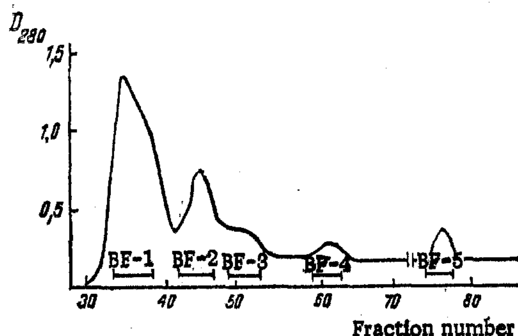

Fig. 1. Gel filtration of a cyanogen bromide hydrolysate of rat transferrin on a column (2.5 × 100 cm) of Sephadex G-50. Elution with 35% acetic acid at a rate of flow of 15 ml/h with collection of 5-ml fractions.

BF-2, and BF-3. As can be seen from Fig. 1, BF-5 was eluted in the form of an individual symmetrical peak. On electrophoresis we observed here an individual peptide fragment with a molecular weight of 6000 dalton. Fraction BF-1 was rechromatographed. Analysis by electrophoresis in PAAG of an aliquot from fraction BF-1 subjected to rechromatography showed the presence of a peptide with a molecular mass of about 26,000 dalton. Fraction BF-1 was saturated with iron ions with the aid of a soltuion of FeCl# and with the addition of trace amounts of NaHCO#. An aliquot from this fraction was subjected to electrophoresis, and after electrophoresis the gels were treated by a method proposed by Kh. S. Rafikov [4] for determining iron-containing proteins. The presence of a brown band in the gel is confirmation of the fact that the component with a molecular mass of 26,000 dalton contains an iron-binding center.

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LIPOLYTIC ENZYMES OF THE VENOM OF *Vespa orientalis*

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The venom of the giant hornet *Vespa orientalis* possesses lipolytic activity [1, 2]. We now give information on the isolation and purification of three lipolytic enzymes from the venom.

The fractionation of the whole venom was carried out in the first stage by gel filtration on Sephadex G-50 under the conditions given previously [1]. The fraction with phospholipase activity after demineralization and lyophilization was subjected to chromatography on CM-cellulose CM-52 in Tris-HCl buffer, pH 7.25, using a linear NaCl gradient (up to 0.3 M). Phospholipase activity was possessed by three fractions (CM-II, CM-III, and CM-V) out of the five. CM-III and CM-V catalyzed the hydrolysis mainly of lecithin, and CM-II that of lysolecithin. Electrophoretic analysis [2] showed the inhomogeneity of these fractions. Individual proteins were obtained after the twofold chromatography of CM-II on CM-cellulose under the same conditions, and after the rechromatography of CM-III and CM-V according to the results of disc electrophoresis in 15% polyacrylamide gel and analysis of the terminal amino acids. The yields of CM-II, CM-III, and CM-V were approximately 1.5 and 1.5-2%, and the degrees of purification 100-, 20-, and 70-fold, respectively. Below, we give some properties of the puri-

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fied lipolytic enzymes from the venom of the giant hornet (+, activation present; —, absence of an effect):

Index	CM-II	CM-III	CM-V
Specific activity, μmole/min/μg	1.0	5.76	0.077
pH optimum	7.5	8–9	8.0–9.5
Activation by Ca^{2+} ions	+	+	+
50% inhibition by			
Triton X-100, %	0.25	—	—
Na DOCh, mM	0.5	1	1
Tris, mM	4.0	—	—
Molecular weight			
by gel filtration	26,000	26,000	22,000
by disc electrophoresis	26,000	26,000	20,000
N-Terminal amino acid	Phenylalanine	Phenylalanine	Aspartic acid
Type of enzyme	Lysophospholipase A_1	Phospholipase A_2	Phospholipase A_2

The hydrolysis of various substrates (synthetic dipalmitoyllecithin, 1-palmitoyllysolecithin, egg lecithin), and some other properties of the enzymes obtained (see above) permitted the conclusion that CM-III and CM-V were phospholipases A_2 and CM-II was a lysophospholipase A_1. The bases for this conclusion, apart from their various substrate specificities, were the values of the specific activities, the different behaviors in relation to inhibitors, their sensitivites to metal ions, and the differences in their molecular weights and N-terminal amino acid residues.

These three enzymes from the venom of the giant hornet also differ in their toxicity. The LD_{50} value on intravenous injection into white mice was 0.58 mg/kg for the phospholipase A_1 (CM-III) and 0.88 for the lysophospholipase A_1, and in the case of the other phospholipase A_2 (CM-V) the animals did not die even with the injection of 5 mg/kg.

Thus, all the proteins that we have isolated are completely different enzymes, although they all belong to the lipolytic enzymes and two of them are phospholipases A_2.

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