DEPENDENCE OF ULTRASTRUCTURE OF THE FIBRIN FIBER ON ACTIVITY OF FACTOR XIII

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Inactivation of factor XIII in the blood plasma disturbs the fibrin ultrastructure: the characteristic cross striation of the fibrin fibers disappears and they become shorter. Addition of factor XIII restores the ultrastructure of the fibrin fibers.

The structure of the fibrin fiber as seen under the electron microscope was first described in 1939 [10, 11]. A characteristic feature distinguishing the ultrastructure of fibrin, according to these workers, is its cross striation consisting of the alternation of dark and light bands or periods throughout the length of the fibrin fiber. Subsequent work showed that the width of each period is 230±20 Å [5, 6, 9]. The electron-dense, dark areas of the fiber arise as the result of intersection between the fibrin molecules and salts of the heavy metals (uranium, lead) [6].

Besides fibrinogen, thrombin, and calcium salts, the formation of fibrin fibers also requires the presence of the Laki–Lorand factor (fibrin-stabilizing factor, factor XIII), which is evidently a transaminase catalyzing interaction between the amino group of one fibrin molecule and the carboxyl group of another fibrin molecule. By the action of this factor, cross-linkages are formed between the fibrin molecules, ensuring the stability and insolubility of the fibrin fiber in 5 M urea and monochloroacetic and acetic acids [7, 8].

The authors have previously discovered a definite relationship between the activity of factor XIII and the plasma tolerance of the fibrin clot, and between the activity of factor XIII and aggregation of the platelets [1, 2].

This paper describes results indicating that the ultrastructure of the fibrin fiber is also dependent on activity of factor XIII.

EXPERIMENTAL METHOD

Experiments were carried out on 40 Wistar rats aged 3 months and weighing 180-240 g. Blood was taken from the jugular vein and rapidly mixed with 1.34% sodium oxalate solution in the ratio of 9:1, and centrifuged at 200 g and 4°C for 5 min. The plasma obtained from it was separated into three equal portions and the activity of factor XIII determined [3].

The first portion of plasma was used as the control: activity of factor XIII was 100%. In the second and third portions the activity of factor XIII was reduced [4] to 30% and 10% of its initial level respectively. Fibrin clots were obtained from each portion.

A solution of factor XIII of high activity was added to the plasma with 10% activity of factor XIII. This solution was obtained from the plasma of healthy rats by gel filtration through Sephadex G-200, which increased the activity of factor XIII to its original level (physiological saline was added to the control).

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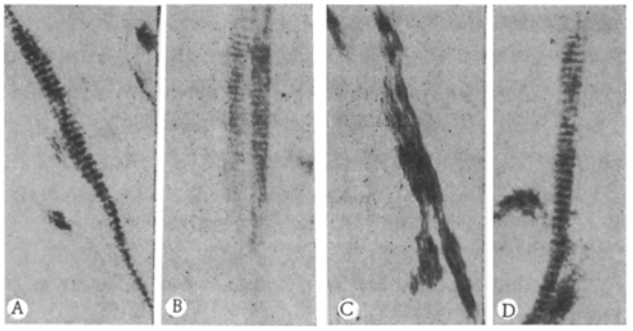

Fig. 1. Relationship between ultrastructure of fibrin fiber and activity of factor XIII: A) electron-micrograph of fibrin fiber obtained from plasma with 100% activity of factor XIII; B) electron-micrograph of fibrin fiber obtained from plasma with 30% activity of factor XIII; C) electron-micrograph of fibrin fiber obtained from plasma with 10% factor XIII activity; D) electron-micrograph of fibrin fiber obtained from plasma with factor XIII activity restored to 100%.

The fibrin clots were obtained by adding 25 units thrombin and 0.4 ml of 0.277% $CaCl_2$ solution to 0.2 ml of plasma, and then by incubating the mixture for 45 min at 37°C. The fibrin clots were cut into pieces measuring about 1 mm^3 and fixed for 60 min in Palade's solution at pH 7.2. After dehydration in alcohols, the clots were stained with 1% uranyl acetate solution. The material was embedded in Araldite, sections were cut and applied on grids, shadow-cast with 2% lead nitrate, and examined under the electron microscope.

EXPERIMENTAL RESULTS

The ultrastructure of fibrin fibers obtained from the original plasma showed cross striation with a period width of 220±20 Å (Fig. 1A). The length of the fibers varied from 12,000 to 18,000 Å and their width from 1500 to 2000 Å.

When the activity of factor XIII was lowered to 30%, fibrin fibers whose ultrastructure was the same as that in the original plasma could be seen in the field of vision, but equally there were fibrin fibers whose ultrastructure was marked by the absence of cross striation. The length and width of these fibers were the same as those of fibrin formed from plasma with 100% activity of factor XIII (Fig. 1B).

When the activity of factor XIII was lowered to 10% the ultrastructure of the fibrin fibers showed substantial changes: cross striation was absent in all fibers, the length of the fibers was reduced to between 7000 and 9000 Å and their width was 1500 to 2500 Å (Fig. 1C).

Addition of factor XIII to inactivated plasma restored the ultrastructure of the fibrin fibers. The cross striation reappeared, and the fibers almost regained their original width and length (Fig. 1D).

This investigation thus showed that factor XIII participates in the formation of the cross striation of the fibrin fiber as revealed by electron microscopy. With a decrease in activity of this factor, the structure of the fibrin clot is disturbed and the ultrastructure of the fibrin fiber is altered.

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