Desmin Is a Specific Marker for Rhabdomyosarcomas of Human and Rat Origin

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Putative human rhabdomyosarcoma (RMS) has been divided into two groups according to desmin content. Twenty-five tumors with histologic features consistent with but not necessarily sufficient to prove a diagnosis of RMS were desmin-positive. More than 95% of the tumor cells were desmin-positive, suggesting a muscle origin and supporting the diagnosis of RMS. Nine tumors for which the preferred first histologic diagnosis was also RMS were desmin-negative. Reexamination of the original histologic slides together with results from intermediate filament typing resulted in a diagnosis other than RMS for all tumors in this second group, and in several instances other tests were used to prove the correctness of the final diagnosis. The results on human material were extended to a rat model system in which RMS was induced by nickel sulfide. Again, all 24 tumors tested were desmin-positive. Vimentin was coexpressed in a varying percentage of tumor cells in RMS of human and rat origin. The results show that desmin is an excellent marker for rhabdomyosarcoma, yielding few if any false-positive or false-negative results in frozen or alcohol-fixed material. (Am J Pathol 1985, 118:85-95)

RMS IS THE MOST common soft tissue sarcoma in childhood.[1,2] The embryonal type[3] accounts for 50–65% of childhood RMS, and in this type the tumor cells are loosely packed and cross-striations are only rarely present. Sarcoma botryoides, a special variant of embryonal RMS, is distinguished by its polypoid growth. The alveolar subtype is typically found in adolescents and is characterized by a pseudoalveolar pattern in which connective tissue trabeculae are lined by undifferentiated rhabdomyoblasts. Pleomorphic RMS is an extremely rare neoplasm occurring in adults and often characterized by multinucleated giant cells. Progress in therapy for childhood RMS has been achieved with the use of a combination of surgery, radiation, and chemotherapy (for reviews see Maurer et al[4] and Treuner and Niethammer[5]). However, successful treatment is dependent on a correct morphologic diagnosis, especially because embryonal and alveolar RMS must be distinguished from other pediatric round-cell tumors, including neuroblastomas, malignant lymphomas, and extraskeletal Ewing's sarcoma.

In the last few years several immunohistochemical procedures have been introduced in an attempt to increase the accuracy in diagnosis of round-cell tumors in childhood, in particular, RMS. These include the use of antibodies to actin[6,7] or to myosin.[8] These investigations were necessarily disappointing, because structural proteins such as actin or myosin are not muscle-specific and are therefore found not only in RMS but also in other tumors. Antibodies to myoglobin have also been used[9] but do not identify all RMS.[10,11] A more interesting possibility is the use of antibodies in determining the intermediate filament protein type present in normal tissues and in pathologic material. Such methods yield information about the histogenetic origin of different cell types. In particular, it has been shown by studying histologic specimens of several hundred human tumors that carcinomas are keratin-positive, the majority of sympathetic derived tumors are positive for neurofilaments, astrocytomas express GFA, and non-muscle sarcomas as well as leukemias and malignant lymphomas contain only vimentin.[12–15] Of particular relevance to the current study is the finding that RMS

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Table 1 – Desmin-Positive Tumors

No.	Age (yrs)	Sex	Location of primary tumor	Material/ Fixation	Cytokeratin	Vimentin	Desmin	Neurofilaments	Diagnosis
1	10	F	Abdomen	B/F	–	+	+	–	Embryonal RMS
2	11	M	Parotid	B/F	–	+	+	–	Embryonal RMS
3	13	F	Orbit	B/E	–	(+)	+	–	Embryonal RMS
4	20	M	Foot	C/BM	–	(+)	+		BM infiltration, alveolar RMS
5	17	M	Abdomen	C	–	(+)	+	–	Embryonal RMS
6	5	M	Unknown*	B/E	–	–	+	–	Lymph node metastasis, embryonal RMS
7	11	F	Retropharyngeal tumor	B/E	–	(+)	+	–	Embryonal RMS
8	12	M	Urinary bladder	B/E	–	(+)	+	–	Embryonal RMS
9	15	M	Unknown†	B/E	–	+	+	–	Lymph node metastasis, alveolar RMS
10	15	F	Abdomen	B/C	–	–	+	–	Embryonal RMS
11	7	M	Urinary bladder	B/E	–	(+)	+	–	Embryonal RMS
12	8	M	Upper arm	C/BM	–	–	+	–	BM infiltration, embryonal RMS
13	9	F	Nasal cavity	C	–	+	+	–	Embryonal RMS
14	12	F	Orbit	B/E	–	(+)	+	–	Embryonal RMS
15	5	M	Prostrate	B/E	–	–	+	–	Embryonal RMS
16	5	F	Urinary bladder	B/E	–	(+)	+	–	Embryonal RMS
17	9	F	Abdomen	B/E	–	+	+	–	Embryonal RMS
18	7	M	Unknown†	C	–	+	+	–	Lymph node metastasis, alveolar RMS
19	0.5	M	Bile duct	B/E	–	–	+	–	Sarcoma botryoides
20†	4	M	Foot	B/E	–	+	+	–	Embryonal RMS
21	7	M	Sinus maxillaris	B/E	–	(+)	+	–	Embryonal RMS
22‡	10	F	Orbit, sinus maxillaris	B/E	–	–	+	–	Embryonal RMS*
23	15	F	Paravertebral soft tissues	C/BM	–	+	+	–	BM infiltration, alveolar RMS
24‡	5	M	Retroperitoneum	B/E	–	(+)	+	–	Embryonal RMS
25‡	7	F	Thigh	B/E	–	+	+	–	Embryonal RMS

+, all tumor cells positive (>95%); (+), 5–50% of tumor cells positive; B, biopsy; C, cytologic smears; E, ethanol; F, frozen section; BM, bone marrow.

* Lymph node metastasis in neck.

† Lymph node metastasis, supraclavicular.

‡ Cases described originally in Altmannsberger et al.[12] In this study Case 22 was listed as an undifferentiated sarcoma, probably rhabdomyosarcoma. In view of its desmin positivity, we now classify it as a rhabdomyosarcoma.

is desmin-positive.[12] Thus, RMS, like other neoplasms, appears to retain the intermediate filament type characteristic of the cell type of origin.

Because RMSs are relatively rare tumors, it is important to have an experimental animal system in which neoplasms with comparable morphologic characteristics can be studied. RMS in animals can be induced by inoculation with sarcoma viruses,[16] by treatment with chemical carcinogens,[17,18] or by implantation of different heavy metals or their derivatives, including nickel or nickel sulfide (Ni_3S_2).[19] When injected in bone or subcutaneously, nickel powder, as well as other nickel compounds, produces fibrosarcoma; but when injected in muscle, RMS develops (for a review see Furst and Haro[20]).

The two major objectives of this study were 1) to present our immunohistochemical findings on a large number of childhood RMSs which demonstrate that desmin is an excellent marker for this tumor; 2) to investigate the intermediate filament types present in Ni_3S_2-induced RMS in rats.

Materials and Methods

Childhood Rhabdomyosarcomas

Diagnosis, age, sex, location and method chosen for fixation are shown for the tumors included in the current study in Tables 1 and 2. Tumor samples were either fixed in ethanol and embedded in paraffin or were frozen directly in liquid nitrogen. In some cases specimens were obtained by fine-needle aspiration biopsy or from bone marrow metastases, and cytologic smears were prepared. Touch imprints were also used in two cases. The sections were stained with hematoxylin and eosin (H&E). Giemsa, Gomori, and periodic acid–Schiff (PAS); and the cytologic smears and touch imprints

Table 2—Desmin-Negative Tumors

No.	Sex	Age (yrs)	Location	First diagnosis*	Material/ Fixation	Cyto-keratin	Vimen-tin	Desmin	Neuro-filaments	Final diagnosis†
1	M	17	Lower leg	Alveolar RMS	B/E	–	+	–	–	Round-cell liposarcoma
2	M	18	Paravertebral	Pleomorphic RMS	B/F	–	+	–	–	Pleomorphic liposarcoma
3	M	1	Testis	Embryonal RMS	B/E	–	+	–	–	Malignant histiocytosis‡
4	F	13	Fibula	Embryonal RMS	B/E	–	+	–	–	Ewing sarcoma
5	F	0.5	Thigh	Embryonal RMS	B/E	–	+	–	–	Infantile fibrosarcoma
6	M	0.1	Orbit	Embryonal RMS	B/E	–	+	–	–	Unclassified nonmuscular sarcoma
7	M	5	Abdomen	Embryonal RMS	TI	–	+	–	–	Non-Hodgkin's lymphoma‡
8	F	4	Thorax	Embryonal RMS	B/E	–	+	–	–	Ewing sarcoma
9	F	3	Retroperitoneum	Embryonal RMS	TI	–	–	–	+	Neuroblastoma§

B, biopsy; E, ethanol; F, frozen section; TI, touch imprint.
* First diagnosis made by routine pathologic study from conventionally stained slides.
† Final diagnosis made from immunocytologic results and reexamination by routine pathologic study of the conventionally stained slides.
‡ Later confirmed by the use of surface markers specific to lymphatic cells or histiocytes.
§ Later granules were shown in electron microscopy, and elevated catecholamine levels were demonstrated.

were stained by the May–Grünwald–Giemsa (MGG) procedure.

Nickel Sulfide-Induced Rhabdomyosarcomas

Tumors were induced in 8-week-old male Wistar rats with an average weight of 80 g (Winkelmann, Borchenkirchen, FRG). The animals were divided into four groups, and all substances were injected into the gastromeric muscle.

Group 1

Fifteen animals were given injections of 0.2 ml Ni_3S_2 suspension (100 mg Ni_3S_2 in 1 ml physiologic saline).

Group 2

Fifteen animals were given injections of 0.2 ml Ni_3S_2 penicillin G (100 mg Ni_3S_2, 200,000 IU penicillin G in 1 ml physiologic saline).

Group 3

Five animals were given injections of 0.2 ml physiologic saline.

Group 4

Five animals were given injections of 0.2 ml penicillin G (200,000 IU penicillin G in 1 ml NaCl).

Twenty-four of 30 animals in Groups 1 and 2 developed tumors 2–3 cm in diameter at the site of injection after 4–6 months. No tumors were detected in the Groups 3 and 4 in the same time interval.

Tumor material was either ethanol-fixed and embedded in paraffin or, in a few cases, frozen directly in isopentane cooled to −140 C with liquid nitrogen. H&E-, Masson's trichrome-, and PAS-stained slides prepared from this material to further subdivide these experimental RMSs into different categories.

Immunohistology

Ethanol-Fixed Paraffin-Embedded Material

Sections, nominally 1–2 μ thick, were cut and dried for 1–2 hours at 37 C. They were subsequently deparaffinized with xylol and an alcohol series. All specimens were fixed in acetone at −10 C for 10 minutes. After a wash with phosphate-buffered saline (PBS), 10 μl of the first antibody was added and the slides were incubated for 45 minutes at 37 C. After having been washed well with PBS, 10 μl of the appropriate FITC-, rhodamine- or peroxidase-labeled second antibody was added, and the samples incubated for another 30 minutes at 37 C. Specimens incubated with FITC- or with rhodamine-labeled second antibodies were mounted in Mowiol 4-88 (Hoechst, Frankfurt, FRG). Specimens incubated with peroxidase-labeled second antibodies were treated with diaminobenzidine and H_2O_2 for development of the peroxidase stain, counterstained with hematoxylin, dehydrated, and mounted in Vitroclud.

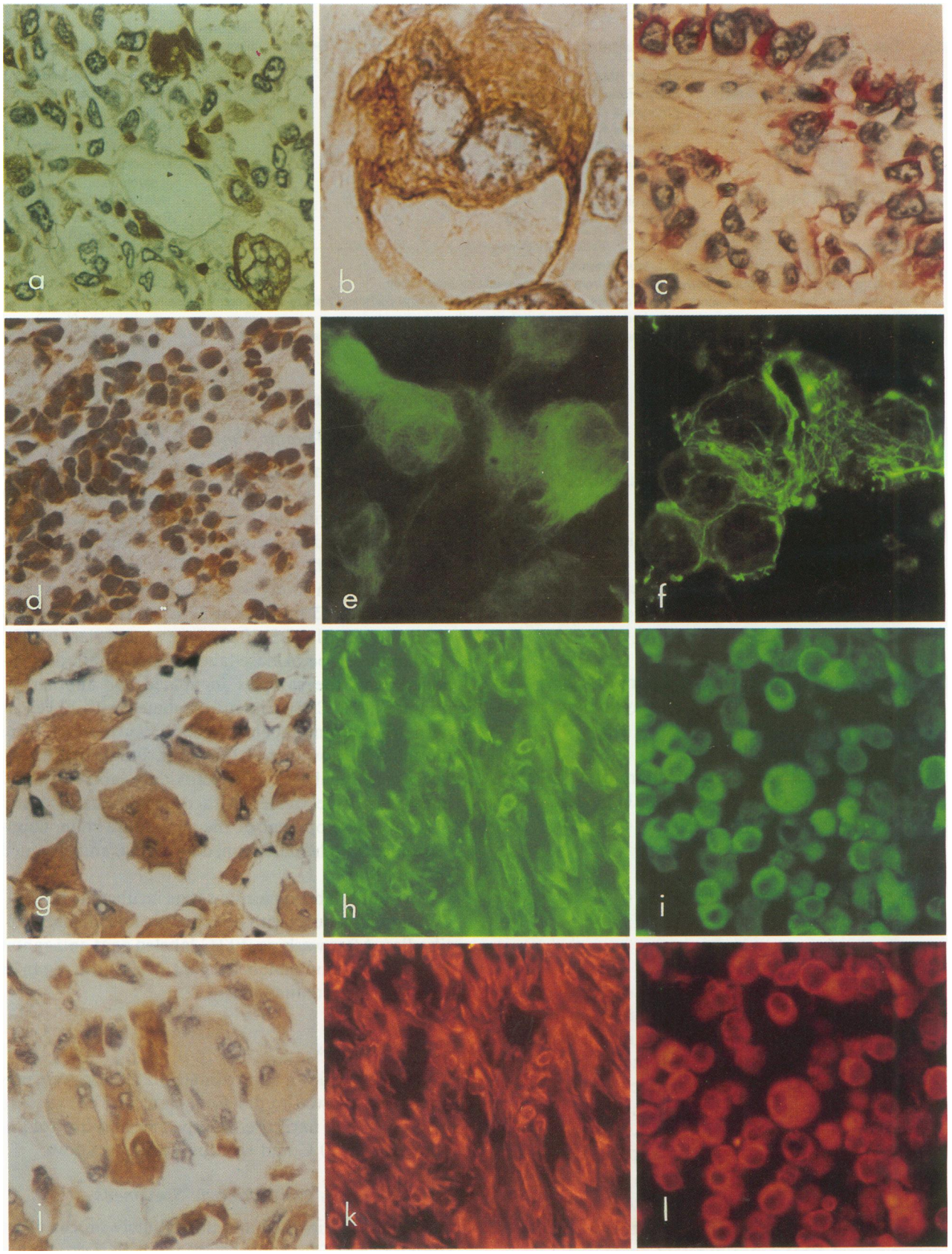
For double labeling sections were incubated after fixation simultaneously with both desmin and vimentin antibodies. After washing, FITC-labeled goat anti-rabbit IgGs and rhodamine-labeled goat anti-guinea pig IgGs were applied simultaneously.

Frozen Sections

Cryostat sections, approximately 5 μ thick, were prepared and allowed to dry for 1 hour at 37 C or were held at −70 C until use. All specimens were fixed in acetone at −10 C and then processed as above.

Touch Imprints and Cytologic Specimens

These were air-dried and immediately processed or stored at −20 C for some days if immediate processing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was impossible. Smears were fixed in cold acetone for 10 minutes and then processed as above.

Antibodies

Conventional and monoclonal antibodies each specific for one intermediate filament type have been described in detail elsewhere. Those used in the current study included the following.

Conventional Antibodies

1) Antibody was prepared in rabbits against desmin purified from chicken gizzard.[21] 2) Antibody was prepared in sheep or in guinea pig against vimentin from rabbit chondrocytes.[21] 3) Keratin antibody was made in guinea pig against keratin purified from cow snout.[21] 4) Antibody was prepared in rabbits against glial fibrillary acidic protein.[21] 5) Antibodies prepared in rabbits against neurofilaments purified from rat sciatic nerve which specifically recognized 68K or 200K polypeptide were used.[22]

First antibodies were used at 50 μg/ml; second antibodies were used at 400 μg/ml.

Monoclonal Antibodies

Monoclonal antibodies directed against desmin which do not recognize other intermediate filament types have been described.[23] In the current study DE-B-5 was used.

The supernatant was used directly, and the second antibody in this case was FITC-labeled goat anti-mouse IgGs (Cappel Laboratories, Cochranville, Pa).

Results

Tables 1 and 2 list the cases sent to us in the last 3 years as possible RMS. Immunohistologic study, or in some cases immunocytologic study, with desmin antibodies allowed the classification of these tumors into two groups. Those that were desmin-positive are listed in Table 1, and those that were desmin-negative are listed in Table 2.

Human RMS: All Positive for Desmin

Of those tumors classified as desmin-positive in more than 50% of the cases a diagnosis of RMS was apparent with the use of the usual pathologic criteria[3] without recourse to immunohistologic study. However, the remaining cases, although desmin-positive, showed morphologic features typical of round-cell tumors; without the use of the desmin stain these might initially have been classified as Ewing's sarcoma, lymphoma, or neuroblastoma, rather than as RMS.

Different histologic types of childhood RMS, all of which stained positively with antibodies to desmin, are shown in Figures 1 and 2. Thus, the tumor cells in embryonal RMS (Figure 1a–c), as well as the sarcoma botryoides subtype (Figure 2e), were strongly desmin-positive; and in the instances we have studied >95% of the tumor cells were desmin-positive, whereas cells in the stroma were negative for desmin. In the 4 cases of alveolar RMS the tumor cells were also strongly desmin-positive (Figure 1d, Table 1). Demonstration of desmin in tumor cells of RMS was possible not only in histologic sections but also in cytologic specimens obtained by aspiration biopsy cytologic study. Figure 2c shows a smear obtained by fine-needle aspiration biopsy of an intranasal RMS stained by antibodies to desmin. By the combination of May–Grünwald–Giemsa stain and immunocytologic study a definitive diagnosis could be made in this case and in the other cases cytologically studied, which are listed in Table 1. A further important use of desmin immunocytology is the detection of single tumor cells in bone marrow of patients with Stage IV RMS. This is illustrated in Figure 1e, where desmin-positive tumor cells in bone marrow were found.

Coexpression of desmin and vimentin could be found in varying numbers of RMS tumor cells (eg, Figure 2a–d). For example, in the embryonal RMS shown in Figure 2a and b all tumor cells coexpress desmin and vimentin. In contrast, for the embryonal RMS shown in Fig. 2c, d, while all tumor cells are positive for desmin only a few coexpress vimentin. Examination of Table 1 shows that in the 25 cases studied vimentin was coexpressed in all tumor cells in 9 cases, in 5–50% of

Figure 1—RMS of human (**a–e**) and rat (**g–l**) origin stained with antibodies to desmin (**a–e** and **g–l**) or to vimentin (**j–l**). Note the strong staining of the tumor cells in the human embryonal RMS (**a–c**), human alveolar RMS (**d**), as well as in the bone marrow infiltration (Stage IV) of embryonal RMS shown in **e**. Not only the large rhabdomyoblasts (**a** and **b**), but also the small tumor cells (**a**, **c**, and **d**) are desmin-positive. Shown for comparison in **f** is a touch imprint from a neuroblastoma stained with an antibody to neurofilaments. Staining with desmin and vimentin antibodies of the three types of RMS induced by nickel sulfide are shown in **g**, **j**; **h**, **k**; and **i**, **l**), respectively. Whereas the tumor cells in all three types are desmin-positive, the tumor cells of the three types differ in their vimentin content. Thus, in the rhabdomyoblastic type (**g** and **j**), only the less differentiated small cells are vimentin-positive (**j**). In contrast, in the spindle-cell (**h** and **k**) and in the round-cell types (**i** and **l**) all tumor cells show coexpression of desmin and vimentin. (**a–d** and **g–i**, ethanol-fixed and paraffin-embedded; **e**, cytologic smear; **a**, **b**, **d**, **g**, and **j**, peroxidase; **c**, alkaline phosphatase; **e**, **f**, **h**, and **i**, fluorescein- and **k** and **l**, rhodamine-labeled, second antibodies)

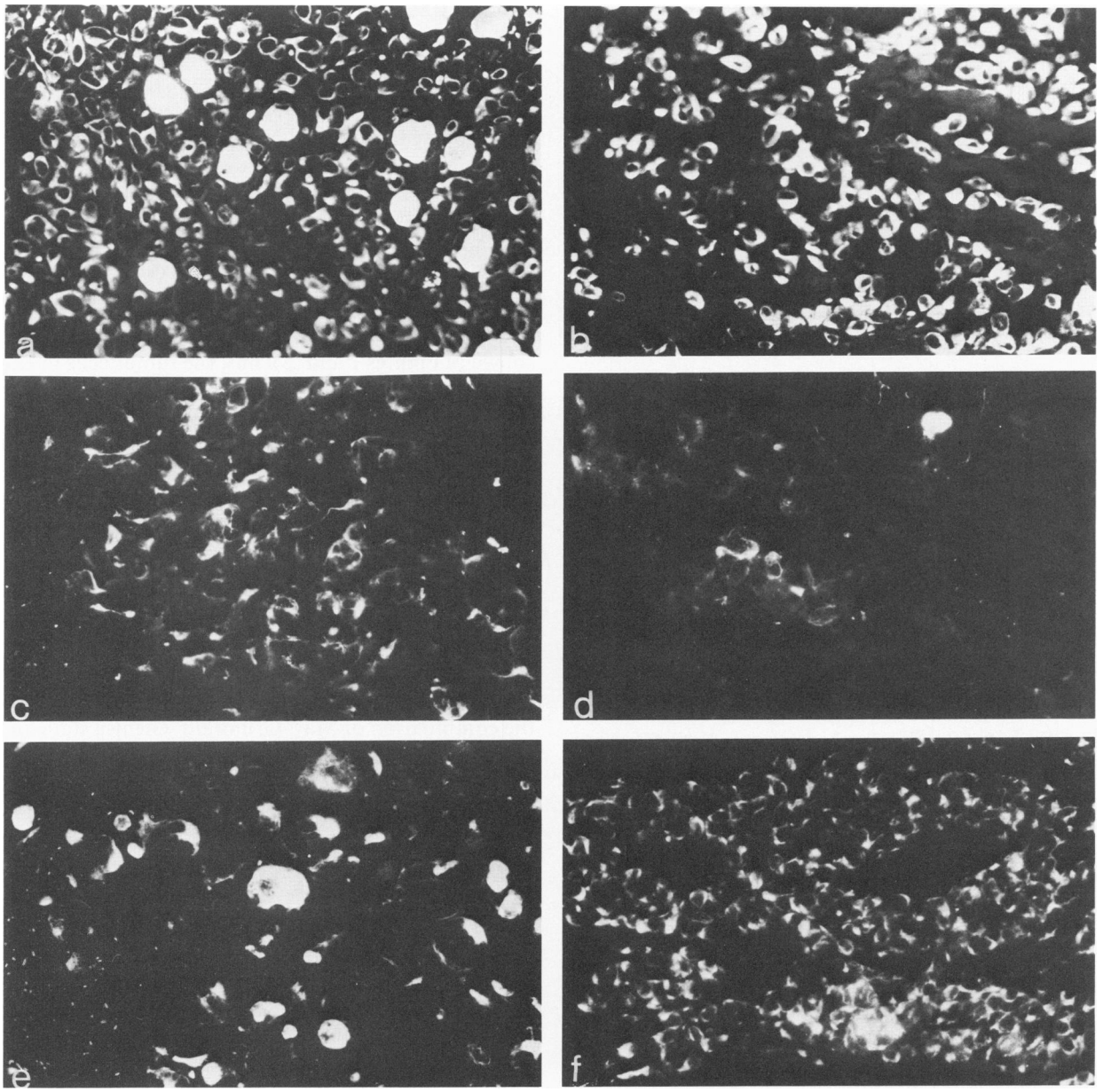

Figure 2—Human embryonal RMS (**a**–**d** and **f**) and human sarcoma botryoides (**e**) stained by conventional antibodies to desmin (**a**, **c**, and **e**) and vimentin (**b** and **d**) or by monoclonal antibodies to desmin (**f**) and viewed with immunofluorescence. All types of RMS are clearly desmin-positive. In the tumor shown in **a** and **b** all tumor cells show coexpression of desmin and vimentin, in the tumor shown in **c** and **d** all cells are desmin-positive, while only a few coexpress vimentin. (**a**, **b**, **e**, and **f**, ethanol-fixed and paraffin-embedded; **c** and **d**, cytologic smear)

the cells in 10 cases, and not observed in 6 cases. As expected, cells in the stroma were vimentin-positive.

Round Cell Tumors Other Than RMS Negative for Desmin

Table 2 lists tumors where on first diagnosis by conventional pathologic techniques RMS was the favored diagnosis, although in several instances other round-cell tumors such as neuroblastoma, lymphoma, and Ewing's sarcoma could not be excluded. After intermediate filament typing the cases listed in Table 2 were desmin-negative but positive for either vimentin (Cases 1–8) or for neurofilaments (Case 9). These findings resulted in a reevaluation of the normal histologic slides by pathologists expert in the diagnosis of pediatric neoplasms and unconnected with the immunohistologic aspects of this study. This reevaluation, taken together with the immunohistologic findings, resulted in the final diagnosis listed in the last column of Table 2. Tumors

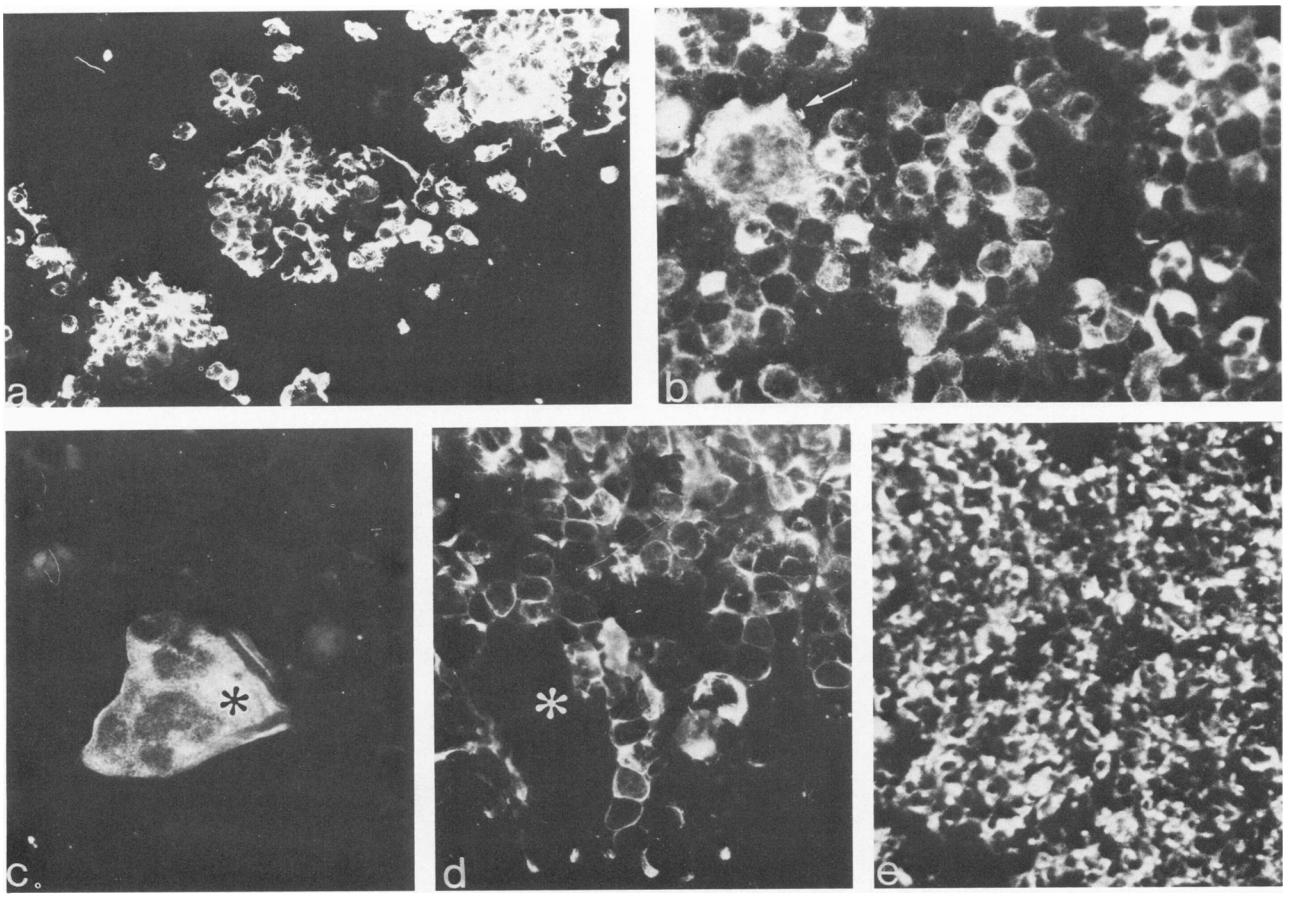

Figure 3a–e — Lesions and tumors other than RMS identified with the use of intermediate filament antibodies in immunofluorescence microscopy in human material. **c** — Hepatoblastoma stained with a broad specificity keratin antibody. Note the coherence of the cytokeratin-positive tumor cells. **b** — Hodgkin's disease stained with a vimentin antibody. Note that the Hodgkin cells (*arrow*) as well as the accompanying lymphatic cells are strongly vimentin-positive. **c** and **d** — Branchial cleft cyst, parallel smears stained either with a broad-specificity antibody to keratin (**c**) or to vimentin (**d**). Note the two different parts of this lesion. Thus, the squamous epithelial cells are cytokeratin-positive and vimentin-negative (asterisks), while the lymphatic cells are cytokeratin-negative, vimentin-positive. **e** — Ewing's sarcoma stained with vimentin antibodies. The tumor cells are strongly positive. (**a–d**, cytologic smears; **e**, ethanol-fixed, paraffin embedded)

such as round-cell liposarcoma, Ewing's sarcoma, infantile fibrosarcoma, and non-Hodgkin's lymphoma have been previously shown to contain only vimentin intermediate filaments, whereas in neuroblastomas neurofilaments have been reported.[12–15] In three instances proof that the final diagnosis was correct was subsequently provided with the use of additional assays. Thus, in the case of non-Hodgkin's lymphoma, surface markers specific to lymphatic cells were demonstrated; and in the case of malignant histiocytosis, surface markers specific to histiocytes were shown. In the case of neuroblastoma, granules were observed by electron microscopy, and an elevated catecholamine level was demonstrated.

The current study, as well as previous work, clearly demonstrates that intermediate filaments are useful markers for differentiation of round-cell tumors of children. This is further illustrated in Figures 1 and 3. Thus, tumor cells in at least some neuroblastomas contain neurofilaments (Figure 1f); whereas Ewing's sarcoma, Hodgkin's disease, and lymphoma contain only vimentin filaments. Figure 3e shows a case of extraskeletal Ewing's sarcoma which stains positively with vimentin antibodies. Figure 3b shows a case of Hodgkin's disease with positive staining by vimentin antibodies. In nephroblastomas blastema cells either coexpress cytokeratin and vimentin[24] or in some instances express only vimentin.[24,25] In hepatoblastoma the tumor cells are keratin-positive (Figure 3a). Finally, the squamous epithelial cells in lateral residual cysts are cytokeratin-positive (Figure 3d and e). In all these tumors, cells in the stroma are vimentin-positive.

Nickel Sulfide-Induced Rat RMS

Eighty percent of the rats in Groups 1 and 2 which had been given nickel sulfide developed a circumscribed tumor at the site of injection after 4–6 months. The

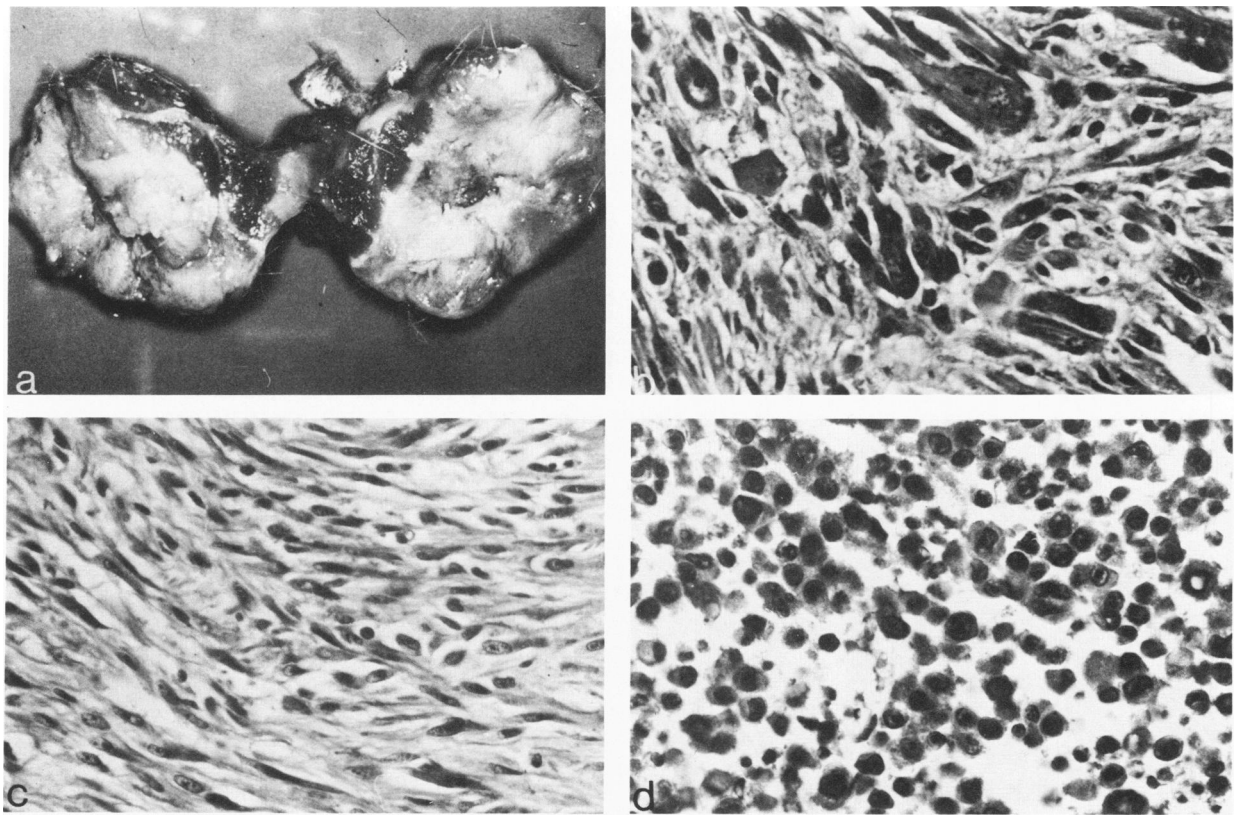

Figure 4 — Nickel sulfide-induced RMS in the rat. **a** — Macroscopic view of pseudoencapsulated gray-white tumor cut to reveal a central area with hemorrhage and necrosis. **b–d** — Three different light microscopic types of rat RMS as revealed by the Masson's trichrome stain on ethanol-fixed paraffin-embedded material. **b** — Rhabdomyoblastic type. Note the large multinucleated cells with eosinophilic cytoplasm. **c** — Spindle-cell type, elongated small cells with spindle-shaped centrally located nuclei. **d** — Round-cell type with round or oval-shaped tumor cells with eccentric nuclei and strongly eosinophilic cytoplasm.

animals in Groups 3 and 4 did not develop tumors. Most of the tumors in the gastronemic muscle were encapsulated macroscopically and on cutting showed surface hemorrhaging and necrosis (Figure 4a). The histologic features of these tumors demonstrated three different rhabdomyoblastic types (Figure 4b–d). The first type (Figure 4b) is characterized by the presence of highly differentiated myoblasts with three or four nuclei and a large eosinophilic cytoplasm. In some tumor cells cross-striations were evident. The second tumor form (Figure 4c) consisted of spindle cells with elongated nuclei and a small eosinophilic cytoplasm. When compared with the rhabdomyoblastic type, the mitotic rate was increased, whereas cross-striations were not detected. The third, round cell type (Figure 4d) showed the greatest similarity to childhood RMS (compare, for instance, Figures 4d with 1c and 2b). Tumor cells were round or oval and had one or two eccentrically located nuclei. The cytoplasm was eosinophilic, the number of mitoses was increased, and a high number of atypical mitoses were present. In this tumor type necrosis was very common. Only a few tumors were composed of a single histologic type, and most showed a combination of the three histologic types.

When the nickel sulfide-induced rat RMSs were tested with antibodies to intermediate filaments tumor cells in all specimens were strongly positive with antibodies to desmin. More than 95% of the tumor cells were desmin-positive in the 24 tumors studied (Figure 1g–i). A coexpression of desmin and vimentin, such as is found in immature muscle, could be demonstrated in the small cells present in the rhabdomyoblastic type as well as in almost all tumor cells in the spindle-cell and round-cell RMS studied by this technique. Figure 1g and j show similar sections of a rhabdomyoblastic tumor stained with antibodies either to desmin (Figure 1g) or to vimentin (Figure 1j). When the desmin antibody was used, the multinucleated tumor cells, as well as the small tumor cells, are clearly positive (Figure 1g). In contrast, when the vimentin antibody was used, the large cells were negative and the small cells were positive (Figure 1j). Coexpression of desmin and vimentin in the spindle-cell type is demonstrated in Figure 1h and k. The spindle-shaped tumor cells reacted strongly with the

desmin antibody (FITC, Figure 1h) and also with the vimentin antibody (rhodamine, Figure 1k). Similar results were also found in the round-cell tumors that were studied. Again, tumor cells were brightly labeled by the desmin antibody (FITC, Figure 1k), and the same cells were labeled by the vimentin antibody (rhodamine, Figure 1l).

Use of Monoclonal Antibodies to Desmin

We have recently described the isolation and characterization of a series of monoclonal antibodies specific for desmin.[23] These monoclonal antibodies identify tumor cells in RMS of human (Figure 2g) and rat origin.

Discussion

Our results demonstrate that desmin is a very useful marker, allowing a positive identification of RMS and distinguishing this tumor from the other round-cell tumors of children. In the cases listed as positive in Table 1 >95% of the tumor cells were positive. All 25 desmin-positive cases in Table 1 have features in conventional histology consistent with a diagnosis of RMS, although in up to 50% of these cases an unambiguous diagnosis of RMS cannot be made from the routinely stained slides. The 9 cases listed as examples in Table 2, although originally classified by conventional histology as RMS, were desmin-negative, and these tumors could on reexamination be reassigned to another tumor type. In some of these cases further examination of the original histologic slides already suggested an error in diagnosis; in other cases, the final diagnosis could not have been made without the results obtained from intermediate filament typing. In three of the cases in Table 2 confirmation that the change in diagnosis was correct was obtained with the use of accepted markers for lymphatic cells or histocytes, by the use of electron microscopy for demonstration of granules, or by the demonstration of an increased catecholamine level.

The use of desmin as a marker specific for RMS and for leiomyosarcoma would be consistent with the finding from cell biologic studies that desmin expression is an early event in the differentiation of skeletal and smooth muscle cells.[21,26,28] Thus, the finding that not only the large rhabdomyoblasts but also the more numerous smaller, less well differentiated tumor cells are desmin-positive[12] is perhaps expected. However, although the use of desmin as a marker for RMS has been confirmed in another study,[29] other groups using different desmin antisera have not always been able to identify RMS reliably. Thus, in the 1 case studied by Gabbiani et al,[13] and in 2 further cases studied with the use of the same antibody,[30] only the large rhabdomyoblasts were desmin-positive. In addition, Kahn et al[31] report that only 8 out of 25 of their RMSs were desmin-positive, a result which may be attributable to the different fixation methods used (M. J. Phillips, personal communication) or could perhaps be due in some instances to difficulties in diagnosing RMS from the morphologic characteristics alone. As shown here and previously,[23] monoclonal antibodies to desmin stain both rhabdomyoblasts and the smaller cells present in rhabdomyosarcomas of human and rat origin positively and specifically, and these monoclonal antibodies therefore appear to be of use in human tumor diagnosis, although clearly more tumors have to be tested. Desmin antibodies do not allow the separation of RMS from leiomyosarcoma or from myoma,[32] but this can generally be done first on the grounds of morphology and secondly by the fact that leiomyosarcoma and myoma are extremely rare in childhood.

Our results with desmin (25 of 25 RMSs desmin-positive, small cells as well as rhabdomyoblasts desmin-positive) can be compared with data in which myoglobin has been used.[9-11,31] These results show that in one study[11] 13 of 17 RMSs were myoglobin-positive; whereas in a second study,[10] although 11 of 12 proven RMSs with cross-striations were myoglobin-positive, only 5 of 24 cases of undifferentiated embryonal sarcomas were myoglobin-positive. Kahn et al report that 11 of 25 cases of embryonal rhabdomyosarcoma were myoglobin-positive. A further and more serious problem with the use of myoglobin as a marker is that in many instances relatively few of the tumor cells are positive, in contrast to desmin, where >95% of the tumor cells are positive. Thus, it is easier to distinguish putative RMS from other tumors in which muscle differentiation in single cells is found, eg, in müllerian tumors, in nephroblastomas,[24] and in malignant Triton tumors,[33] with desmin rather than with myoglobin as a marker. Similarly, it is easier to distinguish true RMS from other tumors or reactive processes in which single pleomorphic muscle cells are present with the desmin antibody. We assume this is because desmin is expressed early in muscle development,[26,27] whereas myoglobin is expressed only later in skeletal muscle development. This explanation is not consistent with the finding of three RMSs by Kahn[31] that are myoglobin-positive and desmin-negative, and we would assume that the formalin fixation used by Kahn may have decreased the number of desmin-positive cases. Again, such differences seem to emphasize the necessity for taking fixation methods into consideration in assessing immunocytochemical results.

Intermediate filament typing of Ni_3S_2-induced rat RMS showed that the tumor cells in 24 of 24 instances were desmin-positive. Previous descriptions of Ni_3S_2-induced RMS[34-36] have shown two main groups of

tumor cells, ie, round or spindle-shaped undifferentiated immature tumor cells and differentiated "strap" or "tadpole" cells with or without cross-striations. Ultrastructural studies have demonstrated that Z-lines and myofibrils can be detected only in better differentiated myoblasts or myotubes, whereas round or spindle-shaped cells are characterized by randomly arranged microtubules and 70 Å filaments.[36] In addition, in neoplastic cells the myotubes, myoblasts, sarcomeres, and myofibrils are seldom completely normal in structure. Our studies by light microscopy allow the classification of the tumor cells in three groups: round-cell type, spindle-cell type, and rhabdomyoblastic type. In the majority of cases the three types are present within one tumor. The round cells and the spindle cells were found within the less differentiated proliferating tumor regions, as documented by the high number of mitoses present. In contrast, the rhabdomyoblastic type usually exhibited more advanced maturation, as indicated by multinucleation and by the presence of cross-striations.

Do the results with intermediate filament typing help to further subdivide RMS? In the rat model system most tumor cells in the round and spindle types coexpress desmin and vimenntin, whereas in the more mature rhabdomyoblastic type only a minor fraction of the cells coexpress vimentin. In human RMS this coexpression is variable. Thus, vimentin is sometimes seen in all cells, sometimes in 5–50% of the tumor cells, and sometimes not at all (Table 1). Whether the biology and prognosis of tumors which show coexpression are different from those of tumors characterized only by the desmin type of intermediate filaments is not known. However, the rat and human RMS spectra can be viewed as mimicking normal muscle development, in that there appears to be an RMS type in which vimentin is not present in line with the idea that vimentin expression is lost during normal muscle maturation.[21,26] Neither the rat nor the human studies support the idea of a class of primitive RMSs which are vimentin-positive, desmin-negative, in agreement with the finding that skeletal muscle cells *in situ* are desmin-positive.

Although it may seem presumptuous to reclassify tumors on the basis of a single immunologic marker, it should be stressed that in each of the desmin-negative tumors support for the reclassification was obtained either from a reexamination of the original histologic features or from other tests. In addition, the evidence suggesting that intermediate filaments are cell-type–specific markers is now extremely strong, not only for normal tissues but also for tumors (for a review see Osborn and Weber[15]). Thus, epithelial cells as well as carcinomas are without exception keratin-positive if a broad-specificity keratin antibody is used; most, but not all, neuronal cells, as well as tumors derived from such cells, display neurofilaments; glial cells such as astrocytes and Bergmann glia display GFA, as do tumors derived from such cells; cells such as fibroblasts, chondrocytes, etc., contain only the vimentin type of intermediate filaments, as do, for example, nonmuscle sarcomas, lymphomas, and solid leukemias. Desmin is characteristic of skeletal muscle, visceral, and certain vascular smooth-muscle cells and, as demonstrated here and elsewhere, is also found in RMS and leiomyosarcomas. The findings detailed here on RMS provide further evidence encouraging use of antibodies specific for the different intermediate filament types in situations where a degree of uncertainty exists in the diagnosis agreed on by conventional pathologic techniques, and where often the preferred treatment is critically dependent on the diagnosis, eg, in the small round-cell tumors of children[12,24,25,28,29,37] and in cytologic specimens.[38,39]

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