Virchows Arch [Pathol Anat] (1981) 391:217–225

Virchows Archiv A

Factor VIII Related Antigen as an Endothelial Cell Marker in Benign and Malignant Diseases

Maxwell Sehested and Klaus Hou-Jensen
Department of Pathology, Finsen Institute, Strandboulevarden 49, DK-2100 Copenhagen, Denmark

Summary. The presence and distribution of Factor VIII related antigen (FVIIIR:Ag) in formalin fixed, paraffin embedded tissue were studied in benign and malignant vascular tumors, inflammatory vascular diseases, normal tissue from various organs and a number of malignant tumors. The unlabeled peroxidase-anti-peroxidase method was utilized. Immunostaining was observed only in endothelial cells, in tumor cells of endothelial cell origin and in megakaryocytes and platelets. The staining method gave a distinct picture of the vascular pattern in all types of tissue examined. The demonstration of FVIIIR:Ag by means of the immunoperoxidase technique is considered a valuable method in diagnosing tumors of vascular origin. The method also facilitates detection of vascular invasion of malignant tumors in small caliber vessels.

Key words: Factor VIII – Endothelial cells – Malignant tumors

Introduction

The term "Factor VIII" refers to a single protein or complex of related proteins important in initiation of blood coagulation and in platelet function. The purified protein (or proteins) has three functionally distinct components: an antigen, a clot-promoting factor and von Willebrand factor (Jaffe 1977). In 1973 Bloom et al. (1973) and Hoyer et al. (1973) independently demonstrated in indirect immunofluorescence studies that vascular endothelium, megakaryocytes and platelets reacted to rabbit antiserum to "Factor VIII". Tissue culture studies have confirmed the synthesis of Factor VIII related antigen (FVIIIR:Ag) in endothelial cells (Jaffe et al. 1973; Shearn et al. 1977).

The main purpose of this study was 1) to determine whether FVIIIR:Ag could be detected in formalin fixed, paraffin embedded tissue, 2) whether FVIIIR:Ag could be used as an endothelial cell marker in normal tissue as

Offprint requests to: Maxwell Sehested

0340-1227/81/0391/0217/$01.80

well as in inflammatory and neoplastic diseases, 3) whether the ability to stain for FVIIIR:Ag was retained in malignantly transformed endothelial cells.

Material and Methods

The files of the Pathology Department of the Finsen Institute were searched for cases diagnosed as haemangiopericytoma, Kaposi's sarcoma and haemangioendotheliosarcoma. The medical notes on these patients were studied in order to obtain clinical confirmation on the histological diagnosis. Thereafter additional cases were selected at random from the files as shown in Table 1. New sections were cut from the paraffin embedded tissue blocks and subjected to the unlabeled antibody enzyme method (PAP) according to Sternberger (1974) with modifications as follows:

1) deparaffination through 99% ethanol, 2) 0.1% celloidin, 3) through 99%, 96%, 70% ethanol to distilled water, 4) 0.1% pronase (protease type 7, Sigma corp. 5255) at 37 degrees centigrade, 5) ice-cold Tris buffer, 6) 1% H2O2 in methanol, 7) normal swine serum, 8) rabbit anti-human FVIIIR:Ag 1:100, 9) swine anti-rabbit IgG 1:20, 10) peroxidase-anti-peroxidase (PAP) 1:40, 11) 3-amino-9-ethylcarbazole, H2O2, 12) counterstain with Mayer's haematoxylin and mount with Aquamount (R). After stages 6, 8, 9 and 10 the sections were rinsed with Tris buffer and after stages 11 and 12 with water. All stages were carried out at room temperature except when otherwise stated. The dilution of antiserum to FVIIIR:Ag was chosen after testing serial dilutions from 1:50 to 1:1,000 with and without prior trypsination (stage 4).

The antiserum to FVIIIR:Ag and the pure human factor VIII used in the controls were kindly supplied by Dr. Agnete Ingild (Dako-immunoglobulins a/s, Copenhagen). The antiserum was monospecific in crossed immuno-electrophoresis against plasma and did not react with serum from patients with von Willebrands disease. The other immunological reagents were commercially obtained from Dako-immunoglobulins a/s.

The following controls were performed: 1) nonimmune rabbit serum was substituted for the anti-FVIIIR:Ag in stage 8 on a seperate section from every fifth case. No staining was seen. 2) 100 microliters of antiserum to FVIIIR:Ag was absorbed with the following amounts of pure human factor VIII-400, 600, 800, 1,000, 2,000 and 4,000 micrograms. Absorption with 2,000 micrograms of antigen clearly diminished the brown immunostain and absorption with 4,000 micrograms completely abolished the immunostaining.

Table 1. Histological diagnosis and number of cases studied

Diagnosis	No of cases
Normal skin	3
Normal liver	2
Normal lung (autopsy)	2
Normal kidney	1
Normal spleen	2
Normal lymph node	3
Vasculitis	4
Normal bone marrow	2
Haemangioma – capillary type	3
Haemangioma – cavernous type	3
Kaposi's sarcoma	2
Haemangiopericytoma	1
Haemangioendotheliosarcoma	6
Non-Hodgkin malignant lymphoma	3
Hodgkin's disease	12
Carcinoma of the breast	10
Small cell carcinoma of the lung	3
Malignant melanoma	8

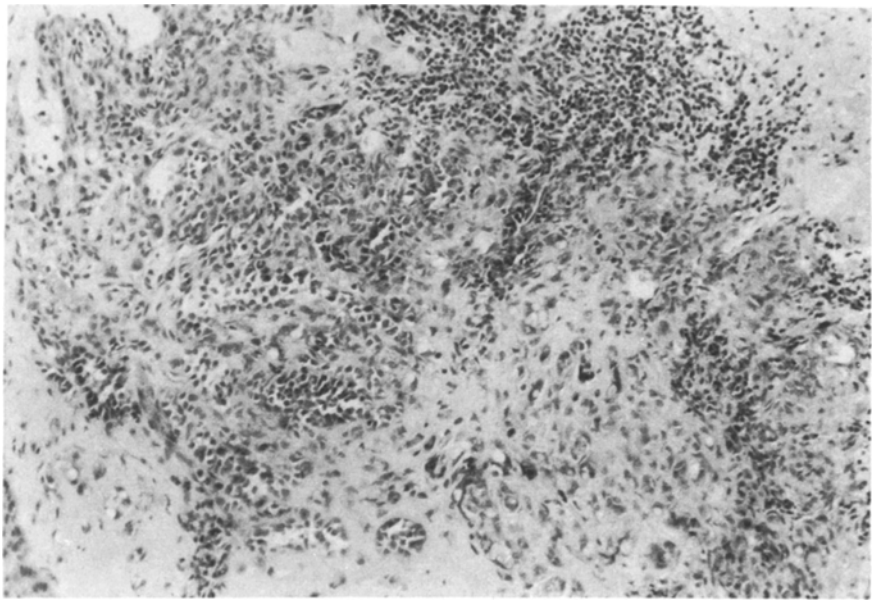
Results

In all the sections examined we observed a positive diffuse or sometimes slightly granular staining reaction for presence of FVIIIR:Ag in the cytoplasm of the endothelial cells lining the lumen of capillaries, lymphatic vessels, arteries and veins. Endothelial cells in glomeruli and in the sinusoids of liver and spleen also showed a positive staining reaction. The only cell type to show positive intracytoplasmatic staining apart from endothelial cells was the megakaryocyte and platelet. Prior trypsination greatly enhanced the staining reaction as a dilution of antiserum of 1:1,000 with prior trypsination gave a stronger staining reaction than a dilution of 1:100 without trypsination.

The endothelial cells in all four cases of vasculitis examined stained distinctly for presence of FVIIIR:Ag, even when necrosis of the vessel wall was noted.

In the cases of haemangioma the endothelial cells in both the capillary and cavernous types stained distinctly. In the four sections from the two cases of Kaposi's sarcoma a heavy staining reaction was seen in the endothelial cell component of the tumorous tissue lining vascular slits while the spindle cell component failed to stain. The case diagnosed as a haemangiopericytoma showed slit-like capillaries lined by endothelial cells positive for FVIIIR:Ag surrounded by tumor cells which were negative.

Six cases diagnosed as haemangioendotheliosarcoma were available for study. In one of the cases only a few scattered tumor cells showed a positive staining



Figs. 1–6. Case of haemangioendotheliosarcoma

Fig. 1. Section with central area consisting of tumor. Low power view. (Haematoxylin and eosin, ×100)

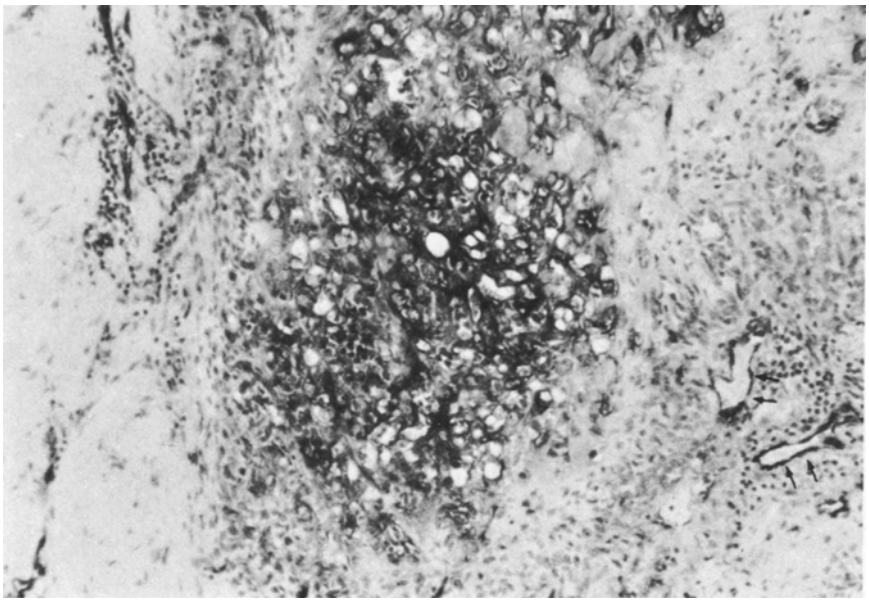


Fig. 2. Same tumor as in Fig. 1. Heavy positive immunostaining of tumor cells. Note positive immunostaining of endothelial cells in normal vessels in lower righthand corner (arrows) (FVIIIR : Ag – PAP, counterstain haematoxylin, × 100)

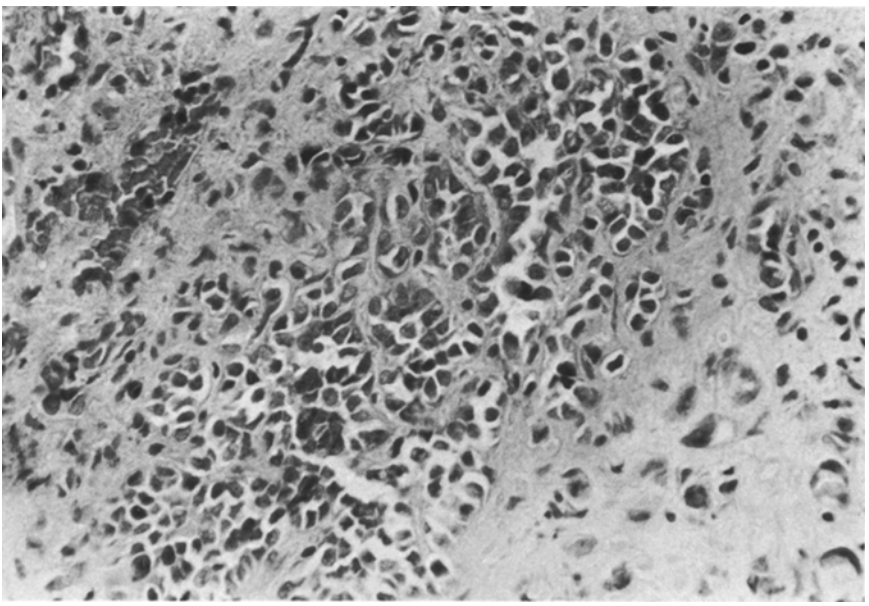


Fig. 3. Medium power view of tumor in Fig. 1. Pleomorphic tumor cells lining vascular channels. (Haematoxylin and eosin,. × 250)

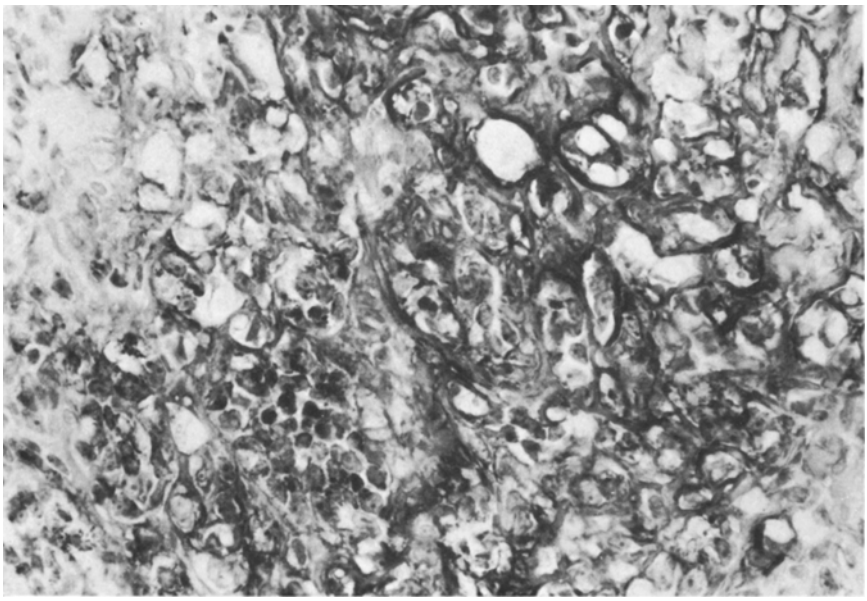


Fig. 4. Medium power view of section shown in Fig. 2. Positive immunostaining of tumor cells, (FVIIIR:Ag – PAP, counterstain haematoxylin, × 250)

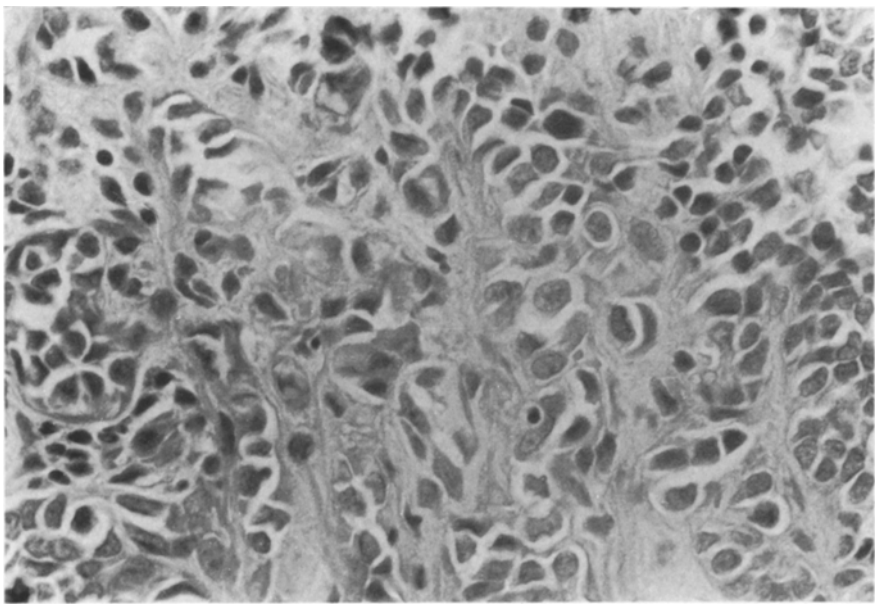


Fig. 5. Control section of tumor. High power view, (non-immune rabbit serum – PAP, counterstain haematoxylin, × 400)

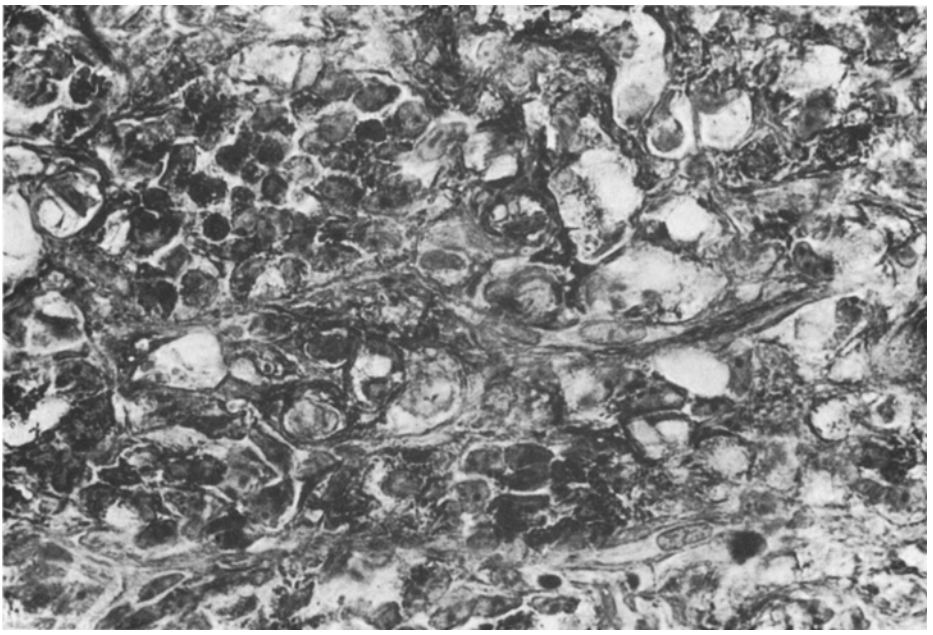


Fig. 6. High power view of section shown in Fig. 2. Finely granular intracytoplasmatic immunostaining of tumor cells, (FVIIIR:Ag – PAP, counterstain haematoxylin, ×400)

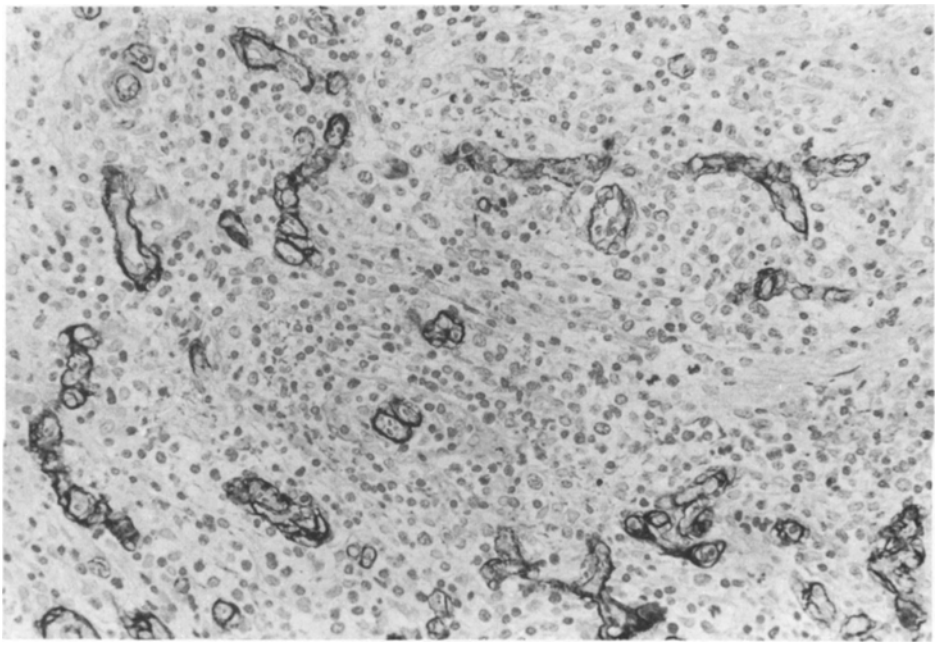


Fig. 7. Case of malignant lymphoma, diffuse undifferentiated type. Immunostaining of endothelial cells demonstrates the microvascular pattern, (FVIIIR:Ag – PAP, counterstain haematoxylin, ×150)

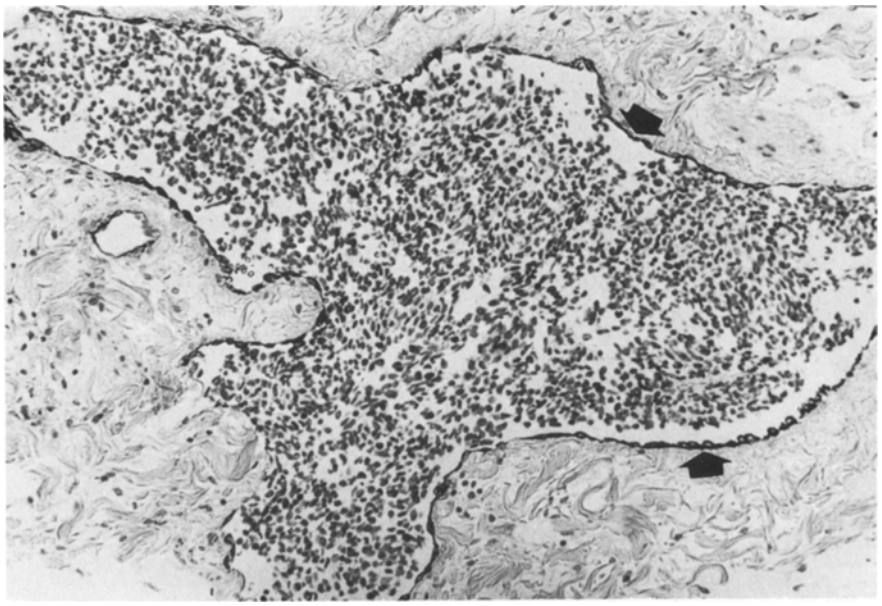


Fig. 8. Case of small cell carcinoma of the lung. Immunostaining of endothelial cells (arrows) lining dilated thinwalled vessel containing numerous tumor cells, (FVIIIR:Ag – PAP, counterstain haematoxylin, × 100)

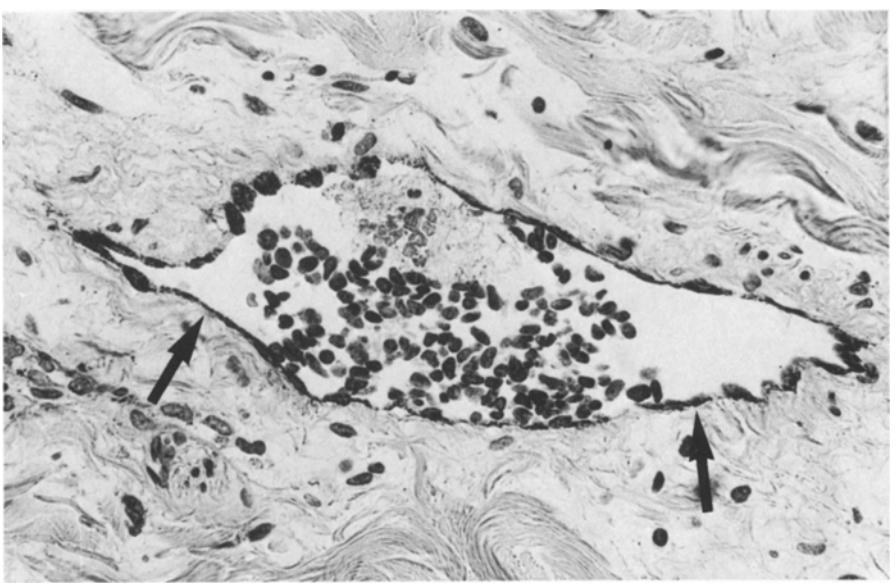


Fig. 9. Same case as Fig. 8. Immunostaining of endothelial cells (arrows) lining small caliber vessel containing tumor cells, (FVIIIR:Ag – PAP, counterstain haematoxylin, × 400)

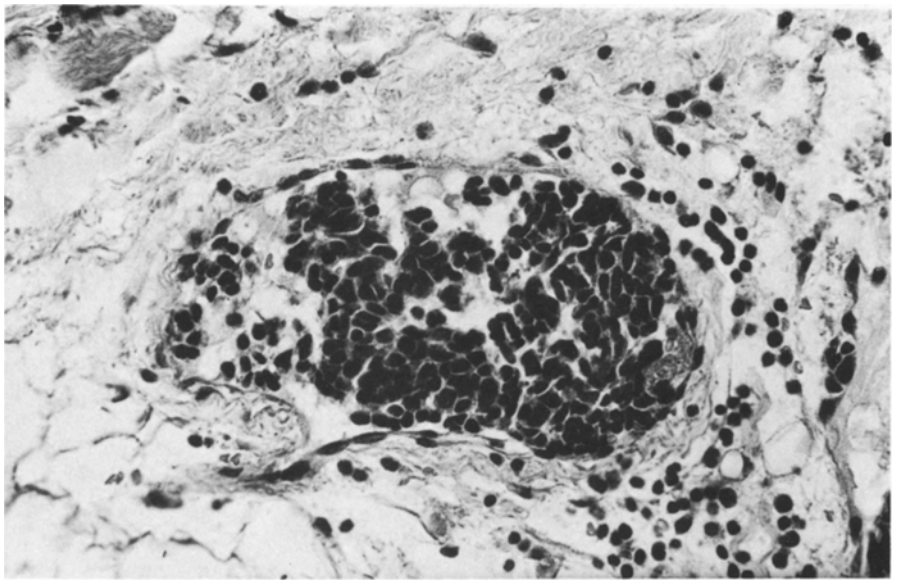


Fig. 10. Serial section of vessel shown in Fig. 9 stained with haematoxylin and eosin (×400)

reaction for presence of FVIIIR:Ag. A second case had approximately 5–10% positive tumor cells while the remaining four cases demonstrated a larger proportion of positive tumor cells amounting to 25–90% of the tumor cell population (Figs. 1–6). Positive tumor cells were seen both lining vascular clefts as well as in solid areas. Though positive staining was observed in several undifferentiated tumor cells with marked nuclear pleomorphism the staining for FVIIIR:Ag was more often found in tumor cells with a more moderate degree of nuclear pleomorphism.

In the cases studied of malignant tumors of non-vascular origin only endothelial cells were stained and the microvascular pattern was easily apparent (Fig. 7). This facilitated the detection of vascular invasion in small caliber and thinwalled vessels by tumor cells (Figs. 8–10).

Discussion

The role of the endothelial cell in the production of FVIIIR:Ag has been firmly established (Bloom 1979). The present study has utilized this knowledge together with the unlabeled antibody enzyme method of Sternberger (1974) in order to study the endothelial cell in routine histological sections. Our results have confirmed the previous immunofluorescence studies in showing that FVIIIR:Ag is localized in endothelial cells and megakaryocytes. Furthermore we have shown that it is possible to use immunostaining for FVIIIR:Ag as an endothelial cell marker in inflammatory and neoplastic disease.

After completion of the present study our attention was drawn to the newly published report by Mukai et al. (1980) on immunoperoxidase staining of

FVIIIR:Ag in endothelial cells in various organs. The staining results in endothelial cells in arteries, veins and capillaries are similar in the present study and the study of Mukai et al. (1980) However in the study of Mukai et al. (1980) endothelial cells lining lymphatic vessels were negative and endothelial cells in renal glomeruli were generally negative in contrast to the findings in the present study. This difference in staining results could be due to the different antisera used or to the different immunostaining techniques used in the two studies, the main difference in technique being that we used prior trypsination on the sections.

The cases of Kaposi's sarcoma studied confirmed the dual cell nature of this tumor as we observed both a well demarcated endothelial cell component positive for FVIIIR:Ag and a non-staining stromal spindle-cell component.

The reason for the varying staining results for FVIIIR:Ag in tumor cells in the cases of haemangioendotheliosarcoma in the present study is unknown. A possible explanation could be that endothelial cells lose their ability to produce FVIIIR:Ag with an increasing level of dedifferentiation.

The present study has also shown that immunostaining for FVIIIR:Ag in cases of malignant disease gave a distinct picture of the vascular pattern and enabled detection of vascular invasion. The method may therefore be of use as an adjunct to the elastic stain.

Acknowledgement. Ms Lotte Laustsen is thanked for helpful technical assistance.

References

Bloom AL (1979) The biosynthesis of Factor VIII. Clin Haematol 8:53–77

Bloom AL, Giddings JC, Wilks CJ (1973) Factor VIII on the vascular intima: possible importance in haemostasis and thrombosis. Nature (London) New Biol 241:217–219

Hoyer LW, de los Santos RP, Hoyer JR (1973) Antihemophilic factor antigen: localization in endothelial cells by immunofluorescent microscopy. J Clin Invest 52:2737–2744

Jaffe EA (1977) Endothelial cells and the biology of Factor VIII. N Engl J Med 296:377–383

Jaffe EA, Hoyer LW, Nachman RL (1973) Synthesis of antihemophilic factor antigen by cultured human endothelial cells. J Clin Invest 52:2757–2764

Mukai K, Rosai J, Burgdorf WHC (1980) Localization of factor VIII-related antigen in vascular endothelial cells using an immunoperoxidase method. Am J Surg Pathol 4:273–276

Shearn SAM, Peake IR, Giddings JC, Humphrys J, Bloom AL (1977) The characterization and synthesis of antigens related to Factor VIII in vascular endothelium. Thromb Res 11:43–56

Sternberger LA (1974) Immunocytochemistry. Prentice-Hall, Englewood Cliffs, New Jersey

Accepted November 12, 1980