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TECHNICAL METHODS

A Standard for the Determination of Total Protein and Globulin in Biological Fluids

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Determination of Total Protein

Quantitative estimations of total protein in biological fluids may be performed by the micro Kjeldahl reaction with or without distillation (Rappaport and Lasowski, 1943), by a colorimetric method such as the biuret (Weichselbaum, 1946; Kingsley, 1940), by measuring the specific gravity according to the method of Phillips, Van Slyke, Emerson, Hamilton, and Archibald (1944), by the turbidimetric method (King and Wootton, 1956; Bossak, Rosenberg, and Harris, 1949), or according to the Klett manual.

Yeoman (1955) compared the iodometric Kjeldahl and turbidimetric methods using sulphosalicylic acid as reagent and found no significant differences between them, but for small laboratories it is important to use a simple turbidimetric method which is easy to perform. It must be remembered, however, that the turbidimetric methods are dependent on suitable standards.

Rappaport and Loew (1957) used in their colorimetric method a peptone solution as a standard for determining proteins in blood plasma.

A different standard suitable for turbidimetric determination is proposed for use in quantitative determinations of protein in cerebrospinal fluid, urine, etc.

In a similar fashion to Hiss (1904), who used water-diluted serum as nutrient medium for bacteria, it has been found that human sera diluted 1:100 in distilled water may be repeatedly autoclaved without coagulation and turbidity and may thus be used as standards.

The quantity of protein in a given sample of serum is determined before dilution either by the Kjeldahl method (Benhold, Kylin, and St. Ruszynyák, 1938) or by comparison with a peptone standard. Then this serum of now known protein content is diluted 1:100, autoclaved, and, if clear, distributed into vials; the vials are sealed and autoclaved again and may be kept indefinitely as standards. Sera containing abnormal globulin are not suitable for this purpose.

Principle of the Method

One part of cerebrospinal fluid and 6 parts of sulphosalicylic acid are mixed and the turbidity is measured in the improved Klett Summerson colorimeter using a sterile water-diluted serum for standard.

Reagents

(1) **Sulphosalicylic Acid.**—In a 3% aqueous solution, it keeps indefinitely.

(2) **Standard.**—One millilitre of human non-haemolytic serum of known protein content in 100 ml. distilled water, sterilized as described above (30 minutes at 115° C.), is distributed in 2 ml. quantities in vials, sealed, and sterilized again.

Procedure

(1) **Test Proper.**—To 3 ml. of 3% sulphosalicylic acid add 0.5 ml. of cerebrospinal fluid (or if only a small quantity of cerebrospinal fluid is available 1.8 ml. sulphosalicylic acid + 0.3 ml. cerebrospinal fluid).

(2) **Standard.**—To a second tube with 3 ml. of sulphosalicylic acid add 0.5 ml. of standard.

(3) **Blank.**—Three millilitres sulphosalicylic acid +0.5 ml. saline.

The protein concentration is calculated from the optical density read on filter (blue) 420 mn. using contents of tube 3 as blank and of tube 2 as standard.

The improved Klett colorimeter was used for readings (Rappaport and Eichorn, 1955).

The results of the total protein in the undiluted serum according to the colorimetric method of Weichselbaum (peptone standard) and diluted serum by the turbidimetric method (using Hiss water serum as standard) were identical during examination of a series of 40 sera.

Determination of Globulin in Cerebrospinal Fluid

The electrophoresis of cerebrospinal fluid is gaining importance in routine diagnostic work, but large quantities are needed and are not always available, therefore microanalysis, which gives the total protein and the globulin content, is important. In the literature many turbidimetric methods using ammonium-sulphate as reagent are described, and it is well known that the turbidimetric methods are the best when working with minute quantities of protein. The method of St. Ruszynyák (Yeoman, 1955) for the determination of globulin in cerebrospinal fluid was used here.

Principle of the Method.—Equal amounts of cerebrospinal fluid and neutral saturated ammonium sulphate are mixed. The turbidity is measured in the improved Klett-Summerson colorimeter using a sterile water-diluted serum as standard.

Reagents.—(1) Saturated solution of ammonium sulphate as used for the Nonne-Apelt reaction; the *p*H of the solution is corrected to *p*H 7, with sodium hydroxide or ammonium hydroxide.

(2) **Globulin Standard.**—The same as used in the determination of total protein in cerebrospinal fluid.

The globulin content of the standard solution in the vials is standardized either by a Kjeldahl or a biuret determination. When available Lederle (gamma) globulin diluted 1:200 can be used for this purpose as well. The diluted Lederle standard globulin coagulates on heating and so cannot be used as a sterilized standard.

An interesting phenomenon was observed while preparing the sterilized water-diluted serum. The total protein value determined in the diluted serum before and after sterilization remains constant, but the globulin value determined before and after sterilization showed a marked increase by this denaturation procedure. After renewed concentration as used for the electrophoresis of C.S.F. this solution showed only one globulin fraction and no albumin at all.

Procedure.—(1) Mix 1 ml. standard solution with 1 ml. of ammonium sulphate reagent.

(2) Mix 1 ml. of C.S.F. with 1 ml. of ammonium sulphate reagent.

(3) Mix 1 ml. of saline with 1 ml. ammonium sulphate reagent to be used as blank. Read in the colorimeter with a 420 mm. filter after five minutes.

The comparison of the results of globulin determinations in undiluted sera by the Weichselbaum colorimetric method and the turbidimetric one using diluted sera proved very satisfactory.

Summary

A method is described for the determination of total protein and globulin in biological fluids such as cerebrospinal fluid, urine, etc.

The standard is a sterile aqueous solution of serum which can be used for both the total protein and globulin determinations.

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Permanent Sickle Cell Preparations

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The usual methods for detecting sickling of red cells consist of the examination of wet preparations of red cells to which bacteria or a reducing agent are added. It would often be an advantage if stained preparations of sickle cells could be obtained for a "permanent" record, for teaching purposes, and for following the development of the sickling phenomenon in the newborn. Very few techniques have been described for the preparation of permanent films. Two methods are described below, the first simple and the second slightly more complex but even so still easy, rapid, and reliable with some advantages over the more simple technique. English (1958) recently described a simple method employing paraformaldehyde which had the disadvantage of liberating noxious formaldehyde fumes into the atmosphere. A technique without unpleasant fumes would be an advantage, and the first method described below is such a technique. Sherman (1940), while investigating techniques for the differentiation of sickle cell trait from sickle cell anaemia, took venous blood under anaerobic conditions and fixed the red cells immediately, taking care to exclude all oxygen until fixation was complete. This method did not reveal sickle cell trait. The basis of the second method, described below, is similar to the method of Sherman, except that the sickling desired is produced artificially by the addition of a reducing substance to a specimen of blood in an anticoagulant collected by venepuncture without any special precautions. Furthermore, using this method sickle cell trait can also be demonstrated.

Methods

Wet Preparations.—Two methods of making wet preparations to demonstrate the presence or absence of sickling were investigated: (1) using sodium diothionate (Itano and Pauling, 1949); (2) using 2% sodium metabisulphite (Lehmann, 1958).

The second technique proved most reliable and consistent, and was adopted for subsequent use.

Method 1.—Red cells were made to sickle in the usual way by mixing one drop of fresh 2% sodium metabisulphite on the centre of a 3 × 1 in. microscope slide with one drop of blood. A $\frac{7}{8}$ in. × $\frac{7}{8}$ in. coverslip was placed over the preparation and the edges were sealed round with paraffin wax/vaseline mixture. The slide was then allowed to stand on the