Virchows Arch [Pathol Anat] (1983) 401:177–184

Virchows Archiv A

Malignant hemangioendothelioma of the thyroid and factor VIII-related antigen

Madeleine Pfaltz[1], Chr. Hedinger[1], P. Saremaslani[1], and B. Egloff[2]

[1] Institute of Pathology of the University of Zurich, University Hospital, CH-8091 Zürich, Switzerland

[2] Institute of Pathology of the Kantonsspital Winterthur*

Summary. Thirty-six malignant hemangioendotheliomas of the thyroid were examined immunohistochemically using antibody probes to factor VIII-related antigen in order to reevaluate the histogenesis of this neoplasia. The 36 cases were reclassified according to their light microscopic features without prior knowledge of the immunohistochemical results. Three different tumor types were discerned: Group I: classical hemangioendotheliomas (20 cases); Group II: borderline cases between malignant hemangioendotheliomas and anaplastic carcinomas (14 cases) and Group III: anaplastic carcinomas with hemangio-endotheliomatous features (2 cases). Factor VIII-related antigen could be demonstrated in 12 (60%) tumors of group I, 3 (21%) tumors of group II and in neither tumor of group III. Five control cases with the typical histological picture of anaplastic carcinoma of the thyroid were negative for factor VIII-related antigen. The results of our study suggest that at least part of the tumors termed as malignant hemangioendotheliomas are in fact derived from endothelial cells.

Key words: Malignant hemangioendothelioma of the thyroid – Factor VIII-related antigen

Introduction

Malignant hemangioendothelioma (MHE) of the thyroid was first described by Limacher in 1898 under the title "Blutgefässendotheliom der Struma". The same tumor was studied further by Hedinger (1909) and Wegelin (1926), who introduced the term "hemangioendothelioma of the thyroid". The MHE usually develops in a pre-existing goiter and has a typical macroscopic

Offprint request to: Chr. Hedinger at the above address

* This work was supported by grants from the Wilhelm Sander-Stiftung, Neustadt/Donau and the Emil Barell-Stiftung, Basel

aspect. In most cases there is only one tumor node with a necrotic or hemorrhagic center surrounded by grey to red tumor tissue. The microscopic picture is characterized by cleft-like spaces lined by large, polymorphic tumor cells (Fig. 1). These spaces sometimes contain red blood cells. Tumor cells may also be arranged in solid sheets (Fig. 3). Hemosiderin deposits are frequently present in the interstitium. It is still uncertain whether the MHE is derived from endothelial cells, or whether it represents a highly vascular variant of anaplastic or undifferentiated carcinoma of the thyroid. Immunohistochemical demonstration of factor VIII-related antigen allows identification of endothelial cells and neoplastic cells derived from endothelial cells (Mukai et al. 1980; Sehested and Hou-Jensen 1981). We applied this method to 36 cases of MHE in order to obtain further evidence for the endothelial origin of this tumor.

Material and methods

Biopsy and autopsy material from the Institute of Pathology of the University of Zurich, the Institute of Pathology of the Kantonsspital Winterthur, as well as single cases from other Pathologic Institutes in Switzerland (1953–1982), were studied by light microscopy and immunohistochemistry using antibody probes to factor VIII-related antigen. At the time of biopsy or autopsy, all 36 tumors available had been diagnosed as MHEs. In these 36 cases, we had at our disposal thyroidectomy specimens in 24 cases, thyroidectomy and autopsy material in 7 cases, and autopsy material only in 5 cases. In 9 cases metastatic tissue in addition to the primary tumor was available for investigation from the following sites: lymph nodes (3), small intestine (2), brain (1), lung (1), liver (1), pleura (1), adrenals (1), stomac (1).

We reclassified the tumors according to their light microscopic appearance without knowledge of the immunohistochemical staining results and discerned three different tumor types: Group I: classical MHE, Group II: borderline case between MHE and anaplastic carcinoma; and Group III: anaplastic carcinoma with hemangioendotheliomatous features. Five control cases with the typical histological picture of anaplastic carcinoma of the thyroid were also tested with antiserum to factor VIII-related antigen.

For the immunohistochemical demonstration of factor VIII-related antigen a histoset of Immulock (USA) was used. For photographic illustration a few selected cases were tested according to a slightly modified PAP-method after Sternberger et al. (1970) using a factor VIII-related antigen antiserum purchased from DAKO (Denmark). Dilution of the antiserum was 1:50. Sections were not trypsinized prior to incubation with antiserum. Negative controls were established by substituting non-immune rabbit serum for the specific serum.

Results

Data on age and sex were available in 32 of the 36 patients. Twentyfour (75%) were men, 8 (25%) women and the average age was 69 years. The reclassification of the tumors based on their light microscopic appearance resulted in the following distribution:

Group I:	classical MHE	20 cases
Group II:	borderline case between MHE and anaplastic carcinoma	14 cases
Group III:	anaplastic carcinoma with hemangioendotheliomatous features	2 cases

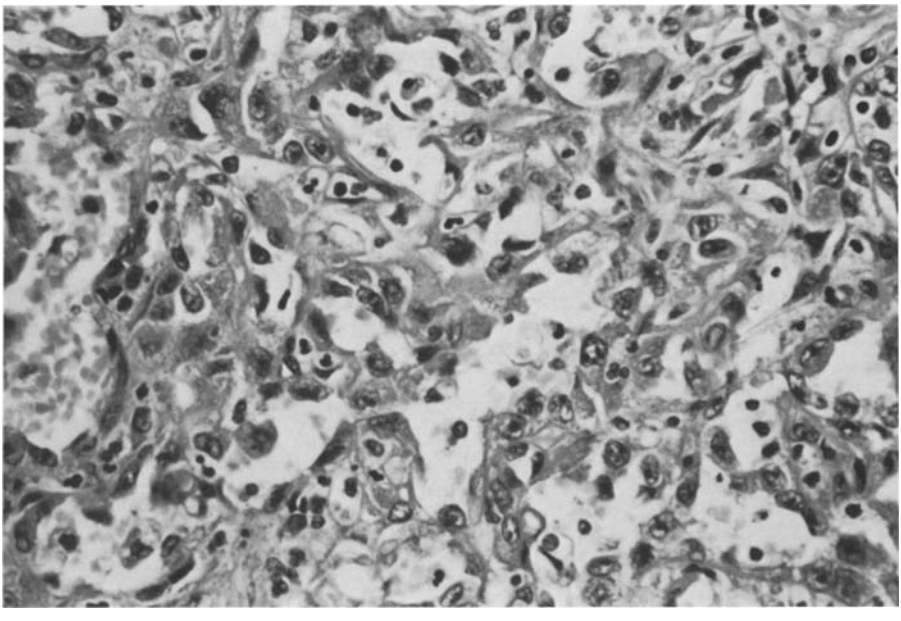
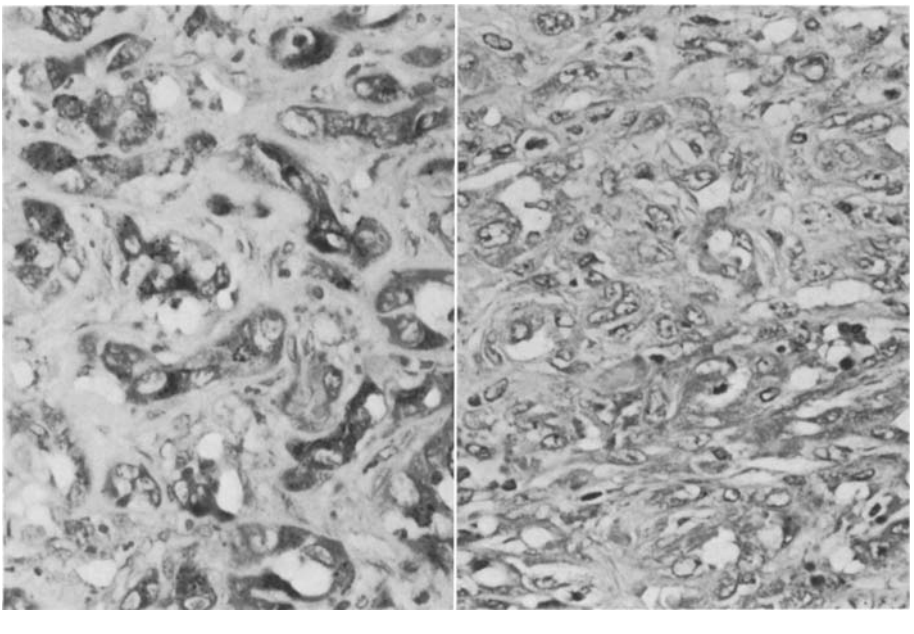


Fig. 1. Classical malignant hemangioendothelioma with large, polymorphic tumor cells lining vascular-like spaces (H&E ×360)



a **b**

Fig. 2a, b. Immunohistochemical demonstration of factor VIII-related antigen (same case as Fig. 1)) (H&E ×360). **a** Positive staining in the cytoplasm of tumor cells. **b** Negative control

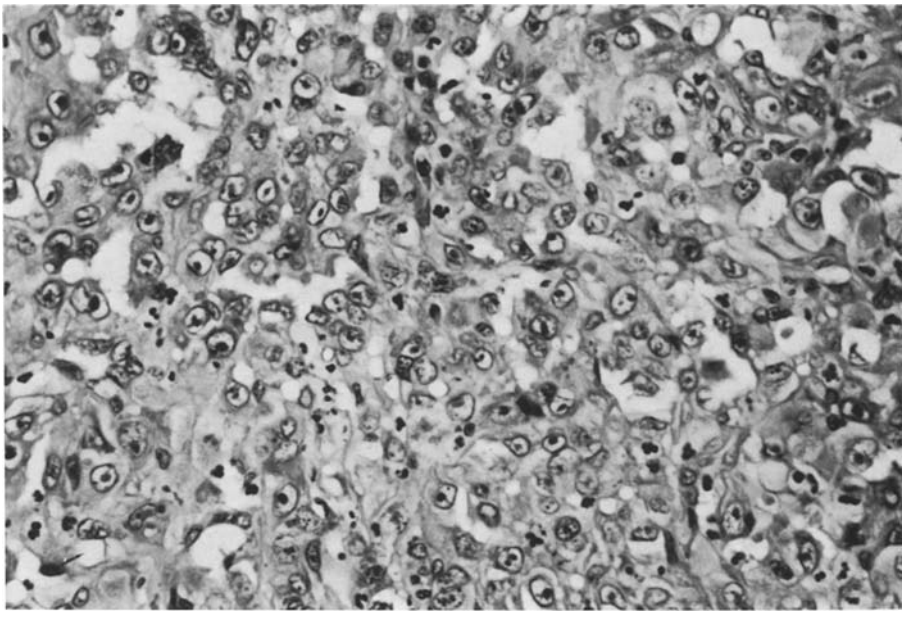
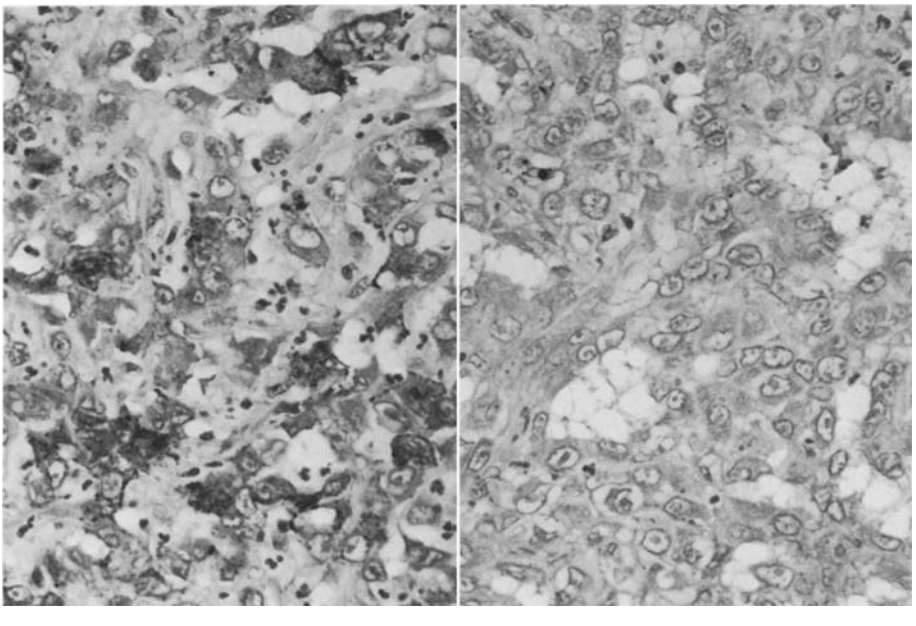


Fig. 3. Malignant hemangioendothelioma with tumor cells arranged in solid sheets (H&E × 360)



a **b**

Fig. 4a, b. Immunohistochemical demonstration of factor VIII-related antigen (same case as Fig. 3)) (H&E × 360). **a** Positive staining in the cytoplasm of tumor cells. **b** Negative control

Table 1. Immunohistochemical results

	Positive primary tumors	Intensity of staining		Proportion of positive tumor cells		
Total:	15 (42%)	weak	strong	+	++	+++
Group I (20 cases)	12 (60%)	6	6	4	6	2
Group II (14 cases)	3 (21%)	1	2	2	1	0
Group III (2 cases)	0 (0%)	0	0	0	0	0

The results of the immunohistochemical reactions are summarized in Table 1. The antisera obtained from DAKO (Denmark) and Immulock (USA) gave identical staining results. In 4 of the 12 positive cases out of group I, additional metastatic tissue was available for investigation. Three of these 4 cases turned out to be positive for factor VIII-related antigen. No metastatic tissue was available in the 3 positive cases of group II. All of the other metastases tested were negative for factor VIII-related antigen. The intensity of the histochemical staining differed from case to case, ranging from a weak but definitely positive reaction, to a very intense staining of the whole cytoplasm (see Table 1). Cells from solid parts of the tumor as well as cells lining cleft-like spaces stained positively; however, in the latter cells, the positive staining reaction prevailed in quantity and quality (Figs. 2, 4).

In 33 of our 36 cases the factor VIII-related antigen could be detected in the endothelium of capillaries and larger vessels. The staining reaction ranged from very weak staining in occasional vessels to an intense reaction in all of the vessels present. In the 3 cases with a negative reaction in vessels away from the tumor, the tumor tissue as well was negative for factor VIII-related antigen.

The anaplastic carcinomas serving as control cases did not show any positive staining of the tumor cells, whereas the vessels away from the tumor stained positively.

Discussion

In the WHO-classification of thyroid tumors the MHE is classified neither as carcinoma nor as sarcoma, but under category "miscellaneous tumors" (Hedinger and Sobin 1974), the histogenesis of this tumor still being uncertain. Krisch et al. (1980) consider the MHE to be a variant of anaplastic carcinoma of the thyroid mainly because of its biological and clinical behavior. The lack of blood group antigens – a characteristic component of endothelial cell walls – in the tumor cells of hemangioendotheliomas is another argument against the endothelial origin of this tumor (Feigel et al. 1976). The presence of Weibel-Palade bodies (Weibel and Palade 1964) in tumor

cells of MHE, however, indicates that this tumor represents a neoplasm derived from vascular endothelia (Egloff 1983). Endothelial cells can be identified as such by the recently developed immunohistochemical demonstration of factor VIII-related antigen, one of three components of the blood coagulant factor VIII. It can be demonstrated specifically in endothelial cells (Hoyer et al. 1973; Jaffe et al. 1973; Jaffe 1977; Piovella et al. 1978; Mukai et al. 1980; Rand et al. 1980; Sehested and Hou-Jensen 1981; Jeanneau and Sultan 1982; McComb et al. 1982), platelets (Howard et al. 1974; Piovella et al. 1978; Sehested and Hou-Jensen 1981), megakaryocytes (Piovella et al. 1978; Sehested and Hou-Jensen 1981) and mast cells (Kindblom 1982). Neoplastic cells derived from endothelial cells also contain factor VIII-related antigen. It has been demonstrated immunohistochemically in cardiac myxomas (Morales et al. 1981), hemangiomas (Mukai et al. 1980; Sehested and Hou-Jensen 1981), adenomatoid tumors (Bell and Flotte 1982), in Kaposi's sarcomas (Guarda et al. 1981; Nadji et al. 1981; Sehested and Hou-Jensen 1981) and in other skin tumors of alleged vascular nature (Burgdorf et al. 1981). Guarda et al. (1982) detected the factor VIII-related antigen in 23 of 28 angiosarcomas and Sehested and Hou-Jensen (1981) in 5 of 6 hemangioendotheliosarcomas. These authors do not mention whether malignant hemangioendotheliomas of the thyroid were included in their series. However, Schäffer and Ormanns (1983) who investigated malignant hemangioendotheliomas of the thyroid for the presence of factor VIII-related antigen reported 4 of their 6 cases to be positive.

The results of our investigation show that 60% of the cases of group I (classical MHEs) are positive for factor VIII-related antigen, whereas only 21% of group II (borderline cases between MHE and anaplastic carcinoma) turned out to be positive. This diverging number of positive cases in the two diagnostic groups can be interpreted as follows: the negative tumors may represent dedifferentiated MHEs which lost their ability to produce demonstrable amounts of factor VIII-related antigen. Along with its dedifferentiation, the tumor is thought to loose the tendency to form vascular spaces. The resulting change in the histological pattern may have led us to classify these dedifferentiated tumors in the borderline group II. On the other hand, unusual anaplastic carcinomas with vascular-like spaces could imitate the histological picture of MHEs.

The influence of artifacts, however, should be taken into account especially with negative results. Storage in formalin for longer periods apparently decreases the antigenicity of the tissue. In one of our cases, paraffin blocks and formalin fixed material stored for several years was available for investigation. Factor VIII-related antigen was detectable only in sections cut from the blocks, whereas the formalin fixed tissue did not show any positive staining reaction. Such an artifact could be the cause of the negative immunohistochemical results of tumor tissue and normal vessels in 3 of our 36 cases. The absence of staining in the metastasis in one of our cases with a positive primary tumor could be due to either inadequate fixation or the above mentioned dedifferentiation of the tumor. Trypsinization prior to incubation with the antiserum did not enhance the staining reaction,

in contrast to the results of other authors (Sehested and Hou-Jensen 1981; McComb et al. 1982).

Other problems may arise from the interpretation of the immunohistochemical staining results as shown by the contradictions found in the literature in reference to investigations with factor VIII-related antigen on adenomatoid tumors. Barwick and Madri (1982), Gould et al. (1982) and Said et al. (1982) describe adenomatoid tumors as factor VIII-related antigen negative, whereas in the study of Bell and Flotte (1982) at least some cases were positive. In our study, therefore, we have considered as positive only cases in which large, definitely polymorphic tumor cells gave a positive immunohistochemical reaction.

Based on the technical problems discussed above and the difficulties that arise from the histological interpretation, immunohistochemical investigations with antiserum to factor VIII-related antigen cannot always give unequivocal results. The high portion of positive tumors in our material (60% in group I), however, indicates that at least a good part of the tumors termed as MHEs are derived from endothelial cells.

Acknowledgement. The authors would like to thank Dr. A.R. von Hochstetter for his kind assistance in preparing the English version of the manuscript and Miss R. Pfister for her excellent secretarial assistance.

References

Barwick KW, Madri JA (1982) An immunohistochemical study of adenomatoid tumors utilizing keratin and factor VIII antibodies. Evidence for a mesothelial origin. Lab Invest 47:276–280

Bell DA, Flotte ThJ (1982) Factor VIII related antigen in adenomatoid tumors: Implications for histogenesis. Cancer 50:932–938

Burgdorf WHC, Mukai K, Rosai J (1981) Immunohistochemical identification of factor VIII-related antigen in endothelial cells of cutaneous lesions of alleged vascular nature. Am J Clin Pathol 75:167–171

Egloff B (1983) The hemangioendothelioma of the thyroid. Virchows Arch [Pathol Anat] 400:119–142

Feigel W, Denk H, Davidovits A, Holzner JH (1976) Blood group isoantigens in human benign and malignant vascular tumors. Virchows Arch [Pathol Anat] 370:323–332

Gould EG, Moskowitz LB, Penneys NS, Nadji M (1982) Adenomatoid tumor: Immunohistologic demonstration of mesothelial origin. Lab Invest 46:30A–31A

Guarda LG, Silva EG, Ordonez NG, Smith JL (1981) Factor VIII in Kaposi's sarcoma. Am J Clin Pathol 76:197–200

Guarda LA, Ordonez NG, Smith JL Jr, Hanssen G (1982) Immunoperoxidase localization of factor VIII in angiosarcomas. Arch Pathol Lab Med 106:515–516

Hedinger E (1909) Zur Lehre der Struma sarcomatosa. 1. Die Blutgefässendotheliome der Struma. Frankfurt Z Pathol 3:487–540

Hedinger Chr, Sobin LH (1974) Histological typing of thyroid tumours. International Histological Classification of Tumours Nr. 11. World Health Organization, Geneva

Howard MA, Montgomery DC, Hardisty RM (1974) Factor VIII-related antigen in platelets. Thromb Res 4:617–624

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, Hoyer LW, Nachman RL (1973) Synthesis of Antihemophilic Factor Antigen by Cultured Human Endothelial Cells. J Clin Invest 52:2757–2764

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

Jeanneau Ch, Sultan Y (1982) Localization of factor VIII/von Willebrand factor antigen by immunoelectron microscopy in human endothelial cells using Fab fragments coupled to peroxidase. J Histochem Cytochem 30:1091–1096

Kindblom LG (1982) Factor VIII related antigen and mast cells. Acta Pathol Microbiol Scan (A) 90:437–439

Krisch K, Holzner JH, Kokoschka R, Jakesz R, Niederle B, Roka R (1980) Hemangioendothelioma of the thyroid gland – true endothelioma or anaplastic carcinoma? Pathol Res Pract 170:230–242

Limacher F (1898) Ueber Blutgefässendotheliome der Struma mit einem Anhang über Knochenmetastasen bei Struma maligna. In: Virchow R (Hrsg) Beiträge zur Geschwulstlehre, Arch Pathol Anat Physiol Klin Med Suppl. Reimer, Berlin Bd 151, S 113–150

McComb RD, Jones TR, Pizzo SV, Bigner DD (1982) Specificity and sensitivity of immunohistochemical detection of factor VIII/von Willebrand factor antigen in formalin-fixed paraffin-embedded tissue. J Histochem Cytochem 30:371–377

Morales AR, Fine G, Castro A, Nadji M (1981) Cardiac myxoma (endocardioma); an immunocytochemical assessment of histogenesis. Hum Pathol 12:896–899

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

Nadji M, Morales AR, Ziegles-Weissman J, Penneys NS (1981) Kaposi's sarcoma; immunohistologic evidence for an endothelial origin. Arch Pathol Lab Med 105:274–275

Piovella F, Nalli G, Malamani GD, Majolino I, Frassoni F, Sitar GM, Ruggeri A, Dell'Orbo C, Ascari E (1978) The ultrastructural localization of factor VIII-antigen in human platelets, megakaryocytes and endothelial cells utilizing a ferritin-labelled antibody. Br J Haematol 39:209–213

Rand JH, Sussman II, Gordon RE, Chu SV, Solomon V (1980) Localization of factor VIII-related antigen in human vascular subendothelium. Blood 55:752–756

Said JW, Nash G, Lee M (1982) Immunoperoxidase localization of keratin proteins, carcinoembryonic antigen, and factor VIII in adenomatoid tumors: Evidence for a mesothelial derivation. Hum Pathol 13:1106–1108

Schäffer R, Ormanns W (1983) Immunohistochemischer Nachweis von Faktor VIII-Antigen in malignen Haemangioendotheliomen der Schilddrüse: Ein Beitrag zur Histogenese. Schweiz Med Wochenschr 113:601–605

Sehested M, Hou-Jensen K (1981) Factor VIII related antigen as an endothelial cell marker in benign and malignant diseases. Virchows Arch (Pathol Anat) 391:217–225

Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG (1970) The unlabeled antibody enzyme method of immunohistochemistry: Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333

Wegelin C (1926) Das Haemangioendotheliom der Schilddrüse. In: Henke F, Lubarsch O Handbuch der speziellen pathologischen Anatomie und Histologie. Springer, Berlin, Bd 8, S 297–302

Weibel ER, Palade GE (1964) New cytoplasmic components in arterial endothelia. J Cell Biol 23:101–112

Accepted June 27, 1983