EFFECT OF IONIZED CALCIUM ON THE ULTRASTRUCTURE OF FIBRIN

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Fibrin clots formed in vitro in the presence of calcium ions differed in their ultrastructure from those formed without calcium ions. Calcium ions do not affect the structure of the fibrin fiber.

Adequate evidence has been obtained for the role of calcium ions in the final stage of the reaction: thrombin + fibrinogen → fibrin S + factor XIII + Ca^{++} → fibrin i. The fibrin formed during clotting of plasma fibrinogen in the presence of calcium ions has been shown to be mechanically stronger, insoluble in 5 M urea solution and in monochloroacetic acid, and its fibers are resistant to the action of alkalies and acids and more resistant to bacterial fibrinolysin than fibrin formed from oxalated plasma [1-6].

The object of this investigation was to study the effect of ionized calcium on the ultrastructure of fibrin; such an effect would demonstrate the participation of calcium in the formation of the fibrin fiber and the fibrin clot.

EXPERIMENTAL METHOD

Experiments were carried out on 60 Wistar rats of both sexes weighing 150-200 g. The effect of ionized calcium on the ultrastructure of fibrin was studied in blood taken from the jugular vein of rats into silicone-coated syringes; the blood was mixed with anticoagulant (1.34% solution of sodium oxalate) in the ratio of 9 : 1. Oxalated plasma was obtained by centrifuging stabilized blood at g = 200 for 15 min.

Fibrin clots were obtained by clotting 0.2 ml oxalated plasma with a thrombin–calcium mixture (0.1 ml thrombin with an activity of 25 sec + 0.4 ml 0.277% $CaCl_2$ solution). In control tests for fibrin clot formation the $CaCl_2$ solution was replaced by 0.4 ml of 0.85% NaCl solution. The reacting mixture was incubated at 37°C, and the fibrin clots which formed 1 h later were fixed in Palade's solution, dehydrated in alcohols, stained with uranyl acetate, embedded in Araldite, and examined in the electron microscope.

EXPERIMENTAL RESULTS

The ultrastructure of the fibrin fiber formed in the presence of calcium ions, illustrated in Fig. 1b, shows strict regularity and cross-striation with a period of 210 ± 10 Å in width. The ultrastructure of the fibrin fiber formed without calcium ions is also characterized by a certain degree of regularity and by cross-striation with a period 210 Å in width (Fig. 1d), or in other words, no significant differences were found between the formation of the ultrastructure of the fibrin fiber obtained in coagulating media containing or not containing calcium ions.

However, the ultrastructure of the fibrin clot formed in the presence of calcium ions (Fig. 1a) differed significantly from that of the fibrin clot formed without calcium ions (Fig. 1c). In the former there was a dense fine-mesh, three-dimensional network with many lateral associations of fibrin fibers, 2000-

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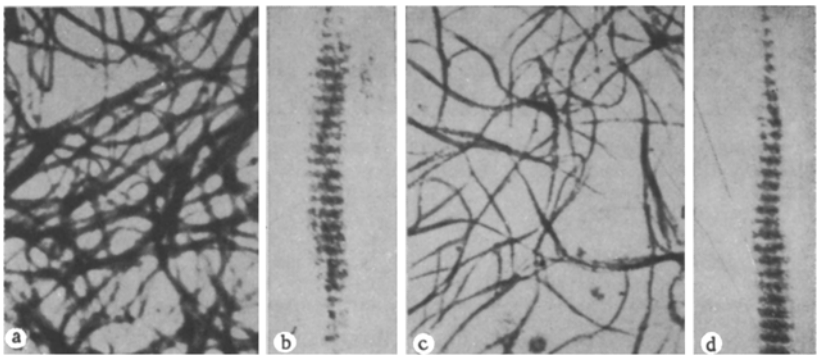

Fig. 1. Effect of ionized calcium on ultrastructure of fibrin: a and b) ultrastructure of fibrin clot and fibrin fiber formed in the presence of calcium ions, respectively; c and d) ultrastructure of fibrin clot and fibrin fiber formed without calcium ions, respectively.

3500 Å in width. By contrast, the ultrastructure of the fibrin clot formed without calcium ions consisted of a coarse-mesh network with only a few lateral associations of fibrin fibers, not more than 2500 Å in width.

These results show that calcium ions participate in the conversion of fibrinogen into fibrin and affect the formation of the ultrastructure of the fibrin clot.

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