A Polysaccharide Component in the Vascular Lesions of Thrombotic Thrombocytopenic Purpura

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The vascular lesions of thrombotic thrombocytopenic purpura (TTP) occur in the terminal arterioles and capillaries of all the tissues of the body. Two distinct histologic components are present in the vascular lesions. The most common is a focal disruption of the continuity of the wall of the vessels by an acellular material, which is covered by endothelium and which partially occludes the lumen of the vessel. In rare instances thrombi are present but are always associated with the lesion described above.

Several interpretations of the nature of the lesions have been presented. Baehr, Klemperer and Schifrin considered the vascular lesions as agglutinated platelets. Altschule suggested that endothelial damage preceded the thrombotic material. Gore pointed out two components of vascular change, degeneration of the vessel wall and agglutination of platelets at the site of the lesion in the vessel wall. He indicated that the change in the vessel wall was the initiating factor for platelet agglutination. Craig and Gitlin, by the use of fluorescein-labeled antibody, have demonstrated the presence of fibrin in the vascular lesions. They could not find evidence of immunochemically reactive platelet material with this technic.

In view of the recent suggestions by Curran and these authors concerning the production of acid mucopolysaccharides by vascular endothelium, the lesions of TTP were reinvestigated. The vascular abnormalities in our cases were similar to those described in the literature and consistently contained an acidic mucopolysaccharide.

Materials and Methods

Four cases of TTP were included in this study, two of which have been reported previously. Tissues from all the abdominal and thoracic organs, brain, skin, skeletal muscle, bone marrow and lymph nodes were examined.

The hematoxylin and eosin stain was used for general histologic detail. Elastic fibers were demonstrated by Weigert's technic and counterstained with the Van Gieson method for collagen fibers.

The periodic acid-Schiff (PAS) method as modified by McManus was used before and after each of the following. A control section was always employed.

1. Testicular hyaluronidase digestion.
2. Alpha amylase digestion.
3. Beta glucuronidase digestion.
4. Hyaluronidase followed by digestion with pepsin.
5. Hyaluronidase followed by peptic digestion followed by digestion with hyaluronidase.
6. Pepsin followed by hyaluronidase digestion.
After each of the following treatments sections were stained by the metachromatic procedure previously described:10
1. None, control section.
2. Sulfate esterification.
3. Testicular hyaluronidase digestion.
4. Hyaluronidase digestion followed by sulfate esterification.
5. Hyaluronidase followed by digestion with pepsin.
6. Hyaluronidase followed by peptic digestion followed by digestion with hyaluronidase.
7. Pepsin followed by hyaluronidase digestion.
Sulfation,11 hyaluronidase digestion and beta glucuronidase digestion12 were done by the methods previously described. Alpha amylase digestion was done according to Pearse.13 The method for pepsin digestion was that of Windrum and Kramer.14

RESULTS

Histology
Vascular lesions typical of TTP in the terminal arterioles and capillaries were found in most of the tissues from each of the cases (fig. 1). Beneath the endothelial lining of the affected vessels was an accumulation of an acellular material, which was confined to only a part of the total subendothelial area. Occasionally this material occupied the entire thickness of the vessel wall (fig. 2). In the region of the lesion the endothelial cells were larger than normal and very often present in multiple layers (fig. 3). Frequently the elastic fibers were interrupted at the site of the acellular material in the larger arterioles (fig. 4). Aneurysmal dilatation of the vessels was also noted.8

Histochemical Reactions
In all instances the amorphous subendothelial material was strongly positive with the PA-S reaction (fig. 5), and was not altered with any of the enzymes or combinations of enzymes used.
Approximately one-third of the affected vessels were metachromatic from pH 4.0 to 7.0 at ionic strength of 0.0025 (fig. 6), but not from pH 2.5 to 4.0. The remaining vascular lesions showed no evidence of metachromasy. When the amorphous material was metachromatic, the endothelial cells frequently had small metachromatic foci within their cytoplasm. After treatment of the tissue with testicular hyaluronidase there was no evidence of metachromasy (fig. 7). Sulfate esterification did not augment the metachromatic reaction, nor did it restore it after treatment with testicular hyaluronidase or produce a metachromatic reaction in those lesions which did not have a positive reaction initially.
When the tissue was digested with pepsin, almost all of the lesions were metachromatic from pH 4.0 to 7.0. Testicular hyaluronidase removed all of this metachromatic material unmasked by peptic hydrolysis. Additional metachromatic substrate was revealed when hyaluronidase-treated sections were digested with pepsin. This material was also hyaluronidase sensitive.

*Metachromasy in this paper is defined as the change in absorption of toluidine blue from its normal absorption in the red to that in the blue. This is visually distinguished as a shift in color from the blue to the red. All observations of metachromasy reported in this paper were made in aqueous media.10
Platelets in the lumen of the vessels were sometimes metachromatic. Here again the metachromatic substrate was completely sensitive to testicular hyaluronidase. Peptic digestion did not affect the metachromatic properties of the platelets.

DISCUSSION

The vascular lesions of TTP have been discussed largely on the basis of the morphologic appearance of the lesion. It was on such evidence that Baehr, Klemperer and Schifrin suggested that the lesion was a diffuse platelet thrombosis in the capillaries and terminal arterioles. This was modified by Altschule, who proposed that the damage of vascular endothelium was re-
sponsible for platelet deposition and that this initial insult preceded the development of the platelet thrombus. This latter view was supported and extended by Gore.3

There have been few reports which have been concerned with the nature of the material in the vascular lesions. Craig and Gitlin4 have shown that these lesions fail to react with labeled rabbit anti-human platelet antibody, which indicated that immunochemically reactive platelet material was not present in these lesions. These authors have also shown that the material in the lesions reacts specifically with fluorescein-labeled rabbit anti-human fibrin antibodies, even though standard dye staining procedures sometimes failed to demonstrate the presence of fibrin. The conclusions of Craig and Gitlin were that the lesions in TTP were composed of a saline insoluble derivative of fibrinogen or fibrin. Orbison5 also found positive reactions for fibrin, but expressed doubt that the aneurysms, which he described in this disease, could be caused by the agglutination of platelets and the development of occlusive thrombi.

The observations made here show an important additional component in
the lesions, which may be accounted for on the basis of stimulation of the endothelial cells. It seems likely that endothelial damage is the first event in the pathogenesis of the lesion. These cells respond by the elaboration of an abnormal amount of mucopolysaccharide, which is intimately associated with protein. If the platelets have any role in the pathogenesis of the lesion, it would seem to be secondary to the initial tissue change.

The presence of a metachromatic substrate from pH 4.0 to 7.0 and the sensitivity of this material to testicular hyaluronidase is consistent with an acidic mucopolysaccharide of the hyaluronic acid or chondroitin sulfate type. The unmasking of a metachromatic, hyaluronidase-sensitive material by pepsin is not surprising; identical results have been obtained in the case of amyloid. By these methods the metachromatic material which appears after peptic digestion is similar to the material which could be demonstrated directly. The presence of a similar mucopolysaccharide associated with platelets is well known. Odell and Anderson have extracted material from the blood platelets of rats that had many of the properties of chondroitin sulfate.

Recent studies by Curran indicate that capillary endothelial cells must be numbered among the cells that can elaborate sulfated mucopolysaccharide. Furthermore, Curran has pointed out that activity of endothelial cells is greatly increased in a variety of tissue responses, such as osteogenesis, repair tissue and in the capillaries of actively proliferating neoplasms. Further evidence for the participation of the endothelial cell in the elaboration of a connective tissue mucopolysaccharide has also been presented by Moore and Schoenberg.

In the light of these experiments it is necessary to propose two interpretations concerning the nature of the vascular lesions in TTP. The question of the participation of the platelets in the deposition of polysaccharide in these sites cannot be completely resolved. Insofar as histochemical procedures can demonstrate, the material in the platelets has similar properties to that found within the vessel walls. However, in view of the fact that vascular endothelium may produce mucopolysaccharides similar to those normally found in connective tissue, it is attractive to propose that these cells are the source of the metachromatic material in the arterioles and capillaries of TTP. It should be pointed out that histochemically identical mucopolysaccharides are normally present beneath the endothelial cells in vessels of similar type.

The excessive production of mucopolysaccharide in the capillaries and terminal arterioles in TTP could be the result of an injury of the endothelial cells. Injury to the endothelium of small vessels often results in an accumulation of ground substance and an increase in the number and size of endothelial cells. In this sense the accumulation of a metachromatic substrate in TTP can be interpreted as an excessive production of mucopolysaccharide by injured endothelium.

**Summary**

Four cases of thrombotic thrombocytopenic purpura have been used in this study. The typical vascular lesions associated with this syndrome were present in all instances. They consistently disclosed an accumulation of acidic poly-
saccharide similar to chondroitin sulfate or hyaluronic acid. This is presented as further evidence for a primary alteration of the vessel walls in this disease.

**SUMMARIO IN INTERLINGUA**

Quatro casos de thrombotic purpura thrombocytopenic esseva usate in le presente studio. Le typic lesiones vascular que es associate con iste syndrome esseva presente in omne le casos. Le patientes monstrava uniformemente un accumulation de polysaccharido acide simile a sulfato de chondroitina o a acido hyaluronic. Isto es presentate como prova additional que le alteration del pariete vascular in iste morbo es de character primari.

**REFERENCES**
