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SEPHADEX G-25 BEADS AS CHROMATOGRAPHIC CARRIER FOR THE FRACTIONATION OF ANIMAL CELLS

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SUMMARY

The suitability of Sephadex G-25 as a chromatographic carrier for fractionation of animal cells was assessed against Degalan V 26 and glass beads. The counts of non-adherent cells, reversibly adhered cells and irreversibly adhered cells were determined in cell suspensions of mouse thymus, spleen, lymph nodes, bone marrow and erythrocytes. The lowest adherence of cells was observed on Sephadex G-25. Practically no cells adhered irreversibly to this carrier.

INTRODUCTION

Improvement in fractionation methods used in the investigation of cells is a problem pressing for a solution. In column chromatographic methods, which seem to have potential for cell fractionation, at present many practical difficulties are experienced, one of them being the retention of a great number of cells in the column.

When using glass bead columns a certain part of the retained cells can be set free by mechanical stirring, but about 30% of the total number of cells applied remain irreversibly adhered to the glass beads[1,2]. It is, therefore, necessary to test more suitable carrier materials for chromatographic fractionation of cells.

Many reports have been published describing the separation of certain types of cells on columns filled with Sepharose beads[3], dinitrophenyl-conjugated Sepharose beads[4], immunoglobulin-coated Degalan V 26 beads[4], nylon wool[5], modified Sephadex G-200 (refs. 6 and 7), and other carrier materials. However, only a few papers describe the adherence of cells to various carrier materials[8] and the conditions leading to cell adhesion[9]. This was the reason why we used Sephadex G-25 in our experiments, as a representative hydrophilic carrier, and Degalan V 26 (polymethylmethacrylate beads), as representative hydrophobic carrier, for the fractionation of mouse cells from several organs by means of adherence chromatography. The results were compared with those obtained during parallel fractionation on glass beads.

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MATERIALS AND METHODS

Solutions

For chromatographic elution we used the phosphate-balanced solution (PBS) with streptomycin, penicillin and 20% calf serum (PBSC), at pH 7.2. The preparation of the phosphate solution has already been described[2].

Cells

In our experiments we used suspensions of cells from A strain adult mice, weight 20–30 g. A suspension of erythrocytes was obtained from mouse blood in a 3.7% sodium citrate solution. Erythrocytes were washed three times with saline, packed red cells were re-suspended in PBSC, and their number adjusted to $75 \cdot 10^6$/ml.

Cell suspensions from spleen, thymus and lymph nodes were prepared from a group of freshly killed mice. The organs used were washed with sterile saline (0.9% NaCl) and then teased with forceps in PBSC solution. The crude cell suspension was filtered through a fine-mesh polyamide sieve.

Bone marrow cells were flushed from femurs of the same group of mice. The crude cell suspension in PBSC was washed twice with PBSC and then suspended in the same medium to give a final concentration of $75 \cdot 10^6$/ml. The cells were immediately used for chromatography.

Chromatography

We used jacketed chromatographic columns (30 × 1.3 cm) connected to a Hoeppler ultrathermostat.

A slurry of 18 ml of glass beads (0.2-mm mean diameter; Batch No. 16; Jablonec Glass Works, Prague, Czechoslovakia), Degalan V 26 beads (0.2-mm mean diameter; Degussa Wolfgang, Hanau am Main, G.F.R.) and Sephadex G-25 beads (100–300 mesh; Pharmacia, Uppsala, Sweden) was placed in the columns.

Pre-treatment of glass beads has already been described[1]. Degalan and Sephadex were treated for 72 h in PBS before filling the columns. The three columns were washed before adherence chromatography by 50 ml of PBS (flow-rate 1 ml cm^{-1} min^{-1}). A 1-ml volume of cell suspension containing $75 \cdot 10^6$ cells was applied and soaked in the columns and then PBSC was applied in two 1-ml portions. After standing for 15 min at 25°, the cells were eluted with 70 ml PBSC. After 10 ml the flow-rate of 0.5 ml cm^{-1} min^{-1} was doubled and was maintained at this level until 70 ml had been collected. This was fraction No. 1: non-adhered cells. We then applied 10 ml of PBSC and the adhered cells were mechanically set free by stirring the suspension for 40 periods of 40 s with a spiral stirrer (platinum wire). This was fraction No. 2. The mechanical stirring was repeated once more and finally 5 ml of PBSC were applied. The total volume of fraction No. 2, reversibly adhered cells, was 25 ml. The amount of cells in each fraction was determined in a Bürker chamber. The amount of irreversibly adhered cells was calculated as the difference between the number of cells applied to the column and the number of cells found in fraction Nos. 1 and 2.

RESULTS AND DISCUSSION

We made three parallel runs of the same cell suspension on three columns filled with beads of glass, Sephadex G-25 and Degalan V 26. The cells were eluted from the columns with PBSC solution and the reversibly adhered cells were then loosened by mechanical stirring. The number of liberated cells in both fractions was determined and in some samples a morphological evaluation was carried out.

Fig. 1 shows the results of fractionations of cell suspensions from mouse thymus, lymph nodes, spleen, bone marrow, and erythrocytes. The cell counts in Fig. 1 are expressed in percents as the average (± 10%) of cell counts of non-adhered, reversibly adhered and irreversibly adhered cells.

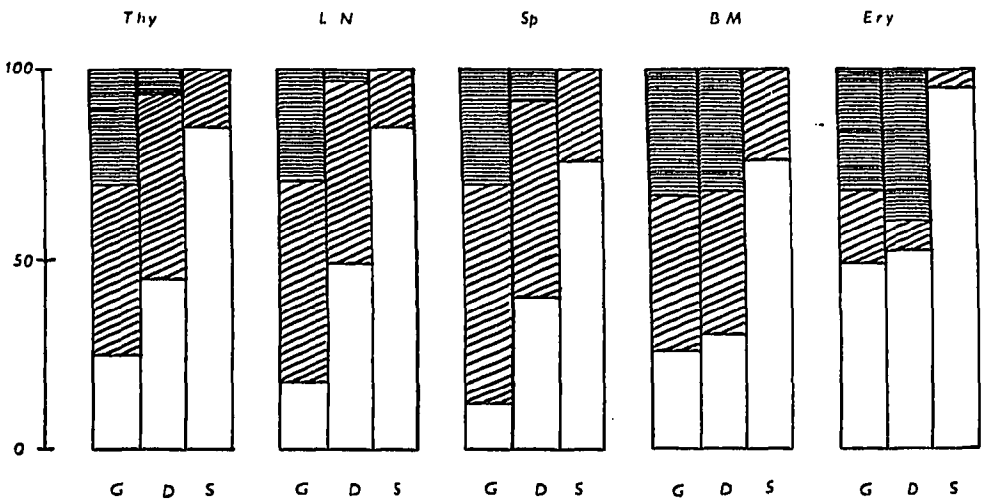

Fig. 1. Adherence chromatography on Sephadex G-25 (S), Degalan V 26 (D) and glass beads (G). Fractionation of cell suspensions from mouse thymus (Thy), lymph node (LN), spleen (Sp), bone marrow (BM) and erythrocytes (Ery). The cell counts are expressed in percents as the average (± 10%) of cell counts of non-adhered (blank areas), reversibly adhered (diagonally hatched areas), and irreversibly adhered (horizontally hatched areas) cells.

It is evident that in comparison with Degalan and glass bead columns there were no irreversibly bound cells on the Sephadex G-25 column. From this column the majority of cells was eluted with PBSC and adhered cells were liberated by mechanical stirring. Thus 15% of the total of applied thymus and lymph node cells, 25% of the spleen and bone marrow cells and 5% of the erythrocytes adhered reversibly. Morphological evaluation showed that erythrocytes and some small lymphocytes passed through the Sephadex column without adhering, and that other lymphocytes and granulocytes adhered reversibly.

On the hydrophobic surface of Degalan V 26 beads a great number of erythrocytes adhered irreversibly (about 40%) and only a few cells from thymus, lymph nodes and spleen (about 6%).

On glass bead columns 30% of the total applied cells adhered irreversibly, irrespective of the origin of the cell suspension. The amount of reversibly adhered

cells was about 50%. During the run through the Sephadex column animal cells were generally fractionated into adherent and non-adherent populations, as can be seen from the number of reversibly adhered cells and the morphological pattern. The viability of cells after chromatography on Sephadex beads was similar to that of cells separated on glass beads (*i.e.*, 85–95%).

When discussing the markedly lower adherence of cells to Sephadex G-25 in comparison to glass and Degalan it should be pointed out that:

(1) Sephadex beads have surface hydrophilic groups with attached water molecules.

(2) The beads are microporous and penetrable by low-molecular-weight substances but not by proteins or cells.

(3) Proteins are not substantially adsorbed on Sephadex G-25. In contrast to this, the compact glass and Degalan beads (which are rather hydrophobic) are known to adsorb proteins spontaneously in thin films so that strong binding can take place between such surfaces and proteins of the cell membrane. These interactions are possibly one of the reasons for the strong and irreversible binding of certain cell populations to glass and Degalan.

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