Virchows Arch. A Path. Anat. and Histol. 370, 113—128 (1976)

Globular Hyaline Microthrombi — Their Nature and Morphogenesis*

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Received November 30, 1975

Summary. The ultrastructure of globular hyaline microthrombi (GHM) is characterized by a spherical space lattice of frequently interconnected bundles of fibres of different width, with a periodic transverse striation and the fibrin-characteristic axial periodicity of 23 nm. These are surrounded by plump or slender bundles of fibres spreading radially over the surface which are only occasionally interlinked. These filamentary formations of the so-called corona are also characterized by the fibrin-characteristic periodicity. Part of the GHM, however, lacks this axial periodicity, and periodic striation is then only visible in the radially extending fibrils of the corona. The spherical space lattices with their plump or slender fibrillary fibrin bundles are also replaced by mosaic-like or nearly amorphous fine-grained precipitates. All intermediate stages between these main types of GHM can be found. The disappearance of the axial periodicity and of the fibrillary structure of the spherical space lattices is considered to be the morphological equivalent of secondary fibrinolysis, here called endolysis, in the centre of the GHM. The morphogenesis of the GHM in states of shock of different aetiologies is discussed.

Globular hyaline microthrombi (GHM) are intravascular coagulation products which occasionally reach 100μ in diameter, but are usually in the range 3 to 60 μ. They occur individually or in groups, and when stained may be homogenous or inhomogenous. They are eosinophilic in haematoxylin-eosin preparations, strongly PAS positive, and can be shown to contain fibrinogen or fibrin by fluorescent techniques. Known in the literature as fibrin balls (Fibrinballen Manasse, 1892), fibrin globules (Fibrinkügelchen Apitz, 1938, 1942), Siegmund-Schindler globules (Siegmund-Schindlersche Kugeln, Schindler, 1938), hyaline microthrombi (Skjørten, 1964), or globules (Hardaway, 1966), they have come to be considered as a characteristic of all forms of circulatory shock and part of the pathology of any kind of generalized intravascular coagulation activation or consumption-coagulopathy (Lasch *et al.*, 1961, 1971). The morphogenesis, the patho-physiological importance and the functional significance of these GHM are unexplained. In particular no evidence has been produced to support the theory that GHM are the morphological equivalent of a simultaneous activation of coagulation *and* fibrinolysis, i.e. equivalent to the formation of defective polymers from fibrin monomers and fibrinogen- or fibrin degradation products (Skjørten, 1964, 1969). However, it seems that Apitz (as early as 1938, 1942) thought of the possibility of a 'pathological' coagulation causing the formation of GHM.

* Supported by Deutsche Forschungsgemeinschaft — Sonderforschungsbereich 90, "Cardiovasculäres System", University of Heidelberg.

The following histomorphological and electron microscopic investigations were directed towards the ultrastructure, the morphogenesis and the metamorphosis of these GHM in autopsy cases where death was caused by shock of different aetiologies.

Method

Given our present day knowledge of intravascular coagulation it is not possible to predict with a sufficient degree of accuracy whether typical GHM are formed in autopsy cases where death was caused by shock. We therefore made use of the so-called KMU-technique (Rossner, 1971) which consists of the combined use of the light and electron microscopes for the investigation of formalin-fixed paraffin slices which had previously been stained with haematoxylin-eosin and had shown typical globules.

Thanks to the KMU-technique it is possible, firstly to restrict the investigations to those autopsy cases, organs and vascular areas where shock and the shock induced activation of the coagulation had actually led to the formation of GHM, and secondly, the method permits a direct comparison between light- and electron microscopic structures, an advantage which is of considerable help in determining the ultrastructure of these extremely heterogeneous GHM.

After removing the coverglass the conventional histological slices were initially subjected to a careful process of rehydration and osmation. They were embedded in silicone-caoutchouc moulds before being removed from the original slide in the polymerized synthetic resin bloc, by means of a rapid change in temperature. After being thus re-embedded, the histological slices were cut with a razor-blade into reconstitutable detailed sections, and the individual samples obtained in this way were cut partly into ultra-thin sections and partly into semi-thin sections. Further details regarding this method can be found in the original description (Rossner, 1971). Semi-thin slices were stained with toluidine blue for investigation with the light microscope whereas ultra-thin slices were contrasted with uranylacetate and lead citrate for electron microscopic investigations. The electron microscope used was a ZEISS EM 9A.

Results

1. Light Microscopic Findings

The histomorphological picture of the GHM is variable. Alongside thrombi with a dense homogeneous fine-vacuolar or fine-grained internal structure and a smooth surface, other microthrombi can be found that possess this appearance only at the periphery while irregular, faggotlike, fibrillary structures appear in the centre (Fig. 1). In part of the GHM these fibrillary structures are strictly confined to the actual centre of the GHM, but they may also permeate the whole globular body.

Frequently the same fibrillary structures are also seen on the surface of the fibrin globules. In many instances they cover the outside of the globules like a uniform ‚corona' (Fig. 2), even though the length and width of these more or less densely packed fibrillary structures can vary considerably. On further magnification it becomes clear that these filamentary structures can basically continue into the inner part of the GHM, whereas on other occasions they may only line the surface with the centre presenting the fine-grained or homogeneous intimate structure already described. In the immediate vicinity of such GHM other fibrillary structures occasionally become visible. They resemble a loose ball of fibres not yet having the appearance of a dense central globular body (Fig. 2).

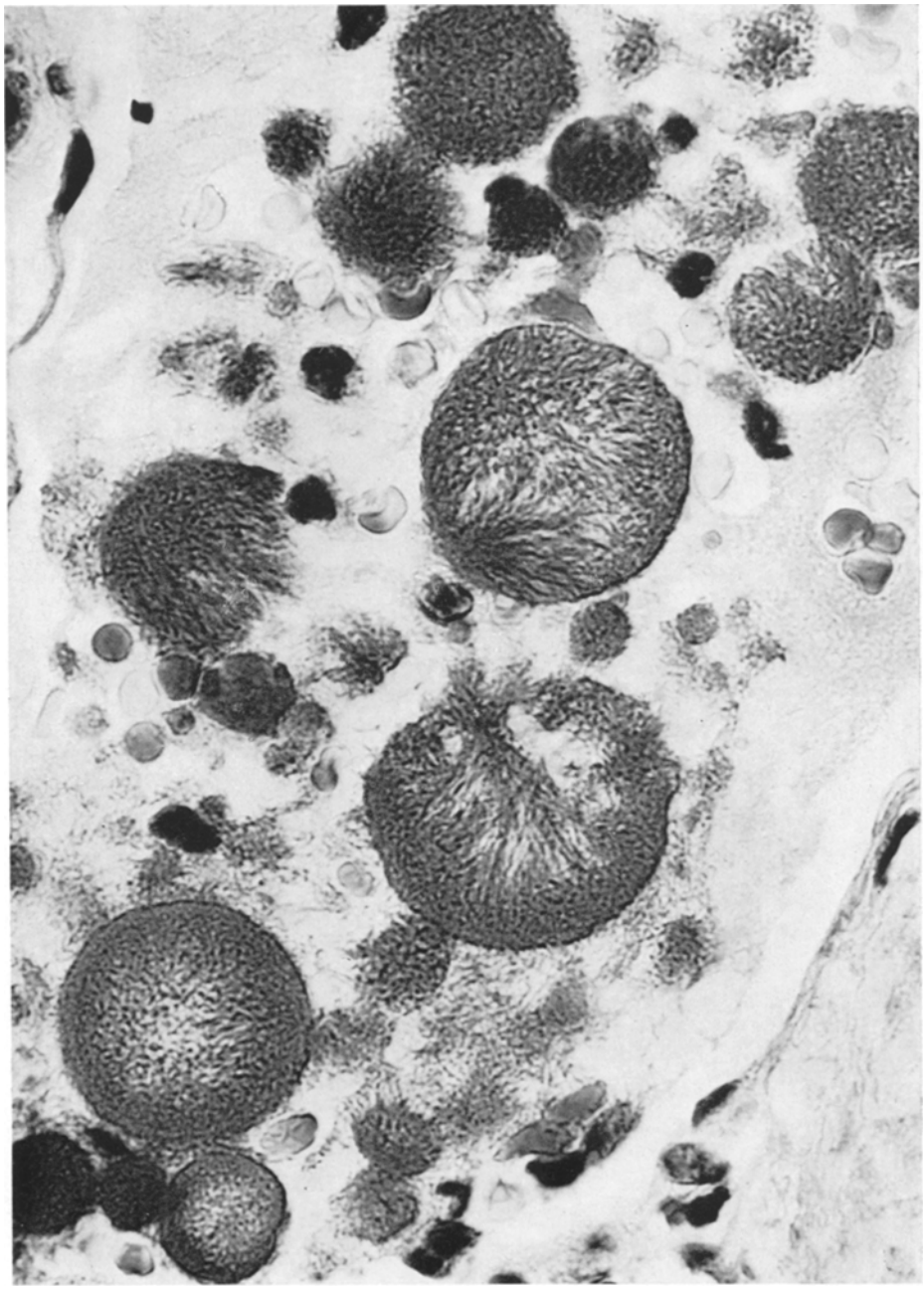

Fig. 1. GHM with characteristic fine-grained structures in the periphery and fibrillary structures in the centre of the globular body. Formalin, paraffin, H. and E., Magn. × 160

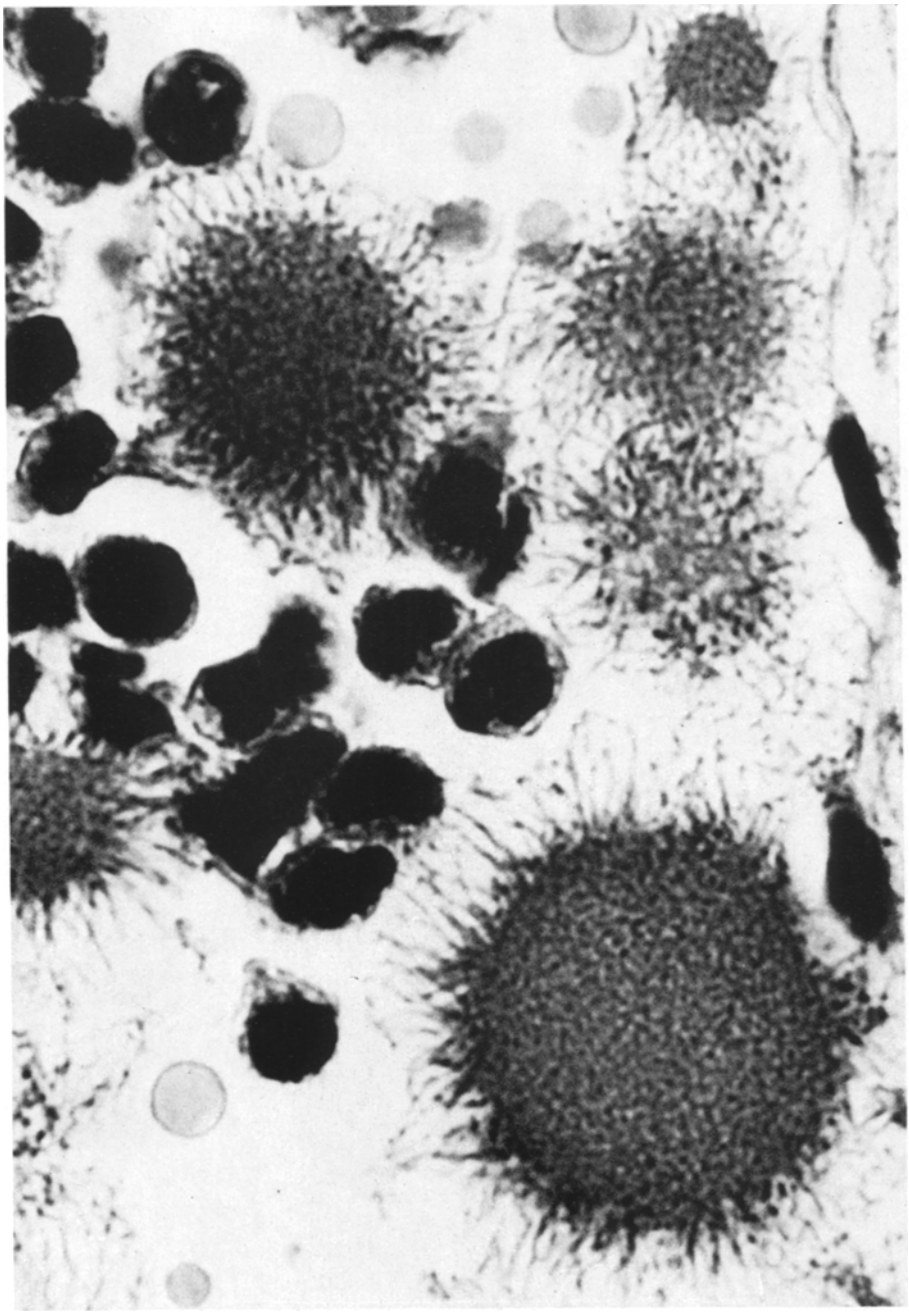

Fig. 2. GHM with typical fibrillary structures of the so-called 'corona' on the surface and fibrillary or densely packed fine-grained structures in the centre. The fibrillary structures of the 'corona' seem partially to continue into the centre of the GHM. Formalin, paraffin, H. and E., Magn. $\times 160$

All these types of GHM can normally be found with the conventional highly fibrinous microthrombi that are considered to be the characteristic morphological representation of the so-called DIC-syndrome (disseminated intravascular coagulation) and of the consumption coagulopathy. In the immediate vicinity of the GHM there may also occur the familiar star- and needle-shaped precipitations that have, since the investigations of Zenker (1895) and Apitz (1942) been considered as the morphological equivalent of a post-mortem fibrinogen-fibrin conversion in the vascular bed, i.e. as the equivalent of a post-mortem activation of the coagulation.

2. Electron Microscopic Findings

The electron microscopic picture of the GHM also lacks uniformity. The majority of these GHM consist of a network of frequently interconnected bundles of fibres of different widths that enclose multiangular or nearly round spaces of varying dimensions and spread throughout the inner part of the GHM in the manner of a spherical space lattice (Fig. 3). Generally speaking this network seems to be more closely knit in the peripheral area of the GHM than in the centre, furthermore the bundles of fibres in the periphery often seem to be plumper and the spaces separating them smaller. The borderline between the fibrillary structures of the spherical lattice and the interposed spaces is usually relatively blurred, the fibrillary bundles of fibres are invariably surrounded by a collar of fine-grained precipitates that fill the adjoining spaces in varying degrees of density (Fig. 5). Other spaces appear empty.

The outer circumference of the GHM is for the most part characterized by a more or less extended 'corona' consisting of either plump or slender bundles of fibres that spread radially over the surface and are occasionally interlinked. They extend into the dense bundles of fibres of the globular periphery and seem to be connected to these peripheral bundles. In some instances the GHM are even linked to each other by means of such radially arranged starlike bundles of fibres (Fig. 4). Only on very are occasions are the bundles forming the 'corona' completely missing, although their number can be relatively small.

Further magnification shows that the frequently interlinked bundles of fibres of the spherical space lattice are marked by a completely uniform transverse striation with axial periodicity, as are those of the bundles of the so-called 'corona' though this cannot be established in all fibres (Fig. 5).

Repeated measurements with parallel sections of these fibres yielded similar results at the centre and the periphery of the GHM as well as in the region of the fibre formations of the so-called 'corona', i.e. a period ranging from 19 nm to 25 nm resulting in a mean value of 23 nm for the period-coincidentally arranged fibrillary formations.

Not all GHM showed this monotonous picture in the ultra-thin slices, consisting of bundles of fibres varying only in their widths and degree of interlinkage, but distinguished by the uniformity of their axial periodicity of 23 nm. Part of the thrombi lacked this periodicity in respect of the fibre formations inside the GHM although the basic fibrillary structure of the microthrombi and the webbing of their fibre formations into spherical space lattices seemed to be intact (Fig. 6). The only evidence of periodic striation, as far as these

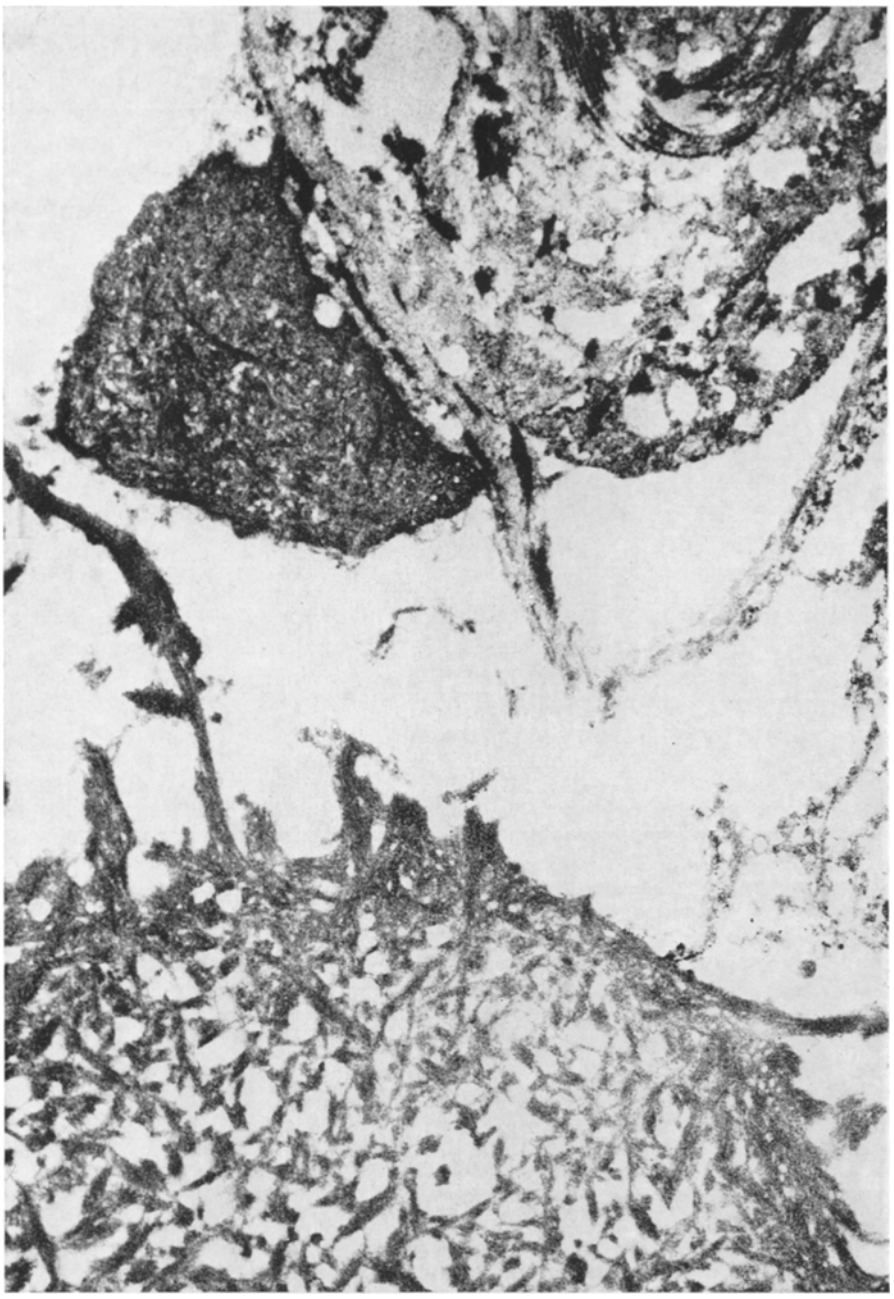

Fig. 3. GHM in the vicinity of a pulmonary endothelial cell with the characteristic spherical space lattice consisting of intricately knitted bundles of fibres that enclose multiangular 'empty' spaces. KMU-technique, Magn. × 5,000

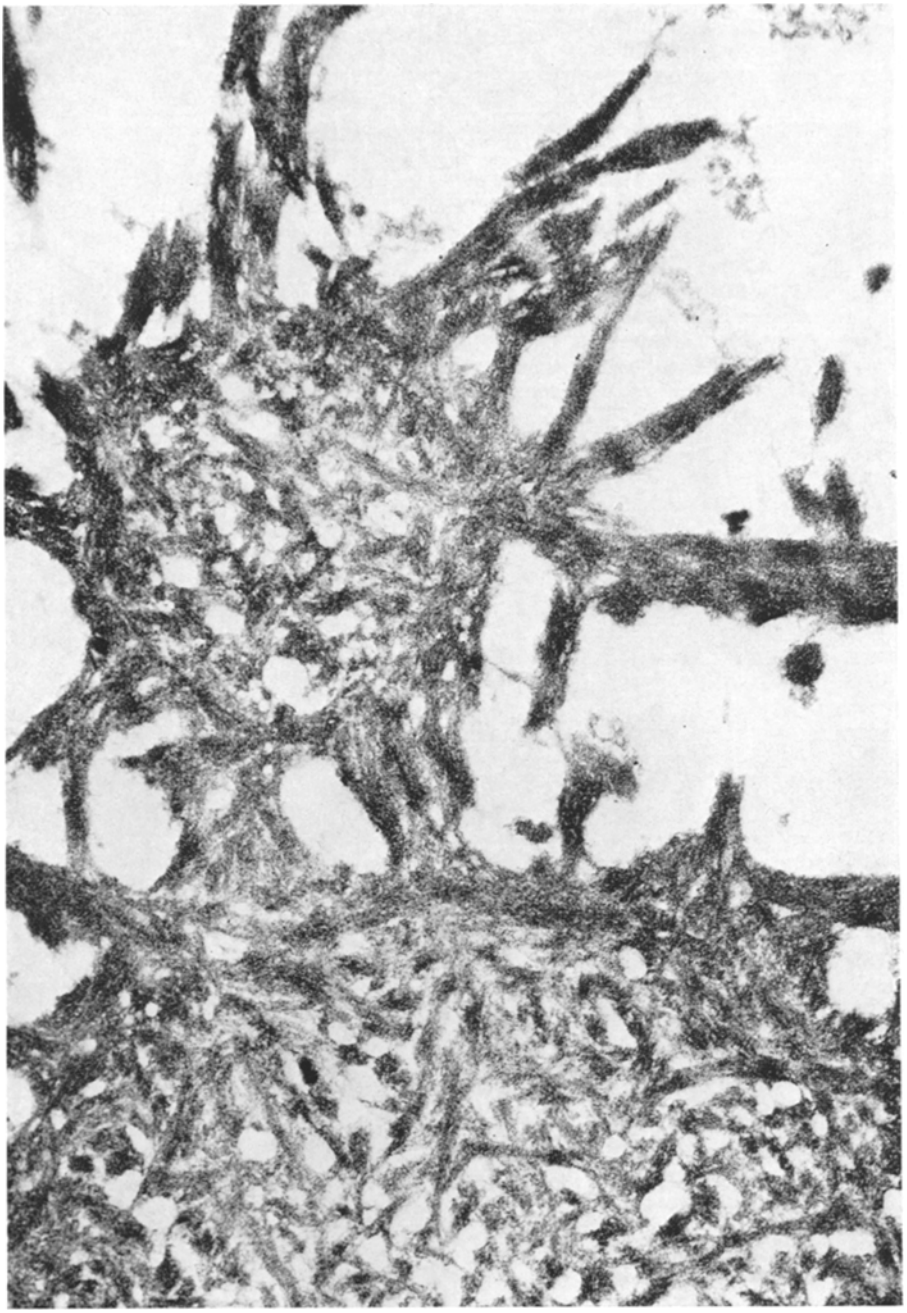

Fig. 4. Two typical GHM with more or less densely packed spherical space lattices which are interlinked by plump or slender bundles of fibres of the so-called 'corona'. KMU-technique, uranylacetate—lead citrate, Magn. × 9,500

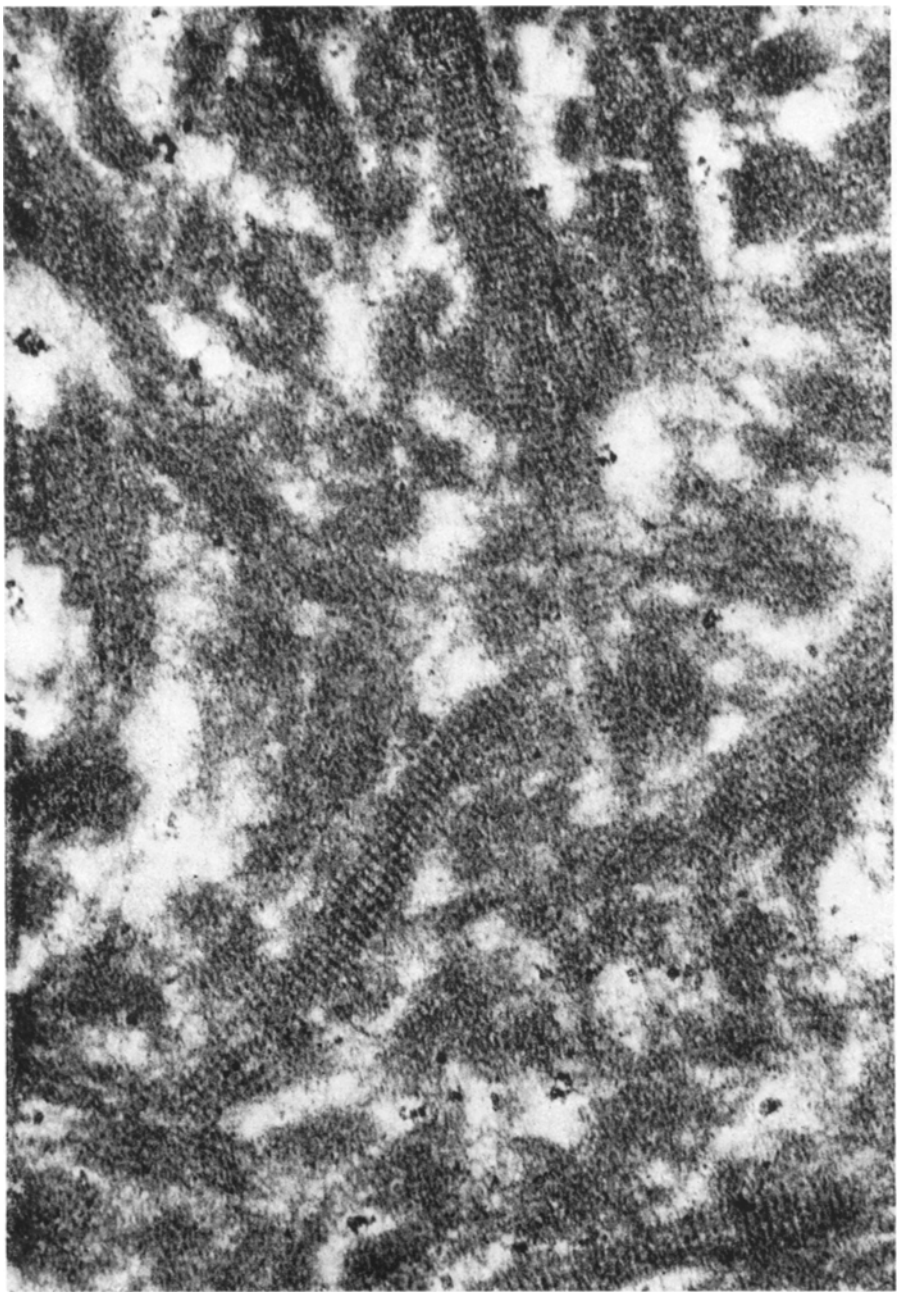

Fig. 5. Uniform transverse striation of the fibrillary structures with a periodicity of 23 nm in the centre of a pulmonary GHM. KMU-technique, uranylacetate—lead citrate, Magn. $\times$ 40,000

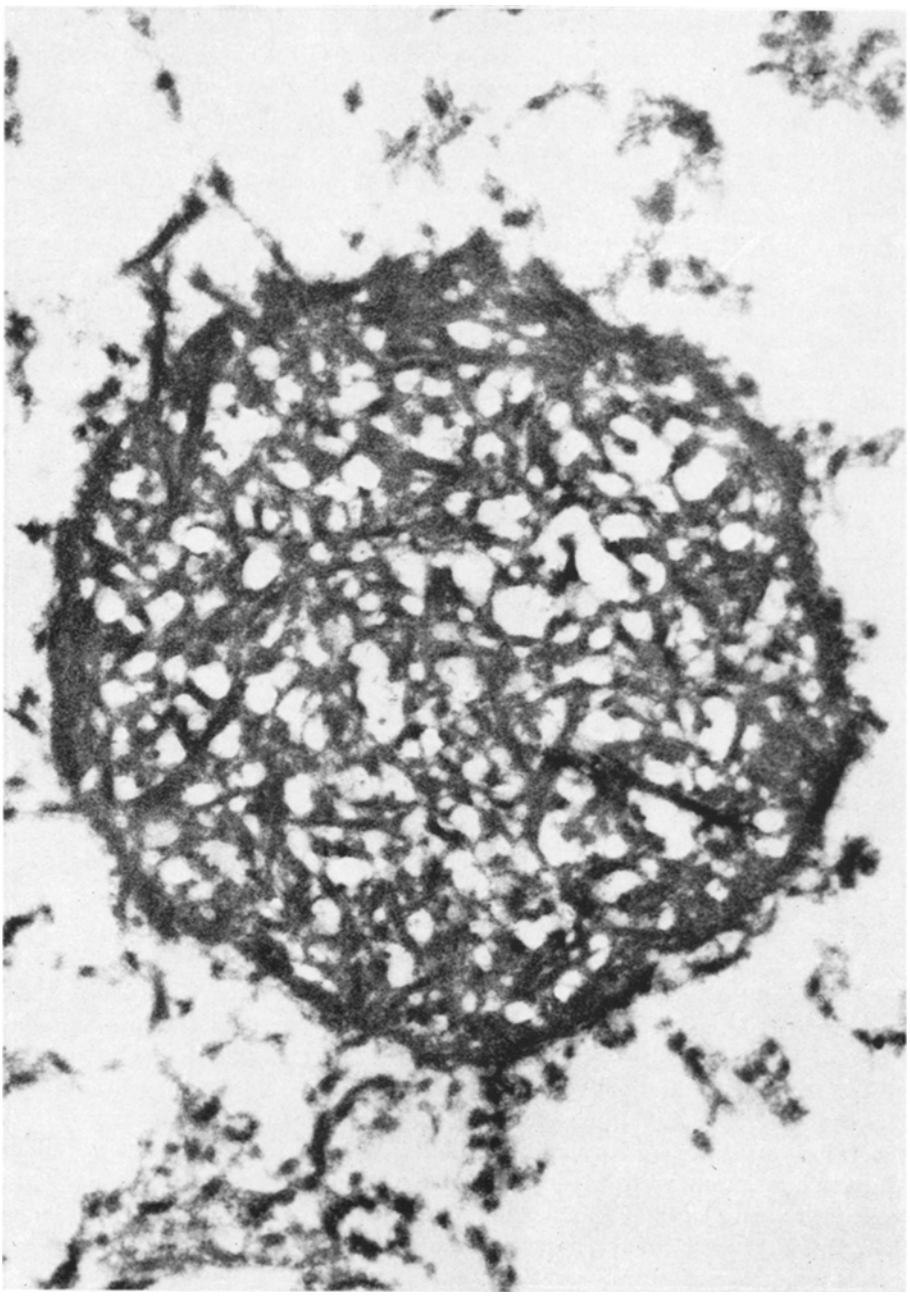

Fig. 6. GHM without the signs of a periodic axial striation of the fibre formations of the spherical space lattices. KMU-technique, Magn. × 5,000

microthrombi are concerned, was visible in the radially extending fibrils and the bundles of the 'corona'. In a further group of GHM even the characteristic basic fibrillary structure of the spherical space lattice was no longer apparent. In these instances the centre of the GHM was characterized by fine-grained precipitates more or less densely packed together, permeating the internal part of the GHM, instead of the irregular network of interlinked and interconnected fibre formations (Fig. 7). But even these finegrained precipitates seemed to be arranged into spherical space lattices around spaces that were poorly structures or even empty, so that a mosaic-like pattern was sometimes visible. The borderline between these fine-grained lattice structures and the intermediate space was, however, much more blurred than in the case of the microthrombi, where the basic fibrillary structure was preserved. Very occasionally there was evidence of residual fibrillary structures with or without the characteristic periodicity of 23 nm amongst the fine-grained precipitates inside the GHM. On the surface, however, even these GHM retained the pattern of radially arranged bundles of fibres with the characteristic periodic striation.

A very small number of GHM lacked even the mosaic-like pattern of fine-grained lattice structures seen in the ultrathin slice. In these GHM the internal part of the globule was filled with densely packed precipitates (Fig. 8) so that the outline of the residual lattice structures and the intermediate spaces could no longer be distinguished. Filamentary structures with or without periodic striation could not usually be made out inside these GHM, but they were occasionally still linked to each other by means of superficial bundles of fibres.

Between the two main types of GHM, i.e. those with fibrillary spherical lattice structures and fibre formations with a typical periodicity of 23 nm, and those with non-fibrillar, fine-grained globular centres, all intermediate stages could generally be found. Furthermore, the different types of GHM often occurred simultaneously and immediately next to each other in the same autopsy case.

Discussion

The present investigations into the ultra-structure of the GHM show firstly that these GHM consist mainly of fibrin, like the classic microthrombi described by Bohle *et al.* (1957, 1959) in the Sanarelli-Shwartzman-phenomenon. Thus the electronmicroscopic findings confirm previous immuno-fluorescent studies carried out by Skjørten (1968) with fluorescein-marked antihumanfibrinogen sera. According to studies by Kay and Cuddingham (1967) fibrin has a very uniform transverse striation with an average axial period of 22 nm, Hall and Slayter quoted an axial period of 24 nm, Bang (1965) arrived at an average axial period of 25 nm. In the case of the measurements stated in this paper the axial periods of the period-coincidentally arranged fibre formations inside the thrombus as well as those of the bundles of fibres belonging to the so-called 'corona' varied between 19 nm and 25 nm, i.e. values that come very close to those quoted by Bang, Hall and Slayter and Kay and Cuddingham.

In contrast to the bundles of fibres of the highly fibrinous intravascular microthrombi of the Sanarelli-Shwartzman-phenomenon which were described by Bohle *et al.* (1957, 1959) as coarse, mostly arranged parallel to their axis

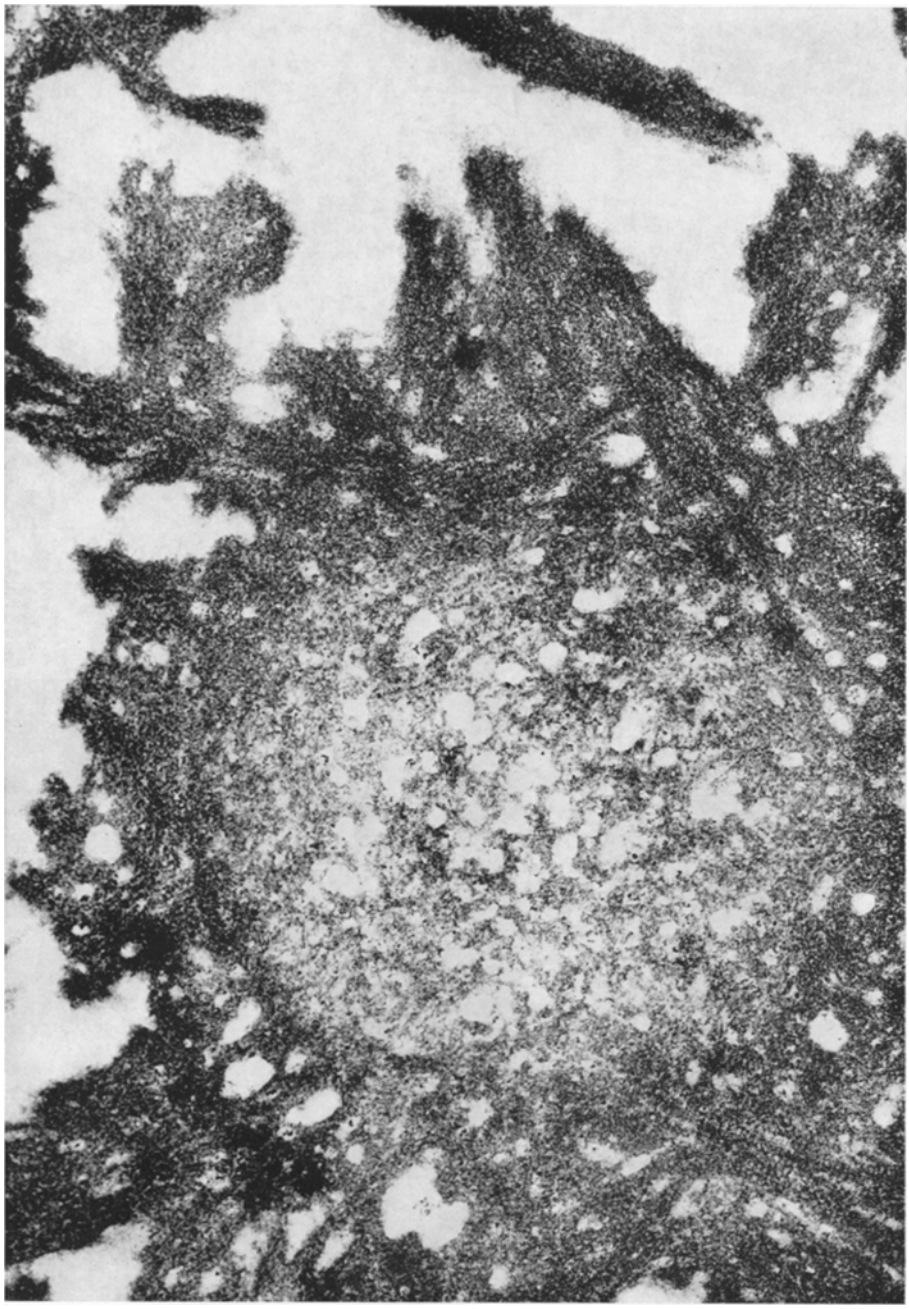

Fig. 7. Instead of the irregular network of interlinked fibres the centre of the GHM is characterized by fine-grained precipitates showing a mosaic-like pattern without evidence of periodic striation. KMU-technique, Magn. × 20,000

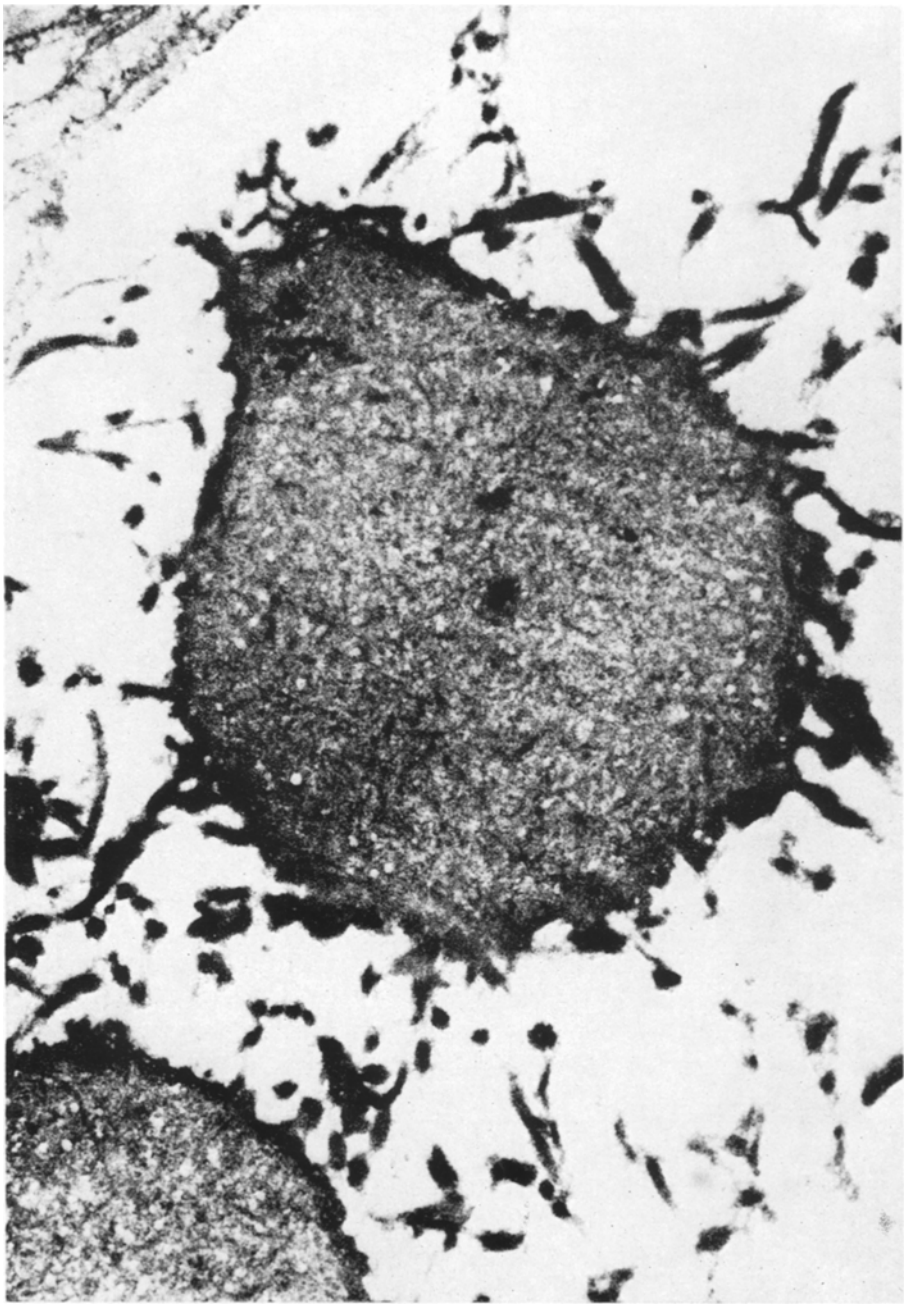

Fig. 8. The pattern of the spherical space lattice has disappeared in this GHM, but even this microthrombus still shows some superficial bundles of fibres with a periodic striation. KMU-technique, Magn. × 6,000

with little or no ramification, the fibrin fibre formations of the GHM appear as relatively slender bundles of fibres intricately knitted together to form fibrillary space lattices of varying dimensions. They enclose intermediate spaces that have hardly any structure or are completely empty. The tendency shown by filamentary fibrin derivatives to form period-coincidental lateral aggregations with the help of coarse fibrils and bundles of fibrils is obviously less pronounced in the GHM than in the microthrombi of the Sanarelli-Shwartzman-phenomenon.

The morphogenesis of the GHM can be deduced from the characteristic ultra-structure of these fibrillary space lattices. GHM apparently originate from the interlocking and internetting of intravascularly preformed, partly polymerized, filamentary intermediates of fibrinogen-fibrin conversion in the *flowing blood.* Exceptionally the formation of the fibrillary space lattices from such filamentary preformed intermediates of the fibrinogen-fibrin conversion can even be seen through the light microscope (Fig. 1). There is considerable evidence that the intermediates of the fibrinogen-fibrin conversion circulating in the plasma with their distinctive filamentary preformations and period-coincidental arrangement, trap plasma proteins in the spaces of the fibrillary spherical lattices, i.e. causing a so-called plasmatic occlusion (Regoeczi, 1968).

Apart from the generalized activation of intravascular coagulation the decisive factor for supporting such a morphogenesis, is the effect of continued circulation of the blood. Circulation alone enables the activated coagulation products and intravascular intermediates of the fibrinogen-fibrin conversion to be continually carried away and simultaneously permits these intermediates to become interlinked and interconnected into spherical space lattice structures. That is the reason why GHM are fundamentally different from the conventional highly fibrinous microthrombi of the classic animal-based Sanarelli-Shwartzman-phenomenon and of circulatory shock. The morphogenesis of the conventional highly fibrinous microthrombi also depends on sufficient movement of fibrin monomers and partly polymerized intermediates of the fibrinogen-fibrin conversion, but in this case the period-coincidental arrangement of the filamentary fibrin derivatives into fibrils, and the knitting, webbing and bunching together of these fibrils into coarse microthrombi must take place under the conditions of a far more pronounced reduction in blood flow (inadequate capillary perfusion, Hardaway, 1965) than in the case of the GHM. In other words: the formative stimulus of blood flow is of far greater importance for the morphogenesis of GHM than for the conventional highly fibrinous microthrombi. GHM must, therefore, be considered as the vital equivalent of a generalized activation of coagulation. Zinck had this in mind (as early as 1940) when the took the view GHM originated in *flowing* blood.

The fibrils and bundles of fibrils with the characteristic striation that are visible on the surface and in the immediate vicinity of the GHM as the 'corona'—just like the well-known intravascular and extravascular fibrin stars, are principally the expression of the stagnation of a plasma rich in fibrin monomers and fibrin intermediates. They are, therefore, not subject to the formative stimulus of the blood stream. By far the largest portion of these fibrils and bundles of fibrils are probably formed as a result of the generalized intravascular activation of coagulation which caused the development of these GHM, persisting throughout the agonal period and post mortem. The same highly polymerized intermediates of the fibrinogen-fibrin conversion that were previously knitted together into spherical lattices

under the influence of the blood stream are obviously also responsible for the formation of the 'corona' under the conditions of the stagnation of the blood stream.

In view of the great uniformity of the ultrastructure of the microthrombi with fibrillary spherical lattice structure it is not possible at this stage to give a conclusive answer to the question whether *polymerizable intermediate fibrin degradation products of high molecular weight*, (in particular the thrombin clottable high molecular weight fragment X) can be incorporated into the spherical lattices during morphogenesis of the GHM, thus leading to the formation of the so-called *defective polymers*. High molecular weight degradation products are the expression of the early stage of plasmin-mediated intravascular fibrinolysis. In the case of a simultaneous activation of coagulation *and* fibrinolysis these early high molecular weight derivatives of plasmin digestion are in principle able to complex and to polymerize with circulating fibrin monomers. This results in highly organized, though abnormally fibrillary, fibrin polymers. High molecular weight fibrin degradation products of the fragment X type show (according to the electron microscopic investigations into the so-called paracoagulation phenomenon) the same pattern of striation with a periodicity of 23 nm, after their incorporation into highly polymerized filamentary fibrin derivatives as do the intermediate products of the fibrinogen-fibrin conversion that are free from degradation products (Stewart, 1971). This is the reason why until now it has not been possible to arrive at a morphological differentiation between the various polymerizable high molecular weight fibrin degradation products. However, the morphological findings presented here do not exclude the possibility that polymerizable fibrin degradation products may be incorporated into the fibrillary lattice structures of the GHM.

Numerous GHM lacked in part or completely the filamentary basic structure of the spherical lattice and the characteristic striation of the fibrils with the typical periodicity of 23 nm. The filamentary structure was replaced in these GHM by lattices consisting of more or less fine-grained precipitations occasionally arranged in a mosaic-like pattern in ultra-thin sections, between which ill-defined structures or even empty spaces of irregular size were visible. Short, plump, sometimes singularly lancet-shaped filamentary residual structures with or without striation could sometimes be made out between the fine-grained precipitations of these GHM. However, the vast majority of GHM with the fine-grained basic structure lacked even these filamentary residual structures. Even the fine-grained precipitates of these GHM were quite frequently found to be still surrounded by collar-like filamentary structures with or without periodic striation. The ultrastructure of this second non-fibrillary typeof GHM corresponded in detail to the electron microscopic findings of Skjørten (1968) obtained in connection with GHM and interpreted as being morphologically typical of all GHM.

The morphogenesis of this second type of GHM is difficult to interpret since fibrinogen, combinations of fibrin monomers and fibrinogen, and the majority of fibrinogen- and fibrin degradation products arising from the plasmin-mediated fibrinolysis (fragment Y, fragment D and E) appear as fine-grained or globular precipitates in electron microscopic investigations into the phenomenon of the so-called paracoagulation (Bang, 1963, Stewart *et al.*, 1969, 1971). In view of the

electron microscopic findings presented here one cannot exclude the possibility that the above mentioned second type of GHM originate from the polymerization of filamentary intermediates of the fibrinogen-fibrin conversion with such fine-grained precipitating fibrinogen- and fibrin degradation products. The formation of this second type of GHM would, therefore, not only be proof of a coagulation activation of intravascular origin but at the same time be symptomatic of a simultaneous activation of the fibrinolysis with abnormal primary fibrin polymerization.

A more probable explanation is, however, that the second type of GHM are primarily thrombi free from degradation products, consisting of highly polymerized fibrin derivatives with characteristic fibrillary lattice structures and a typical periodicity of 23 nm, whose fibrils and bundles of fibrils have been exposed to a more or less extended secondary fibrinolysis, (endolysis) starting from the centre and progressing towards the periphery. The main finding in favour of this explanation is that between the fibrillary and fine-grained GHM all possible transitional forms can be found, and also, that the fine-grained form of these GHM shows initially the typical basic structure of a spherical lattice. In the advanced stages of fibrinolysis the clear division between these fine-grained lattice structures and the nearly vacant intermediary spaces seems to disappear and be replaced by a diffuse precipitation in the centre of the globule. This explanation would also mean in the final analysis that the fine-grained precipitates in the centre of the GHM represent fibrin degradation products of lower molecular weight. However, the appearance of these fibrin degradation products would basically have nothing to do with an abnormal primary fibrin polymerization, but would be equivalent to a secondary fibrinolysis of highly polymerized fibrin derivatives.

References

Apitz, K.: Pathologische Physiologie der Blutgerinnung. Kolloid-Z. **85**, 196 (1938)

Apitz, K.: Die intravitale Blutgerinnung. I. Teil: Physiologische Grundlagen und Besonderheiten der intravitalen Gerinnung. Ergebn. inn. Med. Kinderheilk. **61**, 54 (1942)

Bang, N. U.: A molecular structural model of fibrin based on electron microscopy of fibrin polymerization. In: Hunter, R. B., F. Koller and F. Beck, Fibrinogen and fibrin. Turnover of clotting factors. S. 73. Stuttgart: F. K. Schattauer 1965

Bohle, A., Krecke, H.-J., Miller, F., Sitte, H.: Über die Natur des sogenannten Fibrinoides bei der generalisierten Shwartzmanschen Reaktion, S. 339. In: Grabar, P. und P. Miescher (Hrsg.), Immunpathology. Basel-Stuttgart: Benno Schwabe 1959

Bohle, A., Sitte, H., Miller, F.: Elektronenmikroskopische Untersuchungen am Glomerulum des Kaninchens beim generalisierten Shwartzman-Phänomen. Verh. dtsch. Ges. Path. **41**, 326 (1957)

Hall, C. E.: Electron microscopy of fibrinogen and fibrin. J. biol. Chem. **179**, 857 (1949)

Hardaway, R. M.: Syndromes of disseminated intravascular coagulation. Springfield (Ill.): Charles C. Thomas 1966

Kay, D., Cuddingham, B.: The fine structure of fibrin. Brit. J. Haemat. **13**, 341 (1967)

Lasch, H. G., Huth, K., Heene, D. L., Müller-Berghaus, G., Hörder, M.-H., Janzarik, H., Mittermayer, C., Sandritter, W.: Die Klinik der Verbrauchskoagulopathie. Dtsch. med. Wschr. **96**, 715 (1971)

Lasch, H. G., Krecke, H.-J., Rodriguez-Erdmann, F., Sessner, H. H., Schütterle, G.: Verbrauchskoagulopathie (Pathogenese und Therapie). Folia haemat. (N.F.) **6**, 325 (1961)

Manasse, P.: Über hyaline Ballen und Thromben in den Gehirngefäßen bei akuten Infektionskrankheiten. Virchows Arch. path. Anat. **130**, 217 (1892)

Rossner, J. A.: Morphologische Untersuchungen im Licht- und Elektronenmikroskop mit der kontinuierlichen morphologischen Untersuchungstechnik (KMU-Technik). Verh. dtsch. Ges. Path. **55**, 824 (1971)

Schindler, K.: Gewebliche Veränderungen nach intravenöser Verabfolgung von Bakterienkulturfiltraten. Inaug.-Diss., Kiel 1938

Skjørten, F.: Bilateral renal cortical necrosis and the generalized Shwartzman reaction, II. Acta path. microbiol. scand. **65**, 405 (1964)

Skjørten, F.: On the nature of hyaline microthrombi. A light microscopical, immunfluorescent and ultrastructural study. Acta path. microbiol. scand. **73**, 489 (1968)

Stewart, G. J.: An electron microscope study of the polymerization of fibrinogen and its derivatives. In: M. Verstraete, J. Vermylen and M. B. Donati, Fibrinogen degradation products. Copenhagen: Munksgaard 1971

Stewart, G. J.: An electron microscopic study of ethanol gelation. In: M. Verstraete, J. Vermylen and M. B. Donati, Fibrinogen degradation products. Copenhagen: Munksgaard 1971

Stewart, G. J., Niewiarowski, S.: Non-enzymatic polymerization of fibrinogen by protamine sulfate: an electron microscope study. Biochim. biophys. Acta (Amst) **194**, 462 (1969)

Zenker, K.: Über intravasculäre Fibringerinnung bei der Thrombose. Beitr. path. Anat. **17**, 448 (1895)

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