

AN INVESTIGATION OF THE VENOM OF THE VIPER

I. BIOLOGICAL ACTIVITY AND FRACTIONATION

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The bulk of the whole venom of the viper *Vipera ursini renardi* Ch. consists of proteins (~84%), and in addition to proteins the venom contains nucleotides and sugars ($E_{1\%}^{260} = 28$, $E_{1\%}^{230} = 27$). The whole venom possesses proteolytic (1325 units/mg of venom) and phospholipase activities (in a dilution of 1:1000 it increases the time of coagulation of egg yolk to 90 min in comparison with 2 min in the control), and has hemorrhagic (on subcutaneous administration 1.2 mg/kg gives a hemorrhage zone with $l = 1.53$ cm) and toxic actions [$LD_{50} = 3.85$ (3.48–4.23)/kg body weight of the mouse]. The value of LD_{50} of the whole venom is high in comparison with the literature reports [1], which can be explained by the use in our experiments of batches of venom stored for 5 yr. Furthermore, a variation in the toxicity of the venoms connected with the season of their collection must be taken into account [2].

By gel filtration on Sephadex G-75, the viper venom was separated into four fractions: A, B, C, and D. Proteolytic activity was found in the high-molecular-weight fractions A and B, but fraction D, containing components with small molecules, showed little such activity. Phospholipase A was found only in fraction C. When the material was administered to white mice, it was found that the fractions possessing high proteolytic activity—A and B—had a hemorrhagic effect, and fractions A and C had lethal activity. Electrophoresis revealed a considerable heterogeneity of the fractions obtained.

The LD_{50} value was determined by the method of Litchfield and Wilcoxon [3], the hemorrhagic effect was evaluated by Kondo's method [4] 30 min after the administration of the dose, the proteolytic activity by Kunitz's method on a case in substrate [5], and the phospholipase activity from the time of inhibition of the coagulation of egg yolk [6]. Gel filtration on Sephadex G-75 and disk electrophoresis [7] was performed by the usual methods.

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