CHROM. 5394

Gel chromatography of modified bovine serum on Sepharose

Modified bovine serum (MBS) belongs to one type of blood volume expanders[1-3] which have an important role as infusion material, especially in emergency situations. The preparation of MBS[3,4] investigated in this institute is based on heat denaturation and formolation of a mixture of bovine serum and partially degraded gelatine, followed by partial oxidation by hydrogen peroxide.

MBS represents a complex mixture of molecules and particles of different qualities and sizes. Several methods are used to check the standard physicochemical quality of the product before biological application, *e.g.* electrophoresis[5], viscosimetry[4], gel chromatography on Sephadex G-200[6] and on pearl condensed agar[5,7], etc. However, the latter two techniques served as a rather rough assay of MBS. It is not feasible to fractionate the high-molecular-weight aggregates of molecular weights above about 5×10^5 in MBS on Sephadex G-200 because of the narrow pores of the gel; the pore size of 4% agar pearls was more suitable for this purpose, but the material was neither of standard quality nor chemically homogeneous and the fractionation was not altogether satisfactory.

These disadvantages led us to the use of standard Sepharose gels to investigate whether new information about the distribution of macromolecules present in MBS could be obtained by gel chromatography on this material.

Materials and methods

MBS was a standard preparation (DG 469) prepared in this laboratory. Bead forms of agarose Sepharose 2B, 4B and 6B (Pharmacia, Uppsala, Sweden) were used. The columns were 86 × 1.6 cm for Sepharose 4B and 6B and 80 × 1.4 cm for 2B. 0.9% sodium chloride solution was used as the elution solution. 4% protein solutions

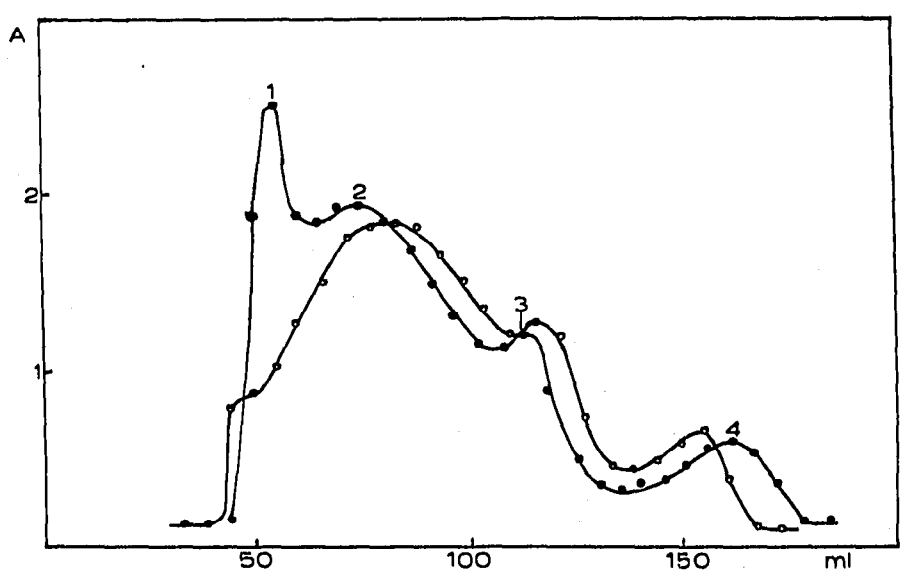

Fig. 1. Gel chromatography of modified bovine serum on Sepharose 4B and 6B. Column, 86 × 1.6 cm; elution solution, 0.9% NaCl. ○, Sepharose 4B; ●, Sepharose 6B.

were applied in 2-ml portions to the top of the column. The flow rate was 5 ml/cm²/h at a temperature of 20–24°. 4-ml fractions were collected and analyzed for proteins by direct photometry at 280 nm.

Results and discussion

The results presented in Fig. 1 indicate that Sepharose 6B and 4B were suitable media for characterizing MBS by four distinct peaks on the elution patterns during gel chromatography. In contrast, Sepharose 2B (Fig. 2) was not suitable since no characteristic fractionation of MBS was achieved, evidently because the sieve structure of this gel was too loose. Chromatography on Sepharose 6B was especially convenient for the analytical check of the standard quality of different batches of MBS. According to the calibration curve based on gel chromatography of standard substances (bovine RNAse, human serum albumin, human γ-globulin and α_2-macroglobulin), a rough estimate of the molecular sizes of the four fractions was made: peak 1 corresponded to proteins having a molecular weight of the order 10^6, peak 2 to about 800,000–1,000,000, peak 3 to about 80,000–100,000 and peak 4 (oxygelatine) to about 10,000–15,000. However, with regard to the differences between the rather compact structure of the calibrating substances and the uncoiled conformation of modified proteins[5–7], the above numbers have only a relative meaning.

The distinct shape of the peaks makes it possible to estimate quantitatively the content and distribution of the four characteristic fractions present in MBS[5,7]. This is very important, especially for the first peak. This fraction consists of the largest aggregates of modified proteins and their condensates with formaldehyde, and its molecular weight is about 10^6. The presence of this fraction seems to be a serious drawback of most MBS preparations because it causes the physical heterogeneity, opalescence and instability of these expanders during storage. It represents also a certain risk to the recipient organism which in some cases might react unfavorably to the infusion of such very high-molecular-weight substances. In this sense, gel

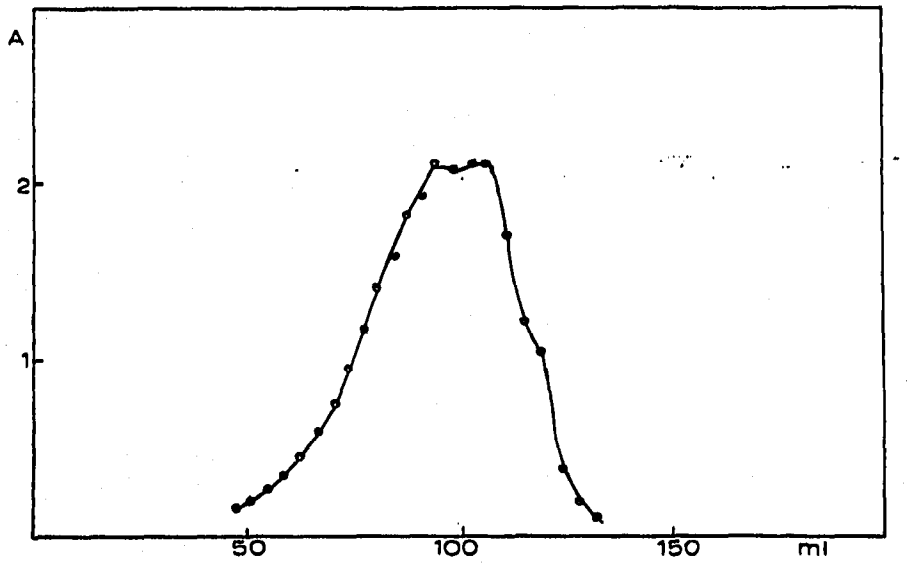

Fig. 2. Gel chromatography of modified bovine serum on Sepharose 2B. Column, 80 × 1.4 cm; elution solution, 0.9% NaCl.

chromatography on Sepharose 6B may be used also as an important means in the search for new methods of preparing MBS of improved quality.

The authors are grateful to Mrs. ZD. NOVOTNÁ for technical assistance.

*Institute of Hematology and Blood Transfusion**, S. ULRYCH
Prague (Czechoslovakia) T. I. PŘISTOUPIL

1 A. G. BORDUAS AND A. FRAPPIER, *Rev. Can. Biol.*, 19 (1960) 395.
2 M. J. PANKRATOVA, *Vopr. Gematol. Perel. Krovi*, 3 (1961) 143.
3 T. I. PŘISTOUPIL AND J. NIKL, *Czech. Pat.*, PV 4562-69 (1969).
4 T. I. PŘISTOUPIL, V.FRIČOVÁ AND E. PALUSKA, *Z. Immunitätsforsch.*, 125 (1963) 50.
5 T. I. PŘISTOUPIL AND S. ULRYCH, *J. Chromatogr.*, 28 (1967) 49.
6 T. I. PŘISTOUPIL, *J. Chromatogr.*, 19 (1965) 64.
7 T. I. PŘISTOUPIL AND S. ULRYCH, *J. Chromatogr.*, 25 (1966) 58.

First received February 16th, 1971; revised manuscript received April 6th, 1971

* Director: Prof. J. HOŘEJŠI.