Hähne: offen: a, b, α, e, d, g, i, j, o, p, s, u, w, y, z, l, m, n; geschlossen: c, β, γ, f, h, k, q, r, t, v, x.

(4) *Einschleusen der abgestellten Fraktion zur weiteren zyklischen Chromatographie*

Weg: Vorratsgefäss, Ventile 1, 2, 21, 7, 8, 10, 11, 13, 15, 17, 18, 19, 3, Pumpe, Säule, Durchflussphotometer, Ventile 4, 5, 6, Pumpe, Fraktionensammler.

Hähne: offen: a, c, e, γ, o, p, s, u, w, y, z, h, g, i, k, m, n; geschlossen: b, d, f, α, j, q, r, t, v, x, l, β.

Sowie die Fraktion in der Säule ist, weiter chromatographieren wie unter (2).

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Separation of myoglobin and hemoglobin on Sephadex thin layers

Sephadex gels have proved very convenient for the separation of substances differing in molecular weight. In chemical pathology it is sometimes necessary to differentiate Mb from Hb* by a simple method.

Although we have succeeded by using conventional paper electrophoretic separation[1,2] we decided to try also Sephadex thin layers (TLC) for this purpose.

TLC was performed on glass plates 18 cm × 4 cm. A suspension of Sephadex was prepared by shaking swollen Sephadex beads by means of an agitator in an 0.1 *M* phosphate buffer, pH 7.4, or in a mixture of this buffer with the same volume of 0.9% NaCl. Sephadex G-50 Superfine, G-75 Superfine and G-200 Superfine were used. The volume ratio of gel solution was chosen according to the instructions of the manufacturer[3]. Layers 0.9 mm thick were spread by means of a glass rod provided with cuffs of adhesive tape (Isolepa). Descending chromatography was performed on glass plates inclined at an angle of 12°, connected with the solvent trough by a bridge of Whatman 3 paper and placed in a closed glass tank. Detection was performed either by the use of reactions specific for the heme groups of Hb and Mb, with benzidine[4] or *o*-dianisidine[5], respectively; or by staining for proteins with bromphenol blue[6].

Fig. 1 shows the separation of Hb from Mb on Sephadex G-50 Superfine in

* Abbreviations: Mb = myoglobin; Hb = hemoglobin.

Fig. 1. TLC on Sephadex G-50 of muscle extract and added Hb. Chromatography was performed with phosphate buffer for 40 min.

phosphate buffer, pH 7.4, after 40 min development. Detection with benzidine–H_2O_2. Similar results were obtained with Sephadex G-75 and with a mixture of the phosphate buffer and 0.9 % NaCl (1:1). The solution of NaCl alone (with any kind of Sephadex) was not suitable. With Sephadex G-200 the chromatography proceeded very slowly and even after 16 h separation of Mb and Hb was not achieved. The minimum detectable amount of Hb by the benzidine–H_2O_2 reaction after chromatography was about 15 μg when a 2 cm long line had been spotted.

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