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Separation of hemoglobin and cytochrome c by electrophoresis and by chromatography on DEAE-Sephadex

In the course of studies on the heme proteins[1,2] a complete removal of hemoglobin from myoglobin and cytochrome *c* preparations was found to be essential. Several procedures for the use of cellulose ion-exchange columns have been developed for the heme proteins, and a method for the chromatography of myoglobin on DEAE-cellulose has been described[3].

This report describes procedures for the separation of hemoglobin and cytochrome *c* by the method of membrane-filter electrophoresis[4] and for the chromatography of these proteins on DEAE-dextran columns.

Cytochrome *c* from horse heart, Batch No. 11, 0.42% Fe, 92% purity, was obtained from Seravac Laboratories, Cape Town, South Africa and Colnbrook, Great Britain. Hemoglobin used for chromatography was prepared in the met-(ferri-)state, in our laboratory by the method of ROSSI-FANELLI[5]. In electrophoresis experiments washed and hemolysed erythrocytes were applied directly to the strips. The applied mixture contained about 20 μg of total protein. Cellulose acetate electrophoresis strips (Oxoid, Oxo Ltd, London) 9 × 2.5 cm were used. The electrophoresis was carried out by the method of KOHN[4], using a veronal buffer (pH 8.6), and alcoholic amidoblack for staining. The chromatographic separation was carried out on DEAE-Sephadex A-50 (Pharmacia, Uppsala, Sweden). The Sephadex material was repeatedly suspended in Tris-buffer (pH 8.6) and decanted after 15 min. After packing into a 0.9 × 10 cm tube the column of Sephadex was washed again with Tris-buffer until the pH was constant. A sample containing cytochrome *c* and a relatively large amount of hemoglobin (about 20 mg of total protein) was allowed to

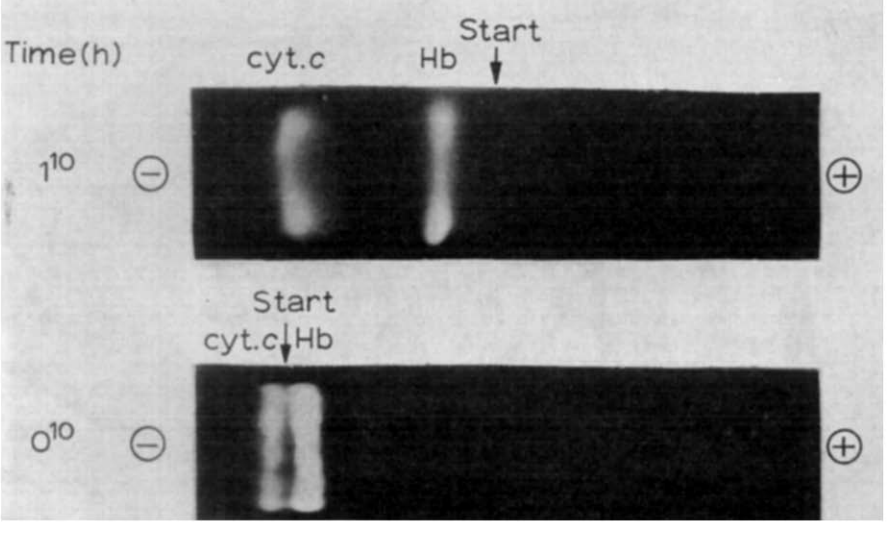

Fig. 1. Micro-electrophoresis of a mixture of cytochrome *c* and hemoglobin. Applied voltage 150 V. Photograph (negatives) obtained by contact printing from stained, uncleared strips.

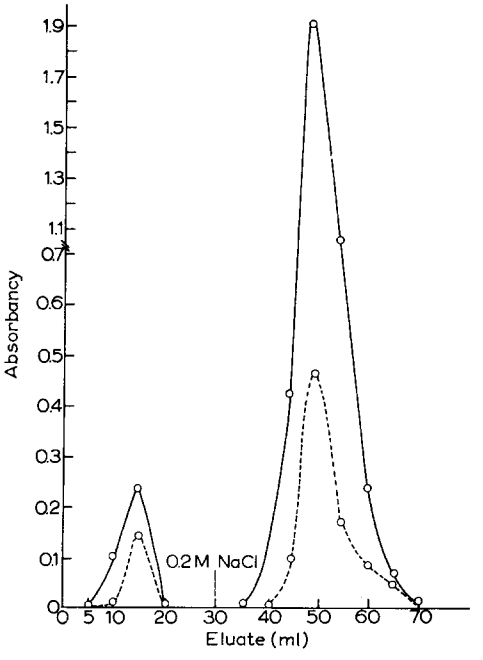

Fig. 2. Chromatography of a mixture of cytochrome *c* and hemoglobin. ———, absorbancy at 280 mμ; — — —, absorbancy at 530 mμ.

percolate into the column by gravity. Cytochrome *c* was eluted with 0.05 M Tris buffer (pH 8.6). Hemoglobin was retained at the top of the column and was removed by 0.05 M Tris buffer (pH 8.6) in 0.2 M NaCl. Flow rate was about 1 ml/min and 5-ml samples were collected. The concentrations of proteins in the effluent fractions were assayed by measurement of absorbancy in a Pulfrich photometer with Elpho-Zusatz 530-mμ filter, and ultraviolet absorbancy at 280 mμ in a Zeiss spectrophotometer VSU 1.

Similar procedures may be used for the separation of hemoglobin from myoglobin[6].

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[1] W. LEYKO, *Lodz. Towarz. Nauk. Wydzial III*, No. 61 (1959).
[2] W. LEYKO, A. DMOCHOWSKI, L. BUCZEK AND R. GONDKO, *Zeszyty Nauk. Univ. Lodz. Ser. II*, 12 (1962) 37.
[3] W. D. BROWN, *J. Biol. Chem.*, 236 (1961) 2238.
[4] J. KOHN, *Clin. Chim. Acta*, 3 (1958) 450.
[5] A. ROSSI-FANELLI AND E. ANTONINI, *Arch. Biochem. Biophys.*, 58 (1955) 498.
[6] R. GONDKO AND M. SCHMIDT; reported at *2nd Polish Conf. Chromatog., Lublin, Sept. 1963*, in preparation.

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