THE SEPARATION OF LIPASE AND PHOSPHOLIPASE ISOLATED FROM COR. PYOGENES VAR. HOMINIS BY GEL FILTRATION

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In previous reports our group described pathogenic properties of Corynebacterium pyogenes var. hominis (1, 2). First, dermonecrotic and lethal activity of a filtrate of a bacterial culture in the rabbit was described (3, 4). Practically simultaneously the phenomenon of inhibition of staphylococal alpha lysin by means of adsorption of a component in the filtrate onto erythrocytes was discovered (5, 6). *Souček and Součková* (7) found on immunological analysis of both components that toxin and the inhibitor are identical. Besides, they found the filtrate to be active in that it dissolves egg yolk and lecithin, and splits Tween 20 with formation of a lister of fatty acids. Both activities were later defined chemically (8) by chromatographic determination of degradation products—choline and phosphatidic acid, or fatty acids as described in another communication (9).

It was found that the component which splits choline of molecules of lecithin is phospholipase D and is identical with the component responsible for the dermonecrotic and adsorption—inhibition activities. It was designated as alpha —component and direct methods for the determination of all its activities were elaborated (10). Purification of filtrate by adsorption onto sheep erythrocytes, followed by precipitation with ammonium sulfate does not impair the activities of alpha—component while the lipase activity is abolished. The component responsible for the hemolytic activity was described earlier and designated beta—component. A further component produced by this microbe, designated gamma—component, is active against Tween 20 and phosphatidic acid which is formed from lecithin by phospholipase D.

It has been attempted to separate these components by gel filtration. Beside confirming that the components responsible for the phospholipase and lipase activities are not identical, this method served to prepare partially purified components. Sephadex G-100 was used, although G-75 also separated phospholipase while hemolysin and lipase passed through feely. A column 40 cm long and 2 cm in diameter and a collector of fractions were used. Filtrate was eluted with buffered physiological saline. The alpha-component was determined a) by the dermonecrotic reaction in the rabbit (10), b) by determining inhibition of staphylococcal alpha-lysin by the 50% hemolysis method (6), and c) by measuring zones of clarification in egg yolk agar (10). Hemolysin was determined by the 50% hemolysis method (6) and lipase by titration in agar with Tween 20 (7). Fig. 1 shows the results of separation of filtrate. The lighter alpha-component was completely separated from the heavier beta- and gamma-components which passed trough. This procedure was used to separate

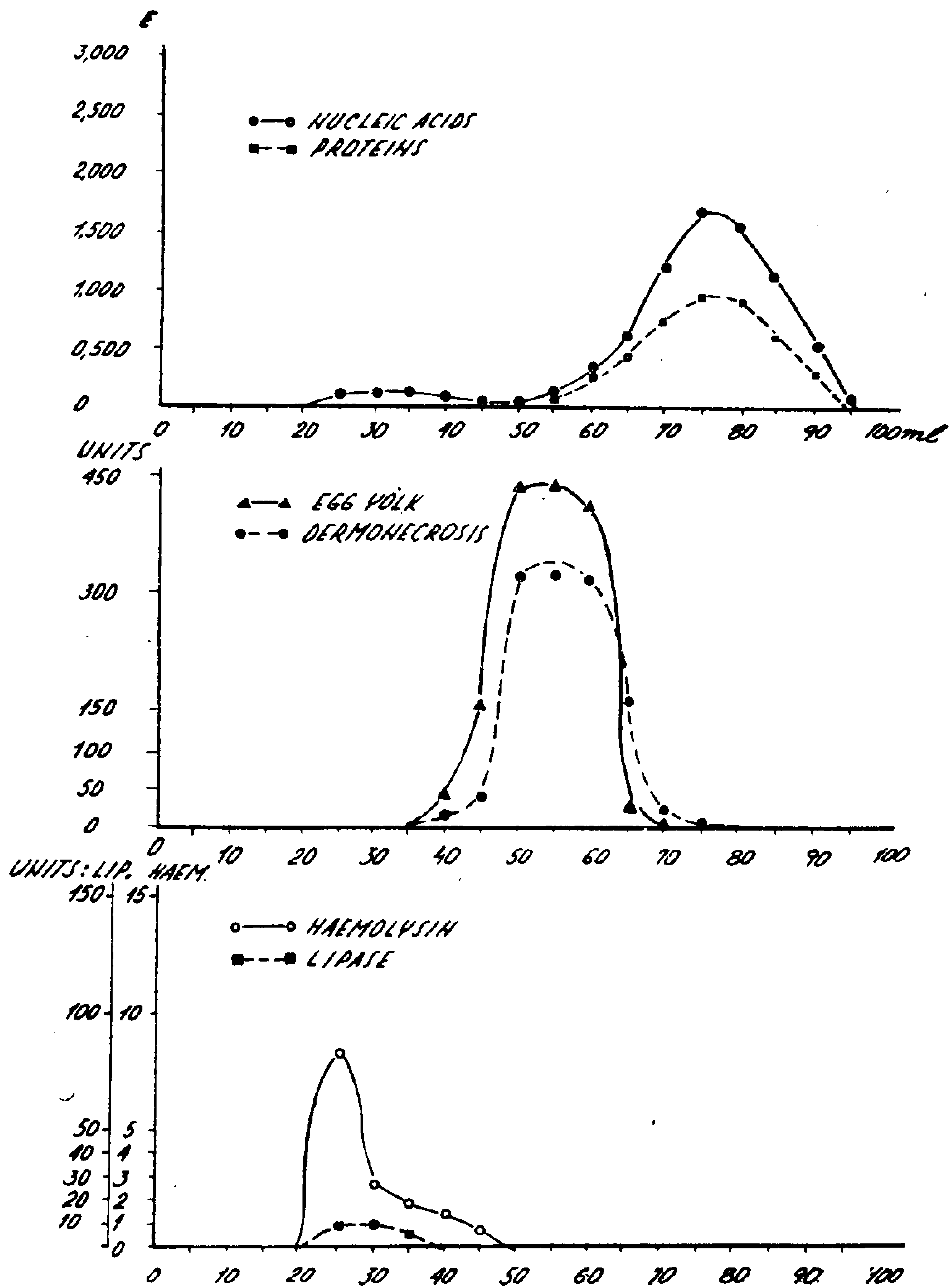

Fig. 1. Separation of Filtrate of Cor. pyogenes var. hominis strain K-29. Nucleic acids and Proteins determined by Optical Density at 260 nm, 280 nm respectively. Other Activities as described in Text.

phospholipase from lipase, followed by precipitation of the phospholipase-containing fraction with ammonium sulfate and reelution in physiological saline.

It was attempted to prepare lipase from endoplasmatic material which has a high content of this enzyme. Bacterial cells were washed by centrifugation and disintegrated in Novotný's mechanical disintegrator with balotino. Supernatant after centrifugation at 10.000g was passed trough a Sephadex column. Separation of components is show in fig. 2. The fractions containing lipase also possessed phospholipase activity which was not present in these fraction derived from filtrate. The fraction was relatively less active in its dermonecrotic action in comparison with phospholipase activity.

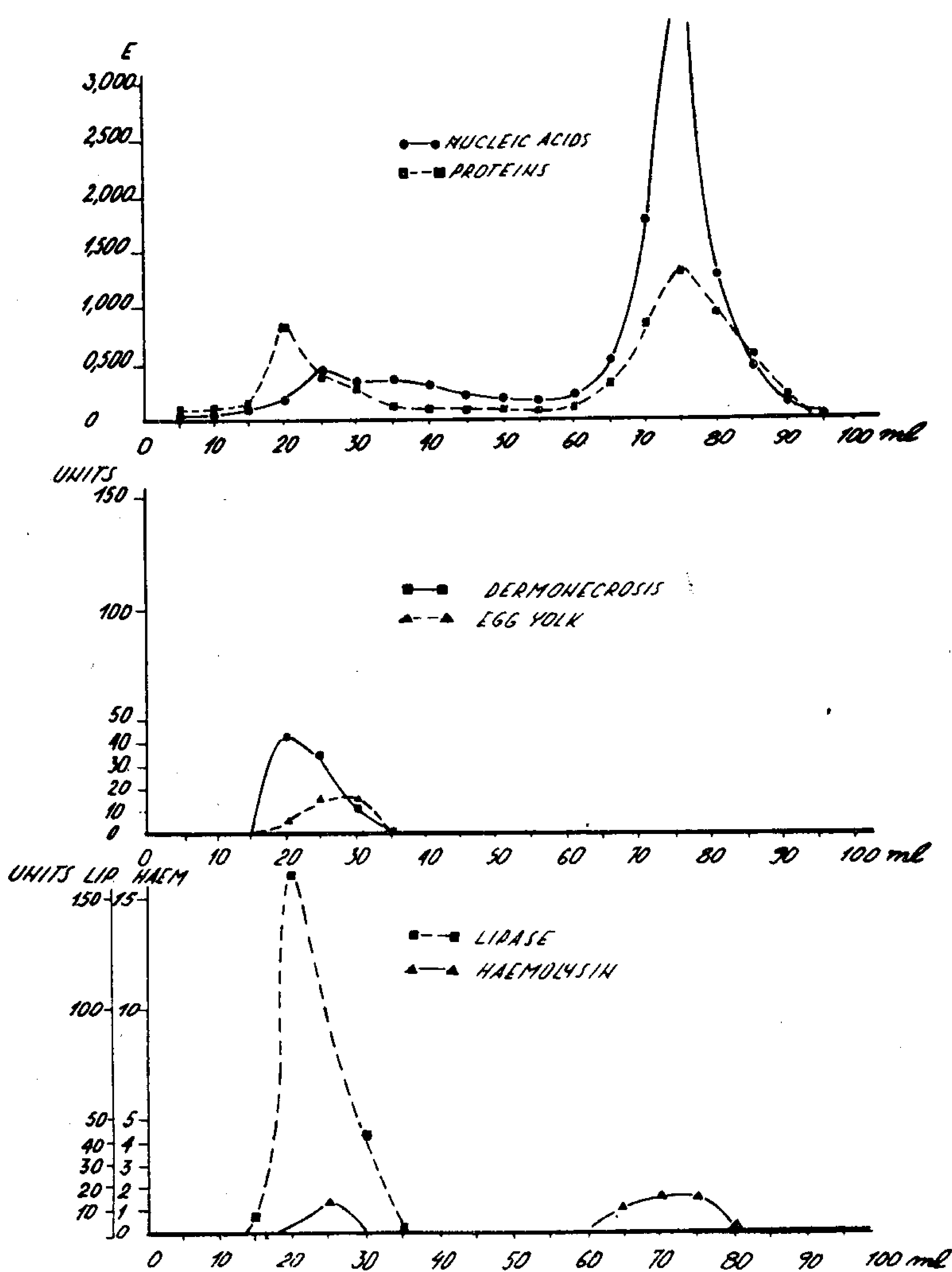

Fig. 2. Separation of the endoplasmatic Material of Cor. pyogenes var. hominis strain K-29 cultivated 48 hours in Todt-Hewitt broth with 2 % rabbit serum. All Activities and Nucleic Acids and Proteins determined as described above.

The same result was obtained when the culture was cultivated under different anaerobic conditions and medium (Vf broth). Fig. 3 shows the results of separation of this endoplasmatic material. This new component is under further investigation. If the possibility of phospholipase activity is eliminated in lipase, we may be dealing with a moleculary larger precursor which is degraded in the bacterial cell and diffuses into the medium.

No further separation was observed in Sephadex G-200. It was attempted to concentrate lipase from filtrate by repeated separation and lyophilization of

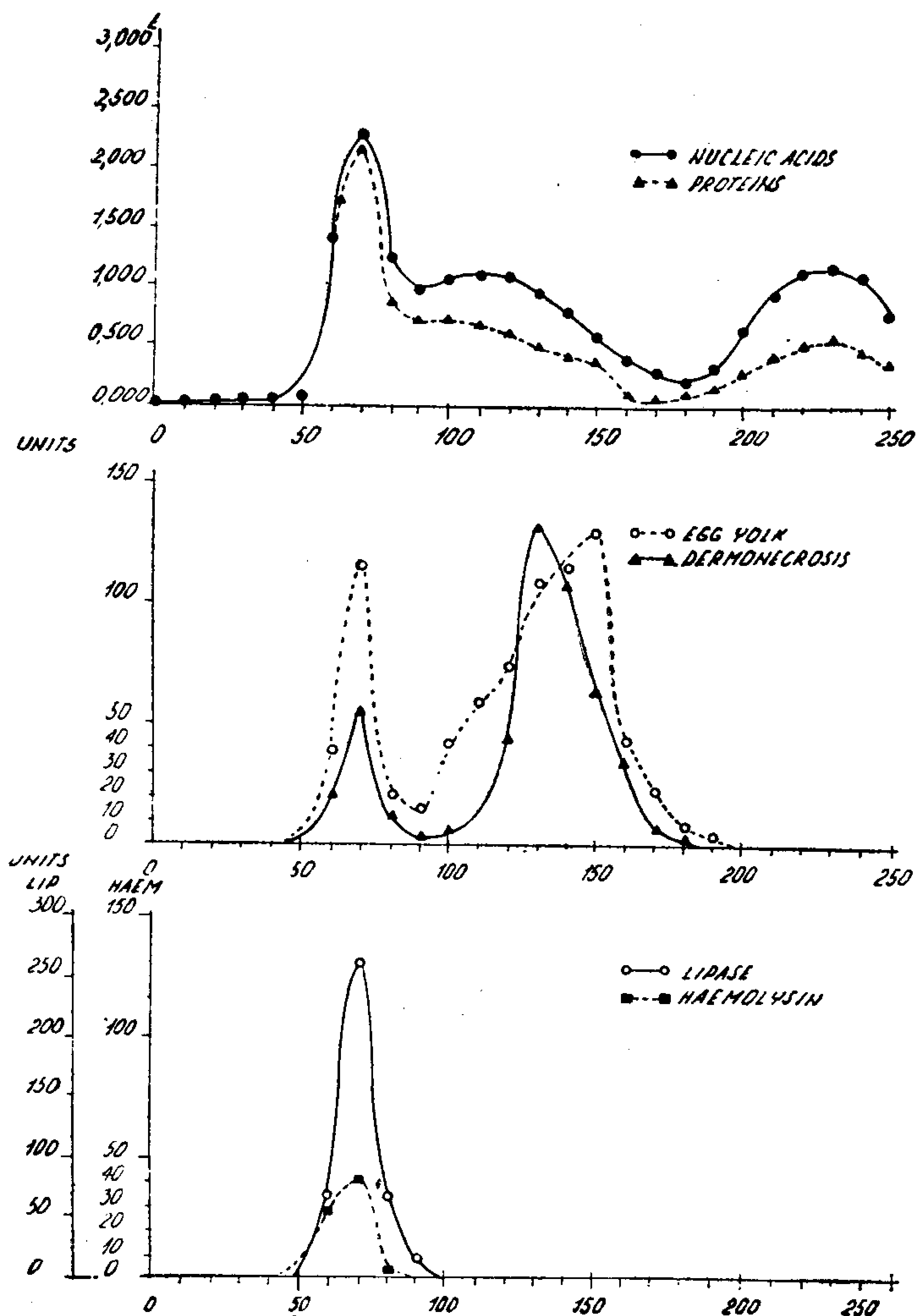

Fig. 3. Separation of the endoplasmatic material of Cor. pyogenes var. hominis strain K-29 cultivated 18 hours anaerobically in Vf broth. All Activities, Nucleic Acids and Proteins determined as described above.

fractions. Its low concentration and greater lability during preparation did not lead to any final results.

It was experimentaly confirmed that phospholipase and lipase activities of filtrate of Cor. pyogenes var. hominis are distinct and specific. We succeeded in purifying phospholipase for detailed studies of its activity. A new component was found which possesses phospholipase activity and is present only in endoplasmatic material. It differs from alpha-component in its molecular weight.

Translated by M. Chýle

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