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T Antigen of *Streptococcus pyogenes*: Isolation and Purification

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A T antigen preparation free of trypsin was obtained by application of CNBr-Sepharose linked to pure trypsin. Purification on an ion exchange chromatograpy column results in an electrophoretically homogeneous preparation of T protein.

Present knowledge about T antigen remains very fragmentary. This is obviously due to the difficulties in isolating the T protein in large quantities and to the concomitant lack of data about its biological properties.

Studies on T antigen have been focused mainly on problems related to serological typing. Relatively few investigations were devoted to isolation, purification, and physical and chemical analysis of this substance (2-4).

The classical method for extraction of T protein according to Pakula (4) is based on digestion of cell suspension by pancreatic extract or by trypsin. Application of this procedure results in crude T antigen contaminated with enzymes and other proteins.

Insoluble trypsin linked to CNBr-Sepharose was used to extract T antigen from cells. Trypsin so prepared can be regenerated and reused.

Streptococcus pyogenes type 12 was cultivated for 48 h at 30 C. Bacteria were centrifuged and washed three times with saline and then heated at 80 C for 10 min and digested by trypsin linked to CNBr-Sepharose at 37 C.

An 8-h digestion appeared to be optimal for T protein release from cells (Fig. 1). After an 8-h digestion the suspension was centrifuged at 12,000 rpm, the sediment was discarded, and the supernatant was dialyzed against distilled water and concentrated by polyethyleneglycol. T protein was then purified by ion exchange chromatography on a QAE Sephadex A-50 column equilibrated with 0.01 M phosphate buffer, using a sodium chloride linear elution.

A crude preparation of T antigen obtained by digestion with trypsin (Spofa-Czechoslovakia) linked to CNBr-Sepharose was introduced on a QAE Sephadex A-50 column and separated into three protein peaks (Fig. 2). Only one peak, eluted at 0.2 M NaCl, was active and electrophoretically homogeneous on polyacrylamide gel by the procedure recommended by Davis (1).

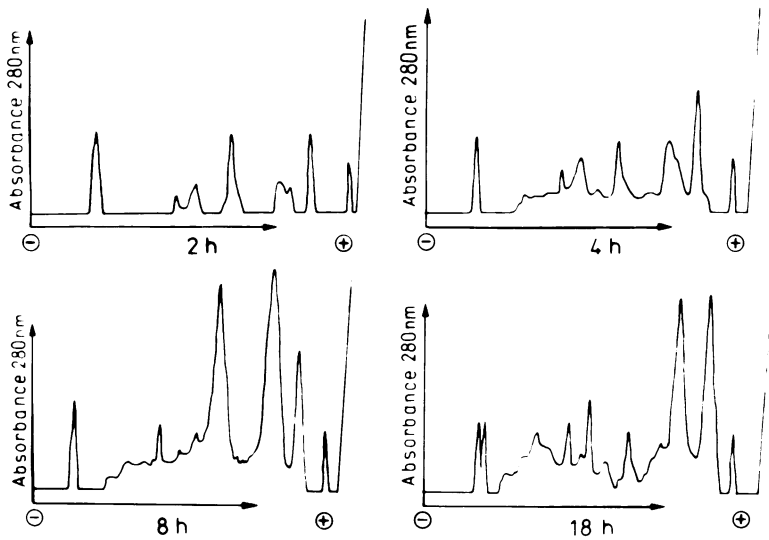


FIG. 1. *Scans of disc electropherograms of streptococcal cells after different times of trypsin digestion.*

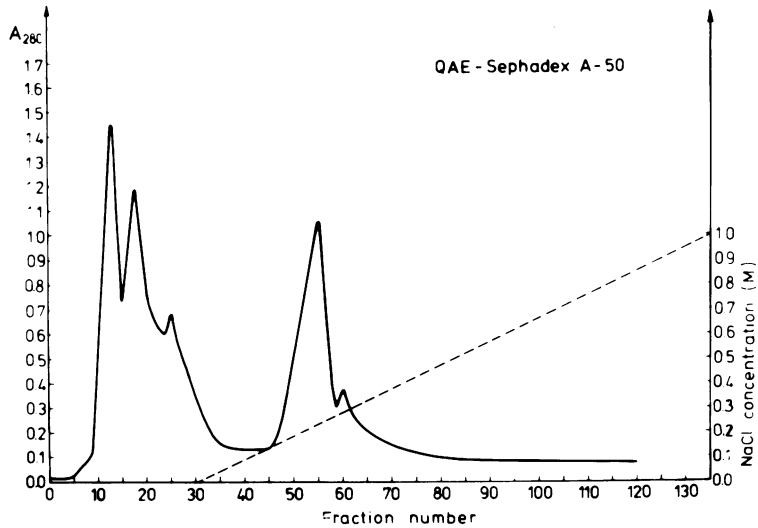
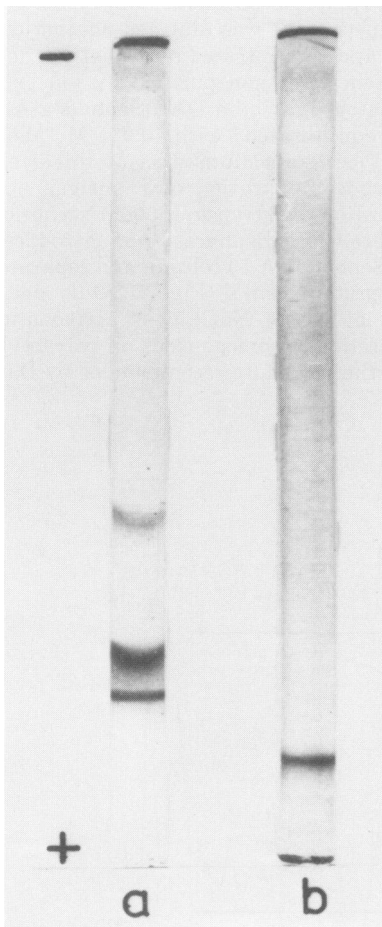


FIG. 2. *Protein peaks obtained after ion exchange chromatography of a crude T antigen preparation.*



The activity was checked by a capillary precipitation test and by a double-immunodiffusion test with anti-T monovalent serum (Fig. 3).

These preliminary results describe what could be an effective method of T antigen isolation and purification. Further research will concern physical and chemical characteristics and development of a quantitative method for T antigen determination.

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FIG. 3. *Column chromatography of (a) a crude T cell preparation. (b) Active protein peak.*