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Blood Group Isoantigens in Human Benign and Malignant Vascular Tumors

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Summary. Paraffin material of 31 benign and malignant vascular tumors was investigated with respect to their blood group isoantigen (BG) content by the mixed cell agglutination reaction (MCAR). In capillary hemangioma, BG was found in endothelial cells as well as in solid buds. Benign hemangioendothelioma found in children differed from that found in adults in that in juvenile cases only endothelial cells expressed BG whereas in adult cases BG isoantigenity was present in endothelial cells as well as in intercapillary cellular elements. In pericytomas only endothelial cells were BG positive, whereas the tumor cells lacked BG. Similar results were obtained with glomus tumors. All but one hemangiosarcoma were BG negative. In one case, however, which probably resembled a "true" malignant hemangioendothelioma (Stout and Lattes, 1967) the tumor cells contained BG in conspicuous amounts.

Key words: Blood group isoantigens — Vascular tumors.

Introduction

It is not yet settled whether or not a benign vascular growth should be regarded as a neoplasm or as a malformation (Albertini, 1974); and in malignant vascular tumors unequivocal diagnostic criteria are still unavailable. This is the reason why tumors of similar histology are still classified differently by different authors (for review see Roth, 1957; Stout and Lattes, 1967).

The present study was designed to test whether tissue-bound blood group isoantigens (BG) could be used to differentiate vascular elements. The existence and distribution of BG on endothelial cells was investigated morphologically by Szulman (1960), by the immunofluorescence technique. Davidsohn and coworkers (for review see 1972) used a modified mixed cell agglutionation reaction (Coombs et al., 1956) on formalin-fixed tissue sections and showed that alcohol soluble BG were preserved in the endothelial cells under these experimental conditions and were readily detected by erythrocyte adherence.

In the present report an attempt was made to answer the following questions:

1. Does blood group isoantigenicity provide a diagnostically useful criterion to differentiate between different varieties of vascular tumors?

2. Is the morphologic detection of BG helpful in the diagnosis of malignant vascular tumors?

3. Is there any relationship between BG isoantigenicity of endothelial cells or their tumor cell equivalents and the degree of differentiation of the malignant tumor?

Material and Methods

1. Material. The number of tumors and the histologic diagnoses are listed in Table 1.

Table 1. Specimens 20 and 22 are metastases of specimens 19 and 21. Primary tumor of specimen 26 is unknown

No. of specimen	Localization	Histologic diagnosis	Erythrocyte BG of patient	Material	Sex	Age (years)
1	Oral mucosa	Capillary	A	bi	♀	26
2	Skin (cheek)	hemangioma	A	bi	♀	14
3	Skin (hand)		B	bi	♀	78
4	Skin (head)		A	bi	♂	48
5	Oral mucosa	Benign	B	bi	♂	75
6	Head, subcutaneous area	hemangio-	A	bi	♀	1
7	Lower lip	endothelioma	A	bi	♂	25
8	Skin (back)		A	bi	♀	3
9	Skin (nose)		A	bi	♂	1
10	Rectum	Cavernous	B	bi	♂	49
11	Skin (knee)	hemangioma	A	bi	♀	11
12	Skin (forearm)		A	bi	♂	10
13	Skin (back)		A	bi	♂	2
14	Oral mucosa		A	bi	♀	60
15	Skin (temporal region)		AB	bi	♀	63
16	Lung	Hemangio-	A	bi	♂	64
17	Retroperitoneal region	pericytoma	B	bi	♂	48
18	Metastasis to the lung		A	bi	♂	34
19	Liver	Angiosarcoma	A	au	♂	67
20	Metastasis to the lung	(malignant	A	au	♂	67
21	Adrenal gland	hemangio-	A	au	♂	64
22	Metastasis to the pleura	endothelioma)	A	au	♂	64
23	Thyroid		AB	bi	♀	81
24	Thyroid		A	bi	♂	66
25	Lung		A	bi	♂	66
26	Metastasis in the thoracic wall		B	bi	♂	65
27	Breast		A	bi	♀	60
28	Forearm and hand		A	amp	♂	40
29	Liver		A	au	♀	78
30	Skin (finger)	Glomus tumor	A	bi	♂	71
31	Skin (finger)		B	bi	♂	37
32	Skin (forearm)	Lymphangioma	A	bi	♀	5

bi = biopsy, au = autopsy, amp = amputation

2. Detection of BG in Tissue Sections. A modified mixed cell agglutination reaction (MCAR) was performed as described previously (Denk et al., 1974a, b). Briefly, deparaffinized tissue sections were washed with Tris-HCl buffer (pH 7.45) and subsequently covered and incubated with commercially available BG antisera (Ortho Diagnostics) for 15 min at room temperature in a moist chamber. The sera were concentrated prior to use to a titer in hemagglutination of 1:500–1:1,000 and were used in this concentration. After incubation, the slides were washed in 3 changes of Tris-HCl buffer (pH 7.45), covered with a 1% suspension of washed human

BG isologous erythrocytes, and again incubated at room temperature for 30 min in the moist chamber. Thereafter, the not specifically adherent erythrocytes were allowed to detach from the slide after inversion of the slide in buffer and the specimens were finally fixed by addition of glutaraldehyde to the buffer to a concentration of 1–5%. With this procedure a good fixation of the specifically bound indicator red cells was achieved and the subsequent hematoxylin eosin (H-E) staining was possible without detachment of the cells. In addition to H-E staining Lepehnes peroxidase reaction was performed in some instances in order to achieve better contrast between indicator erythrocytes and the underlying structures. A positive reaction is morphologically characterized by adherence of test erythrocytes to BG-containing structures. This is not only a feature of endothelial cells but also of preexisting erythrocytes, mucosal cells, squamous epithelium. It is easy to distinguish preexisting erythrocytes and test erythrocytes by looking into the microscope since they are found in differently focused planes (the test erythrocytes are on a higher plane). On the other hand, photoreproduction is often difficult due to the rather weak contrast of the indicator erythrocytes on stained slides.

To assure the specifity of the reaction, a number of controls were necessary. These included BG isologous antisera and nonisologous erythrocytes, and vice versa, as well as nonisologous antisera and nonisologous red cells. In additional control experiments, the BG antisera were absorbed with the respective BG isologous erythrocytes prior to use.

Specimens from patients with BG-O were not included in our study since MCAR with *Ulex europeus* extract gave inconsistent results in our hands.

Results

Capillary Hemangioma

In 4 cases of classical capillary hemangioma most of the vessels were dilated to a variable extent and were lined by flat endothelial cells (Fig. 1 A). Solid areas as well as regular arteries and veins were less often seen. The connective tissue between the vessels was infiltrated by varying amounts of inflammatory cells. BG were found in association with the endothelial cells and cells located in the center of solid buds (Fig. 1 B). Pericytes, smooth muscle cells, and cells of the connective tissue were BG-negative.

Five specimens showed a higher degree of cellularity and, hence, were regarded as benign hemangioendotheliomas (Stout, 1967) ("hemangioma capillare hypertrophicum", Albertini, 1974). Two of them were taken from adults (25 and 75 years of age, respectively) and three from children (1–3 years of age). The light-microscopic appearance of all cases was similar (Fig. 2 A, C). The capillary lumina were narrowed by surrounding large cuboidal or polygonal cells. Despite similar morphology of these intercapillary cells, the mixed cell agglutination disclosed fundamental differences in that the intercapillary cells contained BG in the adult (Fig. 2 D), but lacked BG in the juvenile cases (Fig. 2 B).

Cavernous Hemangioma

In the 6 cavernous hemangiomas studied, BG were confined to the endothelial cells (Fig. 1 C).

Hemangiopericytoma[1]

Two of the three hemangiopericytomas examined were benign. One was located in the lung and one in the retroperitoneal region. The lesions consisted of

1 We wish to thank Dr. St. Wuketich (Dept. of Pathology, Vienna City Hospital, Lainz) for permission to include these three cases of hemangiopericytoma in this study

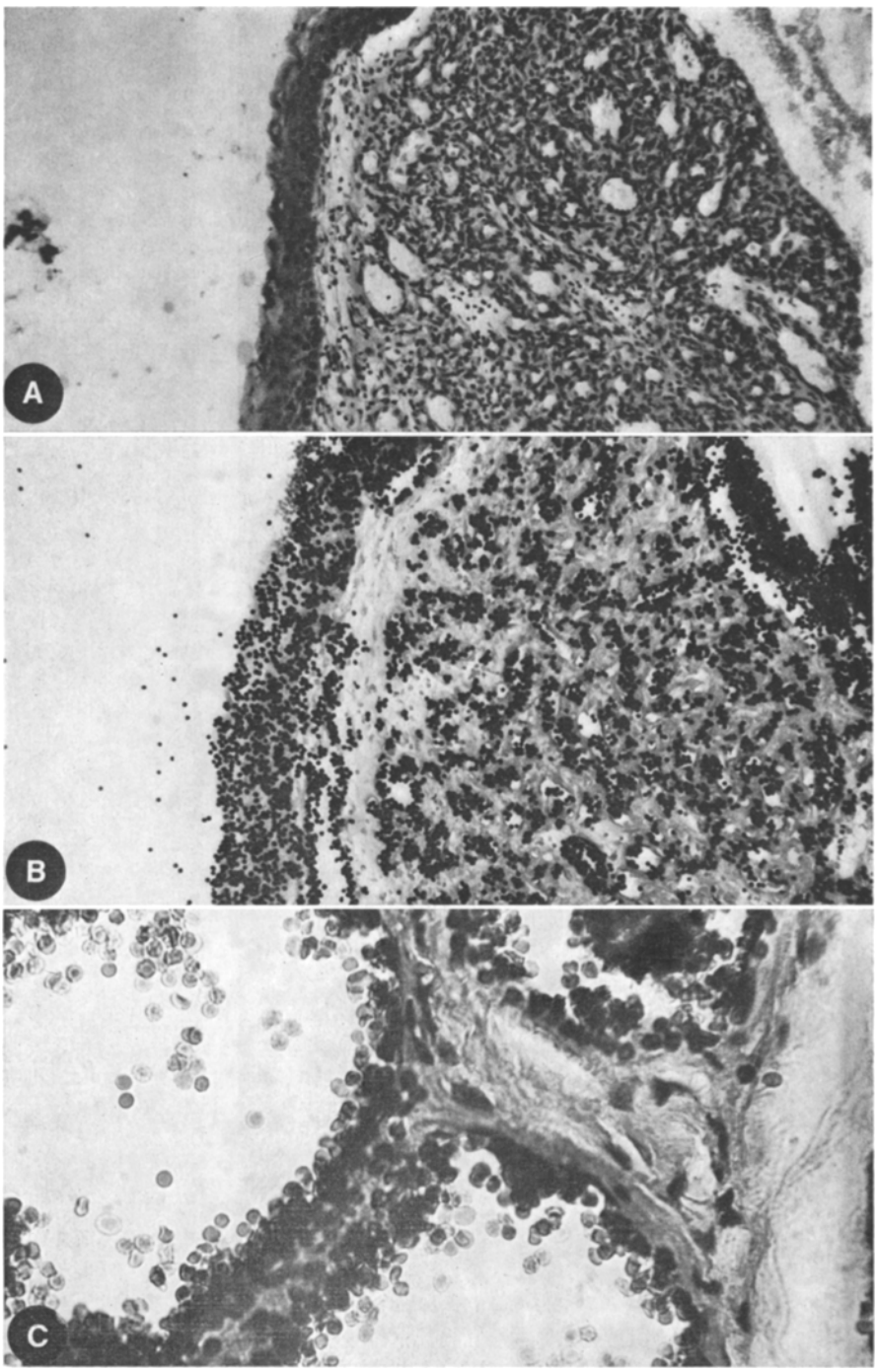

Fig. 1. (A) Capillary hemangioma (Case No. 2), BG A. Negative control specimen. Hematoxylin-eosin ×100. (B) Capillary hemangioma, same case. Positive reaction on endothelial cells and erythrocytes. Lepehne's peroxidase reaction ×100. (C) Cavernous hemangioma (Case No. 10), BG B. Endothelial cells with positive reaction, stroma cells negative. H-E ×400

numerous capillaries surrounded by irregularly arranged pericytelike cells (Fig. 3A). The third was malignant and metastatic tissue from the lung, the site of the primary tumor was unknown. The pericytelike cells showed the histologic criteria of malignancy and were arranged around endothelial cell lined slits (Fig. 3B). In the benign as well as in the malignant cases the endothelial cells were BG positive (Fig. 3A and B), whereas the pericytelike cells lacked BG.

Glomus Tumor ("tumeur glomique" Masson)

Two specimens of glomus tumor of the skin were examined. BG were confined to endothelial cells whereas the epitheloid (= glomus) cells were negative.

Lymphangioma

One lymphangioma included for comparison in this study showed positive endothelial cells.

Hemangiosarcoma (Malignant Hemangioendothelioma)

Hemangiosarcomas represent a rather ill-defined group of malignant neoplasms characterized by blood-filled cavities and channels lined by an atypical tumor endothelium (Albertini, 1974). The endothelial cells may be arranged either in a single layer or in several layers. The tumor cells were BG-negative in all cases but one. Occasionally normal looking endothelial cells containing BG were found in close proximity to the tumor cells. These cells may have been derived from preexisting vessels which were invaded and destroyed by the neoplasm. In addition, normal capillaries with BG-positive endothelial cells were found within the neoplastic tissue presumably representing vessels of the preexisting tissue as well as tumor stromal vessels (Fig. 4B). One specimen, a hemangiosarcoma located in the soft tissue of the forearm in a 41-years-old male differed from the others in that all its tumor cells gave a positive reaction for BG (Fig. 4C1, C2). It should be emphasized, however, that this tumor showed a different histology: blood-filled cavities, bordered by round moderately pleomorphic tumor cells, were seen (Fig. 4C 3). Focally, the tumor cells were arranged in vessellike structures surrounded by a basal membrane with occasional irregular central lumina.

Discussion

Endothelial cells contain BG as cell wall antigens (Szulman, 1960; Davidsohn, 1972) and this feature was tested in the present investigation for its value as an additional criterion to discriminate different cell types in vascular tumors. As far as the benign hemangiomas with a higher degree of cellularity (benign hemangioendothelioma according to Stout and Lattes, 1967; hemangioma capillare hypertrophicum according to Probst, 1964; Albertini, 1974) are concerned, two different types could be discriminated. In the *juvenile* type, these cells were BG-negative, what favors but does not prove the assumption that these cells are not related to endothelial cells. In contrast, in tumors from adults of similar appearance light microscopically (*adult* type) the intercapillary BG-positive cells appear as more or less atypical endothelial cells which proliferate but lack the ability to form

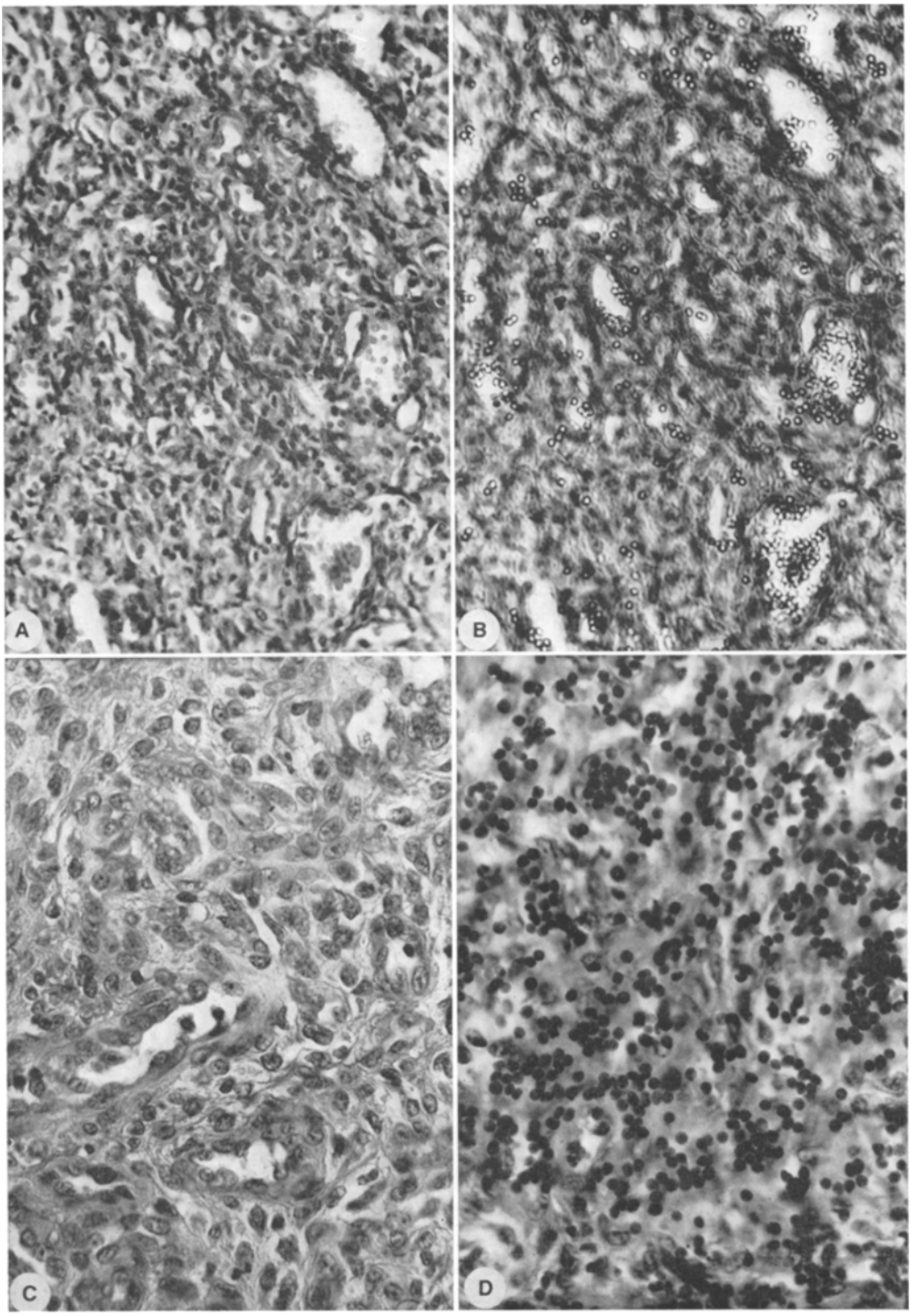

Fig. 2

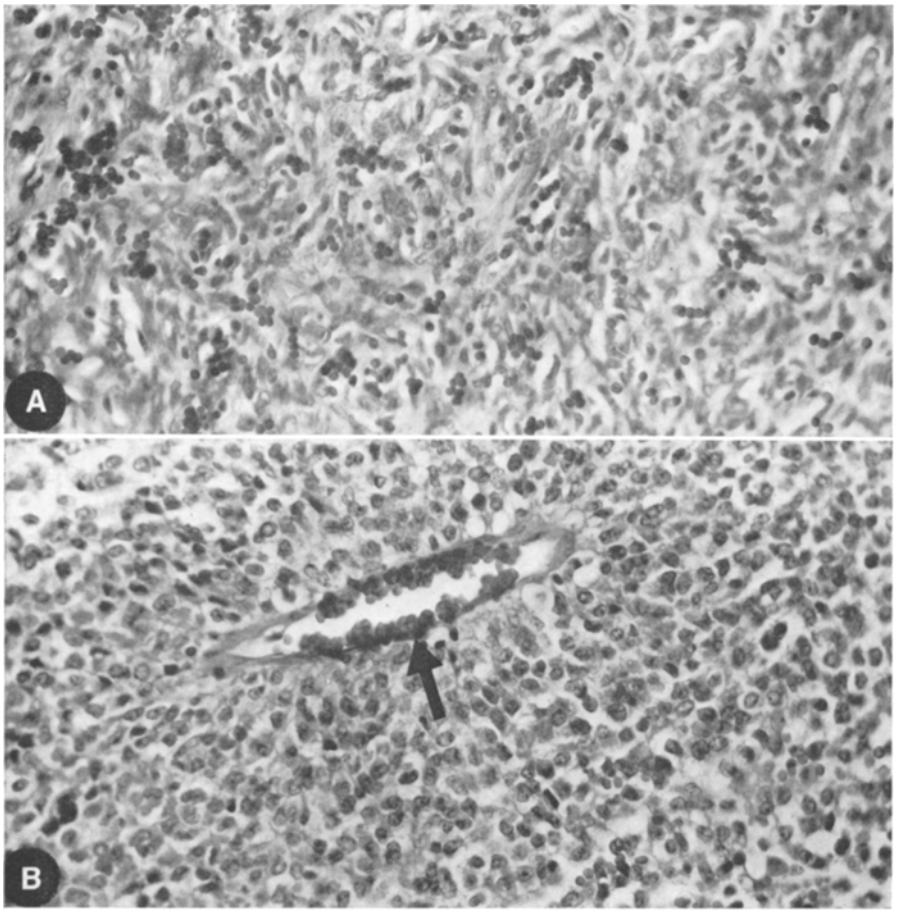

Fig. 3

Fig. 2. (A) Benign hemangioendothelioma, 1-year-old child (Case No. 6), BG A. Erythrocyte adherence to endothelial cells. H-E ×250. (B) Identical specimen after focusing on adhering erythrocytes in order to enhance the contrast. (C) Benign hemangioendothelioma, male, 75 years old (Case No. 5), negative control specimen. H-E ×400. (D) Benign hemangioendothelioma, same case, BG B. Note intensive positive reaction of most tumor cells. Lepehne's peroxidase reaction ×400

Fig. 3. (A) Benign hemangiopericytoma (Case No. 17), BG B. Capillary endothelial cells positive, surrounding cells (pericytes) negative. H-E ×250. (B) Malignant hemangiopericytoma (Case No. 18) BG A. BG negative tumor cells around a positive reacting vessel (arrow). H-E ×250

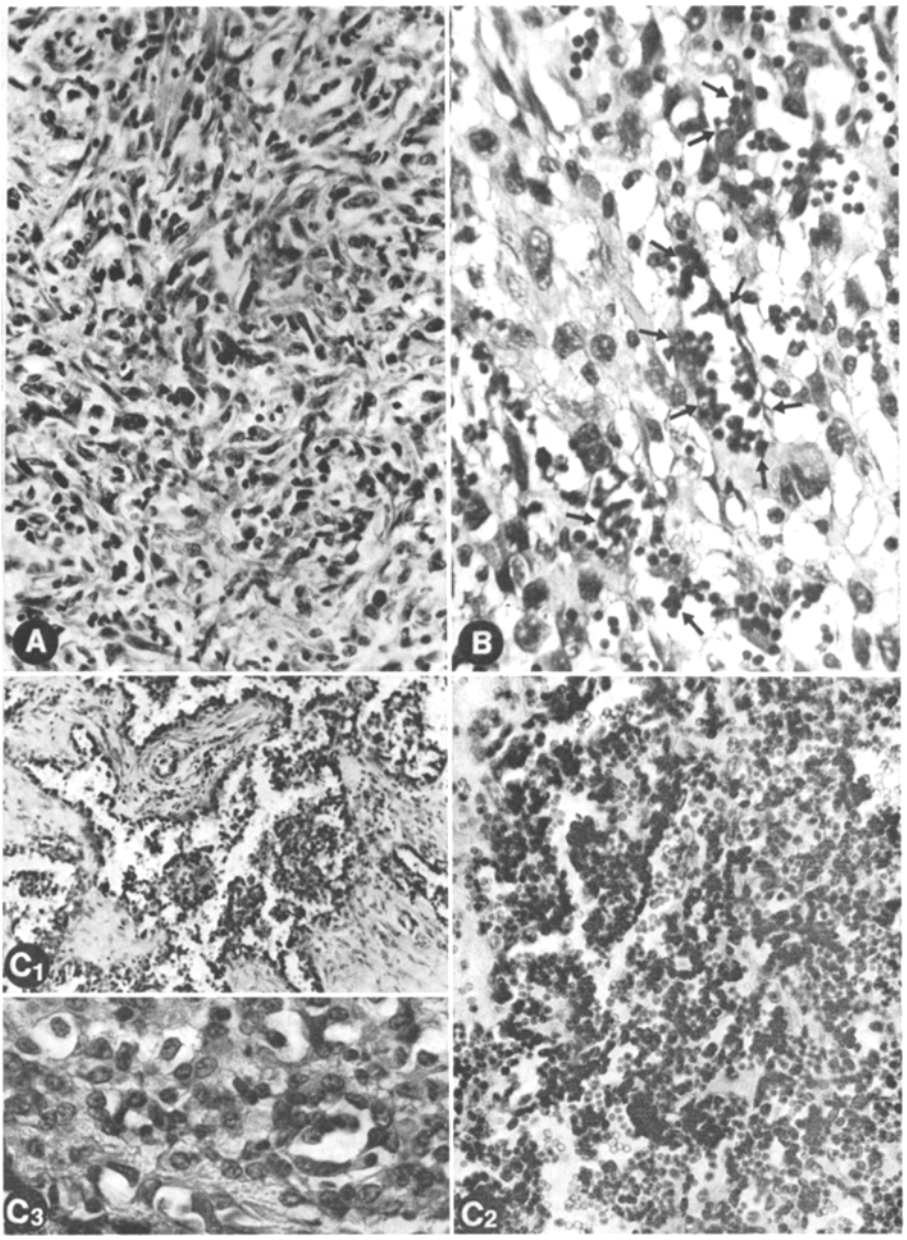

Fig. 4. (A) Angiosarcoma, liver (Case No. 19), BG A. Spindle-shaped tumor cells negative H-E ×250. (B) Angiosarcoma, thyroid gland (Case No. 24), BG A. BG-negative pleiomorphic tumor cells, elongated capillaries with positive nonneoplastic endothelial cells (arrow). H-E ×400. (C1) Angiosarcoma of forearm with BG-positive tumor cells (Case No. 28) BG A. × 100. (C2) Same case, H-E ×250. (C3) Same case, high magnification of typical area (control). H-E ×400

vessels. Although we do not want to draw any definitive conclusions at the present time because of the limited number of cases studied, we are inclined to believe that the juvenile type may be a malformation of the vascular system (Mahnke, 1969), the BG-negative cells being immature mesenchymal elements or perivascular structures (pericytes or fibroblasts). The adult type, however, may be truly neoplastic. This does, however, not exclude the possibility that one type may convert into the other under certain circumstances. These results only partly support the concept of Borst (1936), Geschickter (1958), and Stout (1967) who considered the benign hemangioendotheliomas to be exclusively the result of a proliferation of endothelial cells. But clearly this hypothesis requires further supporting studies.

In hemangiopericytoma, mixed cell agglutination reaction facilitates the discrimination of endothelial cells from pericytes. This technique, however, did not, as expected, allow the differentiation of hemangiopericytoma from glomus tumors.

A contribution to the diagnosis of malignant hemangioendothelioma was one of the major goals of the present investigation. The results indicate that this group of tumors is not homogeneous and needs further evaluation:

1. Most of the specimens studied (derived from liver, thyroid, adrenal, lung, and breast) did not express BG on their tumors cells. This lack could be interpreted either by the fact that these tumors arise from BG-negative cells, such as cells from the reticuloendothelial system, or that BG activity is lost during malignant transformation (Davidsohn, 1972). The continuity between normal Kupffer cells and tumor cells observed in experimentally induced liver angiosarcomas (Herrold, 1967; Lesch et al., 1967) indicates that the first assumption may be correct, at least in the liver. Kupffer cells can be distinguished from endothelial cells by electron microscopy and by their phagocytic capacity (Rappaport, 1972). Moreover, in contrast to sinusoidal endothelial cells, which are BG positive, Kupffer cells are devoid of BG (unpublished observation).

2. Case No. 28 behaved differently with respect to BG isoantigenicity and may be considered a malignant tumor of real endothelial origin. BG reactivity was retained in its solid as well as in its vasoformative parts. This might be one of the few rare cases of true hemangiosarcoma described by Stout and Lattes (1967). Moreover, it supports previous findings (Denk et al., 1974b) that malignant transformation does not necessarily lead to a loss of BG.

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