Alpha₂-macroglobulin levels in disease in man

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SYNOPSIS Serum α_2 -macroglobulin levels were measured in normal subjects and in patients with various diseases by an immunochemical method.

The values for normal men were 284 mg./100 ml. (± 89.6 mg./100 ml.) and for normal women 350 mg./100 ml. (± 94.5 mg./100 ml.).

Men with rheumatoid arthritis had normal levels, but the levels in women were depressed. There was no relationship to the concentrations of the acute phase reactive proteins, haptoglobin, and C-reactive protein.

In chronic liver disease, the levels in men were significantly higher than normal and were slightly higher than normal in women.

A small group of patients with nephrotic syndrome had very high levels.

No significant variations from the normal were found in sera from a group of patients with miscellaneous diseases.

Experimental studies indicate that serum α_2 -macroglobulin binds trypsin (Haverback, Dyce, Bundy, Wirtschafter, and Edmondson, 1962; Mehl, O'Connell, and DeGroot, 1964), plasmin (Schultze, Heimbürger, Heide, Haupt, Störko, and Schwick, 1963), and thrombin (Lanchantin, Plesser, Friedman, and Hart, 1966), and may also transport insulin (Zahnd and Scheidegger, 1963) and growth hormone (Hadden and Prout, 1964). Certain other α -globulins, the acute phase reactive proteins, increase in concentration in inflammatory disease such as rheumatoid arthritis and in neoplasia (Petermann, 1960). The possibility that α_2 -macroglobulin takes part in the acute phase reaction was suggested by Hitzig's (1961) observation of increased levels in rheumatic patients. However, this was not confirmed by James, Johnson, and Fudenberg (1966) in patients with rheumatoid arthritis, and, in addition, nor was any increase in α_2 -macroglobulin detected by Crockson, Payne, Ratcliff, and Soothill (1966) in patients showing the acute phase protein reaction after surgery.

This paper reports the immunochemical measurement of serum α_2 -macroglobulin in 54 patients with rheumatoid arthritis; simultaneous measurements were made of the levels of two known acute phase proteins, namely, haptoglobin (Owen, Smith, Padanyi, and Martin, 1964) and C-reactive protein (Petermann, 1960). α_2 -Macroglobulin levels have

also been measured in patients with chronic liver disease, who frequently show quantitative serum protein abnormalities (Sherlock, 1963), in a small number of nephrotic patients, in whom high levels have been reported (Schultze and Schwick, 1959; Steines and Mehl, 1966), and in a group of patients with miscellaneous diseases.

MATERIALS AND METHODS

SERA Normal sera were obtained from 16 male and 19 female laboratory staff or blood donors.

Disease sera were obtained from 23 males and 31 females with rheumatoid arthritis; seven males and 18 females with chronic liver disease (16 with active chronic hepatitis, three with primary biliary cirrhosis, and six miscellaneous); three males and five females with the nephrotic syndrome; nine males and 13 females with miscellaneous diseases (including eight with thyroid diseases, six with connective tissue diseases, and two cases of carcinoma).

All normal subjects and patients were more than 20 years old except for four of the nephrotics who were aged 4, 11, 14, and 19 years respectively.

α_2 -MACROGLOBULIN FOR IMMUNIZATION PURPOSES This was prepared from normal human serum as follows:

1 Low density lipoproteins were precipitated by dilution of the serum with 9 volumes of calcium barbitone buffer pH 9.0 and the addition of 0.4 volumes of 0.5% dextran sulphate, molecular weight 2×10^6 (a modification of the method of Walton and Scott, 1964);

the precipitate was spun down at 4,000 r.p.m. for 30 minutes.

2 The supernatant was dialysed against distilled water, lyophilized, and redissolved in approximately the initial (serum) volume of 0.06 M veronal buffer pH 8.6.

3 Preparative electrophoresis was carried out in Pevikon-Geon (Fahey and McLaughlin, 1963) and the α_2 -macroglobulin-containing fractions (identified by Ouchterlony tests using a specific antiserum) were pooled and concentrated by ultrafiltration.

4 The α_2 -macroglobulin was finally isolated by fractionation on Sephadex G200.

Serum, 20 ml., yielded on average 12 mg. of α_2 -macroglobulin (representing about 25% recovery), which was pure on immunoelectrophoresis against a polyvalent antihuman serum (Burroughs Wellcome).

ANTI- α_2 -MACROGLOBULIN SERUM This was raised in rabbits by immunization with the above preparation (6 mg. total) in complete Freund's adjuvant and rendered specific by absorption with concentrated material from the pooled second and third peaks from a Sephadex G200 fractionation of normal human serum.

α_2 -MACROGLOBULIN FOR CALIBRATION OF STANDARD SERUM This was obtained by repeated ultracentrifugation of α_2 -macroglobulin-rich Cohn fraction III -O preparations in a sucrose density gradient. No contaminating proteins were detected on immunoelectrophoresis. The protein concentration was measured by the micro-Kjeldahl method (Ma and Zuazaga, 1942).

IMMUNOCHEMICAL ESTIMATION OF SERUM α_2 -MACROGLOBULIN LEVELS The estimation was performed by a modification of the method of Mancini, Vaerman, Carbonara, and Heremans (1963). Of a 1 in 4 dilution of anti- α_2 -macroglobulin serum in 0.3 M phosphate buffer pH 8.0, 6 ml. was heated to 56° C, mixed with an equal volume of molten 3% agar in the same buffer, and poured on to a 3½ in. × 3½ in. glass slide. When the agar had set, 2 mm. holes were cut with a tubular steel cutter and accurately filled with test solutions or sera. After 24 hours' incubation at 4° C the diameters of the precipitin rings were measured to the nearest 0.1 mm. using a scale magnifier. Initially, in order to calibrate the standard serum, a series of known concentrations of the α_2 -macroglobulin preparation obtained by density gradient ultracentrifugation was put up on an antibody plate and the concentrations were plotted against the corresponding ring diameters; the α_2 -macroglobulin concentration of the standard serum, put up on the same plate, was calculated from this graph. In the subsequent estimations on normal sera and disease sera the α_2 -macroglobulin values were obtained from the graph of a series of dilutions of the standard serum on each plate. Test sera were diluted 1 in 3 and those giving very small or very large rings were re-examined at 1 in 2 or 1 in 4 dilutions respectively. Repeated estimations on a single serum sample gave a coefficient of variation of 9%.

HAPTOGLOBIN CONCENTRATION This was estimated by the serum haemoglobin-binding capacity on Sephadex G100 (Ratcliff and Hardwicke, 1964). The normal values for this method are: men, mean 106.8 mg./100 ml. and SD 25.8; women, mean 82.7 mg./100 ml. SD 20.2.

C-REACTIVE PROTEIN This was measured by a gel-diffusion precipitin method (Crockson, 1963). The results could be expressed in absolute units (μ g./ml.) since the C-reactive protein content of the standard serum used in the estimations had been measured by the antibody neutralization technique (Wright, 1959) using known concentrations of pure C-reactive protein isolated from human ascitic fluid (Crockson, personal communication).

SERUM ALBUMIN AND TOTAL GLOBULIN These were measured by the method of Weichselbaum (1946).

The results were evaluated statistically by calculation of the standard error of the difference between means or the Student *t* test as applicable.

RESULTS

The mean and individual values for serum α_2 -macroglobulin in the various groups are shown for males and females separately in Figures 1 and 2.

Normal men had a mean value of 284 mg./100 ml. with a standard deviation of 89.6 mg./100 ml. The mean for normal women was significantly higher at 350 mg./100 ml. ($P < 0.05$) with a standard deviation of 94.5 mg./100 ml.

The mean level in male rheumatoid patients, 287 mg./100 ml., was almost identical to that for normal men. Female rheumatoid patients had a mean value of 308 mg./100 ml., which was considerably lower than their normal mean but just failed to reach significance at the 5% level. The individual levels bore no relationship to haptoglobin or C-reactive protein in either men or women (Figs. 3 and 4). (Forty of the 54 patients had haptoglobin values more than two standard deviations above the normal mean for their sex (Fig. 3), and all patients had detectable C-reactive protein, values ranging up to 400 μ g./ml. (Figure 4).)

In both men and women with chronic liver disease the mean α_2 -macroglobulin level was higher than normal at 390 mg./100 ml. and 380 mg./100 ml. respectively. The difference was statistically significant in men ($0.05 > P > 0.02$) but not in women.

The levels in the nephrotic patients were extremely high, the mean for males being 520 mg./100 ml. and for females 590 mg./100 ml.

In the miscellaneous group there was no significant variation from normal levels; the mean value for men was 272 mg./100 ml. and for women 307 mg./100 ml.

FIG. 1. α_2 -Macroglobulin levels in males. Means, and standard deviation in normal subjects, are indicated.

FIG. 2. α_2 -Macroglobulin levels in females. Means, and standard deviation for normal subjects, are indicated.

FIG. 3. α_2 -Macroglobulin and haptoglobin levels in rheumatoid arthritis.

- = Women
- = Men
- (a) = mean for normal women
- (b) = mean for normal men.

FIG. 4. α_2 -Macroglobulin and C-reactive protein levels in rheumatoid arthritis.

- = Women
- = Men

DISCUSSION

The α_2 -macroglobulin levels in the normal subjects agree well with the results of James *et al.* (1966) and Schultze and Schwick (1959) but are higher than those reported by Ganrot and Scherstén (1967). The reported finding of significantly higher levels in normal women than in normal men (James *et al.*, 1966; Ganrot and Scherstén, 1967) is confirmed. This sex difference remains unexplained but in view of the even higher levels found in pregnant women (Schumacher and Schlumberger, 1963) it is perhaps more likely to be related to the possible functions of α_2 -macroglobulin in hormone transport than to its role in enzyme processes.

The low level in female rheumatoid patients confirms the findings of James *et al.* (1966) and, although the results presented here are not by themselves statistically significant, combining the results from the two series shows a highly significant ($P < 0.01$) difference from the mean for normal women. However, depression of α_2 -macroglobulin levels is not specific for rheumatoid arthritis as low values have also been reported in multiple myeloma (James *et al.*, 1966). That α_2 -macroglobulin does not take part in the acute phase response is indicated by its failure to increase in rheumatoid arthritic patients who showed the acute phase reaction, as demonstrated by high haptoglobin levels and the presence of C-reactive protein.

The significance of the raised level in chronic liver disease is unknown. It could conceivably be part of a general increase in plasma globulin synthesis to compensate for the hypoalbuminaemia which is frequently present (Sherlock, 1963). If this were the case, then α_2 -macroglobulin levels might be expected to be roughly proportional to total serum globulin and inversely proportional to serum albumin. However, there was no evidence of such relationships in the present study: in fact there was some suggestion that α_2 -macroglobulin was directly proportional to serum albumin (Fig. 5). α_2 -Macroglobulin levels did not appear, either, to be related to the type of chronic disease present, although the numbers were too small to draw firm conclusions. Fourteen of the 25 liver disease patients were on ACTH or corticosteroid therapy but this was not related to α_2 -macroglobulin concentration either in this group or in the patients with rheumatoid arthritis. If, however, α_2 -macroglobulin is involved in oestrogen metabolism, as the higher levels in normal women and especially in pregnant women suggest, then the marked increase in men with chronic liver disease could reflect failure of oestrogen inactivation (Tagnon, Lieberman, Schulman, and Brunschwig, 1952). There is clinical evidence of

FIG. 5. α_2 -Macroglobulin and albumin levels in liver disease.

○ = Women
● = Men

FIG. 6. α_2 -Macroglobulin and total globulin levels in liver disease.

○ = Women
● = Men

oestrogen excess in male, but not female, cirrhotics (Sherlock, 1963).

The very high values in the nephrotic sera support previous findings (Schultze and Schwick, 1959; Steines and Mehl, 1966; Kluthe, Hagemann, and Kleine, 1967). Statistical comparison of this group with the normal subjects could not be carried out because four of the nephrotic patients were aged less than 20 years, whereas all of the normal subjects were more than 20 years old; α_2 -macroglobulin levels are known to be higher in children, falling to adult values in the third decade (Ganrot and Scherstén, 1967; Weiss, 1965). Nevertheless, six of the eight nephrotics had values of 500 mg./100 ml. or more which, even allowing for age as indicated by the published figures, puts them above the upper limit of normal. There is no satisfactory explanation for the raised plasma α_2 -macroglobulin in the nephrotic syndrome. Kluthe *et al.* (1967), investigating α_2 -macroglobulin turnover in nephrotics, have reported normal absolute catabolic rates (and inferred normal absolute rates of synthesis) and suggest that the high plasma levels result from diminution of plasma volume and selective retention of high molecular weight proteins by the kidney. However, it is improbable that these factors alone could produce levels of the order found in this study or in the series of Kluthe *et al.* (up to 735 mg./100 ml.); it seems more likely that other factors are operating such as prolonged half-life (as indeed Kluthe *et al.* found) or increased rate of synthesis.

The lack of significant changes in α_2 -macroglobulin levels in the patients with miscellaneous disorders indicates that there is no non-specific change in response to disease processes.

Changes in the levels of an individual serum protein in different disease states may give information about its function but the results of this study do not suggest any further role for α_2 -macroglobulin.

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REFERENCES

- Crockson, R. A. (1963). *J. clin. Path.*, **16**, 287.
- , Payne, C. J., Ratcliff, A. P., and Soothill, J. F. (1966). *Clin. chim. Acta*, **14**, 435.
- Fahey, J. L., and McLaughlin, C. (1963). *J. Immunol.*, **91**, 484.
- Ganrot, P. O., and Scherstén, B. (1967). *Clin. chim. Acta*, **15**, 113.
- Hadden, D. R., and Prout, T. E. (1964). *Nature (Lond.)*, **202**, 1342.
- Haverback, B. J., Dyce, B., Bundy, H. F., Wirtschafter, S. K., and Edmondson, H. A. (1962). *J. clin. Invest.*, **41**, 972.
- Hitzig, W. H. (1961). *Int. Arch. Allergy*, **19**, 145.
- James, K., Johnson, G., and Fudenberg, H. H. (1966). *Clin. chim. Acta*, **14**, 207.
- Kluthe, R., Hagemann, U., and Kleine, N. (1967). *Vox Sang (Basel)*, **12**, 308.
- Lanchantin, G. F., Plesset, M. L., Friedmann, J. A., and Hart, D. W. (1966). *Proc. Soc. exp. Biol. (N.Y.)*, **121**, 444.
- Ma, T. S., and Zuazaga, G. (1942). *Ind. Engng Chem. analyt. Edn.*, **14**, 280.
- Mancini, G., Vaerman, J. P., Carbonara, A. O., and Heremans, J. F. (1964). *Protides Biol. Fluids*, **11**, 370.
- Mehl, J. W., O'Connell, W. J., and DeGroot, J. (1964). *Science*, **145**, 821.
- Owen, J. A., Smith, R., Padanyi, R., and Martin, J. (1964). *Clin. Sci.*, **26**, 1.
- Petermann, M. L. (1960). In *The Plasma Proteins*, vol. 2, edited by W. F. Putnam, vol. 2, p. 309. Academic Press, New York and London.
- Ratcliff, A. P., and Hardwicke, J. (1964). *J. clin. Path.*, **17**, 676.
- Schultze, H. E., and Schwick, G. (1959). *Clin. chim. Acta*, **4**, 15.
- , Heimbürger, N., Heide, K., Haupt, H., Störko, K., and Schwick, H. G. (1963). In *Proc. 9th Congr. europ. Soc. Haematol.*, Lisbon, 1963, vol. 2, p. 1315. S. Karger, Basle and New York.
- Schumacher, G., and Schlumberger, H. D. (1963). *Dtsch. med. Wchschr.*, **88**, 645.
- Sherlock, S. (1963). *Diseases of the Liver and Biliary System*, 3rd ed. Blackwell, Oxford.
- Steines, W. J., and Mehl, J. W. (1966). *J. Lab. clin. Med.*, **67**, 559.
- Tagnon, H. J., Lieberman, S., Schulman, P., and Brunschwig, A. (1952). *J. clin. Invest.*, **31**, 346.
- Walton, K. W., and Scott, P. J. (1964). *J. clin. Path.*, **17**, 627.
- Weichselbaum, T. E. (1946). *Amer. J. clin. Path.*, **16**, Tech. Sect., 40.
- Weiss, W. A. (1965). *Klin. Wchschr.*, **43**, 273.
- Wright, S. T. C. (1959). *Nature (Lond.)*, **183**, 1282.
- Zahnd, G. R., and Scheidegger, J. J. (1963). *Helv. med. Acta*, **30**, 506.