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Identification of Soluble Fibrinogen Fibrin Monomer Complexes by Non-Enzymatic Polymerisation in the Tissue*

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Summary. In states of plasmic hypercoagulability and consumption coagulopathy ethanol favours the non-enzymatic polymerization of circulating soluble fibrinogen fibrin monomer complexes (FFMC) in vitro. The ethanol-gelation test of Godal and Abildgaard makes use of this phenomenon, called paracoagulation. The present studies show that it is also possible to visualize soluble FFMC by means of ethanol-gelation. In the electron microscope, FFMC, polymerized non-enzymatically by ethanol in the spleen, are characterized by plump or slender mycelioid fibrillar precipitates that show a uniform rhythmic transverse striation, a period-coincidental filamentary arrangement and an average periodicity of 23 nm. The ultrastructure demonstrates these ethanol-induced filaments to be in vitro-polymerized fibrin monomer derivatives. Paracoagulation with ethanol allows the identification of soluble FFMC in the tissue prior to the formation of highly polymerized fibrin-rich microthrombi, the established equivalents of the DIC-syndrome.

The electron microscope studies also show the existence of a second type of fibrillary structure in the tissue polymerized by ethanol. This second type lacks the characteristic periodicity of fibrin and the period-coincidental arrangement of the filamentary structures, but is characterized by closely packed or chain-like aligned, irregularly sized spherical bodies. There is some evidence that these spherical bodies in vitro represent non-enzymatically polymerized complexes of fibrin monomers and fibrin degradation products (FDP), the equivalent of a limited local or generalized fibrinolysis in vivo.

Key words: Soluble fibrinogen fibrin monomer complexes – Dissiminated intravascular coagulation – Fibrin degradation products (FDP) – Reticuloendothelial system (RES).

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Introduction

Soluble fibrinogen fibrin monomer complexes (FFMC) are plasmic intermediates of the fibrinogen-fibrin conversion which are formed when fibrinogen molecules are exposed to low thrombin concentrations. Soluble FFMC in the flowing blood demonstrate an enhanced turnover of fibrinogen and other plasmic coagulation factors necessary to the generation of thrombin. Soluble FFMC thus are the equivalent of a plasmic hypercoagulability in the course of a localized or generalized activation of the coagulation system.

Soluble FFMC can be determined qualitatively in plasma by the ethanol-gelation test according to Godal and Abildgaard (1966) as well as by the protamine-sulfate precipitation test (Lipinski and Worowski, 1968; Niewiarowski and Gurewisch, 1971). Both methods refer to the same phenomenon, the non-enzymatic polymerization of soluble intermediates of the fibrinogen-fibrin conversion, called paracoagulation. Quantitative results, however, have until now been obtained only by the method of gelfiltration after concentrating FFMC by β-alanin-precipitation (Fletcher et al., 1970; Hafter et al., 1976).

Previous studies provided some evidence to support the hypothesis that in the animal experiments using low ethanol concentrations soluble FFMC can be demonstrated histomorphologically by paracoagulation (Bleyl et al., 1969), and that it is possible to visualize intravascular coagulation before the formation of highly polymerized coagulation products, the disseminated intravascular microthrombi of the so-called DIC-syndrome. The following investigations were carried out to demonstrate the ultra-structure of these soluble FFMC after a generalized activation of intravascular coagulation. They modify former microscopic findings but support the conclusions drawn from them.

Method

A continuous infusion of thrombin (Thrombinum purum, Behring-Werke, Marburg – 180 NIH-U/kg/h) and aprotinin (Trasylol, Bayer AG, Leverkusen – 50,000 KIU/kg/h), diluted in isotonic saline (50 ml/kg/h), was given to adult female rabbits via a polyvinyl-catheter. The infusion was stopped after 60 min. Two hours after the end of the infusion plasma samples of the animals were examined by the ethanol-gelation test to trace circulating FFMC. Three hours after the end of the infusion the animals were killed by an evipan-injection via the polyvinyl-catheter. Immediately after death the spleen of the animals was cut into halves and one half of each was fixed in 10% formalin and 96% ethanol. Both parts of the organ were embedded in paraffin and exposed to the periodic acid-Shiff (PAS-) reaction. Paraffin slices that showed mycelioid filamentary structures, characteristic of soluble FFMC, were subjected to the KMU-technique (Rossner, 1971). This method makes it possible to trace structures found in conventional histological slices at the level of semi-thin slices and to follow up their ultrastructure. Further methodical details regarding this technique can be found in the original communication of Rossner (1971). Semi-thin slices were stained with toluidin-blue, the ultrathin slices were contrasted with uranyl-acetate and lead-citrate. The electron-microscopic investigations were carried out with a Zeiss EM 9A.

Results

Continuous infusions of thrombin lead to the generation of intravascularly circulating soluble FFMC. These soluble FFMC can be demonstrated by the

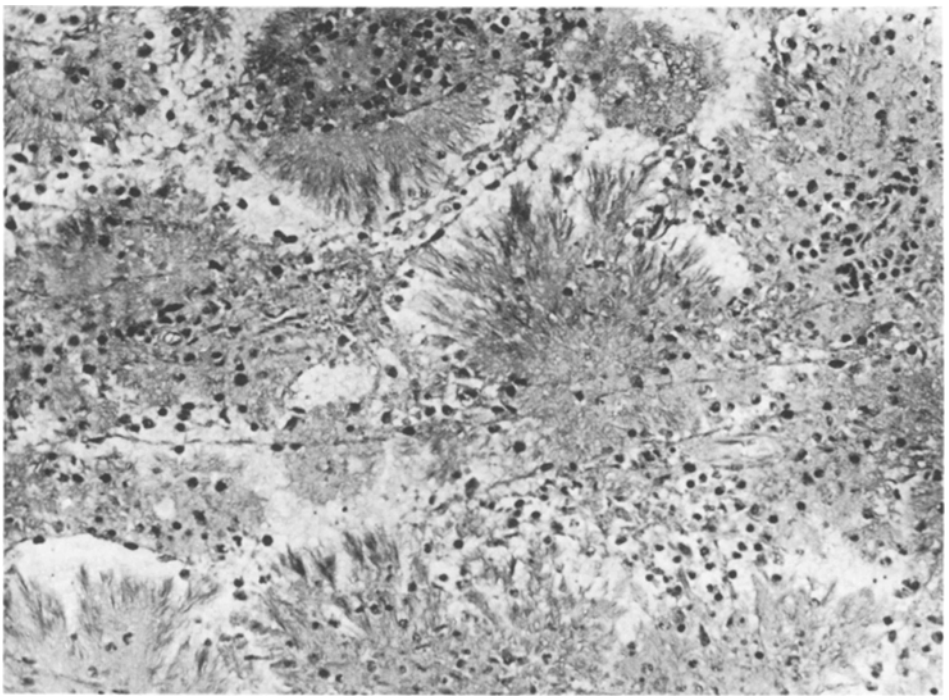

Fig. 1. Ethanol fixation of the spleen 4 h after a continuous infusion of thrombin and aprotinin. Mycelioid, PAS-positive fibrillar precipitates, extending in bunches into hyperemic sinusoids. Paraffin, PAS-reaction. Magn. 210:1

ethanol-gelation test (Godal and Abildgaard) 3 h after beginning of the infusion. Apparently soluble FFMC can be eliminated from the circulation almost without complications and without having polymerized to highly fibrinous microthrombi (Müller-Berghaus et al.,1973). Polymerization and precipitation of soluble FFMC as microthrombi happen only when fibrinolysis is blocked or exhausted. This may explain the fact that simultaneous infusion of thrombin and aprotinin (as an inhibitor of fibrinolysis) causes multilocular, highly fibrinous microthrombi in different organs. In our experiments the appearance of microthrombi in the lungs, liver, spleen, and kidneys of the rabbits is adequadely explained by these experimental conditions, and results concerning this part of the work have already been published (Bleyl et al., 1969; Theiss et al., 1970).

Besides highly polymerized microthrombi other characteristics of the DIC-syndrome in the form of unusual, mycelioid, PAS-positive precipitations can be identified in the ethanol-fixed slices of the spleen (Fig. 1). These are not found in conventionally formalin-fixed samples of the same tissue (Bleyl et al., 1969). The precipitates form irregularly stretched, plump or slender fibres and extend in bunches into overflowing hyperemic sinusoids. An interknitting of the fibrillar precipitates to form globular hyaline microthrombi (Bleyl and Rossner, 1976) is absent. The endothelium of the sinusoids and the reticulum cells close to the sinusoids are frequently surrounded by fibrillar precipitates. Occasionally erythrocytes can be found between the mycelioid precipitates. Remnants

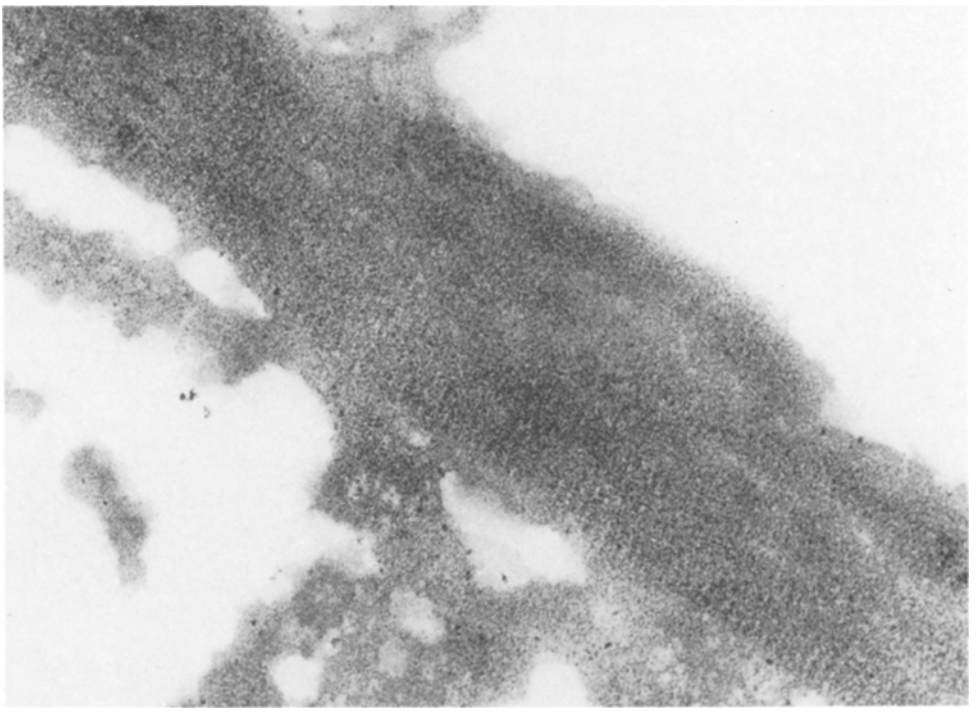

Fig. 2. Ethanol-induced paracoagulation in the spleen 4 h after a continuous infusion of thrombin and aprotinin. Non-enzymatically polymerized filamentary precipitate with uniform rhythmic transverse striation identical with that of the normal fibrin fiber. KMU-technique: electron microscopic investigation of an ethanol-fixed slice. Magn. 40,000:1

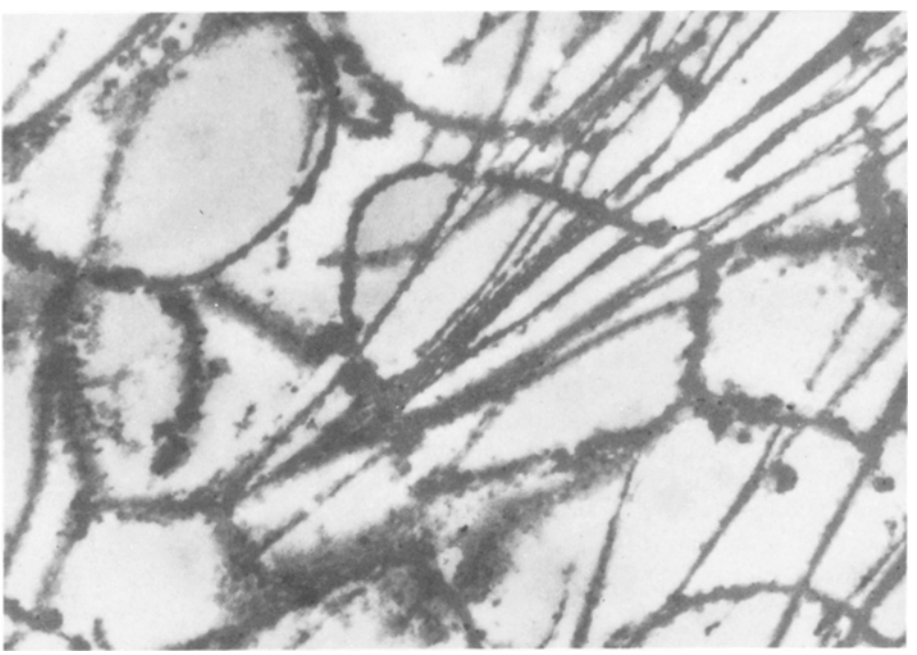

Fig. 3. Ethanol-induced paracoagulation in the spleen 4 h after a continuous infusion of thrombin and aprotinin. Bundles of fibres with irregularly sized, chain-like aligned globular aggregates forming a disorganized network that sometimes surrounds non-fibrous aggregates. KMU-technique. Magn. 6200:1

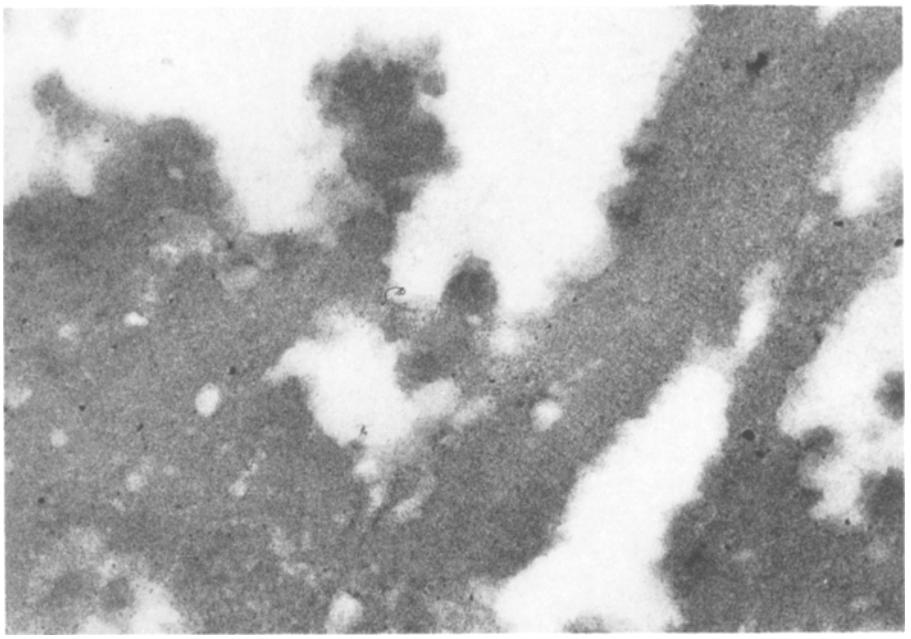

Fig. 4. Ethanol-induced paracoagulation in the spleen 4 h after a continuous infusion of thrombin and aprotinin. Filamentary precipitates with the characteristic axial periodicity of fibrin fibres built into a network of chain-like aligned globular aggregates or surrounded by non-fibrous globular aggregates. KMU-technique. Magnification 18,000:1

of platelet aggregates and megakaryocytes can also be demonstrated between the fibrillar structures.

Electron microscopic investigations show that these mycelioid precipitates are characterized by a monotonuous basic filamentary structure (Fig. 2). Most of the filamentary precipitates show a uniform rhythmic axial striation with a periodicity ranging from 19 to 25 nm; the mean values are about 23 nm, characteristic of highly polymerized fibrin derivatives (Hall, 1949; Kay and Cuddingham, 1967). The precipitates are further characterized by a strong tendency to form irregularly plump fibres with a period-coincidental lateral aggregation.

Bundles of fibres may be found that entirely lack this rhythmic transverse striation or demonstrate it only in part; they seem to be formed of irregularly sized, closely packed or chain-like aligned globular aggregates (Fig. 3) with a mean diameter of approximately 110 nm. These aggregates form irregular, disorganized networks, including other non-fibrous globular aggregates. Remnants of filamentary precipitates with a uniform rhythmic axial striation identical with that of normal fibrin fibres occur only occasionally between these chain-like aligned globular aggregates (Fig. 4). However, non-fibrous globular aggregates sometimes appear on the surface of filamentary precipitates.

Discussion

In plasma, fibrin monomers form dimeric or oligomeric complexes with fibrinogen and remain soluble in these complexes. The prevalent binding of the monomers to fibrinogen molecules happens as long as no high molecular weight fibrin degradation products (FDP) are circulating (Sasaki et al., 1966). In the present studies the fibrinolytic activity was inhibited, at least temporarily, by the proteinase-inhibitor aprotinin; circulating soluble FFMC were thus generated during the one-hour thrombin infusion. These FFMC could be demonstrated by the ethanol-gelation test of Godal and Abildgaard (v. Theiss et al., 1970).

Besides highly polymerized intravascular microthrombi, the morphological equivalents of disseminated intravascular coagulation, we found in the spleen of the rabbits after ethanol-fixation unusual, mycelioid, bunched precipitates that could not be identified in formalin-fixed samples of the same tissue. In the electron microscope these precipitates were similar to in vivo polymerized microthrombi (Bohle et al., 1959) and characterized by a basic filamentary structure with regular transverse striation and a uniform axial periodicity of 23 nm, thus showing themselves to be high molecular weight derivatives of fibrin. In pilot studies Stewart et al. (1971) had shown that soluble FFMC which had polymerized and gelatinized in vitro, under the influence of low-concentrated ethanol formed filamentary structures with the typical axial striation and a 23 nm periodicity that could not be distinguished from the thrombin-mediated highly polymerized fibrin. With regard to these pilot studies the fibrillar precipitates that can only be identified after ethanol-fixation must be considered to be in vivo soluble FFMC which polymerize non-enzymatically in vitro in the course of the fixation.

Ethanol-fixation thus allows – in animal experiments – the identification of circulating soluble FFMC prior to the generation of highly polymerized fibrin-rich microthrombi. A high local concentration of FFMC in the circulation must however pre-exist. It has been shown that the RES of the spleen is of great importance for the clearance of soluble FFMC from the circulation (Bleyl et al., 1969).

Besides filamentary precipitates with the fibrin-characteristic periodicity the ethanol-fixed slices showed fibrillar precipitates which seemed to be composed of chain-like arranged spherical bodies, without evidence of periodic axial striation. Filamentary precipitates with typical transverse striation could be found occasionally between irregularly aligned spherical precipitates without axial striation. The same spherical precipitates could be identified surrounding filamentary structures with periodic axial striation.

The present investigations do not permit us to draw conclusions on how this second type of fibrillar structure is generated. But there is strong evidence that these spheric precipitates represent high molecular weight degradation products of fibrin (FDP), split products of the plasmin-induced fibrinolysis. The enzymatic break-down of fibrin to high molecular weight FDP is known to be a gradual digestion with three main stages (Pizzo et al., 1972; Furlan et al.,

1972, 1975; Marder and Budzynski, 1974). During the first stage FDP of the family of fragment X predominate (stage 1 digest), which in a second stage are split to the intermediate fragments Y and D (stage 2 digest) and in the third stage fragment Y is degraded to a further fragment D and fragment E (stage 3 digest). High molecular weight FDP of the family of fragment X, like fibrinogen, are able to complex with fibrin monomers. According to the pilot studies of Stewart et al. (1971), mentioned above, the ultrastructure of the non-enzymatic polymers, formed of FDP-fragment X and fibrin monomers after ethanol-gelation, cannot be distingushed from the ultrastructure of non-enzymatic polymers consisting of soluble FFMC. They present the same gelation as soluble FFMC with a basic filamentary structure, rhythmic transverse striation and uniform 23 nm-periodicity. Fragment Y is also able to complex to some extent with fibrin monomers. Mixtures, consisting of the intermediate degradation fragments Y, D, and E, as they are formed during the second and third stage of enzymatic breakdown of fibrin, appear after paracoagulation as precipitates consisting of globules and rudimentary fibrils without periodicity, closely packed or aligned in chains. These globules and rudimentary fibres correspond strikingly with the present electron microscopic findings in our second type of fibrillar structure. Bang (1963) has reported that during fibrin polymerization in the presence of intermediate FDP similar non-fibrous globular aggregates are incorporated into the fibrin network and become an integral part of an 'abnormal' polymer.

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