

TUBERCULIN-ACTIVE FRAGMENTS OF MYCOBACTERIA
OF THE STRAIN VALLEE-58

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In order to deepen the study of the interrelationship of the biological function and chemical structure of allergens, we have isolated five homogeneous components from the mycobacteria of tuberculosis strain Vallee-58.

The autoclaved and defatted mycobacteria were extracted with a 200-fold amount (w/v) of 0.1 N HCl at 37°C for 42 days. The extract was fractionated on columns of the sulfonate cation-exchange resins S2D and S4D synthesized by us previously [1]. The hydrochloric acid extract (0.6 liter) was deposited on a column 1.6 × 60 cm equilibrated with 0.2 M Na citrate buffer, pH 2.2. The column was washed with water. Stepwise gradient elution was performed with 0.2 M Na citrate buffer solutions with pH 3.25 and 4.25, 0.05 M pyridine acetate with pH 5.15, 0.2 M aqueous pyridine solutions, and alkalis (Fig. 1). The rate of elution was 120 ml/h, the fraction size being 25 ml.

The further purification of the quantitatively predominating fractions I, II, VI, IX, and X was performed by gel filtration. The homogeneity of the components was determined by electrophoresis in polyacrylamide gel [2], by gel filtration on Sephadexes G-25 and G-75, and by high-voltage horizontal [3] and vertical [4] paper electrophoresis.

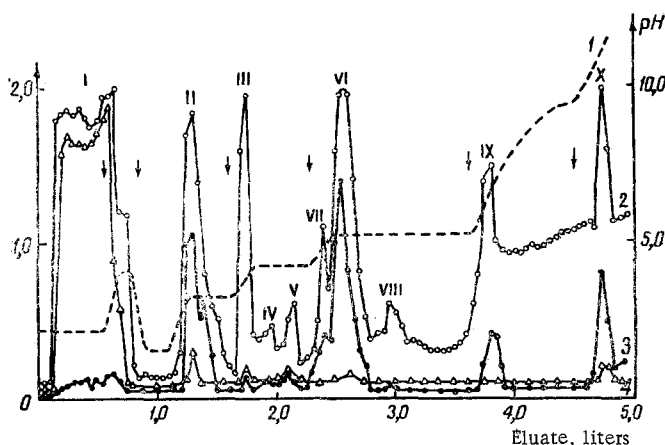


Fig. 1. Fractionation of an HCl extract of mycobacteria of the strain Vallee-58 on S4D sulfonated cation-exchange resin: 1) exchange of eluents and increase in the pH of the eluate; 2) optical density at 280 nm; 3) extinction of the ninhydrin coloration (570 nm); 4) intensity of the coloration with orcinol (540 nm).

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Fraction I was not sorbed by the cation-exchange resin and consisted to the extent of 85.6% of glucose, galactose, and arabinose in a molar ratio of 1:1.6:4.6. In addition, component I contained traces of ribose, rhamnose, xylose, and, probably, tyvelose. Fraction II was found to contain mannose (8.9%) and a small amount of rhamnose.

Components VI, IX, and X consisted of proteins and exhibited a considerable specific activity in sensitized guinea pigs.

Their amino acid compositions were determined by the method of Spackman et al. [5]. The components of VI, IX, and X contained the most frequently encountered proteinogenic amino acids. They were enriched in aspartic and glutamic acids, alanine, and leucine and were impoverished in aromatic and sulfur-containing amino acids and also in tryptophan. The peptide X had larger amounts of arginine and lysine than the others; in the electrophoretically homogeneous component I, only acidic and neutral amino acids were found. It follows from this that the component fractions of the mycobacteria of tuberculosis, which possess qualitatively similar tuberculin activities, have different primary structures.

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