system suggested gives timely warning of the inadequate supply of blood to the inlet of the resistograph, and the pressure stabilizer at the inlet of the instrument enables its error to be eliminated.

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MOLECULAR WEIGHT FRACTIONATION OF PROTEINS WITHIN THE RANGE 10^{-7}-10^{-8} g BY MICROGEL CHROMATOGRAPHY

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A method of microgel chromatography of proteins on a Sephadex G-75 (superfine) column with a working volume of 110-150 μl is described. By means of the method molecular weight fractionation of proteins can be carried out within the range 10^{-7}-10^{-8} g in the course of 45-60 min.

KEY WORDS: microgel chromatography; proteins.

The use of gel chromatography on the macroscale for the molecular weight fractionation of proteins and enzymes of tissue microhomogenates (1-5 mg) is difficult for the following reasons: 1) because of dilution of the small amounts of protein on the column to concentrations which cannot be recorded by the ordinary optical instruments; 2) the considerable volume of the eluted fractions, containing a small quantity of radioactivity, makes radiometric measurements in an anhydrous scintillator difficult. These difficulties can be overcome if the procedure of protein chromatography is converted to the microscale, as was first suggested for fractionation of nucleic acids within the range 10^{-8}-10^{-9} g [1, 2]. The same workers suggested a special technique for measuring the optical density of the microeluate and this was used in the present investigation also.

The procedure of microgel chromatography of proteins is not yet fully worked out, and for that reason the investigation described below was carried out.

The chromatographic columns were made from thick-walled glass capillary tubes with an internal diameter of 1-1.5 mm and a length of 10-15 cm (internal volume of the column 100-150 μl). The bottom end of the column was drawn out over a burner. Quartz sand was placed in the bottom of the column. The column was filled with Sephadex G-75 gel (superfine). The preliminary treatment of the gel was carried out in the usual way [3]. Three volumes of working buffer (0.06 M Tris-HCl, pH 7.1) was run through the filled column. To calibrate the column the following "marker" polymers with known molecular weights were used: blue dextran (2,000,000), bovine serum albumin (68,000), egg albumin (45,000), trypsin (24,000), chymotrypsin (22,500), lysozyme (17,500), ribonuclease (13,600), cytochrome c (13,000), and 5'-AMP (480). The "marker" was applied to the surface of the gel in a concentration of 10 μg in 3 μl. After absorption of the sample into the gel by means of a special micropipet the upper part of the column was filled with buffer and connected to an automatic syringe, supplying eluting buffer at the rate of 3 μl/min. The bottom end of the column was connected by a

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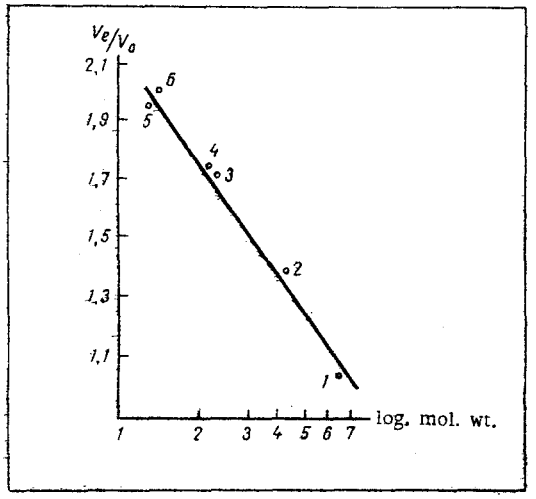

Fig. 1

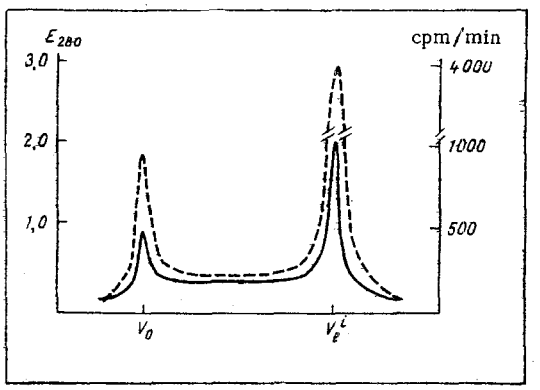

Fig. 2

Fig. 1. Calibration curve of microcolumn: 1) bovine serum albumin; 2) egg albumin; 3) trypsin; 4) chymotrypsin; 5) ribonuclease; 6) cytochrome c. Abscissa, logarithm of values of molecular weight; ordinate, ratio V_e/V_0.

Fig. 2. Determination of V_0 and V_e^i by optical and radiometric methods. Leucine-^{3}H injected in a dose of 50 μCi into heart region of chick embryo. Hippocampal tissue (2 mg) removed 1 h after injection, homogenized in 3 volumes of a solution of 0.25 M sucrose + 0.5% Triton X-100 and centrifuged, after which 3 μl of supernatant applied to column. Continuous line indicates optical elution profile at 280 nm (E_{280}), broken line concentration of leucine-^{3}H in eluate (in cpm).

polyethylene adaptor to the continuous-flow recording cuvette, 2 μl in volume (width 1.5 mm), of the MFSP-1 microspectrophotometer. The comparison cuvette contained the eluting buffer. The optical density of the eluates was recorded continuously by a KSP-4 potentiometer. By means of this method 60 ng protein in the cuvette can be reliably recorded, given a signal-to-noise ratio of the instrument of 5 : 1. The elution volumes (V_e) were determined by specially calibrated capillary tubes. Since V_e/V_0 is a linear function of the logarithm of the molecular weights of the marker polymers (Fig. 1), this indicates that molecular weight fractionation of proteins on the microscale is effective. The error of the method was determined by measuring V_e three times for each of the marker compounds and in no case did it exceed 2%.

The results of molecular-weight fractionation of proteins of a weighed microsample (2-3 mg) of hippocampal tissue from a 19-day-old chick embryo are given in Fig. 2. Repeated measurement of V_0 and V_e^i (elution volume of the polymer of known molecular weight with a column of known internal volume) based on optical density and radioactivity gave identical results, i.e., the basic parameters of the column were independent of the method of their measurement.

The suggested method thus enables the radioactivity of protein fractions and of the reserves of precursor to be calculated in one experiment and, consequently, enables the specific radioactivity of the fractionated proteins of a microsample of test tissue to be determined. The essential feature is that the whole procedure of protein fractionation when this method is used takes only 45-60 min.

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