In the process of separation, the 5'-nucleotidase was separated from the nonspecific alkaline monophosphatase and the phosphodiesterase and was focused in a narrow pH zone from 7.3 to 8.15. Phosphodiesterase activity was detected throughout the course of separation with a small peak at pH 5.2-6.3, and a low activity of the nonspecific alkaline monophosphatase was focused at pH 4.8-5.8 (Fig. 1).

After the end of isoelectric focusing, the fraction containing the 5'-nucleotidase was collected separately. Calculation of the activity in the fraction showed that the yield of 5'-nucleotidase was only 10% and, moreover, a phosphodiesterase was detected in the same fraction, its yield amounting to 1% of the total phosphodiesterase activity of the whole venom. Practically no nonspecific alkaline monophosphatase activity was detected in the 5'-nucleotidase fraction.

The low activity of the enzymes of the fractions can be explained by their inactivation at the isoelectric point on separation in an ampholine pH gradient. The separation of the phosphodiesterase over a wide pH range (from 4.0 to 9.0) is possibly connected with a molecular heterogeneity of this enzyme. The existence of numerous forms of phosphodiesterase in snake venoms has been deduced from the distribution of the phosphodiesterase into several fractions when snake venoms are separated with the aid of chromatography or other protein-fractionating methods [5]. The acidic properties of the nonspecific monophosphatase at pH 4.8-5.8 find confirmation in the results of an electrophoretic study of the venoms of various Central Asian snakes [6].

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KINETICS OF THE ENZYMATIC HYDROLYSIS OF PHOSPHATIDYLCHOLINE BY VARIOUS SNAKE VENOMS. II.

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UDC 547.953.665.37

Earlier [1] for the enzymatic hydrolysis of phosphatidylcholine (PC) we used as sources of phospholipase A_2 the venoms of the snakes *Naja oxiana* Eich (cobra), *Vipera lebetina* L. (kufi), *Echis carinatus* Schneir (saw-scaled viper), *Ancistrodon halus* (Pall) (mamushi) and *Vipera berus* (common adder). There is information according to which phospholipase A_2 acts both in an alkaline and in an acid medium, but a pH of from 7.0 to 10.0 is used most frequently [2-4]. The object of the present investigation was to study the kinetics of the hydrolysis of PC at a pH of from 7.0 to 11.0 by the venoms of the saw-scaled viper, kufi, mamushi, and common adder.

The hydrolysis of the PC was carried out with the aid of a TTA3 instrument (Autotitrator, Holland). For each reaction the cell was charged with 10 mg of PC dissolved in 10 ml of diethyl ether, 5 ml of Tris buffer, 1 ml of venom (the venom was dissolved in 0.1 M Tris buffer at pH 7.0, the concentration of venom being 0.15 mg/ml), 0.3 ml of 0.1 M $CaCl_2$, and 1 ml of Triton X-100. As the titrant we used 0.1 N KOH. In view of the fact that 70% hydrolysis took place even in the first few minutes [4], the time of reaction was

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Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 403-404, May-June, 1984. Original article submitted February 3, 1984.

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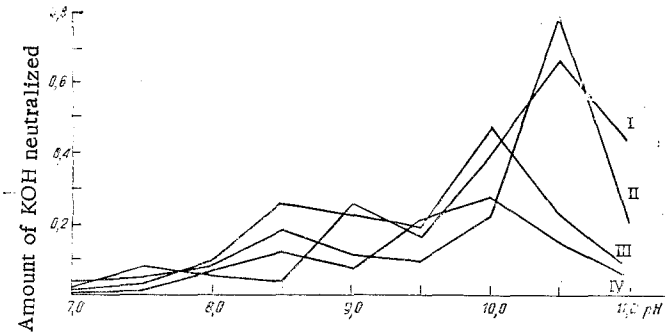

Fig. 1. Hydrolysis of phosphatidylcholine by the venom of: I) saw-scaled viper; II) common adder; III) kufi; IV) mamushi.

made 5 minutes. The Tris buffer was acidified to the required pH with 0.25 N HCl. After each addition of venom, the incubation mixture in the blank variant was monitored, and no absorption of alkali was detected. To stop the reaction, 4 ml of ethanol was added. The results obtained are shown in Fig. 1.

The venoms of the common adder, the kufi, and the mamushi each had two pH optima (8.5, 10.5; 8.5, 10.0; and 8.5, 10.0; respectively), and that of the saw-scaled viper had maxima at pH 7.5, 9.0, and 10.5. This is connected with the fact that the venoms of various snakes contain a whole family of phospholipases A_2 [5, 6] and this probably explains the different behaviors of the venoms.

It must be mentioned that it is just in the pH range of 10.0-10.5 that the action of the venom studied reaches its maximum, after which the activity falls sharply. By comparing the results obtained with literature information [5, 6] it is possible to conclude that the corresponding pH optimum for the hydrolysis of phospholipids should be selected for each concrete venom.

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