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Demonstration of Amyloid-Degrading Activity in Normal Human Serum (37806)

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In systemic amyloidosis, the rare instances of recovery following the successful treatment of a predisposing disease present a challenge in the management of an otherwise fatal disorder (1,2). Notwithstanding, experimental endeavor has been directed largely toward unravelling the nature of amyloid and its pathogenesis rather than the biological mechanism by which this fibrillar protein may be removed from the tissues (3). The following experiments indicate that normal human serum contains a heat-stable component capable of degrading amyloid *in vitro*.

Procedure. In principle, one portion of a standard suspension of water-soluble amyloid (4) was incubated with two portions of human serum in a test tube for 2 hr at 37°. Following incubation the precipitate obtained by centrifugation for 30 min in a cooled PC-2 Sorvall Centrifuge at 15,000 rpm was collected, twice washed in cold saline, and recentrifuged as above, resuspended in normal saline, and its total protein content determined by the Biuret method at 555 nm. Following the same procedure, incubation separately of the above portions of the amyloid suspension and of the serum (each tube made up to same initial volume with normal saline) permitted calculation

$$\text{percent of amyloid degraded} = \left(1 - \frac{AS - S}{A}\right) \times 100, \text{ where}$$

AS = total protein in ppt from incubation of amyloid with serum

S = total protein in ppt from incubation of serum with Saline

A = total protein in ppt from incubation of amyloid with Saline.

Materials. Normal human serum was obtained from 13 healthy blood donors and two of the investigators.

FMF-serum was obtained from three patients suffering from familial Mediterranean fever (FMF), all of them with amyloidosis.

Denatured serum was prepared by heating at 100° for 15 min the sera obtained from three healthy blood donors and two of the investigators and collecting the supernatant after centrifugation in a Spinco model L at 30,000 rpm for 60 min.

Water-soluble amyloid was extracted separately by the method of Pras *et al.* (4) from the spleens obtained postmortem from a case of FMF and one of Hodgkins' disease. Each preparation was precipitated in 0.9% NaCl and resuspended to a concentration, maintained homogeneous by shaking, of 4 mg protein per ml as determined by the Biuret method. These were the standard water-soluble amyloid suspensions used in the incubation experiments. Due to limitation of the quantity available, Hodgkins' amyloid was incubated only with the normal human sera.

Results. Incubation of FMF- and Hodgkins' Amyloids with Normal Sera. The precipitate yielded by 5 ml of FMF-amyloid suspension incubated alone contained 19.3 mg protein. After incubation of the 5 ml of FMF-amyloid with 10 ml of each of the normal human sera, the protein contents of the precipitates ranged from 3.7 to 7.7 (mean 5.5) mg. The contribution of the sera, as determined from their incubation alone, to these precipitates ranged from 0.4 to 1 mg. Incubation of FMF-amyloid with normal human

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TABLE I. Incubation of 10 ml Normal Human Sera and 5 ml Amyloid Suspensions.

Serum no.	Total protein (mg) in precipitate after incubation of [a]: Serum alone (S)	Serum with FMF-amyloid (AS)	Serum with Hodgkins' amyloid (AS)	Amyloid degraded %: FMF-	Hodgkins'
1.	0.4	6.6	7.3	68	59
2.	0.7	7.9	9.0	63	58
3.	0.6	5.6	7.4	74	68
4.	0.2	3.9	4.9	81	76
5.	0.9	6.1	5.6	63	76
6.	0.5	4.0	5.2	82	75
7.	0.6	5.1	5.8	77	73
8.	0.6	4.4	6.9	80	67
9.	1.0	4.5	5.7	82	76
10.	0.7	5.5	6.2	75	71
11.	0.4	5.6	6.7	73	67
12.	0.4	4.3	6.5	80	74
13.	0.9	6.9	8.2	69	62
14.	1.0	7.7	8.6	65	60
15.	0.3	4.4	5.7	79	72

[a]FMF-amyloid alone (A) — 19.3 mg total protein; Hodgkins' amyloid — 19.5 mg.

sera, therefore, resulted in a mean loss of 74% (range 63–82%) protein from the precipitate. These data are recorded in Table I. This table includes the results of parallel incubations using the same sera and the Hodgkins' amyloid suspension. The mean loss of protein from the Hodgkins' amyloid was 69%.

Incubation of FMF-amyloid with FMF-amyloidosis Sera. Results of the incubation of FMF-amyloid with each of the sera obtained from three amyloidotic FMF patients are shown in Table II. The mean loss of protein in the precipitate was only 22.2% (range 20.0–24.4%).

Incubation of FMF-amyloid with Denatured Sera. The precipitates obtained by incubation of 1.5 ml FMF-amyloid suspension with 3 ml of each denatures serum, contained 1.2–1.4 mg protein (Table III). Since incubation of the FMF-amyloid alone yielded a precipitate containing 5.8 mg protein and of the denatured sera alone yielded no precipitate, this indicates a degradation of 78% of the amyloid. The supernatant of one of the FMF-amyloid-denatured serum incubation tubes was shown to contain only small molecules by identical OD_{280} readings before and after Amicon XM-100 filtration. Electron micros-

TABLE II. Incubation of 10 ml FMF-Amyloidosis Serum and 5 ml FMF-Amyloid.

Serum no.	Total protein (mg) in precipitate after incubation of [a]: Serum alone (S)	Serum + amyloid (AS)	Amyloid degraded %
1.	1.3	16.3	22.3
2.	1.0	15.6	24.4
3.	0.6	16.1	20.0

[a]FMF-amyloid alone (A) — 19.3 mg protein.

TABLE III. Incubation of 3.0 ml Denatured Normal Human Serum and 1.5 ml FMF-Amyloid.

Serum no.	Total protein (mg) in precipitate after incubation of [a]: Serum + amyloid (AS)	Amyloid degraded %
1.	1.2	80
2.	1.3	78
3.	1.3	78
4.	1.4	76
5.	1.2	81

[a]Denatured sera alone (S) yielded no precipitate; FMF-amyloid alone (A) — 5.8 mg protein.

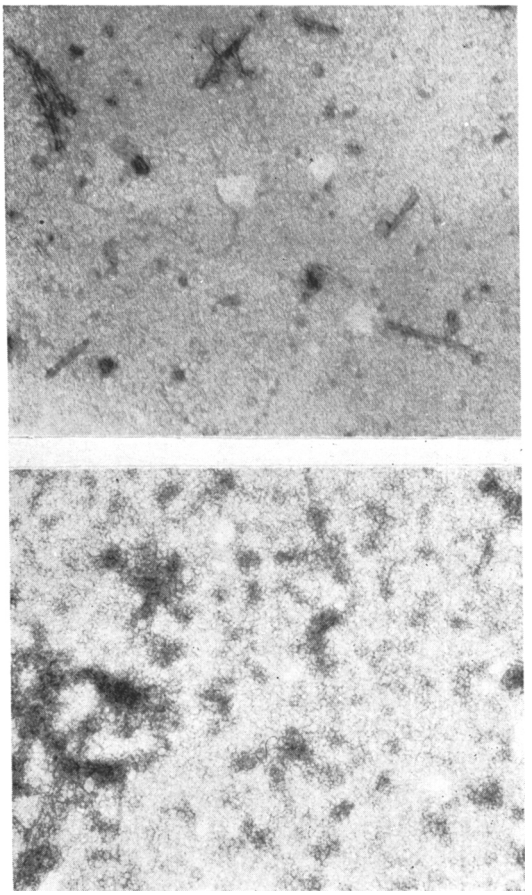

FIG. 1. Increase of amorphous matter after incubation of FMF-amyloid suspension with denatured human serum: above—supernatant of amyloid incubated withs aline alone; below—supernatant of amyloid incubated with denatured serum. Negative staining with uranyl acetate; ×45,000.

copy of the supernatant after negative staining with uranyl acetate revealed an increase of amorphous material (Fig. 1).

Discussion. This study establishes that normal human sera are capable of degrading amyloid and that this activity is not affected by heating to 100°C for 15 min. The action of a presumed amyloid-degrading component (ADC) was demonstrated against two amyloids of perireticulin ("typical") distribution, one of genetic (FMF) origin and the other acquired (Hodgkins) (5,6). It remains to be shown whether it will also degrade amyloid of pericollagen ("atypical") distribution which may be of different molecular structure (7–9).

Interestingly, ADC activity was much less marked in the serum of three amyloidotic FMF patients. Could this hint at a role in *in vivo* amyloid metabolism? Certainly a genetic disorder like familial Medierranean fever, which is usually diagnosed prior to penetrance of the amyloid trait, provides an ideal situation for investigation (10).

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