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Antigenicity of Macroglobulin 19S in the Serum of Newborns and Infants

By

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In earlier ultracentrifugation studies it has been shown that the serum macroglobulin level, which is high at birth (5.4±1.75 per cent) rises still higher (7.41±1.64 per cent) until the end of the first trimester[1]. Electrophoresis reveals macroglobulin 18—20S, homogeneous in the ultracentrifuge, to consist of two differently mobile proteins (gamma M and alpha$_2$ M), and the present investigation had the purpose to determine which of the two fractions was responsible for the observed rise, since the earlier results seemed to justify the conclusion that the fraction alpha$_2$ M was the responsible factor. That this is so can be demonstrated

(i) by immune electrophoresis of the whole serum, and

(ii) by immune electrophoresis of the serum fractions.

The great number (18 to 24) of precipitation bands makes it difficult to evaluate the immune-electrophoretic picture of whole serum, whereas the number of components is contiderably less in the individual fractions and this facilitates interpretasion. The method of gel filtration was employed in order to separate the protein fractions to be examined.

Human serum, filtered on Sephadex G-200 gel in the presence of a suitable solvent (e.g. a pH 8 solution containing 0.2 M NaCl and 0.1 M Tris buffer), gives three peaks. As evidenced by analytical ultracentrifugation, the first peak contains mainly 18—20S, the second 6—7S, and the third 4—4.5S components. Immune electrophoresis of the first peak settles the question as to whether neonatal macroglobulin 19S consists largely of alpha$_2$ globulin and whether it is the increase of this fraction which elevates the level of macroglobulins in the first three months.

Method

Gel filtration of 1 to 2 ml infantile serum was done by the method of Killander and Flodin [9], and that of Roskes and Thompson [12] on two Sephadex-G-200 (Pharmacia, Uppsala) columns. One column, 2.12 cm in diameter and 31.5 cm high, contained 105 ml, while the other, identical in diameter and 54 cm high, contained 180 ml, of Sephadex. Elution diagrams were based on the extinctions of 2.5 to 5.0 ml fractions measured at 280 mμ. Layer thickness was 0.5 cm. A VSU-type

Zeiss spectrophotometer was used. Sedimentation was done in a Phywe type U 77 ultracentrifuge. A MOM polar planimeter was used for indicating on the sedimentation diagrams the proportion of components with different sedimentation coefficients.

Dissociation of the macroglobulin fraction lasted 20 minutes and was effected at room temperature in 2-mercaptoethanol applied at a final concentration of 0.1 M [15].

Immune electrophoresis was carried out with antihuman horse serum by SCHEIDEGGER's micromethod [14].

RESULTS

Sephadex G-200 filtration of infantile serum yielded three fractions under the given experimental conditions. In the elution diagram, as presented in Fig. 1 a—b, the highest elution peaks of the macroglobulin fraction amounted to 40 and 31 per cent, respectively, of the volume of the column.

The immune electrophoretic pattern of fraction M is illustrated in Fig. 2a—d.

(a) Infant of 3 months. There are three precipitation bands in the bottom part of the plate. That of alpha$_2$ M in the middle is sharply defined; the band of gamma M is to the right, that of albumin-like contamination to the left of it. (The latter was identified in the ultracentrifuge, too.) (Fig. 3a) The upper parts show the immune electrophoretic pattern of serum obtained from a healthy control baby.

(b) Fraction M of the serum of a patient suffering from gamma-M-macroglobulinaemia. Contamination towards the albumin zone can be seen.

(c) Mixture of fraction M from the serum of the macroglobulinaemic patient under (b) and that from the serum of a three-month-old healthy baby under (a). (a : b = 3 : 1).

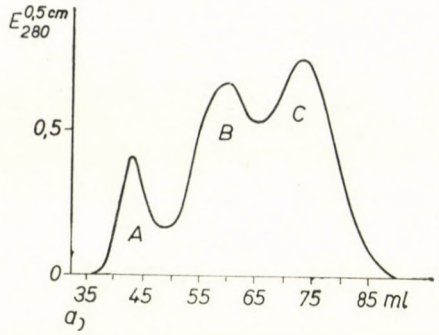
(d) Fraction M of serum of two-day-old infant. No gamma M is visible.

Fig. 3a shows the homogeneity of the ultracentrifuged fraction M from the serum of the three-month-old infant. As in the cases illustrated in Fig. 2, a component with albumin-like sedimentation coefficient (4 to 4.5S) was present beside macroglobulin 18—20S.

DISCUSSION

Fraction M of the serum of mature newborn babies and three-month old infants has been subjected to immune electrophoresis. No presence of gamma M globulin was observed immediately after birth, although it has repeatedly been demonstrated in newborn babies [1, 2, 3, 4, 5, 8, 9, 11, 13]. This apparent contradiction may be due to that only a few cases have so far been examined by our methhod, and, besides, gamma M globulin cannot invariably revealed immediately after birth even if whole serum is used. Additional investigations, including those made with monovalent immune sera, are necessary to settle the problem.

The present results have confirmed our earlier assumption that the macroglobulin 18—20S of newborns and young infants consists largely of a



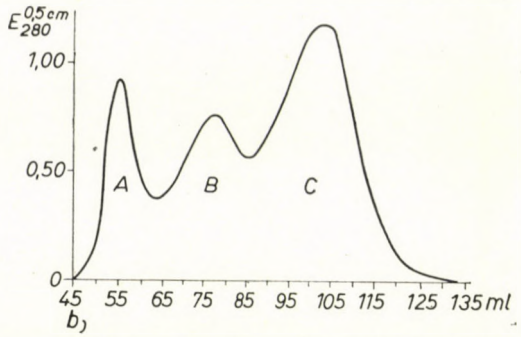


FIG. 1a). Elution diagram of serum made with a 31.5 cm high, b). that made with a 54 cm high, Sephadex G-200 column. A-peak = macroglobulin; B-peak = globulins; C-peak = mainly albumin. Ordinate: extinction at 280 mμ; abscissa: volume (in ml) of solution dripping off the column

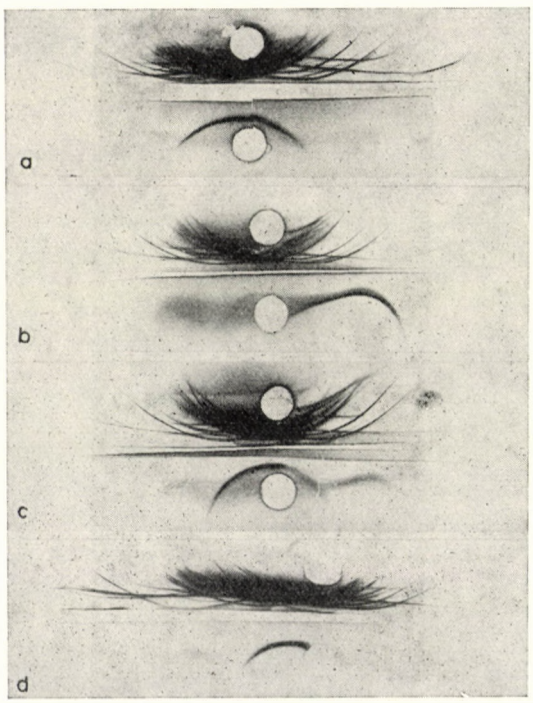


FIG. 2. Immune electrophoretic pattern of fraction M. (Further details in the text)

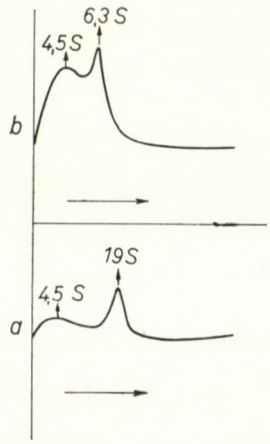


FIG. 3a). Sedimentation diagram of fraction M isolated from the serum of a 3-month-old infant. b). Same as Fig. 3a, in the presence of 0.1 M 2-mercaptoethanol. Direction of sedimentation: ⟶

substance of alpha$_2$ M antigenicity. Observations made on three-month old infants show the gradual appearance of gamma M globulin in the course of the first three months. Problems regarding the interproportion of the two kinds of macroglobulin require further investigation.

We have succeeded in demonstrating by treatment with 2-mercaptoethanol that, — apart from alpha$_2$ M macroglobulin which constitutes its greatest part, — fraction M contains a second component of low sedimentation coefficient (6 to 6.5S) (Fig. 3b). It is known [6] that alpha$_2$ M globulin isolated from adult serum can likewise be dissociated by 2-mercaptoethanol treatment. This suggest the probability that alpha$_2$ macroglobulin is composed of the same subunits in both young infants and adults.

Sephadex G-200 gel was found by KILLANDER and FLODIN [9] as also by ROSKES and THOMPSON [12] to be suitable for obtaining fraction M in a pure state. Although our observations in connection with immune electrophoresis and ultracentrifugation do not support this statement, we regard the gel-filtration method as suitable for the separation of serum fractions and for the study of their antigenicity. It is in any case less laborious than other current methods (e.g. density gradient ultracentrifugation, preparative electrophoresis, zone electrophoresis, etc.) which require great technical skill and consume much time.

SUMMARY

The serum of newborns and three-month-old babies has been fractionated on Sephadex G-200 gel; the macroglobulin fraction so obtained was then subjected to immune electrophoresis.

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