INCORPORATION OF PLASMA PROTEINS INTO AMYLOID

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With the aid of pure antibodies labeled with fluorochrome and peroxidase, amyloid, occurring in various situations in patients with secondary amyloidosis, was found regularly to contain γ-globulin, fibrinogen, and albumin. The possible mechanisms of accumulation of proteins in amyloid are discussed.

KEY WORDS: amyloid; plasma proteins; fluorochrome; peroxidase.

The basic substrate of amyloid is known to consist of a heterogeneous protein complex and plasma participates in its formation [3, 5, 8, 13]. The spectrum of plasma proteins and their relative proportions in amyloid have not been precisely established, partly on account of the difficulty of interpreting data obtained on different objects and of the absence of definite standardization of the experimental methods used.

To determine the precise antigenic composition of amyloid, pure antibodies against the main components of plasma were used in the present investigation, instead of antiserum as in previous studies, to secure greater reliability.

The test material consisted of the spleen, liver, and kidneys taken at autopsy from patients with tuberculosis complicated by amyloidosis. Pure rabbit antibodies against human immunoglobulin G, fibrinogen, and albumin and donkey antibodies against rabbit immunoglobulin G, eluted from the corresponding immunosorbents by the method of Avrameas and Ternynck [1, 7], were used. Antibodies were labeled in the usual way with fluorescein isothiocyanate for fluorescence microscopy and with horseradish peroxide (Serva) by Avrameas' method [4, 6], previously verified in the writer's laboratory, in order to observe the antigen–antibody reaction in the light microscope. The working dilution of antibodies and conjugates was equivalent to 0.5 mg/ml protein determined by Lowry's method. Serial cryostat sections were fixed for 10 min in 96° ethyl alcohol and treated by the direct and indirect Coons' method.

The incubation time of the sections with native and fluorchrome-labeled antibodies did not exceed 30 min, but with peroxidase-labeled antibodies it was 2-3 h. Peroxidase activity was detected by benzidine (Chemapol) in the presence of hydrogen peroxide. The diagnosis of amyloidosis was made on the basis of examination of histological sections stained with Congo red, methyl violet, and thioflavine T.

EXPERIMENTAL RESULTS

In all five cases, irrespective of the method of treatment of the sections, the results were the same. No difference was found in the distribution of plasma proteins in amyloid from different situations. Everywhere the amyloid had a high content of γ-globulin, and only in the center of massive deposits was the specific fluorescence (or staining) slightly weakened (Figs. 1a and 2a). Fibrinogen–fibrin was detected in the amyloid in a lower concentration (Figs. 1b and 2b). Conglomerates of amyloid almost free from immunologically active fibrinogen were found. Albumin, on the other hand, infiltrated the amyloid diffusely, but its content was always low (Figs. 1c and 2c).

Comparison of the experimental and control preparations (Figs. 1d and 2d) and the results of histological investigation showed that proteins, mainly albumin, penetrate beyond the boundaries of the amyloid into the surrounding tissues in the kidneys in cases of severe nephrosis.

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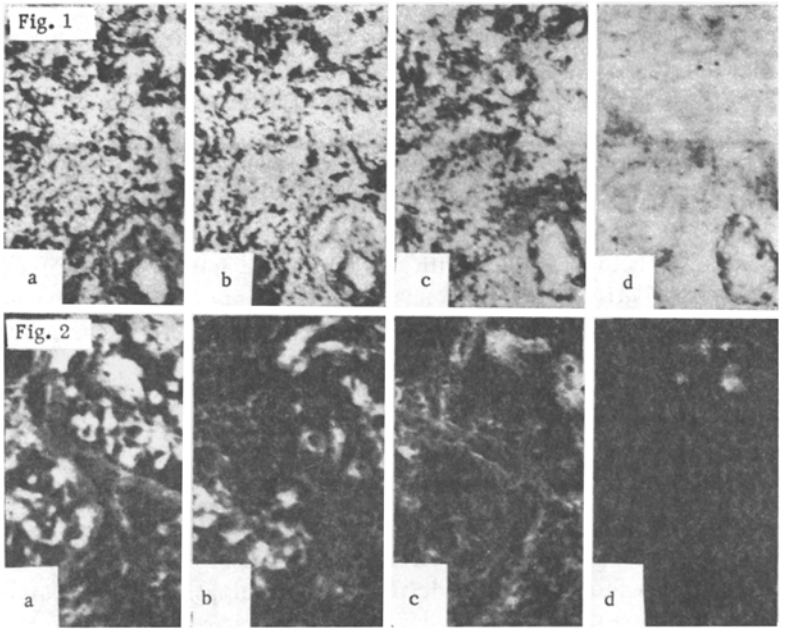

Fig. 1. Sections through human spleen: detection of γ-globulin (a), fibrinogen–fibrin (b), and albumin (c) in amyloid masses. Treatment with corresponding rabbit antibodies, except control section (d), followed by donkey antibodies against rabbit γ-globulin, labeled with horseradish peroxidase. Peroxidase activity detected as brown color (dark on the photographs). Light microscope, 60 ×.

Fig. 2. Sections through human kidney: fluorescence of γ-globulin (a), fibrinogen–fibrin (b), and albumin (c) in amyloid located in glomeruli. Treatment by indirect Coons' method with corresponding rabbit antibodies, except control section (d), then by donkey antibodies against rabbit γ-globulin labeled with fluorescein isothiocyanate. Fluorescence microscope, 100 ×.

Thus, in secondary amyloidosis in man, as in induced amyloidosis in mice [3], γ-globulin was found to be an invariable component of the amyloid, followed by fibrinogen and albumin in order of decreasing concentration. The hypothesis of the origin of amyloid from immunoglobulin light chains has so far been confirmed only for primary [11, 16] amyloidosis, but not for secondary [12, 15]. The facts are overwhelmingly against an immune or autoimmune origin of the amyloid [2, 3, 10]. At the present stage, therefore, the mechanism of incorporation of γ-globulin, as of other proteins, into amyloid can most easily be explained by the tendency of these substances to form complexes and their ability to be adsorbed on fibrillary structures [5, 14]. There are also grounds for the suggestion that, although they share a common ultrastructural organization [9, 13], the various forms of amyloid at the same time differ from each other by the particular plasma (tissue) protein which they contain. In other words, amyloidogenesis cannot be regarded purely as specific fibrillogenesis without regard to differences in the metabolism of the tissues in which it takes place.

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