Blood Vessel Tumorigenesis by 1,2-Dimethylhydrazine Dihydrochloride (Symmetrical)

Gross, Light and Electron Microscopic Descriptions. I.

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Administration of 0.001% 1,2-dimethylhydrazine dihydrochloride, symmetrical, in the drinking water of 7-week-old randomly bred Swiss mice for the remainder of their lifetime induced blood vessel tumors and enhanced the incidence of lung neoplasms. Ninety-eight percent of the females and 92% of the males developed vascular lesions, whereas among the controls the incidence was 3% in the females and 1% in the males. In addition, the incidence of lung tumors rose from 12 to 44% in the females and from 10 to 24% in the males, as compared with the controls. The occurrence of the vascular tumors in order of decreasing frequency was as follows: muscle, pararenal, fat, liver, parametrial, paraepididymal tissues, etc. Gross, light and electron microscopic examinations of vascular lesions revealed the characteristic appearance of angiosarcomas. The type and extent of macroscopic and histologic involvements of the various tissues by the tumors are presented. The ultrastructural descriptions of hemorrhagic areas, vascular spaces, neoplastic endothelial cells, their cytoplasms and organelles are illustrated in detail.

In conclusion, whereas hydrazine enhanced the development of lung tumors, when the dimethyl group was attached to it at symmetrical positions, it evoked vascular tumors. Thus, the present study provides evidence for the possible relationship between chemical structure and tumor induction at specific organ sites. (Amer J Path 64:585–600, 1971)

STUDIES using substituted hydrazines to determine the possible relationship between chemical structure and tumor development at specific organ sites have been in progress in this laboratory since 1968. This class of chemicals was selected because it was postulated that the tumor types could possibly be determined by the radicals attached to the original hydrazine molecule. These compounds were thought to be particularly suitable for such a study since hydrazine by itself and as the sulfate was found to be an inducer of lung tumors in mice.[1–3] Hydrazine was also quite suitable chemically

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since it seemed to be relatively easy to attach radicals to it and it is a stable compound. Thus, in contrast to other classes of chemicals, such as nitrosamines,[4,5] where the base compound is unstable and therefore could not be utilized for biologic experiments, the chemicals used in our studies appeared to offer clear advantages.

The current report deals with the oncogenic effect of 1,2-dimethylhydrazine dihydrochloride, symmetrical, in mice. Earlier, several studies of this compound were performed, using subcutaneous injections. This chemical was shown originally to induce intestinal tumors in BD rats[6]; this was later confirmed in Sprague-Dawley and Wistar strains.[7] In golden hamsters, it induced liver stomach, and intestinal tumors[8]; strangely enough in mice at the first attempt it was found to be without any apparent carcinogenic action.[9] This study was followed by another that clearly demonstrated the capability of this compound to induce intestinal tumors in mice.[10]

Materials and Methods

Swiss albino mice from the colony randomly bred by us since 1951 were used. They were housed in plastic cages with granular cellulose bedding, and separated according to sex in groups of 10. Rockland diet in pellets and tap water containing the chemical were given *ad libitum*.

The chemical used was 1,2-dimethylhydrazine dihydrochloride, symmetrical (DMH) (K and K Laboratories, Inc, Plainview, NY). The experimental group and the controls are described below:

Group 1. 1,2-DMH was dissolved in the drinking water as a 0.001% solution and was given continuously for the lifespan of 50 female and 50 male mice that were 7 weeks old (50 days) at the beginning of the experiment. The solution was prepared thrice weekly and the total water consumption containing 1,2-DMH was measured at the same intervals during the treatment period. The average daily consumption of DMH-containing water per animal was 5.8 ml for the females and 8.7 ml for the males. Therefore the average daily intake of 1,2-DMH was 0.058 mg for a female and 0.087 mg for a male.

Group 2. As a control, 110 females and 110 males were untreated.

The experimental and control animals were carefully checked and weighed at weekly intervals, and changes in the skin and subcutaneous structures recorded.

For light microscopic examination, the animals either were allowed to die spontaneously or they were killed with ether when they were found to be in poor condition. Complete necropsies were performed on all animals. All organs were examined macroscopically and were fixed in 10% buffered formalin. Histologic studies were done on liver, spleen, kidneys and at least four lobes of the lungs of each mouse as well as on those organs that showed gross pathologic changes. Sections from these tissues were stained routinely with hematoxylin and eosin and by additional special methods when necessary.

For electron microscopic examination, representative samples of the tumor tissues (approximately 1-mm cubes) were fixed in buffered 1% osmium tetroxide at 4 C.[11,12] The specimen blocks were dehydrated in ethanol and passed through propylene oxide and embedded in araldite (502) epoxy resin mixture.[13] Thick sections (1 μ) were cut from each block and the slides were stained with 0.2% tolui-

dine blue [14] and examined by light microscopy. Thin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome and stained with lead citrate [15,16] and uranyl acetate.[17] Finally, the sections on bare grids were examined at 60 kV in a Philips 300 electron microscope.

Results

The survival rate at 10-week intervals is recorded in Table 1. It is clear from this that DMH treatment substantially reduced the survival compared with the corresponding controls.

Table 2 presents the numbers of various types of tumors, their percentages and latent periods. The two most important lesions are described below in detail.

Tumors of Blood Vessels

In the DMH-treated females, 49 mice (98%) developed such tumors. The average latent period was 45 weeks, the first was found at 28 weeks and the last at the 58 weeks of age. In the DMH-treated males, 46 animals (92%) developed vascular tumors. Their average latent period was 42 weeks, the first was observed at 29 weeks and the last at 58 weeks of age.

Table 3 lists the location of these lesions in the various tissues. In the females, the occurrence of tumors in the order of decreasing frequency was as follows: muscle, liver (Fig 1), pararenal (Fig 2), fat and parametrial tissues, etc, while in males the order was: pararenal, muscle, fat (Fig 3), paraepididymal tissues (Fig 4), livers, etc.

Grossly, the tumor often grows in nodular form, ranging from 3 to 20 mm in diameter and exhibiting soft hemorrhagic consistency (Fig 2–4). Sometimes the lesion grows in an uncircumscribed diffuse

Table 1—Treatment and Survival Rate in DMH-Treated and Control Swiss Mice

Initial No. and sex of mice	No. of survivors												
	Age (weeks)												
	10	20	30	40	50	60	70	80	90	100	110	120	130
DMH - Treated* Group													
50 F	50	49	48	41	14	—	—	—	—	—	—	—	—
50 M	50	49	48	30	7	—	—	—	—	—	—	—	—
Control Group													
110 F	109	109	107	104	96	89	73	57	41	23	11	1	—
110 M	110	95	91	86	67	55	41	22	6	1	1	—	—

* 0.001% DMH in drinking water during entire lifetime.

Table 2—Treatment and Tumor Distribution in DMH-Treated and Control Swiss Mice

Effective No. and sex of mice	Incidence of animals with tumors: Vessel Tumors No.	%	Latent period* (age in weeks)	Lung Tumors No.	%	Latent period* (age in weeks)	Other tumors†	
DMH-Treated Group‡								
50 F	49	98	45 (28–58)	22	44	49 (31–58)	1 Hepatoma (48)	
50 M	46	92	42 (29–58)	12	24	44 (29–58)	1 Liver cell carcinoma (53) 1 Fibrosarcoma, subcutaneous (37) 1 Papilloma of forestomach (44)	
Control Group								
110 F	4	3	68 (42–84)	14	12	90 (64–119)	1 Luteoma (99) 1 Subcutaneous fibroma (87) 1 Papilloma of forestomach (112) 1 Granulosa cell tumor (65) 1 Malignant plasmacytoma (71)	1 Sex-cord mesenchymal tumor (99) 3 Subcutaneous sarcomas (68, 82, 82) 9 Adenocarcinomas of breast (46, 60, 64, 68, 73, 91, 93, 98, 110) 16 Malignant lymphomas (39, 45, 48, 56, 69, 72, 72, 77, 84, 85, 89, 96, 98, 98, 102, 115)
110 M	2	1	76 (72–80)	11	10	74 (47–110)	2 Malignant lymphomas (73, 82)	

* Average and range. † Latent period given in parentheses. ‡ 0.001% DMH in drinking water during entire lifetime.

Table 3—Location of Vessel Tumors in DMH-Treated Swiss Mice

50 Females		50 Males	
Organs or tissue	No. of mice	Organs or tissue	No. of mice
Muscle	40	Pararenal	39
Liver	37	Muscle	37
Pararenal	36	Fat	36
Fat	32	Paraepididymal	28
Parametrial	15	Liver	26
Subcutis	9	Subcutis	15
Ovaries	8	Lymph Nodes	12
Lymph Nodes	4	Pancreas	3
Kidneys	3	Lungs	2
Adrenals	3	Kidneys	2
Lungs	2	Skin	1
Pancreas	1	Adrenals	1
Heart	1	Stomach	1
		Spleen	1
		Heart	1

manner, invading the surrounding tissues with the formation of adhesions (Fig 1). Subcutaneous generalized edema, hemoperitoneum and anemia were commonly associated with development of the tumors.

Light microscopic examination revealed the characteristic appearance of angiosarcomas. The lesion was composed of elongated, flattened, spindle- or polygonal-shaped endothelial cells that were lining the vascular clefts (Fig 5 and 6). Sometimes these neoplastic endothelial cells project into the lumens of blood vessels (Fig 6). The sizes of the vascular clefts varied considerably and often contained blood (Fig 5). Even though some areas within the tumor consisted of closely packed fibroblast-type cells, the main component of the lesion exhibited a vascular pattern. As can be seen from the photographs, the tumor also involved striated muscle (Fig 7) and the lungs (Fig 8).

Electron microscopic study demonstrates the fine structure of the various portions of the vascular lesions. As can be seen (Fig 9), an area is depicted that is composed of elements often occurring in hemorrhages—*ie,* fibrinous material, red blood cells, fibroblasts, histiocytes, lipid droplets, etc. In the next figure (Fig 10), part of a well-developed angiosarcoma is illustrated. Several vascular spaces, which are lined by attenuated endothelial cells, are visible. Many such spaces contain erythrocytes. The neoplastic endothelial cells often, but not always, project into the lumens of blood vessels. The long axes of these

cells run transversly to the vessel wall (Fig 11). Their cytoplasm contains very thin filaments that run parallel to the long axes of the cells. Moderate numbers of mitochondria are evident (Fig 12). Extensive ultrastructural studies have been carried out on the normal vascular system in other species and there are detailed monographs summarizing the findings of other investigators. They were used throughout this investigation for comparative purposes.[18,19]

Lung Tumors

In the DMH-treated females, 22 mice (44%) developed 35 tumors, all of them adenomas. Their average latent period was 49 weeks; the first was found at 31 weeks and the last at 58 weeks of age. In the DMH-treated males, 21 animals (24%) developed 17 tumors of this organ. Out of these, only a single was classified as adenocarcinoma, the others were all adenomas. Their average latent period was 44 weeks, the first was observed at 29 weeks and the last at 58 weeks of age.

The diagnostic criteria for the lung neoplasms were described in a previous publication.[20]

Other Tumors

Table 2 also shows the incidence and latent periods of vascular, lung and a few other types of neoplasm observed in the control and treated animals.

Discussion

The present results clearly demonstrate that, in randomly bred Swiss mice, the lifelong administration of 0.001% DMH in the drinking water, beginning at 7 weeks of age, resulted in the induction of tumors of blood vessels and lungs. Ninety-eight percent of the treated females and 92% of the treated males developed blood vessel tumors, while in the controls the incidence was 3% in the females and 1% in the males. Furthermore, the incidence of lung tumors was increased from 12 to 44% in the females and from 10 to 24% in the males, as compared with the controls. The occurrence of vascular tumors in the order of decreasing frequency was as follows: muscle, pararenal, fat, liver, parametrial, paraepididymal tissues, etc.

Gross, light and electron microscopic examinations of these blood vessel lesions revealed the characteristic appearance of angiosarcomas. The extent of macroscopic involvements of the various tissues by the tumor is tabulated and extensive histologic descriptions of the

lesions are presented. The fine structures of hemorrhagic areas, vascular spaces, the neoplastic endothelial cells, the arrangements of these cells, their cytoplasms and organelles are illustrated in detail.

The spontaneous occurrence of vascular tumors is rare in our untreated control Swiss mice kept until natural death. In 110 females, we observed 1 animal with hemangioma of the ovary and 3 with hemangiomas of the liver; in 110 males, we found only 2 with hemangiomas of the liver.[3] No malignant vascular lesions were seen, however, in these control animals. The induction of blood vessel tumors with varying incidences has been successfully achieved with carcinogenic chemicals in the past. In this laboratory, when dimethylnitrosamine was administered in the drinking water to Balb/c mice it gave rise to 34% hemangiomas and 30% hemangiosarcomas.[20] In Swiss mice, similar results were obtained with this compound.[21] Other investigators also demonstrated that urethane produces blood vessel tumors in several strains of mice.[22-26]

With regard to blood vessel lesions in man, it is known that benign tumors occur commonly, but that malignant ones are observed rarely. Their types are well documented morphologically, although their causative agents remain to be determined.[27]

The relationship, if any, between chemical structure and carcinogenic activity aroused interest in the past among investigators of chemical carcinogenesis. Extensive studies were undertaken with 10 derivatives of 1,2-benzanthracene and 77 derivatives of angular benzacridines. The main concerns of these investigators however, were limited to the responses of skin and subcutaneous tissue and the findings demonstrated that compounds with the various substituents gave rise to tumors with varying incidences in these two tissues.[28,29] The variations in the oncogenic response of different organs to the various nitroso compounds was one of the main objects of the investigations of Druckrey and his co-workers. They have employed over 65 different types of compounds of this class and even though they have claimed that some sort of organotropism exists for the various types of nitrosamines, they could not clearly demonstrate such a phenomenon. Whenever they gave dimethyl, diethyl, *n*-dipropyl, *n*-dibutyl, etc, nitrosamines to BD rats, they induced tumors of liver, ethmoturbinale, esophagus, tongue, bladder, etc. Although some of the induced tumor types varied when one radical was replaced with another, they could not clearly pinpoint which of the radicals was responsible for a certain type of neoplasm.[4]

The present study is part of a systematic investigation aimed at de-

termining the variations in carcinogenic response of different tissues to substituted hydrazines. Hydrazine itself enhanced the development of lung tumors in Swiss mice as did the symmetrical dimethylhydrazine. In addition, the symmetrical dimethylhydrazine evoked blood vessel tumors.[30] Thus, the available results indicate that a possible relationship exists between the chemical structure of hydrazines and tumor development at specific organ sites.

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[Illustrations follow]

Fig 1—Angiosarcoma of liver. Observe the dark neoplastic growth that involves the three largest lobes. Adhesion between lobes is distinct. Male, 32 weeks old (formalin-fixed specimen, × 1.8).

Fig 2—Angiosarcoma of pararenal and parametrial areas. Note the dark nodular tumor growths. Female, 35 weeks old (formalin-fixed specimen, × 1.8).

Fig 3—Angiosarcoma of subcutis. The tumor growth occupies the right inguinal area. Male, 45 weeks old (formalin-fixed specimen, × 1.6).

Fig 4—Angiosarcoma of paraepididymal tissue. Note the dark tumor growths next to testis and epididimydis. Male, 33 weeks old (formalin-fixed specimen, × 1.8).

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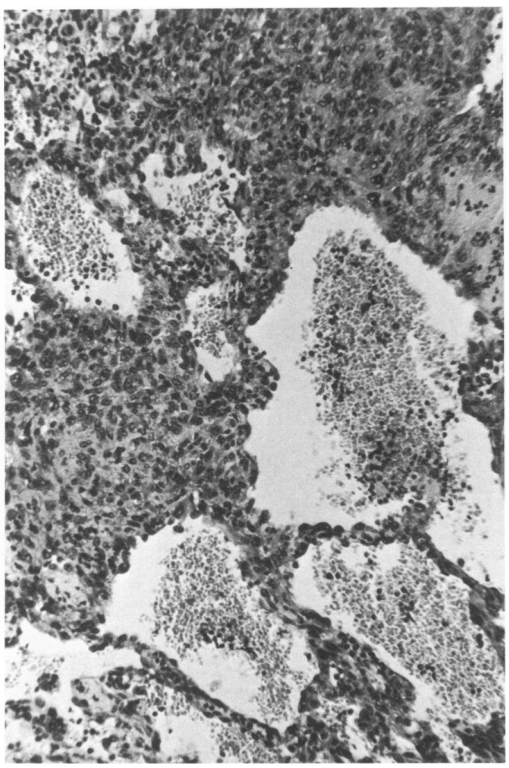
Fig 5—Angiosarcoma of parametrial tissue. Observe the typical appearance of the neoplastic vascular tissue, exhibiting endovascular growth. Female, 52 weeks old (H&E, × 100).

Fig 6—Same as Fig 5. Higher-power view of the tumor. The center of the picture depicts the lumen of a blood vessel. Observe the neoplastic endothelial cells which at the right side project themselves into the lumen (H&E, × 450).

Fig 7—Angiosarcoma involving the muscle. Note the invasion of muscle by the tumor growth. A large area is obliterated by the vascular tumor cells. Female, 48 weeks old (H&E, × 70).

Fig 8—Angiosarcoma of lung. The photomicrograph shows a few vascular nodules in a lobe. Male, 28 weeks old (H&E, × 70).



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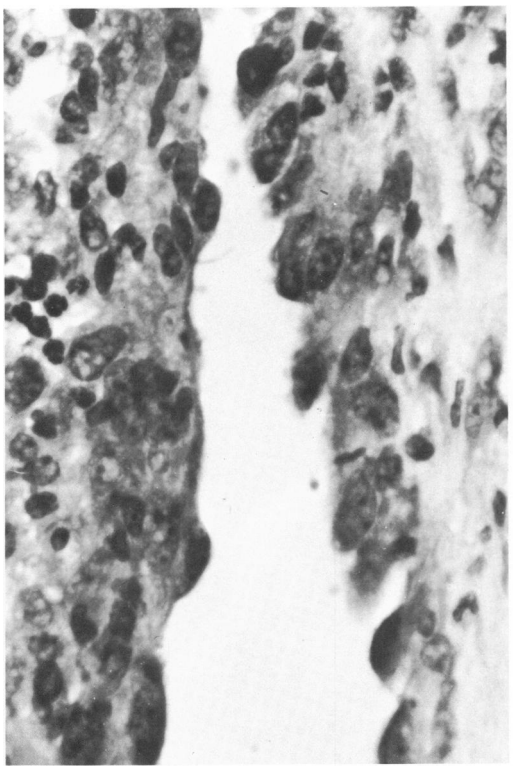

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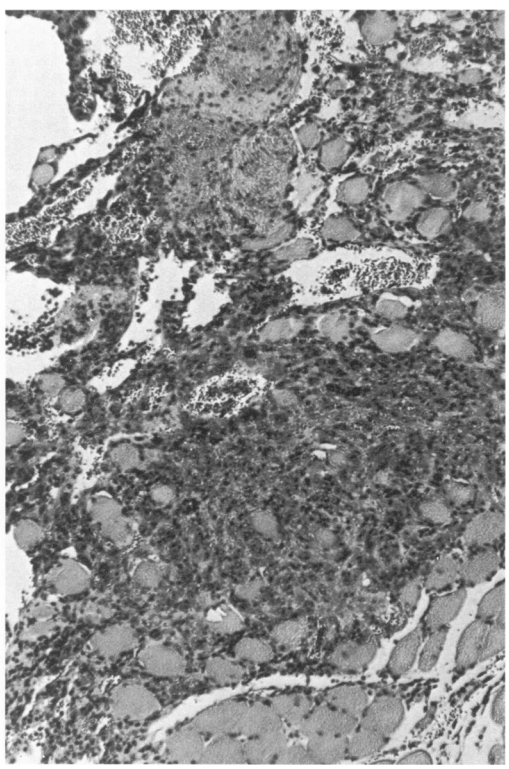

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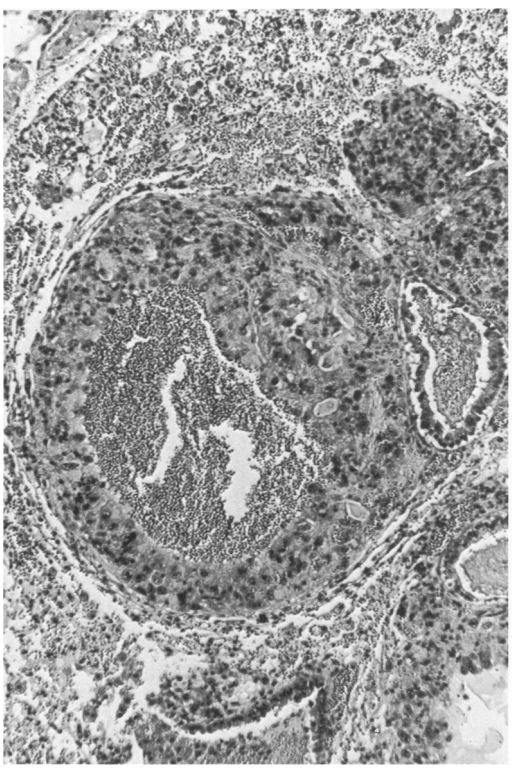

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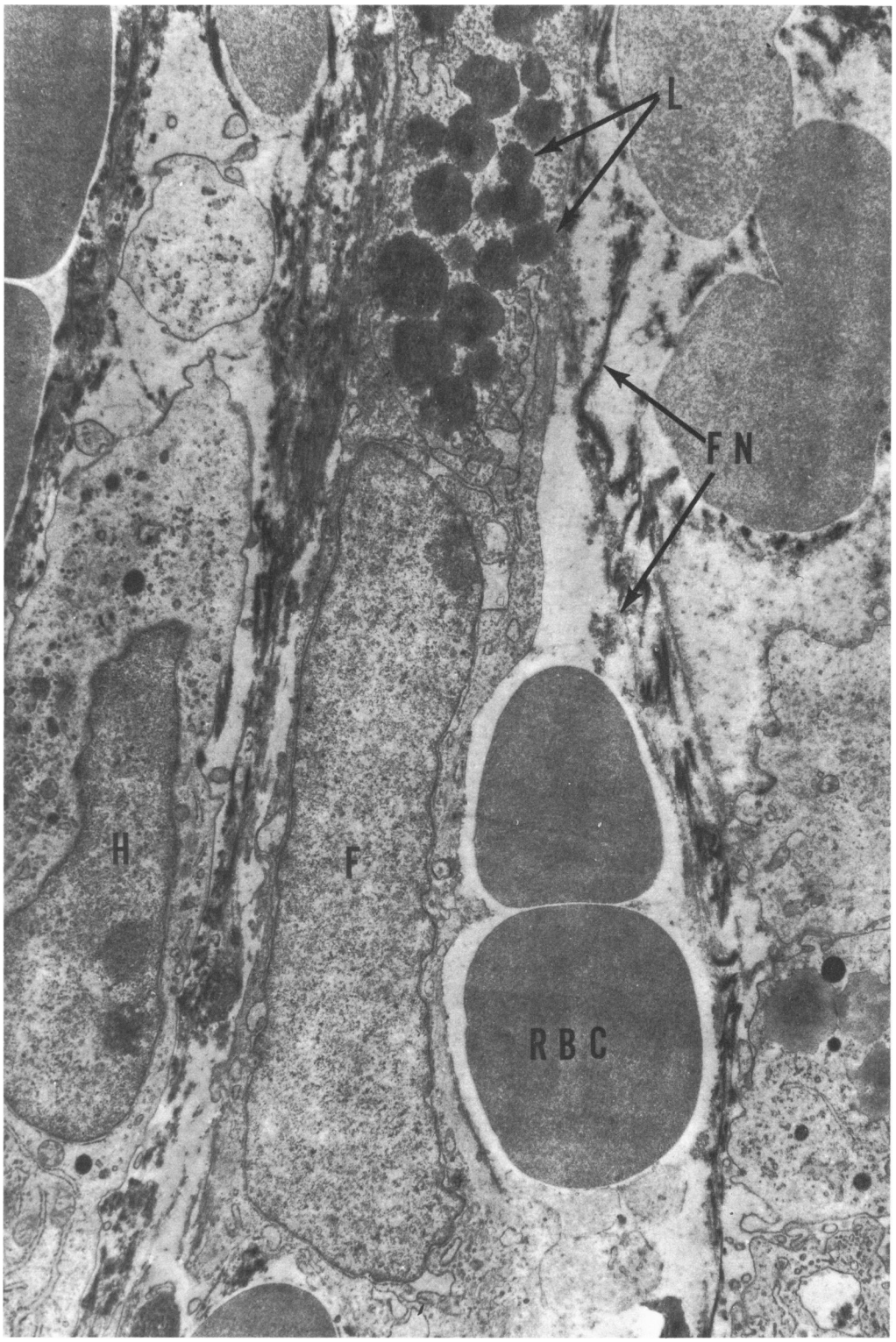

Fig 9—Electron micrograph showing the vascular lesion. Areas as this are sometimes seen in the tumor. Note the red blood cells (*RBC*), the fibrinous materials (*FN*), a fibroblast (*F*), histiocyte (*H*), lipid droplets (*L*) (× 3200).

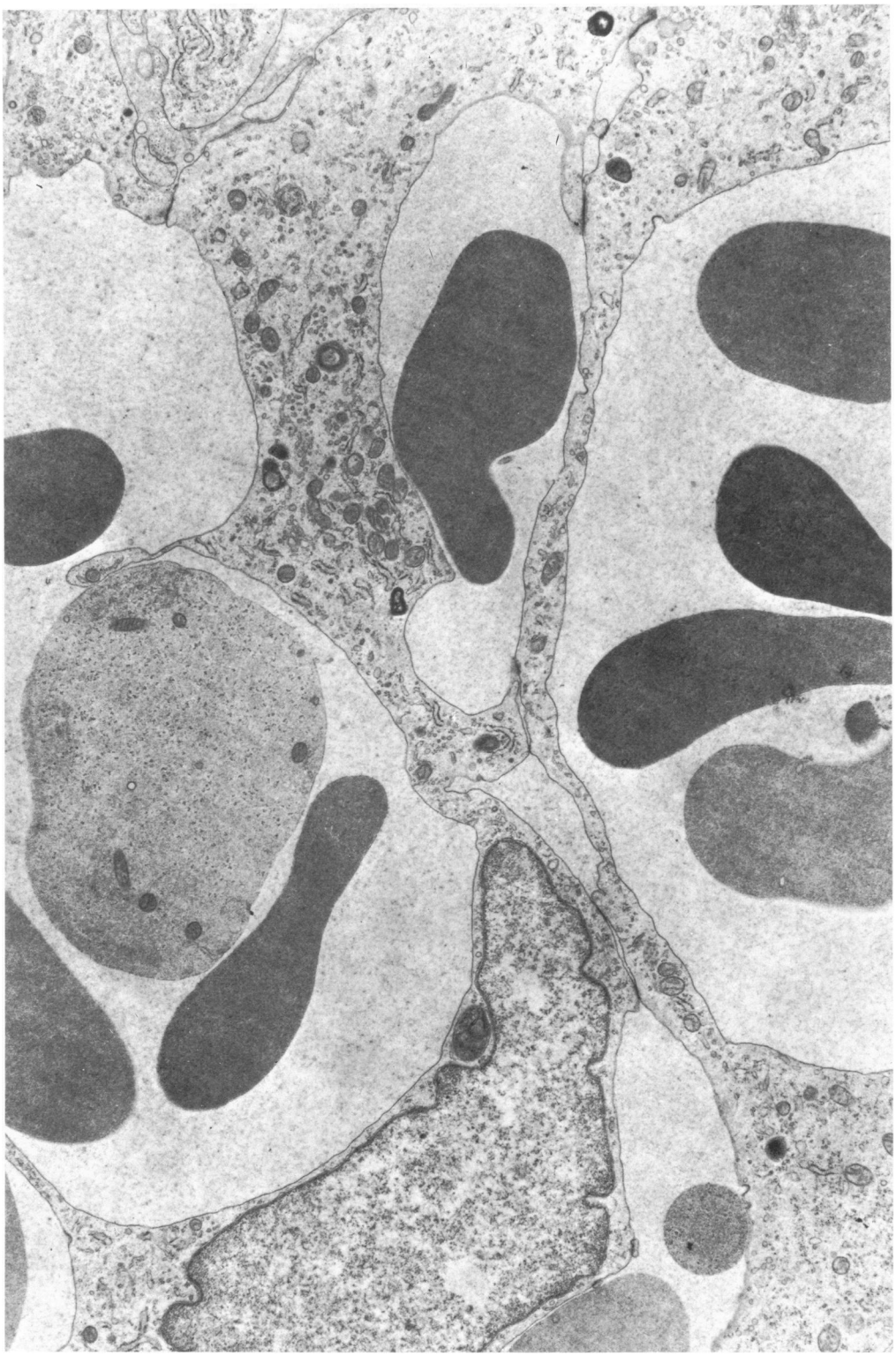

Fig 10—Electron micrograph exhibiting a portion of an angiosarcoma. Observe the numerous vascular spaces lined by attenuated endothelial cells. All contain erythrocytes (× 3200).

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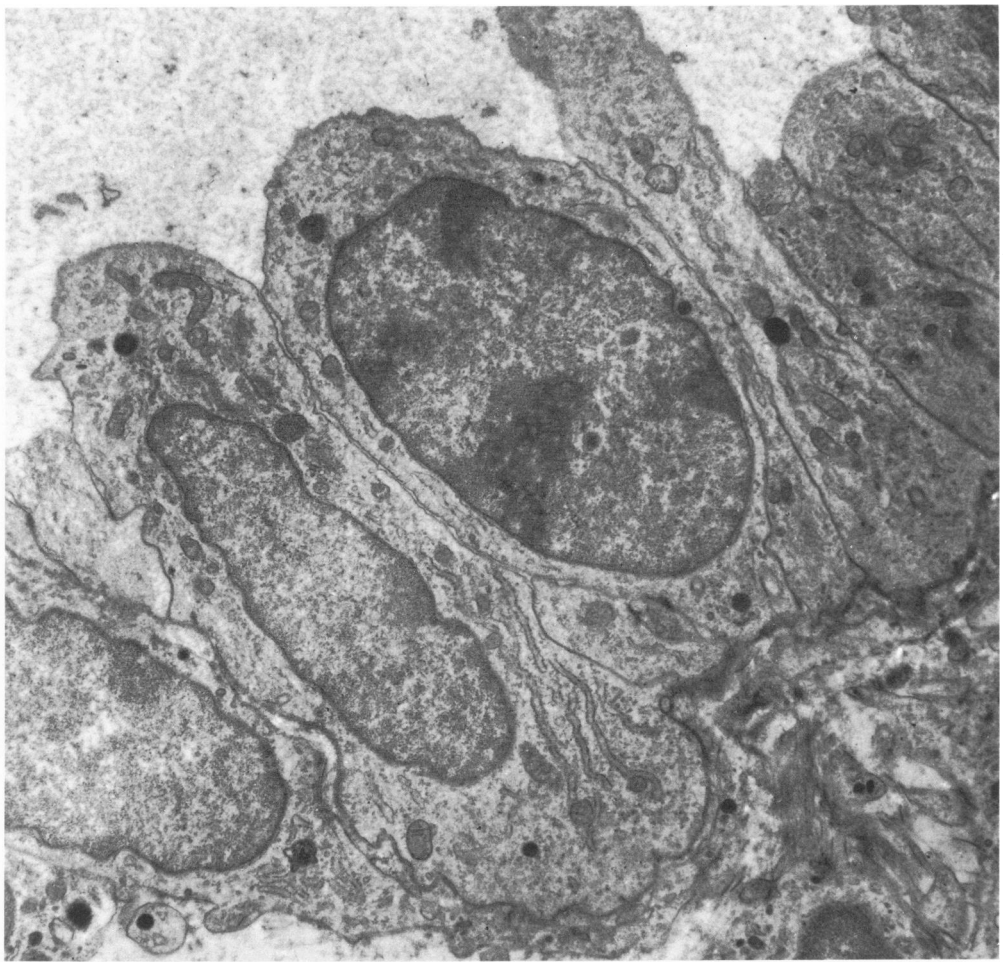

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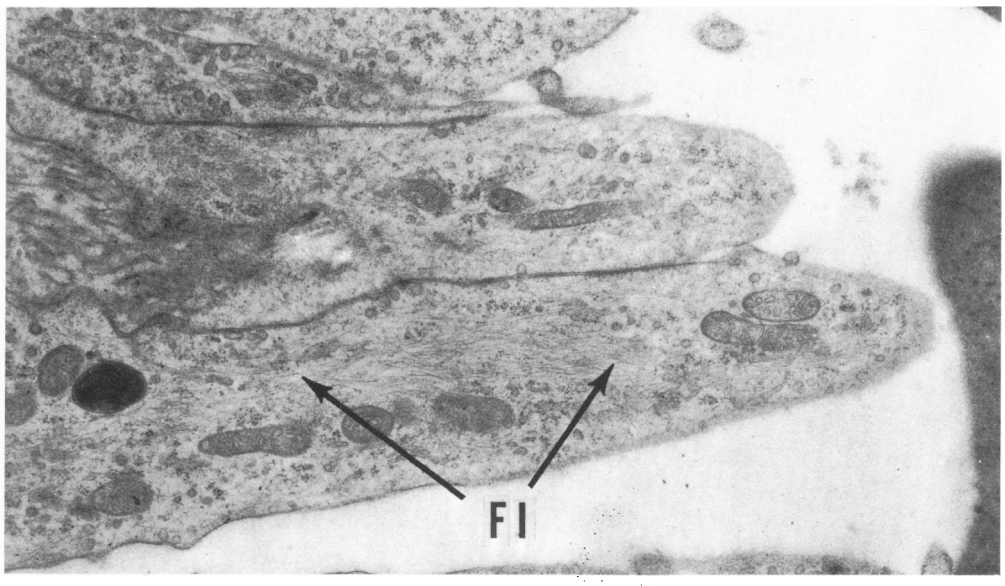

Fig 11—Electron micrograph of the neoplastic endothelial cells, depicted here as they project into the lumen of the blood vessel. The long axes of the cells are running transversly to vessel wall. Observe the closely packed cells. At upper left is lumen of a blood vessel (× 3200). **Fig 12**—Electron micrograph of the cytoplasm of neoplastic endothelial cells. Only their cytoplasms can be seen as they project into the lumen of a vessel. Note the very thin filaments (*FI*) which run parallel to the long axis of the cell. Moderate numbers of mitochondria. At right is the lumen; parts of red blood cells are visible (× 6800).