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A New Abnormal Serum Globulin α_1-Antitrypsin

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A serum has been discovered which contains two α_1-antitrypsin globulins, which are immunologically indistinguishable with conventional antisera but differ in electrophoretic mobility. The anomaly gives rise to an atypical paper electrophoretic pattern and is easily visualized in agar gel electrophoresis.

Schultze *et al.*[1] recently identified Schmid's[2] and Schultze *et al.'s*[3] 3.5 S α_1-glycoprotein (α_1 A-globulin of Burtin[4]) with the α_1-antitrypsin in human serum. Bromphenol blue stains this globulin more intensely than it does the other main constituents of the α_1-globulin fraction obtained on zone electrophoresis in agar or on paper with barbital buffer pH 8–9 and ionic strength 0.1–0.2. In conventional zone electrophoresis the concentration of this globulin is roughly proportional to the colour intensity of the α_1-band (more sharply demarcated in agar than on paper).

In an earlier paper[5] we reported a series of human sera with paper electrophoretic patterns suggesting α_1-antitrypsin deficiency (no visible α_1-band). Further analysis revealed that a subnormal serum content of α_1-antitrypsin (<10 % of normal) may be a manifestation of an inherited predisposition to emphysema.

This paper is concerned with another type of abnormality of the α_1-antitrypsin globulin. The serum was subjected to close analysis because the normally demarcated α_1-antitrypsin band appeared broader and less intense than normally.

METHODS

Serum protein concentration was determined with the Biuret method of Kingsley[6], *quantitative paper electrophoresis* in accordance with Laurell *et al.*[7], *agar gel electrophoresis* in 2 % agar (purified) on cooled (+2°C) glass plates (4 × 40 cm) with calcium containing barbital buffer, a modified Wieme[8] procedure, and *immunoelectrophoresis* as described under agar gel electrophoresis. Rabbit antihuman serum (R.A.H.S.) from Behringwerke was used before and after adsorption with human serum from a patient with only traces of α_1-antitrypsin in her serum.

α_1-antitryptic activity was calculated from the loss in antitryptic activity of serum (or fractions) when treated at 60°C for 20 min after adjustment of pH to 5.1 by addition of 0.5 M acetic acid. The method for estimation of tryptic activity with BAPA (benzoyl DL-arginine p-nitroanilide hydrochloride) as substrate was based on the principles given by Erlanger *et al.*[9].

The electrophoretic distribution of the antitryptic activity in sera was determined after separation in agar (see above). The gel was cut into 0.2 cm segments. The slices were eluted, by agitation over night, in 0.05 M barbital buffer, pH 8.6, at +5°C.

MATERIAL

Serum was prepared from blood drawn from the patient on two occasions and from her three adult children.

Case history. The patient was a Para-III, born in 1900. She had undergone cholecystectomy in 1948. Afterwards she felt well until 1962 when she suffered from dizziness, fatigue and headache. She was admitted to hospital in Nov. 1962, since when her E.S.R. has been continuously elevated (about 50 mm/1 h). The woman was fairly fat but physical examination on admission revealed no signs of a pathological condition. B.P. 220/120 mm Hg. Roentgen examination of the heart and chest showed normal appearances. Intravenous urography gave normal findings. Hgb. 81 %. Red blood cell count 4.3 mill./mm^3. E.S.R. 30–50 mm/1 h and the serum creatinin was 0.72 mg/100 ml.

RESULTS

The paper electrophoretic patterns of serum from the patient and from a healthy subject are presented in Fig. 1 for comparison of the colour distribution in the α_1-zone. In the patient's pattern the normal α_1-band was replaced by a broader, less intensely stained band. Fig. 2 gives the results of agar gel electrophoresis and immunoelectrophoresis of sera from the patient and a control. It is clear from the illustration that the patient's pattern shows a relatively faint band at the site corresponding to the α_1-antitrypsin in the control pattern. However, in contrast to the control serum the patient's serum contains a distinct band in the α_1—α_2 interzone. These two bands of the patient's pattern are of about the same colour intensity. From the immunoelectrophoretic pattern it is apparent that the globulins corresponding to these two bands in the patient's serum share the antigenic determinants normally responsible for the development of the α_1 A precipitation line. The same result was obtained on development of the precipitation lines with the horse antihuman serum. The precipitation line was nearest to the antiserum well at the α_1—α_2 interzone.

The trypsin inhibiting capacity of the patient's serum (990 μg/ml) was normal. That both antigenically related α-globulins had antitryptic activity is clear from Fig. 3, which shows the electrophoretic distribution of the antitryptic activity in the patient's serum in relation to that in a control serum. The antitryptic

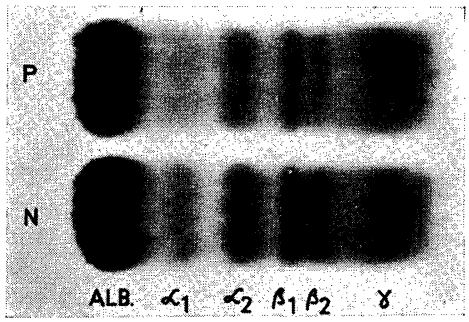

Fig. 1. Paper electrophoretic protein pattern of patient's serum (P) and of normal serum (N).

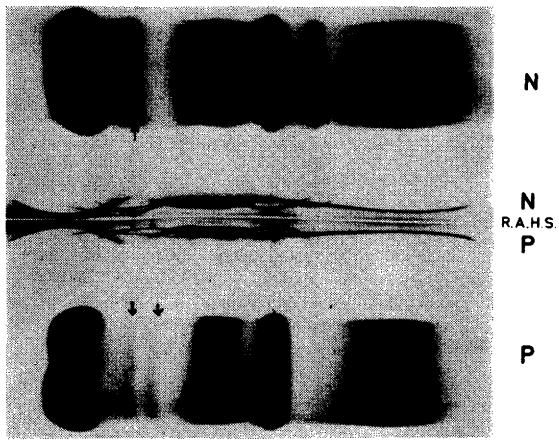

Fig. 2. Protein pattern of patient's serum (P) and of normal serum (N) in agar gel. Immunoelectrophoretic pattern obtained with rabbit antihuman serum (R.A.H.S.) in the well. The arrows indicate the position of the α_1-antitrypsin globulins.

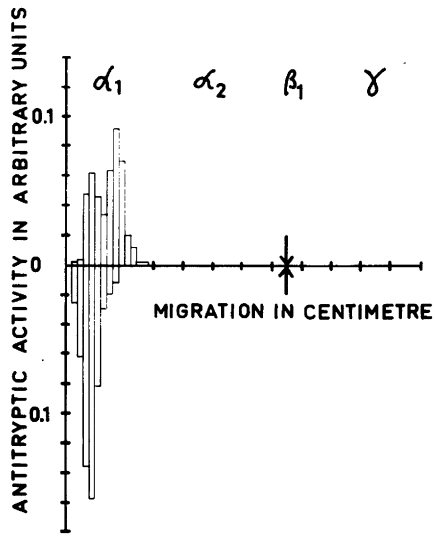

Fig. 3. Distribution of the antitryptic activity after electrophoresis in agar gel. Patient's serum above the abscissa and a normal serum on mirrored scale below the abscissa. Arrow indicates point of application.

activity in the patient's serum caused by the globulins in the electrophoretic α_1- and inter-α-zone disappeared after heating the serum at 60°C for 20 min at pH 5.1, as did the α_1-antitrypsin in the control.

After treatment of the serum with neuraminidase purified from pneumococci the electrophoretic mobility of both α_1-antitrypsin globulins decreased compared with that of the albumin.

DISCUSSION

A serum has been found which, on paper electrophoresis, gives rise to an atypical α_1-globulin pattern. Further analysis revealed that the serum contains two globulins with roughly the same antitryptic activity and the same antigenic properties as the normal α_1-antitrypsin. Patterns of sera from blood samples collected at about one month's interval gave the same atypical pattern, and the patient's disease presumably has nothing to do with the abnormality.

Schultze[10] has shown that the 3.5 S α_1-globulin has much neuraminic acid bound to its surface and that it is rapidly released by neuraminidase. During such treatment the whole α_1-antitrypsin fraction loses in charge and one retarded fraction appears on electrophoresis, but not two globulins, as in our case. That both globulins are synthesized with a different amount of neuraminic acid has not been excluded yet. Neither of the globulins showed increased thermal stability, for both were completely inactivated when heated at 60°C for 20 min at pH 5.1. In our experiments with chromatography of serum and α_1-globulins on DEAE-cellulose in the pH range 5–8 it has repeatedly been found that the α_1-antitrypsin spontaneously loses in charge and somewhat in electrophoretic mobility. Schultze *et al.*[1] have recently suggested that the α_1-antitrypsin during purification has a tendency to dimerization, which may result in a decreased charge.

The most probable explanation for the cleavage of the α_1-antitrypsin in our case is an inborn error of the synthesis of one peptide chain of this globulin. Sera from the patient's three children (all female) have been examined and all showed a normal α_1-antitrypsin band with normal biological activity. The anomaly is probably of no physiological importance since the total antitryptic activity is normal.

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