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Note

New observations concerning the membrane chromatography of cells

In a recent communication[1], we showed the possibility of the rapid chromatographic fractionation of different erythrocytes by using spongy poly(2-hydroxyethyl) methacrylate (HEMA) membranes as the carrier. Since then, new improvements have been made to this microchromatographic technique and new observations have been made on the microstructure of the swollen HEMA membranes and the different chromatographic behaviour of the cells of one individual organism. These results are reported in the present communication.

Material and methods

Fresh erythrocytes and leucocyte-rich plasma were obtained by the spontaneous sedimentation of blood collected in 3.8% sodium citrate solution. Murine and rat spleen cells were suspended in a standard phosphate-balanced solution (PBS) of pH 7.2. Both citrate and phosphate solutions were used as eluants in chromatography. Blue dextran (Pharmacia, Uppsala, Sweden) was used to indicate the moving chromatographic front. HEMA membranes were prepared as described earlier[1]. Swollen HEMA strips, 1 cm wide, 2.5 cm long and 1.5 mm thick, were used for chromatography. About 10 μl of the sample, containing 750,000 cells, was applied to the start with a capillary. Then 2–4 μl of the eluant was applied in order to move the sample about 0.5 mm into the interior of the membrane. The HEMA strip, adhering spontaneously to a supporting glass slide (*cf.* Fig. 1.), was used with the starting-point downwards and rhythmic upward development in repeated ten-step periods, including short downward pulses to prevent clogging, was carried out according to the scheme shown in Fig. 1.

When the zone of blue dextran had travelled over 1 cm from the start, the

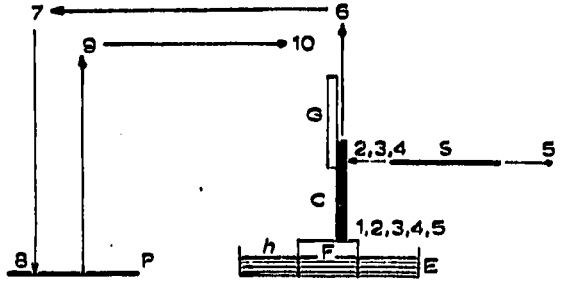


Fig. 1. Scheme of the rhythmic chromatographic development in ten-step periods. E = eluant; F = feeding HEMA membrane; h = distance over the surface of eluant (usually 4–5 mm); C = chromatographic HEMA membrane; G = supporting glass slide; S = dry strip of Whatman No. 3 filter-paper; P = dry Whatman No. 1 filter-paper. The numbers indicate consecutive steps; each step lasted 1 sec. Instructions for each step: 1 = start development, put bottom of C on F; 2 = start suction, contact S with C; 3 and 4 = continue suction; 5 = stop suction, remove S from C; 6 = stop developing, lift C from F; 7 = transfer C above P; 8 = reverse suction, put bottom of C on P; 9 = lift C from P; 10 = transfer C above F again.

HEMA strip was cut into five zones, which were placed on a polyethylene film and wetted slightly with the eluant. The tip of a thin glass capillary designed to drain off the cells was inserted between the supporting polyethylene film and each piece of the HEMA strip. Excess of eluant was then applied to the membranes from another capillary, which simultaneously gently tipped and vibrated the HEMA sponges. A 50–100 μl volume of eluant was usually sufficient to elute a substantial amount of cells. The cells were then fixed, stained and evaluated microscopically by standard techniques.

To investigate the microstructure of swollen HEMA membranes, the material was rapidly frozen at $-70°$ in a fluid propane–butane bath and sliced with a cooled microtome.

Results and discussion

Fig. 2 shows that the solid backbone of the swollen HEMA sponge has the character of partly fused spheres. Different batches differed mainly in the size and size-distribution of the spherical subunits and of the free space. It was stated that the carrier A (Fig. 2), which was most suitable for the chromatography of erythrocytes[1], had a mean size of the sub-units similar to the size of the cells. On

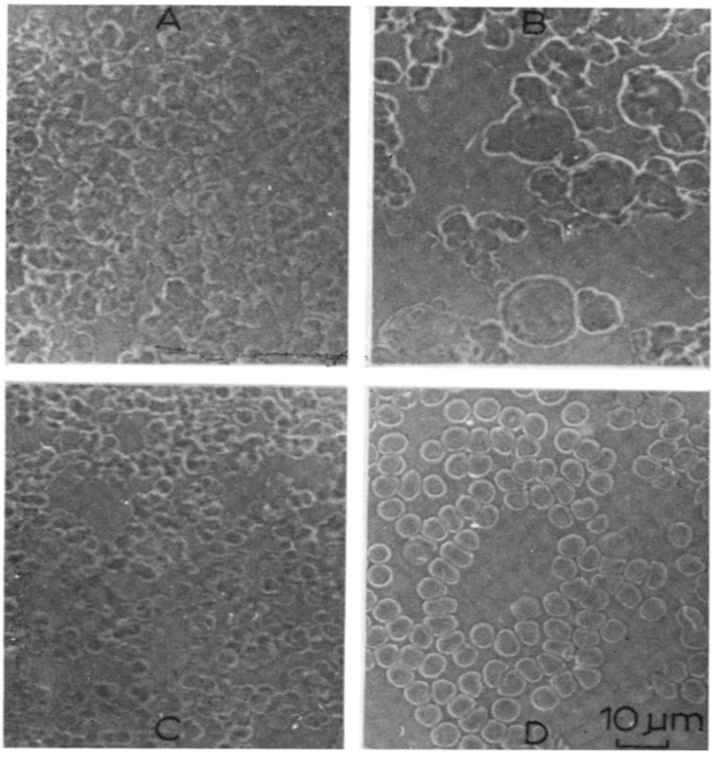
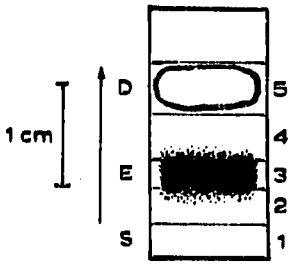


Fig. 2. Microstructure of swollen HEMA membranes. A, membrane prepared from 30% monomer, 70% water, 2% crosslinking agent, fast polymerized[1]. B, membrane prepared from 30% monomer, 70% water, 1% crosslinking agent, fast polymerized. C, membrane prepared from 30% monomer, 70% water, 2% crosslinking agent, slow polymerized. D, murine erythrocytes, unstained smear. All samples were photographed at the same magnification. The slices of HEMA sponges were 10 μm thick.

the other hand, murine erythrocytes D migrated too fast on the carrier B with larger subunits and too slowly on the carrier C with smaller sub-units. Hence the diameters of the capillary communications between the looser lagoons in the spongy structure seem to be a more important factor for the passage of cells than the size of the lagoons themselves.

The result of a typical chromatographic experiment (Fig. 3) shows a partial separation of leucocytes, which remained in fraction 1, erythrocytes, which were concentrated especially in fraction 3, and thrombocytes in fractions 1 (probably aggregates of thrombocytes) and 5. Rat and murine cells in general behaved in a similar manner.



L/100 E	T/100 E	
4	655	
0	15	
0	29	
0	49	
113	223	
14	17	Control

Fig. 3. Chromatography of a mixture of murine blood cells and spleen cells on HEMA membrane A and relative cell counts. D = blue dextran; E = erythrocytes; L = leucocytes; T = thrombocytes; S = start. The counts of leucocytes and thrombocytes are given relative to 100 erythrocytes.

The present results confirm that spongy HEMA membranes of appropriate quality can serve as a suitable carrier for further investigations and separations in the field of chromatographic fractionations of cells, which is dependent predominantly on the size and shape of the cells. Investigations are being carried out to ensure that standard HEMA sponges with a more homogeneous microstructure are prepared.

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