Clin. exp. Immunol. (1984) **57**, 647–656.

'Amyloid degrading activity' of human serum, an *in vitro* clearing effect which does not involve degradation of amyloid fibrils

D. CASPI, MARILYN L. BALTZ, *A. FEINSTEIN, *E. A. MUNN & M. B. PEPYS
*MRC Acute Phase Protein Research Group, Department of Medicine, Royal Postgraduate Medical School, London and *AFRC Institute of Animal Physiology, Babraham, Cambridge, UK*

(Accepted for publication 3 April 1984)

SUMMARY

Clearing of turbid amyloid A fibril containing agarose gels by human serum has been ascribed to 'amyloid degrading activity'. We report here that this optical phenomenon is not due to an enzymatic reaction, does not involve proteolysis of the fibril subunits and is not inhibited by sera of patients with AA amyloidosis. The extent of clearing correlates closely with the serum albumin concentration and, as previously reported by others, serum albumin itself causes clearing comparable to that of whole serum. Furthermore addition of albumin solutions to turbid aqueous suspensions of AA amyloid fibrils causes immediate clearing. Serum albumin is known to clarify turbid non-amyloid fibril containing gels and is used commercially to improve the optical properties of radial immunodiffusion plates. We therefore propose that this property of albumin, the mechanism of which is not yet understood, underlies the so called 'amyloid degrading activity' of human serum and the latter is not therefore likely to be of *in vivo* biological or clinical significance.

Keywords amyloid fibrils amyloid degrading activity serum albumin reactive systemic amyloidosis AA amyloidosis

INTRODUCTION

Amyloidosis is a condition in which abnormal extracellular deposits of protein material accumulate. The deposits consist predominantly of fibrils which are rigid, twisted, non-branching, of indeterminate length and up to approximately 10 nm in diameter. In different diseases associated with amyloidosis, the fibrils are formed from different proteins but always share the same electron microscopic appearance. Amyloid deposits may be localised, focal or systemic and in the latter case are almost invariably associated with dysfunction of the affected organs leading to morbidity and death. Once systemic amyloidosis has been recognized clinically it almost never regresses, the fibrils persist and increase in amount and there is no effective therapy for the amyloid *per se*.

Apart from relatively rare hereditary disorders the two more common forms of systemic amyloidosis are immunocyte dyscrasia associated amyloidosis in which the fibrils (AL) are derived from monoclonal immunoglobulin light chains and reactive systemic amyloidosis in which the fibrils (AA) are derived from, or at least related to, an acute phase reactant known as serum amyloid A protein (SAA). This is an apoprotein of high density lipoprotein. The pathogenesis of amyloid fibril deposition is thought to involve partial proteolytic cleavage of precursor proteins, which are present in abnormal amounts, followed by the polymerization of the cleavage products to form

Correspondence: Dr M. B. Pepys, MRC Acute Phase Protein Research Group, Department of Medicine, Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, UK.

fibrils (Glenner, 1980a, 1980b). Reactive systemic amyloidosis occurs as a complication of chronic active infections, inflammatory or neoplastic diseases in which there are persistently elevated concentrations of acute phase proteins including SAA (Benson & Cohen, 1979; de Beer *et al.*, 1982). However it is not clear why some patients with predisposing disorders develop amyloidosis and others do not, nor why there is so much variation in the rate of deposition or progression of amyloid. One possible mechanism, which has attracted much attention recently, concerns the *in vivo* degradation of amyloid fibrils. It has been suggested that the capacity for and rate of such degradation may be a major factor in the pathogenesis and course of systemic amyloidosis (Kedar, Sohar & Ravid, 1982; Wegelius, Teppo & Maury, 1982; Maury & Teppo, 1982; Maddison *et al.*, 1983).

These concepts have drawn support from experimental observations *in vitro* in which turbid agarose gels incorporating isolated AA type amyloid fibrils (AA-F) are cleared or rendered translucent by incubation with human serum. These findings have been reproduced in several laboratories and it has been confirmed that sera from patients with AA amyloidosis have a significantly impaired capacity to cause clearing (Wegelius *et al.*, 1982; Kedar *et al.*, 1982; Maddison *et al.*, 1983). The activity concerned has been called 'amyloid degrading factor' or 'amyloid degrading activity' (ADA) although no direct biochemical evidence of degradation has yet been published. Evidence has been adduced that the clearing effect is mediated by proteolytic enzyme activity and that this is very closely associated with serum albumin (Kedar *et al.*, 1982; Teppo, Maury & Wegelius, 1982). Most interestingly in view of the poor clinical prognosis of systemic amyloidosis, it has been claimed that various agents, including vitamin C (ascorbic acid), are able to enhance and restore to normal the impaired clearing activity of sera from amyloid patients (Kedar *et al.*, 1982). We report here a study of the so called ADA, of its biochemical nature and of the effects of various agents upon it.

MATERIALS AND METHODS

AA-F. These were extracted with water (Pras *et al.*, 1968) from the spleen of a patient with reactive systemic amyloidosis secondary to long standing classical rheumatoid arthritis and had the characteristic electron microscopic appearance in negatively stained preparations. The AA nature of the fibrils was shown by their response to potassium permanganate treatment (Wright, Calkins & Humphrey, 1977) and was confirmed by specific immunofluorescent staining using rabbit anti-human AA serum (kindly provided by Dr M. Skinner, Boston, Massachusetts, USA) and goat anti-human AA serum (ATAB, Atlantic Antibodies, Scarborough, Maine, USA). The concentration of the water extracted AA-F preparation was assessed by measurement of A_{280}. Aliquots of the AA-F preparation were stored either at $-20°C$, or at 4°C with addition of thymol (Sigma Chemical Co, Poole, UK) 1% wt/vol. as a preservative.

Gel plate assay for ADA. The preparation of AA-F in water was mixed with agarose (1% wt/vol. final concentration in water) (Seakem ME, FMC, Rockland, Maine, USA) at 56°C, following the method of Kedar *et al.* (1982). The concentration of AA-F in agarose which gave optimally cleared areas was determined in preliminary experiments and was in the range of 0·68–1·28 A_{280} units/ml. The suspension was poured onto horizontally levelled Petri dishes to form a gel 1·5 mm thick. Wells of 4 mm diameter were punched in the gel and were loaded with 10 μl samples. After overnight incubation at 23°C the diameter of the cleared area visible to the naked eye around each well was measured with a micrometer and the square of the radius was calculated. A standard calibration curve was established using a pool of serum from five healthy laboratory personnel which was diluted with phosphate-buffered saline, pH 7·2 (PBS) to final concentrations of 25%, 50% and 75% of neat. These standards were run in each experimental gel plate. In the same plates serum pools derived from up to 2,000 different individual healthy volunteer blood donors gave results which were not significantly different from those of the laboratory pool. The results of test sera and samples are expressed as a percentage of this normal value derived from the standard curve.

Clearing of aqueous suspensions of AA-F. Turbid aqueous suspensions (400 μl) of three different preparations of AA-F ($A_{280} = 1·5–2·5$), were mixed with 50 or 100 μl of human serum albumin

(HSA) solution (50 g/l) in PBS and the relative turbidity compared in naked eye observation with control suspensions to which PBS alone was added. After incubation at 23°C for 16–18 h the mixtures were run reduced in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) or SDS-gradient PAGE to examine the subunit composition. In other experiments ^{125}I-AA-F were used and after incubation the mixtures were dialysed against distilled water (150 ml) for 16–18 h and radioactivity in the dialysate was then measured. As a positive degradation control AA-F and ^{125}I-AA-F were incubated separately with pronase and trypsin and then tested as above.

Staining of AA-F in agarose plate. One per cent agarose gels containing AA-F at final concentrations of 1·28, 0·75, 0·38 and 0·19 A_{280} units/ml were poured into wells which had been punched in a plain, non-AA-F containing 1% agarose gel. After setting the plates were stained with Congo red (0·05% wt/vol. or 0·01% wt/vol.) or Sirius red (0·05% wt/vol.) in water and destained in distilled water. Clear differential staining was visible with the naked eye between the plain agarose and the AA-F containing gels. The same staining procedure was therefore adopted for examination of AA-F containing gels which had been cleared by serum.

Inhibition and enhancement experiments. A variety of agents were tested for the capacity to inhibit or enhance ADA by mixing them with sera from normal subjects or from patients with amyloidosis or with solutions of HSA. The concentrations used and the pre-incubation conditions before their assay in AA-F agarose gels are shown in the Results. Each agent was controlled by use of its particular solvent treated in exactly the same way and run in the same gel plates.

Sources of materials. HSA (crystalized and lyophilized), bovine serum albumin fraction V (BSA), hen egg albumin grade III, human plasma alpha-1-anti-trypsin, phenylmethylsulphonyl-fluoride (PMSF) in propan-1-2-diol and trypsin type II (porcine pancreatic) were obtained from Sigma Chemical Co. Pronase from *Streptomyces griseus* and neutral serine protease (Dispase) grade II from *Bacillus polymyxa* were obtained from Boehringer Manheim, Boehringer Corporation Ltd, Lewes, UK. Ascorbic acid injectable was from Evans Medicals, Speke, Liverpool, UK and ascorbic acid pure powder was from Hoffmann-La Roche, Basle, Switzerland. Ethylene diamino tetra-acetate (EDTA), sodium citrate, sodium azide and sodium fluoride were obtained from BDH Chemicals Ltd, Poole. Aprotinin (Trasylol) was obtained from Bayer UK Ltd, Newbury, Berkshire, UK. Cow, dog, guinea-pig, horse, rabbit, rat and sheep sera were obtained from normal animals.

Sera were obtained from six patients with reactive systemic amyloidosis. The AA nature of their amyloid was suggested by their clinical history and by the sensitivity of Congophilia of the deposits to treatment with potassium permanganate. In some cases this was confirmed by specific immunohistochemical staining. The sera were stored in aliquots at −20°C.

Tissue sections. Human kidney tissue was obtained at autopsy from a patient with reactive systemic amyloidosis secondary to rheumatoid arthritis. The tissue blocks were snap frozen in Arcton 12 over liquid nitrogen and stored in liquid nitrogen. AA type amyloid was confirmed by permanganate sensitivity and specific immunofluorescence with goat anti-AA serum (ATAB, Atlantic Antibodies, Scarborough, Maine, USA) and rabbit anti-AA serum (kindly provided by Dr M. Skinner). Cryostat sections were cut at 6 μm and were air dried. The sections were then incubated in serum from normal subjects, serum from a patient with AA amyloidosis or PBS for 4 h at 23°C, washed three times in PBS and stained for amyloid by the alkaline Congo red method (Puchtler, Sweat & Levine, 1962) with haematoxylin counterstaining.

Tissue homogenate. Spleen tissue (0·84g) containing AA amyloid was homogenized with 0·5 ml isotonic saline in a Jencons Uni-Form type I homogenizer (Jencons Scientific Ltd, Leighton Buzzard, UK). Aliquots of 200 μl of the homogenate were then incubated on a shaker at 23°C for 24 h with 400 μl of normal human serum, 400 μl of serum from an amyloidotic patient or 400 μl of PBS, respectively. The suspensions were then spun at 1,500*g* for 10 min. The pellets were washed three times in PBS, smeared on glass slides, air dried, stained with alkaline Congo red and observed by polarized light microscopy.

Iodination of AA-F. AA-F were iodinated using Iodogen (Pierce & Warriner UK Ltd, Chester, UK) following the method of Salacinski *et al.* (1981). A solution (1·3 ml) of AA-F in water (A_{280} = 2·7) was mixed with an equal volume of 0·05 M phosphate buffer pH 7·0 and placed in a plastic vial previously coated with 40 μg of Iodogen. Approximately 1 mCi of carrier free ^{125}I Na (IMS-30, Radiochemical Centre, Amersham) was added and the mixture incubated for 10 min at

23°C. It was then diluted 1:2 with phosphate buffer. The efficiency of labelling, determined by precipitation with 20% trichloracetic acid, was 63%. Free iodine was removed by extensive dialysis against water at 4°C after which protein bound activity was found to be 82%.

SDS-PAGE. Gradient SDS-PAGE was performed on Pharmacia PAA 4–30% gels according to the manufacturer's instructions. Discontinuous SDS-PAGE analysis in 15% gels was run according to Laemmli (1970). The gels were calibrated with marker proteins of known M_r (Pharmacia Fine Chemicals GB Ltd, Hounslow, Middlesex).

Electron microscopy, AA-F in aqueous suspension were allowed to absorb to thin carbon films and were then negatively stained with 2% sodium silicotungstate (pH 7·2). Blocks about 1 mm by 2 mm were cut from agarose gels fixed in 2% glutaraldehyde in 80 mM phosphate buffer pH 7·4 for 1 h at room temperature and then overnight at 4°C. The samples were washed with buffer solution, post-fixed for 1 h with 1% osmium tetroxide solution at room temperature and then were dehydrated and finally embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate.

RESULTS

Clarification of AA-F agarose gels by sera and albumin solutions

Normal human serum produced definite discrete circular zones of translucency in the otherwise turbid AA-F agarose gels (Fig. 1). The area of these cleared zones was greater than those produced by sera from patients with AA amyloidosis (mean $\pm$ s.d. of five normal individuals, 219 ± 12 mm^2,

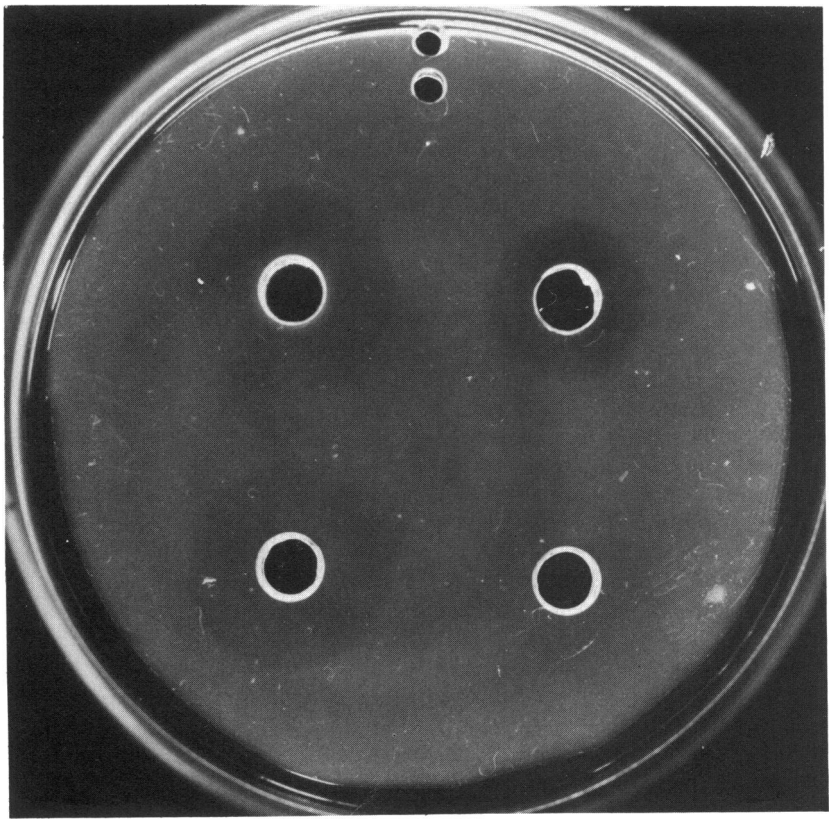

Fig. 1. AA-F agarose gel showing the clearing effect of serum from: normal subject (upper left well) and amyloid patient (upper right well). HSA solution (50 g/l) is in the lower left well.

mean ± s.d. of six amyloid patients, 109 ± 33 mm^2, $P < 0.001$). The albumin concentration in the pooled serum from these same five normal subjects was 55·2 g/l whereas the mean ± s.d. of the albumin concentration in the amyloid sera was 21·0 ± 5·7 g/l. When the area of the cleared zones was plotted against the serum albumin concentrations a linear correlation coefficient (r) of 0·91 was derived, indicating a statistically significant relationship between these two variables ($P < 0.01$). Introduction of 50 g/l solutions, either in water or PBS, of isolated HSA or BSA in place of serum produced zones of clearing of comparable size to those produced by normal serum (Fig. 1). Mixing of amyloid patient serum with an equal volume of 100 g/l albumin solution, that is sufficient to raise the final albumin concentration to the normal range, was associated with enhancement of the clearing capacity of the serum so that it too fell into the normal range.

Sera from a variety of animals including horse, cow, sheep, dog, rabbit, guinea-pig and rat produced zones of clearing similar to those produced by normal human serum.

Staining of AA-F agarose gels

Although the zones of clarification appeared more translucent than the surrounding turbid AA-F agarose gels there was no convincing loss of uptake of the amyloid fibril binding dyes Congo red and Sirius red.

Electron microscopy of AA-F agarose gels

Agarose gels containing AA-F at a concentration of 1·28 A_{280} units/ml were not significantly different in appearance in the electron microscope from agarose gel alone, which itself has a dispersed fibrillar ultrastructure. The apparent thickness and distribution of the fibrils in sections of samples taken from zones cleared by serum or albumin were not significantly different from those in the untreated gel.

Inhibition of serum-induced clarification of AA-F agarose gels

It has been suggested that the reduced clearing activity of sera from patients with AA amyloidosis is due to the presence therein of a low molecular weight inhibitor of the material responsible for clearing (Kedar *et al.*, 1982). Mixtures of equal volumes of normal serum (five individuals) and amyloid patient serum (five individuals) were made in a checkerboard fashion, i.e. 25 different combinations of normal and patient sera. All these mixtures produced areas of clearing greater than those produced by control mixtures of the corresponding normal serum with saline. There was thus no evidence for any inhibitory effect of amyloid patient serum on the clearing phenomenon.

It has also been reported that the clearing effect is inhibited by inhibitors of proteolytic enzyme activity, including $alpha_1$-anti-trypsin, aprotinin and PMSF (Teppo *et al.*, 1982) whilst others have observed inhibition by sodium azide and sodium fluoride (Kedar *et al.*, 1982). However, as shown in Table 1, none of these agents had any effect at all on clearing when compared with identical dilutions of serum made by addition of the solvents used for the materials under test.

Enhancement of serum-induced clarification of AA-F agarose gels

Addition of isolated serum albumin of either human or bovine origin to sera from patients with AA amyloidosis increased the observed area of clearing in a dose-dependent fashion (Table 2).

Table 1. Materials which did not affect the clearing phenomenon

Inhibitor	Final concentration	Pre-incubation conditions
$alpha_1$-anti-trypsin	2–32 μM	30 min, 37°C
Aprotinin	10^2–10^5 u/ml	6 h, 37°C
PMSF	0·5–5·0 mM	Overnight, 23°C
NaN_3	0·01–0·1 mg/ml	30 min, 23°C
NaF	0·1–5·0 mg/ml	30 min, 23°C

Table 2. Enhancement by isolated albumin of clearing capacity of amyloid patient serum

Expt	Serum	Final dilution	Additive	Final albumin concentration (g/l)	Area of clearing (mm^2)
1	NHS	2/3	PBS	33·3	119
	Pt serum	2/3	PBS	12·7	54
	Pt serum	2/3	HSA	55·7	133
	Pt serum	2/3	BSA	55·7	123
2	NHS	1/2	PBS	25	80
	Pt serum	1/2	PBS	9·5	52
	Pt serum	1/2	HSA	34·5	99
	Pt serum	1/2	BSA	34·5	113

Linear correlation coefficient between final albumin concentration and area of clearing $r = 0{\cdot}931$ ($P < 0{\cdot}001$).

In contrast sodium citrate (10 mg/ml final concentration) and ascorbic acid (0·004–50 mg/ml) did not enhance the clearing effect of amyloid patient serum. However either Na_4EDTA (0·1 mg/ml) or a neutral equimolar mixture of Na_2 and Na_4 EDTA (0·1 mg/ml) caused marked clearing of AA-F agarose gels even in the absence of any serum, and this clearing was completely prevented by prior admixture of calcium chloride in a two-fold molar excess.

Effect of temperature

The rate of clearing of turbid AA-F agarose gels by normal human serum (NHS) was compared at 4°C and 37°C (Fig. 2). The difference observed was compatible with accelerated diffusion at the higher temperature but not with the kinetics expected of an enzymatic reaction.

Effect of proteolytic enzymes

Pronase and trypsin solutions in PBS at between 10 μg and 1 mg/ml were placed in wells (10 μl) in AA-F agarose gels instead of serum and the plates were incubated at room temperature. No clearing was observed at any time up to 48 h, although these concentrations of proteinases caused major degradation of the AA peptide subunits of AA-F in the fluid phase (see below).

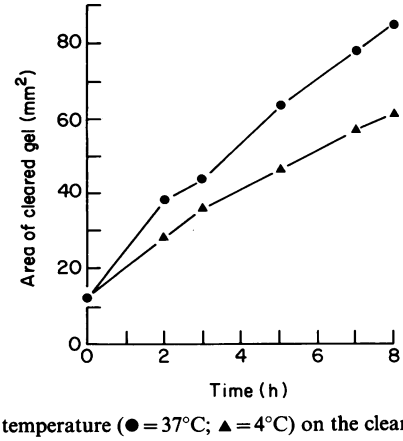

Fig. 2. Effect of temperature (● = 37°C; ▲ = 4°C) on the clearing phenomenon.

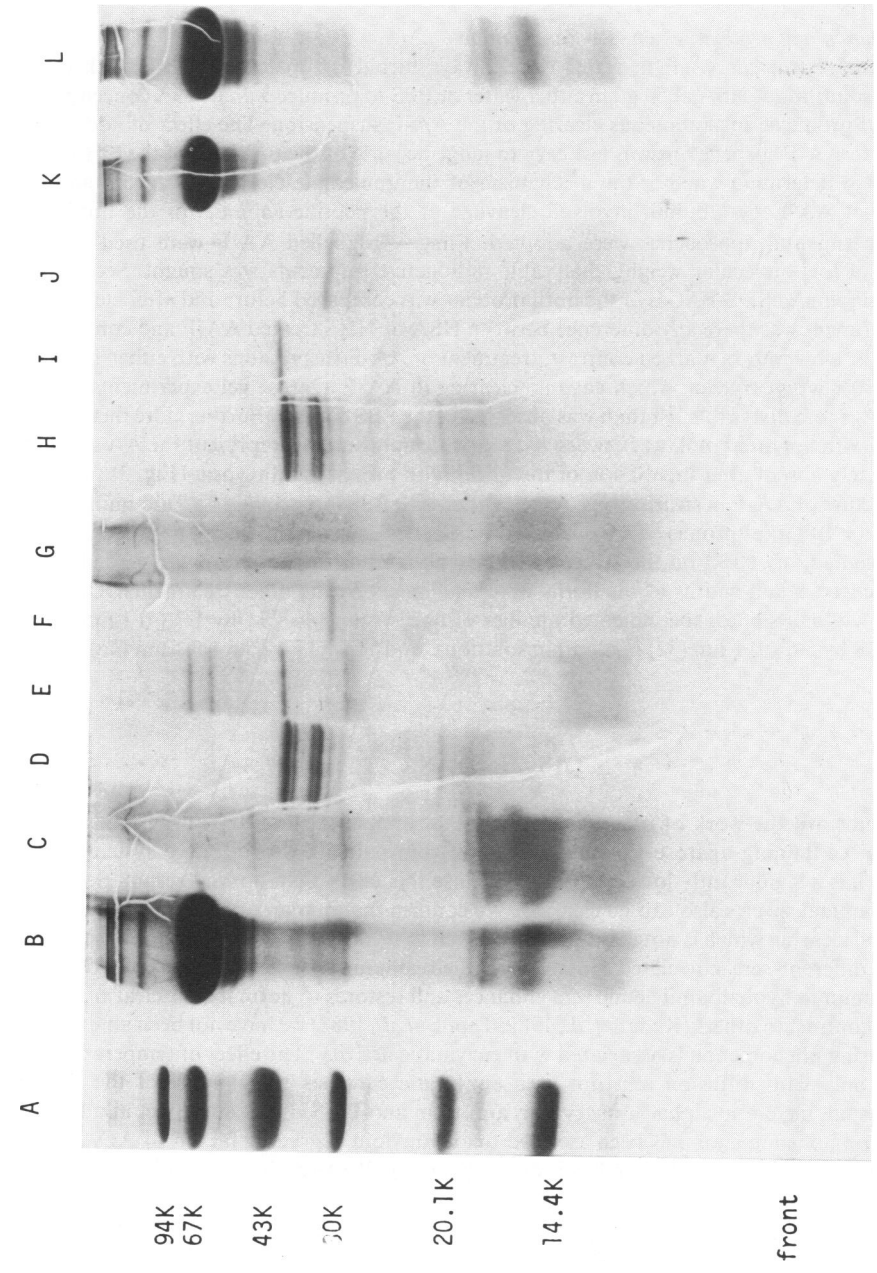

Fig. 3. Analysis by SDS-PAGE (15%) of AA fibrils after incubation with albumin or proteinases. Lane A, marker proteins of known mol. wt; lanes B and L, AA-F plus HSA; lane C, AA-F plus PBS; lane D, AA-F plus pronase; lane F, AA-F plus trypsin; lane H, pronase alone; lane J, trypsin alone; lane K, HSA alone.

Effect of serum on amyloid in tissues

Neither normal human serum nor serum from a patient with amyloidosis had any effect on the Congophilia of AA amyloid deposits in sections of amyloidotic kidney. Similarly incubation of serum with a homogenate of AA amyloid laden tissue did not perceptibly affect the Congophilia of the fibrils.

Effect of albumin and proteinases on AA-F in solution

Suspensions of extracted AA-F in water were always turbid or opalescent to the naked eye. Addition of solutions of HSA at 50 g/l in either water or PBS to produce a final HSA concentration of 5 or 10 g/l produced instantaneous clearing of the AA-F suspension. The effect of addition of whole NHS to AA-F suspensions was less easy to judge because of the turbidity of the NHS itself.

In order to determine whether the mechanism of the immediate clearing of turbid aqueous suspensions of AA-F by albumin involved cleavage of the peptide subunits of the fibrils two different experimental approaches were adopted. First, ^{125}I-labelled AA-F were used and the appearance of low molecular weight, dialysable radioactive fragments was sought. Second, the migration on reduced SDS-PAGE of the fibril proteins was compared before and after clearing. In neither experiment was there any difference between HSA or NHS treated AA-F and control PBS treated AA-F. However, in marked contrast, treatment of AA-F suspensions with either 1% wt/wt pronase or 10% wt/wt trypsin, which gave no clearing in AA-F agarose gel experiments, caused release of 50% more dialysable ^{125}I than was observed in the PBS control. Furthermore the bands in SDS-PAGE with apparent mol. wt between 8–18K, which presumably represent the AA subunits, were completely absent after incubation of the fibrils with pronase and trypsin (Fig. 3).

The structure of AA-F in suspensions treated either with PBS (controls), 10 g/l albumin, pooled normal sera or 10 μg/ml pronase were examined by electron microscopy using negative staining. Only pronase had any effect on the structure, nearly all the fibrils being totally degraded by the enzyme. The length and widths of the fibrils were unchanged by the other treatments. The mean lengths and widths $\pm$ s.e. for the indicated number of fibrils were 144 ± 11 by $5{\cdot}7 \pm 0{\cdot}1$ nm (47) in PBS, 147 ± 18 by $5{\cdot}6 \pm 0{\cdot}1$ nm (32) in albumin solutions, and 154 ± 11 by $5{\cdot}6 \pm 0{\cdot}1$ nm (46) in sera.

DISCUSSION

We have confirmed the work of others in showing that human serum causes optical clearing of turbid AA-F containing agarose gels and that sera from patients with reactive systemic (AA) amyloidosis has a significantly lower capacity to cause this effect than normal serum. Sera from many other animal species also caused clearing. We confirm that human or bovine serum albumin causes optical clearing which is not distinguishable from that caused by whole serum. The extent of clearing by different sera correlated closely with their albumin concentration and addition of isolated albumin to hypoalbuminaemic sera enhances and restores to normal their clearing power. However in contrast to others (Kedar *et al.*, 1982; Teppo *et al.*, 1982) we have not been able to show that the clearing phenomenon is associated with enzymatic activity. The effect of temperature was compatible only with diffusion and not with enzymatic catalysis whilst none of the enzyme inhibitors tested, including alpha$_1$-anti-trypsin, aprotinin and PMSF, had any effect at all.

The clearing phenomenon has been ascribed to an 'amyloid degrading factor' or ADA but no direct evidence for such 'degradation' has been forthcoming. We show here that addition at room temperature of albumin solutions to turbid aqueous suspensions of AA-F produces immediate clearing. These are not the kinetics of an enzymatic reaction. Furthermore even after prolonged incubation of AA-F in aqueous suspension with either albumin alone or whole serum there was no evidence of proteolytic cleavage of the fibril subunits, whereas such cleavage was readily demonstrable following incubation with proteinases which themselves did not cause any clearing in the agarose gel assay system. Incubation of tissue sections or homogenates of amyloid tissue with whole serum or albumin had no demonstrable effect on fibril structure or tinctorial properties.

It has been claimed that sera from AA amyloid patients contain an inhibitor which blocks the

physiological 'amyloid degrading factor' of normal serum and that this inhibition is reversed *in vitro* by addition of vitamin C, a potential therapeutic agent (Kedar *et al.*, 1982). This finding stimulated our interest and was the reason for the present study. Unfortunately we were unable to confirm that sera from amyloid patients inhibited the capacity of normal sera to clear the gels. Mixing experiments gave results compatible only with simple dilution effects and produced clearing in relation to the final albumin concentrations applied to the gels. Ascorbic acid had no detectable effect on clearing by any serum, nor did sodium citrate. However EDTA did affect clearing and could cause clearing on its own, without serum in any form, and this action was inhibited by the presence of an excess of calcium ions.

In conclusion therefore the present results indicate that clearing of fibril suspensions of AA-F in aqueous or agarose gel media is an optical phenomenon, unrelated to enzymatic or other proteolytic cleavage of the fibril protein subunits. The activity is associated with and displayed by commercial preparations of serum albumin and correlates in extent with the albumin concentration of the sera tested. Although the precise biophysical mechanism of the optical phenomenon is not known the capacity of serum albumin to clarify and reduce the turbidity of agarose gels, in the absence of any added fibrils, has long been recognized (Guiseley & Renn, 1977). Indeed major commercial suppliers of antiserum containing agarose gel plates for radial immunodiffusion incorporate additional serum albumin in their gels to improve their optical clarity (D. W. Renn, personal communication).

The biological and clinical speculations regarding amyloid pathogenesis which have hitherto been based on the *in vitro* gel clearing phenomenon are thus probably not sustainable. Certainly despite a short report to the contrary (Chen, Ravid & Kedar, 1983) we have been unable in an extensive study in mice to demonstrate any prophylactic or therapeutic effect of vitamin C on casein-induced or an accelerated model of AA amyloidosis (Baltz *et al.*, 1984). The concept of fibril degradation *in vivo* and the quest for its mechanisms remains, however, of central importance since all hopes of effective therapy of established amyloidosis must rest on the mobilization of such processes.

This work was supported in part by MRC Programme Grant G979/51 to MBP and by a grant from F. Hoffman-La Roche & Co, Basle.

REFERENCES

BALTZ, M.L., CASPI, D., GLATTHAAR, B., MOSER, U. & PEPYS, M.B. (1984) The failure of ascorbic acid therapy to alter the induction or remission of murine amyloidosis. *Clin. exp. Immunol.* **57**, 657.

BENSON, M.D. & COHEN, A.S. (1979) Serum amyloid A protein in amyloidosis, rheumatic and neoplastic diseases. *Arthrit. Rheum.* **22**, 36.

CHEN, B., RAVID, M. & KEDAR, I. (1983) Influence of ascorbic acid and sodium citrate on the formation of amyloid in experimental animals. (Abstract) *Bi-Annual Meeting of Israeli Society of Internal Medicine*, Tel Aviv, 25–26 April 1983.

DE BEER, F.C., MALLYA, R.K., FAGAN, E.A., LANHAM, J.G., HUGHES, G.R.V. & PEPYS, M.B. (1982) Serum amyloid A protein concentrations in inflammatory diseases and its relationship to the incidence of reactive systemic amyloidosis. *Lancet*, **ii**, 231.

GLENNER, G.G. (1980a) Amyloid deposits and amyloidosis. I. The β-fibrilloses. *N. Engl. J. Med.* **302**, 1283.

GLENNER, G.G. (1980b) Amyloid deposits and amyloidosis. II. The β-fibrilloses. *N. Engl. J. Med.* **302**, 1333.

GUISELEY, K.B. & RENN, D.W. (1977) Agarose. Information booklet supplied by Marine Colloids Division, FMC Corporation, Maine, USA.

KEDAR, I., SOHAR, E. & RAVID, M. (1982) Degradation of amyloid by a serum component and inhibition of degradation. *J. lab. clin. Med.* **99**, 693.

LAEMMLI, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680.

MADDISON, P., BACON, P.A., STRACHAN, A.F. & TRIBE, C.R. (1983) A marked deficiency of amyloid degrading factor is associated with a poor prognosis. In *Amyloidosis E.A.R.S.* (ed. by C. R. Tribe & C. A. Bacon) pp. 124–125. John Wright & Sons Ltd, Bristol.

MAURY, C.P.J. & TEPPO, A-M. (1982) Mechanism of reduced amyloid A degrading activity in serum of patients with secondary amyloidosis. *Lancet*, **ii**, 234.

PRAS, M., SCHUBERT, M., ZUCKER-FRANKLIN, D., RIMON, A. & FRANKLIN, E.C. (1968) The characterisation of soluble amyloid prepared in water. *J. clin. Invest.* **47**, 924.

PUCHTLER, H., SWEAT, F. & LEVINE, M. (1962) On the binding of Congo red by amyloid. *J. Histochem. Cytochem.* **10**, 355.

SALACINSKI, P.R.P., MCLEAN, C., SYKES, J.E.C., CLEMENT-JONES, V.V. & LOWRY, P.J. (1981) Iodination of proteins, glycoproteins and peptides using a

solid phase oxidizing agent 1,3,4,6 tetrachloro-3,6-diphenyl glycoluril (Iodogen). *Anal. Biochem.* **117,** 136.

TEPPO, A-M., MAURY, C.P.J. & WEGELIUS, O. (1982) Characteristics of the amyloid A fibril degrading activity of human serum. *Scand. J. Immunol.* **16,** 309.

WEGELIUS, O., TEPPO, A-M. & MAURY, C.P.J. (1982) Reduced amyloid A degrading activity in serum in amyloidosis associated with rheumatoid arthritis. *Br. Med. J.* **284,** 617.

WRIGHT, J.R., CALKINS, E. & HUMPHREY, R.L. (1977) Potassium permanganate reaction in amyloidosis. A histologic method to assist in differentiating forms of this disease. *Lab. Invest.* **36,** 274.