ROLE OF FIBRINOGEN (FIBRIN) IN AMYLOID FORMATION IN EXPERIMENTAL AMYLOIDOSIS

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When sections of the organs of rabbits with experimental amyloidosis are treated with a fluorescent rabbit anti-fibrinogen (fibrin) serum, diffuse specific fluorescence is found at sites of amyloid deposition. The suggestion is made that mainly fibrinogen which has not been converted into fibrin is present in amyloid.

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It was suggested as long ago as in 1859 [13] that amyloid, a substance of protein nature, is modified fibrin. The role of fibrinogen (fibrin) in amyloid formation in certain types of amyloidosis in man has been demonstrated by investigations using the method of fluorescent antibodies [3, 16, 17].

The object of this investigation was to examine the problem of the presence of fibrinogen (fibrin) in amyloid masses in experimental amyloidosis.

EXPERIMENTAL METHOD

Amyloidosis was produced in rabbits by injecting 5 ml of a 10% suspension of sodium caseinate subcutaneously 3 times each week. Altogether 10 rabbits were used and were sacrificed 3 months after the first injection of caseine. The spleen, kidneys, and liver were taken for investigation. The control group consisted of 5 healthy rabbits from which the same organs were investigated. Serial sections were cut to a thickness of 2.5-3 μ on a freezing microtome with a deep-freeze knife [2]. The sections were dried under a fan and then fixed for 3 min in a mixture of equal volumes of 96° ethanol and ether. Having regard to the antigenic identity of fibrinogen and fibrin [10, 12], only one antiserum was obtained by immunization of cocks with rabbit's fibrin homogenate. After exhaustion with rabbit serum proteins, the immune serum gave one precipitation band with citrated rabbit plasma in the agar diffusion reaction (Fig. 1).

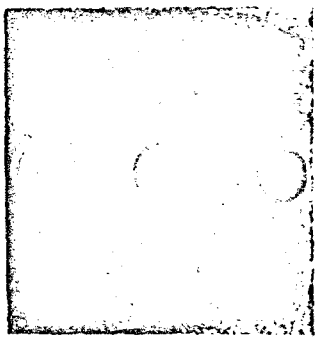

Fig. 1. Agar diffusion reaction. Central well contains antiserum against rabbit fibrinogen, exhausted with serum proteins. 1-6) Rabbit plasma in dilutions of 1 : 1 to 1 : 128.

To obtain good results in the diffusion reaction, the method of preparing the agar had to be slightly modified, because with the usual method [1] plotting of the fibrinogen took place during diffusion into the agar. We prepared a 1% solution of Difco agar in 2.5% sodium citrate solution in which the pH was adjusted to 7.0 by addition of NaOH. The γ-globulin fraction [1] was isolated from the exhausted antisera by precipitation with alcohol in the cold and the γ-globulin thus obtained was treated with fluorescein methylthioisocyanate by the usual method [20]. The conjugate was passed through a column with Sephadex G-25 at pH 9.0, preserved with merthiolate, and stored in a refrigerator at 4°. The sections were incubated with the conjugate for 30 min at room temperature. Control sections were treated with conjugate preliminarily adsorbed with fibrin homogenate, with heterologous labeled antiserum against human serum proteins, and with conjugate complementary to fibrinogen after preliminary treatment

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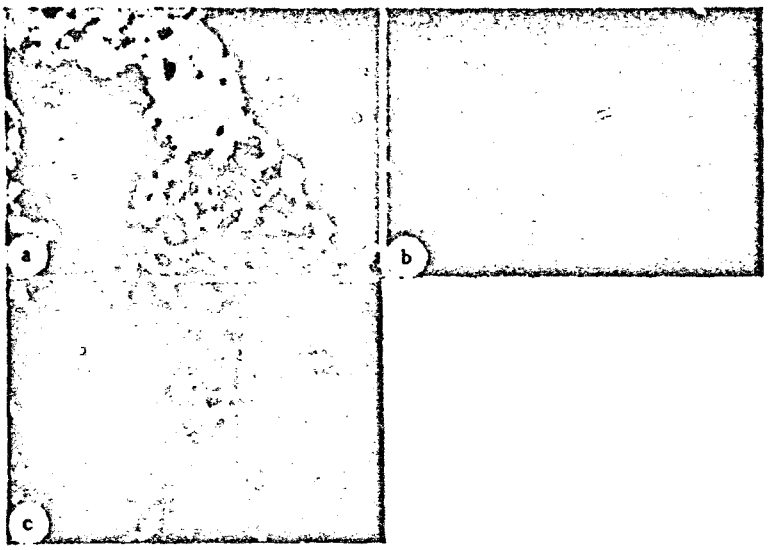

Fig. 2. Amyloid masses in the spleen. a) Specific fluorescence of amyloid after treatment with labeled anti-fibrinogen antibodies; b) serial sections through the same place (control). Incubation with conjugate preliminarily absorbed with rabbit fibrin; c) same section as in control, stained for amyloid with thioflavin T. 30×.

Fig. 3. Kidney (glomerulus). Diffuse fluorescence in amyloid after treatment with conjugate complementary to fibrinogen. Fibrin threads along wall of Shumlyanskii's capsule side by side with amyloid masses. 270×.

of the section with unlabeled fibrinogen antiserum. Sections were also stained with hematoxylin-eosin, and to identify amyloid, with Congo red, methyl violet, crystal violet, and thioflavin T [23].

EXPERIMENTAL RESULTS

Deposits of fibrin in the spleen, liver, and kidneys of the control rabbits were revealed by investigation by Coons' method. In the spleen, fibrin was found as a rule in the small vessels and sinuses of the red pulp. Sometimes it formed a complex network of closely interwoven filaments of varying thickness. Small amounts of fibrin could also be seen in the follicles of the spleen. There were usually tiny strands of fibrin wrapped around the lymphoid cells. Masses of fibrin also were found in the small vessels of the kidneys and liver. Individual strands of fibrin in the hepatic sinuses were often intimately related to their walls. Specific fluorescence was absent from the cytoplasm of the hepatic and Kupffer cells. In the kidneys fibrin was found in the capillaries of the cortex and medulla, and also in the capillaries of the glomeruli and in the cavity of Shumlyanskii's capsule.

Amyloidosis was observed in all the animals receiving caseine. Deposits of amyloid were found in the spleen, at the periphery of the follicles, and in the red pulp, and also in the kidneys, in the glomeruli, and in the ground substance of the pyramids. The amyloid stained orange with Congo red, gave metachromasia with methyl and crystal violet, and after staining with thioflavin T bright green fluorescence of the amyloid masses was observed. Threads of fibrin arranged in the organs in the same way as in the control animals as described above were also observed in the spleen, kidneys, and liver of the rabbits with experimental amyloidosis. On incubation of the sections with labeled anti-fibrinogen (fibrin) antibodies, intense specific fluorescence of the amyloid was seen (Fig. 2). Sometimes threads of fibrin were seen immediately next to masses of amyloid (Fig. 3), but no formed fibers of fibrin could be found in the amyloid itself.

Fluorescence of amyloid in sections treated with labeled anti-fibrinogen (fibrin) antibodies was exceptionally diffuse in character and was present uniformly throughout the mass of amyloid. Fluorescence of this character, in the absence of fibers resembling those of fibrin in the amyloid, suggests that mainly fibrinogen, and not fibrin, was present in the mass of amyloid. Admittedly, fibrin may sometimes clot to form a dense conglomerate in which individual threads cannot be distinguished with the ordinary microscope. We found structures of this type, composed of fibrin and unrelated to amyloid, in the spleen of the investigated animals. There are reports that fibrin deposits lose their fibrous structure during aging [9]. If the fibrin retains its antigenic properties in this case, such fibrin masses will probably fluoresce diffusely when stained by Coons' method. However, the results of a study of amyloid structure with the electron microscope confirm to some extent the view that large quantities of fibrin do not occur in amyloid. Fibrils 100-300 Å in diameter, without cross striation, have been described in amyloid [5-8, 15]. Electron microscopic studies of fibrin have also demonstrated fibrils, although they have a well-defined cross striation with an interval of about 230 Å between the dark zones [14, 22]. Nevertheless, none of the fibrillary structures characteristic of fibrin are found in amyloid. In amyloidosis nonspecific precipitation of fibrinogen from the plasma into already formed amyloid evidently takes place. Possibly a very small part of this subsequently changes into fibrin, but most remains as fibrinogen. In some forms of human amyloidosis, and also in experimental casein amyloidosis, the plasma fibrinogen concentration rises [11, 12, 18, 19]. In all probability the hyperfibrinogenemia facilitates the incorporation of fibrinogen into amyloid.

Deposits in the spleen, liver, and kidneys were observed in both the experimental and control rabbits. The results are analogous to those obtained by investigation of the human spleen [4]. Fibrin formation in the organs possibly takes place during life, reflecting the physiological mechanism of fibrinogen metabolism. However, special investigations are necessary to solve this problem.

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