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METHOD OF PREPARATION OF BETA₂-MACROGLOBULIN OF HUMAN SERUM

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METHOD OF PREPARATION OF BETA₂-MACROGLOBULIN OF HUMAN SERUM

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Abstract — The authors describe a method of preparation of β_2 -macroglobulin IgM of human serum involving three stages:

- (1) Elimination of lipoproteins using dextran sulphate;
- (2) Precipitation of euglobulins using a solution of boric acid.
- (3) Filtration on dextran gel.

In the preparation of β_2 -macroglobulin (Note: Since this article has been edited the World Health Organization recommended the use of the following synonyms: IgM or γ M) we use most often the two properties indicated by the name: its high molecular weight (of the order of a million) and its low electrophoretic mobility.

Consequently we used ultracentrifugation to concentrate heavy molecules of blood serum according to various modalities. The first techniques repeated the ultracentrifugations (see bibliography 18). Important progress was made when we did the centrifugation in a density gradient (13). More recently Filitti-Wurmser and Hartmann (5) recommended a special process of ultracentrifugation called the process of trapping solvent ("procédé sur solvant piégé"). The appearance of filtration on gel (particularly on dextran gel sold commercially under the name of Sephadex G 200) made it possible to obtain comparable fractioning in a much more simple way (7).

These various methods make it possible to collect heavy components but do not bring about any separation of the homogeneous elements in this

connection. Thus, according to Fireman et al (6) we find the following in the same fraction after filtration by Sephadex: α_2 and β_2 -macroglobulin, α_2 and β -lipoprotein, and haptoglobin of the type 2-2 and 2-1. Except for certain immunological studies this summary separation was not sufficient. Consequently we have to add other methods of purification. These methods were reviewed by Walton et al (21). We can use with profit preparative electrophoresis on a block of starch (12), on gelose gel (9), or on powder of synthetic resins (4). We also used chromatography on a column of DEAE-cellulose (3).

We improved the method by enriching the environment of β_2 -macroglobulin, while taking in consideration the fact that this protein is a typical euglobulin. Vaerman et al (20) used dialysis on a pad containing a solution of phosphate of pH = 6 and with a very low ionic strength. We also used the same method (8, 14). We found recently that by diluting the serum with an aqueous solution of boric acid to 7.5 g/l we get the same result faster, more reliably, and on a more selective basis (15).

Another improvement of the method of filtration on Sephadex is that we get rid of the β -lipoprotein which obstructs filtration and contaminates the macroglobulinic fraction. We can achieve this easily by using the precipitation by dextran sulphate according to Burstein and Samaille (1).

The method which we recommend in this article consists of the combination of the following three operations:

- (1) Elimination of lipoproteins by using dextran sulphate.
- (2) Precipitation of euglobulins by using a solution of boric acid.
- (3) Redissolution of this precipitate and filtration on dextran gel (Sephadex G 200).

This method makes it possible to obtain quite fast and in a simple way a preparation of β_2 -macroglobulin which is practically pure.

Material and Methods

We can make this preparation by using normal human serum, but it is obviously of interest to start with a serum which is rich in β_2 -macroglobulin. From this point of view the serum of patients suffering from African trypanosomiasis of *T. gambiense* represents a remarkable source of this protein, because its rate is on the average five to six times higher than normally (17, 2, 16).

1. Precipitation of the β -lipoprotein. — We add to ten ml of serum 0.4 ml of a 10% solution of dextran sulphate (Note: dextran sulphate for analyses: "Biological Equilibrium", Commentary /Allier/, France) and then one ml of a solution of Cl_2Ca M. We shake it and after 15 minutes of rest at laboratory temperature we centrifugate the solution for ten minutes at 6000 g. We collect the float. We eliminate the excess of Cl_2Ca by a short dialysis against distilled water.

2. Precipitation of euglobulins. — We adjust the volume of the float to 200 ml by means of a solution of boric acid at 7.5 g/l. We mix the solution and after half an hour of rest we centrifugate it for ten minutes at 2000 g. The precipitate is washed twice with the same solution of boric acid.

Then we put it in three-four ml of a pad solution of the following composition: Tris hydroxy-methyl-amino-methane 7.96 g, NaCl 58.45 g, HCl 0.033 N q.s.p. 1000 ml, the pH of which is 8.0.

3. Filtration of dextran gel. — Filtration on Sephadex G 200 (Note: Pharmacia, Upsala, Sweden) is carried out according to the suggestions of Hogman and Killander (10) in a column 80 cm high and 2.8 cm in diameter. The absorption curve in X-rays shows the appearance of two peaks. The first one contains macroglobulin. With a little bit of practice we can dispense with the determination of the absorption curve in X-rays, because the solutions containing β_2 -macroglobulin can be recognized by the naked eye because of their strong opalescence. The effluents which correspond to the first peak are collected. At the same time we make sure that we cut down the effluents sufficiently on the side of the second peak in order to maintain a safety margin.

4. Purity control. — After concentration by ultrafiltration in vacuum (19) the preparations are subjected to the following three tests:
 (1) Electrophoresis with cellulose acetate (11).
 (2) Immunological analysis by double diffusion in gelose.
 (3) Immunelectrophoretic analysis.

We used seven different immunization serums in the last two tests.

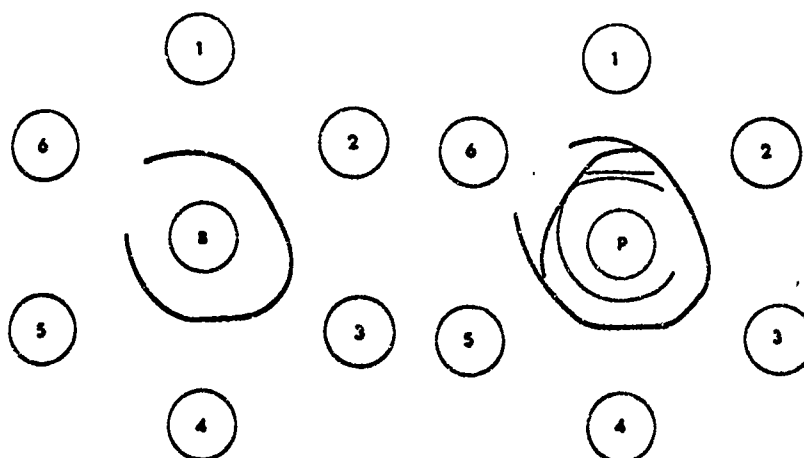


Figure 1. Immunochemical analysis of a preparation of β_2 -macroglobulin, in which the precipitation of euglobulins was achieved by adding boric acid (B) or by dialysis against a solution of pad phosphate of Na 0.005 M of p = H6(P).

- (1) Horse serum normal human anti-serum (Dutch Red Cross, Amsterdam), No. 1621.
- (2) Horse serum human anti- β_2 -macroglobulin (Pasteur Institute, Paris), No. 90.109.
- (3) Rabbit serum normal human anti-serum.
- (4) Horse serum normal human anti-serum (Pasteur Institute, Paris), No. 306.
- (5) Horse serum normal human anti-serum (Pasteur Institute, Paris), No. 13489.
- (6) Goat serum human anti- β_{2A} -globulin (Hyland Laboratories, Los Angeles), No. G206 7 ADS 3163.

Results and Discussion

Electrophoretic analysis shows only one component located at the expected place, in the area of β_2 -globulins. Immunoelectrophoretic analysis confirms this result.

Double diffusion in gelose, which is a more practical method and is used with various immunity serums, shows that frequently the preparation contains only β_2 -macroglobulin which is perfectly pure.

Sometimes, especially when we collected the effluent of filtration by Sephadex too far on the descending slope of the first peak, we could note that it contained some impurity. It was always α_2 -macroglobulin, the arc of which is clear when we use the Ouchterlony method, is hardly visible in immunoelectrophoresis, and does not show at all in electrophoresis on acetate. This indicates that the contamination is minimal in quantitative terms.

The use of boric acid is certainly a progress in comparison to dialysis against phosphate pad which we utilized previously (8, 14). Figure 1 shows immunochemical controls of two preparations made on the basis of the same serum, one involving the use of boric acid, the other involving the use of a solution of phosphate pad 0.05 M of pH 6. We can see that only the preparation made by means of boric acid is pure. However, this improved selectivity does not result in any increase of output.

The method makes it possible to obtain about 30-40 mg of β_2 -macroglobulin from 10 ml of serum of a patient suffering from trypanosomiasis. We can collect 12 mg from 20 ml of serum of a normal individual of black race. The output is of the order of 50%, the losses occur particularly when we collect the effluent during the filtration on gel and when we concentrate the solution by ultrafiltration in vacuum. We can improve this output by concentrating the solution by means of lyophilisation.

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