308 nm using 95% ethanol as solvent. The optical density of an ethanolic solution of allapinine with a concentration of 0.05 mg/ml is measured in parallel.

The amount of lappaconitine with accompanying alkaloids, reckoned as lappaconitine, is calculated as a percentage (X) on the absolutely dry raw material by means of the formula:

$$X = \frac{D_1 \cdot m_0 \cdot C_0 \cdot 0.89 \cdot 100}{D_0 \cdot m\,(100 - w)}$$

where D_0 is the optical density of the allapinine solution;

D_1 is the optical density of the solution under investigation;

m_0 is the weighed amount of allapinine;

m is the mass of the raw material;

C_0 is the amount of allapinine in the standard sample, %;

w is the loss in mass drying, %; and

0.89 is a calculation factor equal to the ratio of the molecular masses of lappaconitine base and lappaconitine hydrobromide.

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ANALYSIS OF THE HEMORRHAGIC PRINCIPLE OF THE VENOM OF *Echis multisquamatus*

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The presence in snake venoms of a large number of interfering components substantially complicates the identification of their active principles [1]. Thus, according to various authors a hemorrhagic lesion may be caused by specific proteinases and by phospholipase A_2 interacting with cytotoxins and, probably, with independent hemorrhagins of protein nature present in snake venoms [2, 3].

The venom of the Central Asian viper Echis multisquamatus, from which individual proteinases and phospholipases A_2 have recently been obtained [4, 5], acts hemorrhagically, but the nature of its hemorrhagic principle is unknown. In the present work we have analyzed the distribution of the hemorrhagic, proteolytic, and phospholipase activities in the fractionation of the venom with the aid of gel filtration and ion-exchange chromatography.

Fig. 1, A, shows the distribution of the whole viper venom in gel filtration on a column of Sephadex G-75. The results obtained indicate differences in the molecular dimensions of the phospholipase A_2 ($PL\text{-}A_2$) and the hemorrhagically acting factor (H) of the venom, which appeared in the same fraction as a casein-hydrolyzing proteinase (or caseinolytic enzyme, CLE). With respect to its molecular mass, H of the venom differs from the specific

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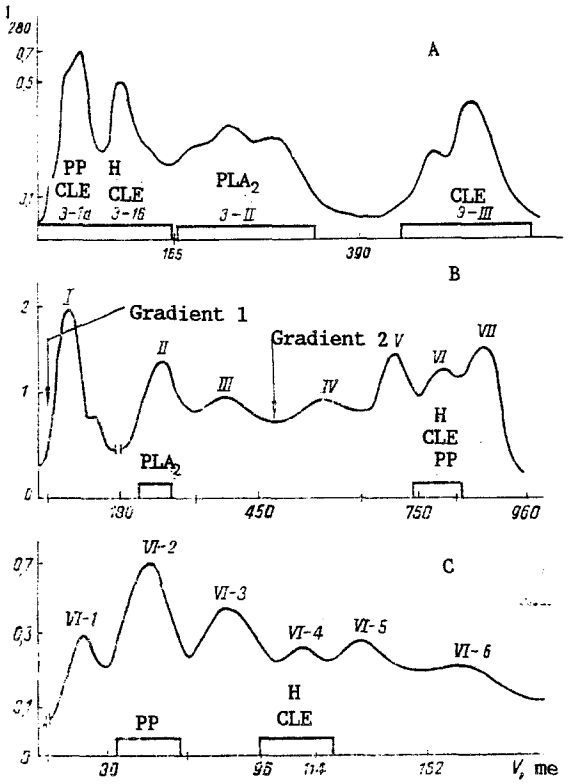

Fig. 1. Graph of the chromatography of whole viper venom on Sephadex G-75 (A) and on CM-Sephadex G-50 (B), and of the active fraction (VI) on Sephadex G-150 (C): H – hemorrhagin; CLE – caseinolytic enzyme; PP – prothrombin-activating proteinase; PLA_2 – phospholipase A_2.

proteinase causing the conversion of prothombin into thrombin (PP). The latter was characterized by greater dimensions than H and was localized in the first fraction. In another variant, the whole venom was subjected directly to chromatography on CM-Sephadex C-50, and then separation of the PL-A_2 from the fractions containing the PP, the CLE, and H was observed (Fig. 1, B). Subsequently, the PP and H present within a single fraction which also possessed CLE activity was separated with the aid of gel filtration on Sephadex G-150 (Fig. 1, C).

The results obtained permit the conclusion that the phospholipase A_2 and prothrombin-activating proteinase have nothing to do with the hemorrhagic effect of the viper venom. More probable is the presence in this venom of a hemorrhagically active proteinase capable of attacking casein, or an independent H, which it will be possible to establish definitively after the isolation of the latter in the individual state.

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