of the injector, separator, and source of ions was 285°C. The conditions for programming temperature of the thermostat were: initial 100°C (6 min), final 280°C (15 min), velocity 5°C/min. The spectrum recording time was 2 sec. The ionizing voltage was –70 eV. The reacting gases were isobutane and ammonia (Finnigan).

EXPERIMENTAL RESULTS

Preliminary investigation of the composition of the diarrhea fluid by electron-impact impact chromato-mass spectrometry showed that metabolites secreted into the intestinal lumen belonged to different classes of chemical substances, and in some cases one chromatographic

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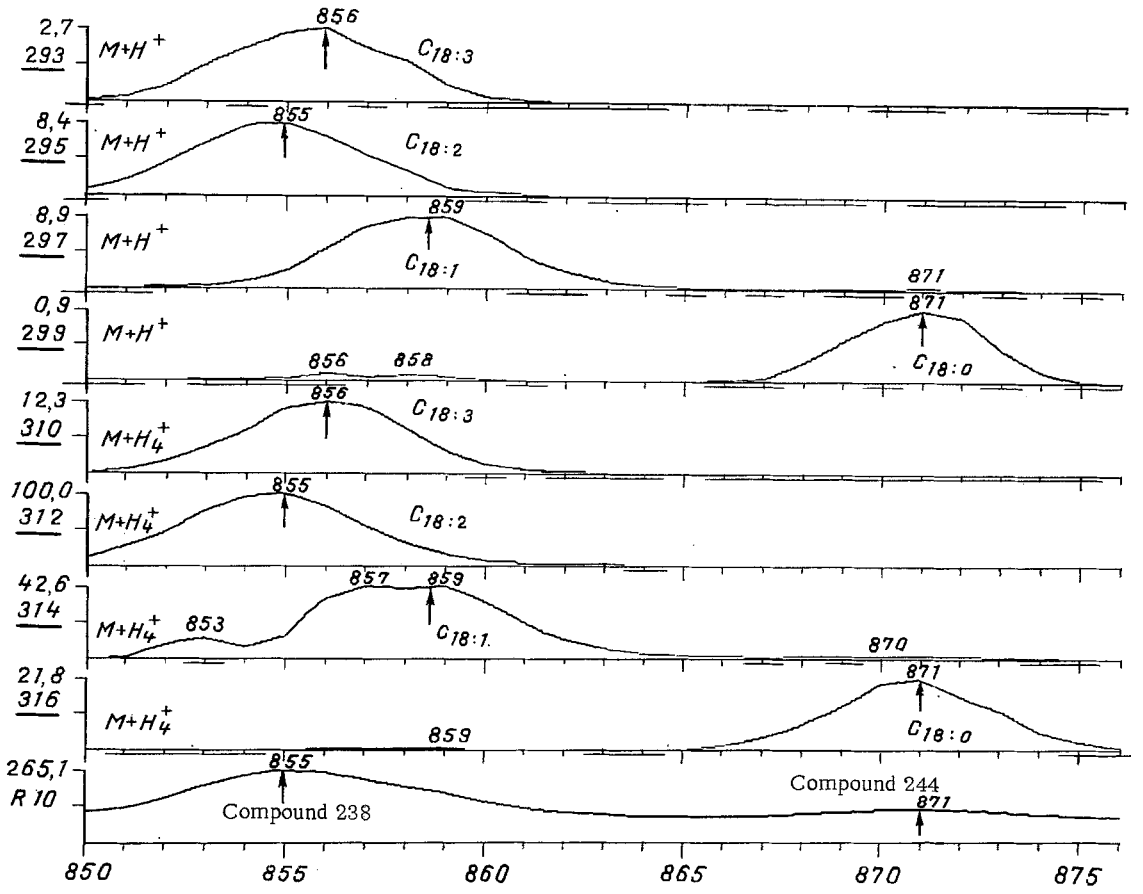

Fig. 1. Mass-chromatograms of peaks with retention time of 23.8 and 24.4 min, for ions with m/e of 293, 295, 297, 299, 310, 312, 314, and 316 (chemical ionization, reactant gas ammonia).

peak was formed by several components. This complicated determination of the structure of the substances sought. To increase the reliability of the mass-spectral study, additional mass spectra of chemical ionization were obtained using reactant gases: isobutane and ammonia, which permit "mild" ionization of molecules of the test compound to be carried out. Under these circumstances quasimolecular ions were formed: $M + H^+$ (isobutane) and $M + H^+$, $M + NH_4^+$ (ammonia). The use of two different reactant gases thus enabled the molecular weight of the original component to be reliably determined; later this simplified their identification, by restricting the choice of possible structures considerably.

The general scheme of the method of establishing structure consisted of the following stages. Initially the individuality of the chromatographic peak was determined by mass-chromatography applied to the principal peaks of the electron-impact and chemical ionization mass spectra. One example of such an investigation of mass spectra of chemical ionization by ammonia for compounds 238 and 244 is given in Fig. 1, which clearly shows that the chromatographic peak with retention time of 23.8 min contained three principal components with quasimolecular ions $M + H^+$ (293, 295, 297) and $M + NH_4^+$ (310, 312, 314). Meanwhile compound 244 ($M + H^+ - 299$, $M + NH_4^+ - 216$) was individual. Subtraction of the background for each component, allowing for these data, enabled the spectrum to be considerably purified, and a correct library search to be undertaken, which revealed the close similarity of the test substances with linoleic (18:2), linolenic (18:3), and oleic (18:1) acids. The presence of three unsaturated fatty acids in one chromatographic peak is in agreement with the known fact that methylsilicone liquid phases of the OV-1 type separate badly according to degree of saturation [4]. The results provided a basis for identification of these substances with synthetic specimens. Although the results of the library search and chemical ionization spectra were fundamental for the choice of one or other structure, in every case various other factors were taken into account. First, this had to be a substance of biological origin. Second, the compound under examination must not contain free hydroxyl, amino, or carboxyl groups, since silylation took

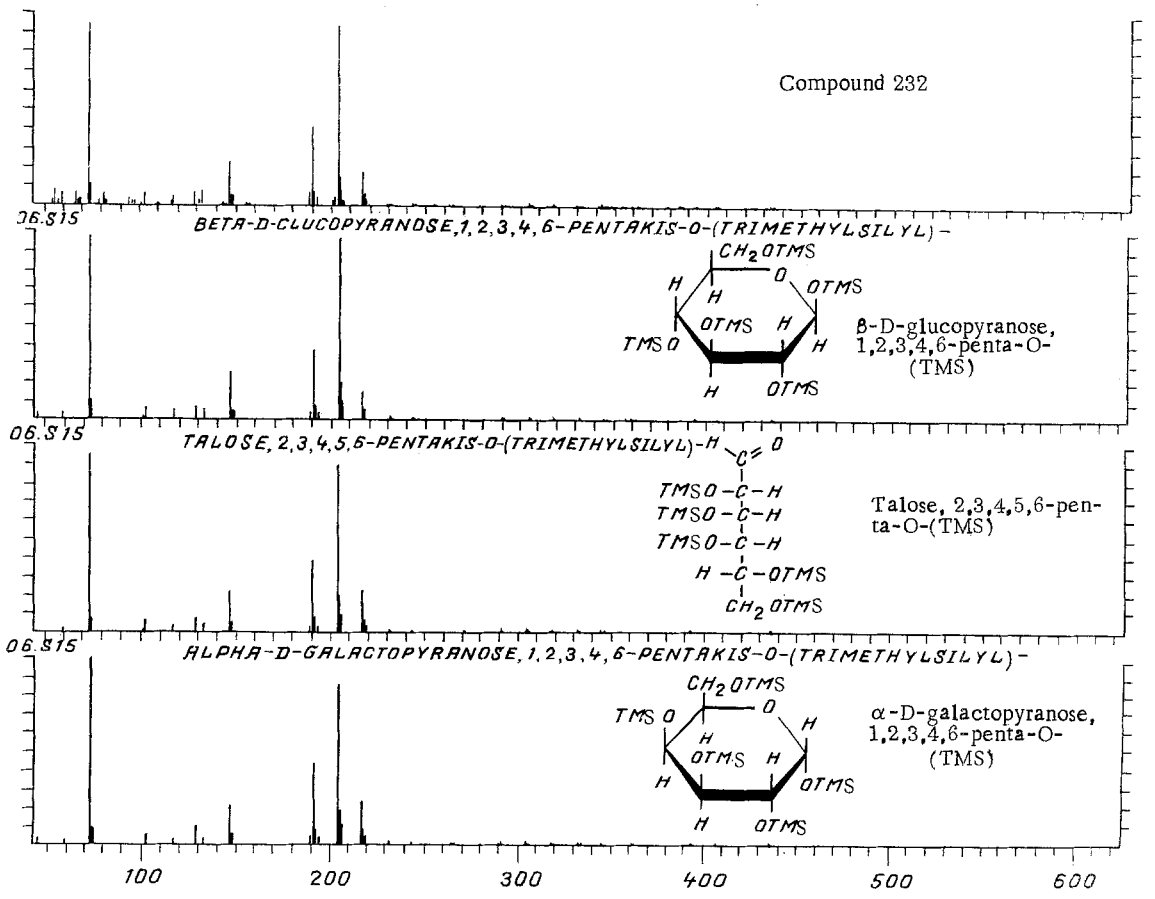

Fig. 2. Comparison of electron-impact mass spectra of trimethylsilyl derivatives of compound 232 and monosaccharides.

place before analysis. In addition, it must not contain such protective groups as methoxime, benzoxime, or trifluoroacyl. Finally, the chosen structure must correspond to the well-known data on correlation between structure and chromatographic properties [3]. Additionally, of course, in doubtful cases the character of fragmentation was analyzed both during electron impact and during chemical ionization, and the chromatographic properties of the test metabolites were considered and compared.

In some cases, however, this information was insufficient for unambiguous identification of structure. For example, spectra of isomeric sugars, both electron impact and chemical ionization, are virtually indistinguishable from each other (Fig. 2). That is why whenever possible, direct comparison with synthetic specimens, both by mass spectra and by retention time, was used. As a result of this investigation, of the 27 components in which we were interested nine were identified, the structure of 14 was suggested, and four remained unidentified.

Quantitative analysis of metabolites in the lumen of the small intestine of the control animals showed that the majority of the components studied were the monosaccharides glucose (232), galactose (211), ribose (196), and hexose (205). The remaining monosaccharides, namely fructose (201), xylose (186) and hexane (274), as well as representatives of other classes of compounds such as fatty acids (238, 244, 262), cholesterol and its precursors (382, 403), polyhydric alcohols (171, 192), higher hydrocarbons and hydroxy-acids (270, 287, 313, 333, 116, 141), were present in the perfusion fluid in very small quantities.

Different periods of action of the toxins in the experimental animals were characterized by accumulation of particular metabolites. Increased entry of unsaturated fatty acids (metabolites 238 and 262) into the intestinal lumen before the beginning of the secretory process must first be noted. Since these metabolites are precursors for prostaglandin synthesis [2], it can be postulated that liberation of these components was connected with activation of prostaglandin synthesis, and it confirms participation of the latter in the mechanism of the

diarrhea process. Furthermore, in the latent period an increase in the fructose concentration was observed, and this continued into subsequent stages of action of the toxin, accompanied by a fall in the level of other monosaccharides (xylose, 186; ribose, 196), evidence of disturbances of carbohydrate metabolism in the mucosal tissues of the small intestine. These changes were analogous in animals of the two experimental groups. However, clear differences were present during the action of the two toxins in the character of changes in concentrations of compounds such as cholesterol (382), dihydrolanosterol (403), hydroxyacetic acid (141), stearic acid (244), and seven higher hydroxy-acids and alcohols (313, 333, etc.).

This investigation thus established the structure of the principal metabolites secreted into the small intestinal lumen. The action of toxins was shown to be exerted primarily on fatty acid metabolism, specifically on precursors of prostaglandins, and later it spreads to metabolism of the majority of low-molecular-weight metabolites.

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