HÆMATOLOGY

Isolation of an α_2-Globulin from Human Plasma

While examining a method for the isolation of haptoglobin by chromatography on *DEAE* cellulose[1] we noticed that several α_1- and α_2-globulins were adsorbed under the experimental conditions used. One of these proteins, an α_2-glycoprotein, has now been obtained at about 90 per cent purity. The protein adsorbed together with haptoglobin, cæruloplasmin, cholinesterase and some α_1-globulins at *p*H 5·0 from human plasma diluted to a freezing point of − 0·15° is the last of these proteins to be eluted at the same *p*H but with increasing molarity of acetate buffer. Indeed, haptoglobin is eluted at 0·1 *M* acetate buffer, cæruloplasmin together with some α_1-globulin at 0·2 *M* and the unknown protein at 0·3–0·4 *M*.

When submitted to starch-gel electrophoresis[2], this protein migrates to the α,β-position. Protein π, as it may be called, is an α_2-glycoprotein containing about 10 per cent hexoses. Protein π is different from these three well-known α_2-globulins: haptoglobin, cæruloplasmin and α_2-macroglobulin. It differs from haptoglobin by its electrophoretic mobility on starch-gel, showing but a single band in the α,β-position (Fig. 1) and a somewhat faster migration on paper strip. Protein π has no hæmoglobin-binding capacity and no immunological relationships with haptoglobin. The new protein is precipitated by rivanol at *p*H 7, whereas haptoglobin is not. The protein under consideration differs from cæruloplasmin in its position on starch-gel at *p*H 8·6. It is colourless and contains no copper and shows no oxidase activity. There is no immunological relationship with ceruloplasmin as studied by the Ouchterlony technique and immunoelectrophoresis. Finally, there is no immunological relationship between α_2-macroglobulin and protein π and again the position on starch-gel of the two proteins is quite different.

The protein separated by chromatography on *DEAE* from whole plasma contains zinc in various amounts. Work is in progress to establish whether zinc is a definite part of the native protein, or a fortuitous contaminant. Protein π has been isolated under the same experimental conditions from fraction III as obtained during routine fractionation of human plasma following a slightly modified Nitschmann technique[3]. Whole plasma contains about 0·2 gm./l. of this protein and some total anti-human-serum gives a precipitation line with the purified protein. A screening for specific enzymatic or clotting activity has not yet given a positive result.

We thank Dr. Fine for immunoelectrophoretic analysis.

M. Steinbuch
J. Loeb *
Centre National de Transfusion Sanguine,
6 Rue Alexandre-Cabanel,
Paris.

* Chargé de recherches au Centre National de la Recherche Scientifique.

1 Steinbuch, M., and Quentin, M., *Nature*, **190**, 1121 (1961).
2 Smithies, O., *Biochem. J.*, **61**, 629 (1955).
3 Nitschmann, H., Kistler, P., and Lergier, W., *Helv. Chim. Acta*, **37**, 886 (1954).

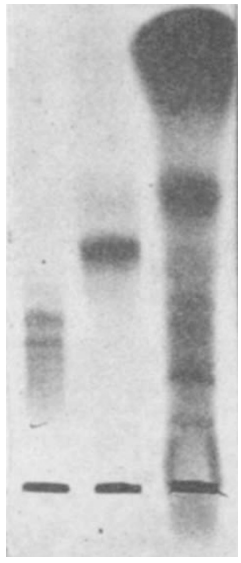

Fig. 1. Position on starch-gel of protein π (in the centre), whole plasma (right), isolated haptoglobin (left)

Quantitative Sub-Groups of the *B* Antigen in Man and their Occurrence in Three Racial Groups

Numerous authors[1–5] have described rare variants of the *B* blood group antigen which may differ qualitatively from other *B* antigens. Formaggio[6] concluded from his examination of 250 samples of *B* agglutinogens that differences within the *B* group were quantitative rather than qualitative. During the performance of 50 per cent end-point hæmagglutination assays of the *B*-anti-*B* system[7], it was noted that *B* cells from a Negroid individual were more strongly agglutinated than were those of Caucasoid origin. The probability–log assay curves in Fig. 1 show the difference observed with four doubling dilutions of anti-*B* serum in an equilibrium system with equal numbers of the two types of *B* cells of equal freshness. The percentages of cells agglutinated were obtained by the free-cell counting technique described in an earlier paper[8] and are plotted against the logarithms of the antiserum concentrations. Since the parallel assay curves indicated that these agglutinogens differed quantitatively rather than quali-

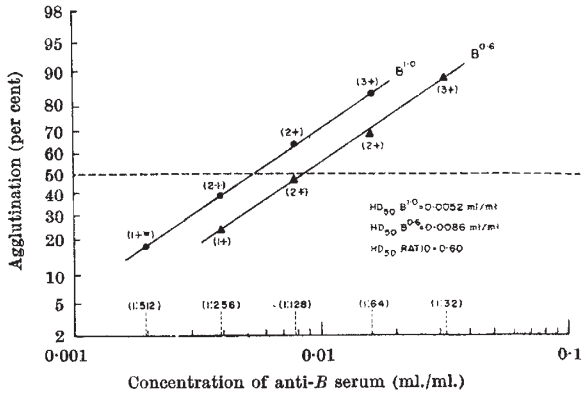

Fig. 1. Hæmagglutination assay curves of red cells of two sub-groups of the *B* antigen.
The degree of gross agglutination is indicated in parentheses. 3+, Large visible clumps of cells with only a few free cells; 2+, macroscopically many small clumps with more free cells; 1+, macroscopically no visible agglutination, microscopically many small clumps of 4–8 cells; 1+w, no visible agglutination macroscopically, microscopically small clumps of 2–4 cells