Histologic Characterization of Renal Tumors (Nephroblastomas) Induced Transplacentally in IIIVO/J and WH/J Rabbits by N-Ethylnitrosourea

GORDON C. HARD, BVSc, PhD, DSc, and
RICHARD R. FOX, PhD

From the Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania, and the Jackson Laboratory, Bar Harbor, Maine

The histologic features of 63 renal tumors induced in 39 rabbits of two partially inbred strains, IIIVO/J and WH/J, by transplacental exposure to N-ethylnitrosourea (ENU) were analyzed. All tumors in the series conformed to nephroblastoma, permitting the establishment of histologic standards for this neoplasm in the rabbit as well as observations on tumor progression. Essentially, nephroblastoma proved to be predominantly an epithelial tumor identifying with metanephrogenic blastema, which was presumed to be the tissue of origin during fetal development. The outstanding features comprised clusters or sheets of undifferentiated blastemalike tissue and differentiation along the epithelial pathway into tubular profiles and structures suggestive of primitive, nonvascularized glomeruli. The latter were frequently of a complex nature, with a papillary configuration. On the other hand, no definitive evidence of bipotential differentiation into malignant secondary mesenchyme was found, there being no recognizable areas of fibrosarcomatous elements or specialized connective tissue such as smooth or striated muscle, adipose tissue, cartilage, or osteoid. Mesenchymelike fascicular disposition of neoplastic cells between blastemal clusters was an acquired feature seen in advanced tumors but not in small early lesions. By light microscopy alone it was not possible to determine whether this represented a conformational change of tumor cells or true bipotential differentiation into neoplastic secondary mesenchyme. However, the reticulin pattern was not characteristic of sarcoma. A conspicuous feature accompanying the growth and development of tumors was the magnitude of host fibrous reaction discernible only as a simple ramifying stroma in the earliest lesions but attaining impressive proportions both within and around the tumor with advancing age. Increasing collagen formation appeared to be associated with ischemic necrosis of tumor tissue. Other features of advanced tumors were the presence of discrete foci of differentiated tubular structures suggestive of mature medullary elements and small islands of squamoid differentiation. Metastases occurred only in rabbits of strain IIIVO/J, which had been subjected to a single dose of the carcinogen, representing an incidence in this subgroup of 25%. Nephroblastomas resulting from transplacental induction in IIIVO/J rabbits, particularly by single, high doses of ENU, appear to provide a suitable model for the predominant histologic form of the Wilms' tumor complex in man. (Am J Pathol 1983, 113:8–18)

NO SATISFACTORY basis has emerged for the histologic identification of nephroblastoma among different species. In man, for example, this childhood tumor has been regarded as an array of neoplasms merging from the purely blastemal to those consisting entirely of neoplastic connective tissue, ie, secondary mesenchyme.[1,2] Recently, however, it has been recognized that some of the so-called mesenchymal variants, for example, congenital mesoblastic nephroma,[3] bone-metastasizing renal tumor of childhood,[4] and malignant rhabdoid tumor of the kidney,[5,6] are probably entities distinct from Wilms' tumor. Recent studies in this laboratory (FRI) have been directed toward elucidating the nature of nephroblastomas in laboratory animals, particularly the rat, in the hope of achieving better understanding of the tumor complex apparent in man.[7,8] Until recently there has been little opportunity to characterize the

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Address reprint requests to Gordon C. Hard, BVSc, PhD, DSc, Fels Research Institute, Temple University School of Medicine, 3420 North Broad Street, Philadelphia, PA 19140.

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nature of this tumor in the rabbit because of its rare occurrence.[9,10]

Following recognition of a genetic predisposition to renal cortical cyst formation,[11] and to neoplasms[12] in certain partially inbred strains of laboratory rabbit developed at the Jackson Laboratory (Bar Harbor, Maine), the almost exclusive induction of renal tumors by transplacental administration of N-ethyl-nitrosourea (ENU) was reported in the IIIVO/J and WH/J strains by Fox and his associates.[13] Renal tumors were induced in these strains regardless of whether the carcinogen was administered during the gestation period as a single high systemic dose,[13] as multiple low systemic doses,[14] or as a combination of the precursor amide and nitrite by the oral route.[15] The tumors induced by these methods were originally identified as renal tubular cystadenomas and cystadenocarcinomas of papillary type, as well as nephroblastomas with Wilms' tumor features.

Nephroblastomas have been produced in high incidence in chickens by substrains of avian myeloblastosis virus[16] and at an almost 30% frequency rate in the North American opossum by postnatal administration of ENU.[17] However, in conventional laboratory mammals this neoplasm has been induced experimentally only in very small numbers,[18,19] not sufficiently high as to provide a suitable working model for effective study of its pathogenesis. Transplacental induction by ENU in rabbits appears to offer some promise in this direction. In contrast to outbred rabbit strains, in which ENU induces kidney tumors after a long latency of 2–2.5 years,[20] the partially inbred strain IIIVO/J represents a particularly applicable animal model, in that frequency of renal neoplasms is high (approximately 90%) and the induction period short, with a mean latency of 3.3 months, depending on the treatment schedule.[13] The time frame of development thus appears to match the childhood predilection of human Wilms' tumor.

The tumors developing with high incidence in the Jackson Laboratory strains after transplacental ENU exposure have not been histologically characterized with respect to the distribution of tumor types. Nor have they been compared with the slower growing lesions recorded in outbred rabbits, originally described as adenocarcinoma, adenocarcinosarcoma, polymorphocellular sarcoma,[20] and later as nephroblastomas[21] by Hänichen and Stavrou. The large series of renal neoplasms acquired by the methods herein described provides an opportunity to determine the range of tumor types induced in the young rabbit by ENU and to standardize the histologic spectrum of nephroblastoma in this species.

Materials and Methods

The tumors examined in this study were produced in partially inbred rabbit strains IIIVO/J and WH/J[22] as follows: 1) administration of a single intraperitoneal injection of ENU (60 mg/kg body weight) dissolved in trioctanoin (Eastman Kodak, Rochester, NY) to pregnant does on Day 18 of gestation (16 IIIVO/J, 3 WH/J)[13] (Hard and Fox, unpublished data); 2) combined administration of ethylurea (Aldrich Chemical Co., Milwaukee, Wisc) and $NaNO_2$ (Fisher Scientific Co., Fair Lawn, NJ) in doses of 100 and 50 mg/kg, respectively to pregnant does on Days 17, 18, and 19 of gestation (1 IIIVO/J, 6 WH/J)[15]; and administration of ENU in trioctanoin intraperitoneally to pregnant does for 10 days at the rate of 10 mg/kg/day commencing on either the 10th, 15th, or 18th day of gestation (10 IIIVO/J, 3 WH/J).[14] The carcinogen was purchased from K & K Laboratories Inc. (New York, NY) or synthesized in the laboratory by addition of excess HCl dropwise to an ice-cold solution of ethylurea and excess $NaNO_2$, with recrystallization of the product from CH_2Cl_2.[23]

For histologic evaluation, tissues were fixed by immersion in Tellyesniczky's fluid as modified by Fekete,[24] in neutral buffered formalin, or, in four cases, by retrograde arterial perfusion with cacodylate-buffered glutaraldehyde. Following conventional tissue processing, sections were stained routinely with Harris' hematoxylin and eosin. Selected tumor sections were also exposed to a variety of special stains, including alcian blue, Bennhold's Congo red, Gomori's reticulin stain, hematoxylin-phloxine-safran, Heidenhain's azan, Jones' periodic acid–methenamine silver, Mallory's phosphotungstic acid hematoxylin, Masson's trichrome, periodic acid–Schiff (PAS), and van Gieson's collagen stain.

Results

Forty-eight renal tumors induced in 27 strain IIIVO/J rabbits and 15 tumors in 12 animals of strain WH/J were examined, ie, a total of 63 tumors in 39 rabbits. The age of the tumor-bearing offspring at the time of spontaneous death or sacrifice ranged from 1.25 to 15.25 months, with 60% of the cases involving rabbits less than 9 months old. The lesions varied in size from 1 mm to 10 cm in diameter. Thus, the series comprised a tumor range from early development to an advanced state.

All 63 ENU-induced renal neoplasms conformed to a single tumor entity classifiable as nephroblas-

Table 1—Histologic Profile of Nephroblastomas Related to the Age of the Tumor Bearer*

	Age of Rabbit at Death (months)												
	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12	12-13	15-16
Number of rabbits in age group	6	4	1	1	4	2	4	1	3	6	4	1	2
Blastema	6	4	1	1	4	2	4	1	3	6	4	1	2
Tubular differentiation	6	4	1	1	4	2	4	1	3	6	4	1	2
Glomeruloid differentiation				1	3	2	3	1	2	4	2	1	2
Squamoid differentiation							4	1	2	6	4	1	2
Reniform foci							1			3	1		2
Mesenchymelike differentiation					2	2	4	1	3	6	4	1	2
Degree of stromal development†													
+	6	4	1		1								
+ +				1	3	2	4		1	3			
+ + +								1	2	3	4	1	2
Association with hereditary cysts	3	2	1										
Rabbits with metastasis					1				1	1			1

* The data are presented as the number of rabbits in each age group that exhibited the particular histologic feature.
† The + designations represent minimal, moderate, and massive development of benign stroma with collagenization.

toma. Apart from the time-dependent variations associated with lesion progression, the tumors were remarkably uniform, presenting a combination of complex histologic features, which were mainly epithelioid, supported by a connective tissue stroma. To better define histotypic standards for this neoplasm, the various elements will be described under separate headings below. In order to envisage the histologic trends that occur with tumor progression, Table 1 summarizes the distribution of histologic features in relation to the age of the tumor bearers.

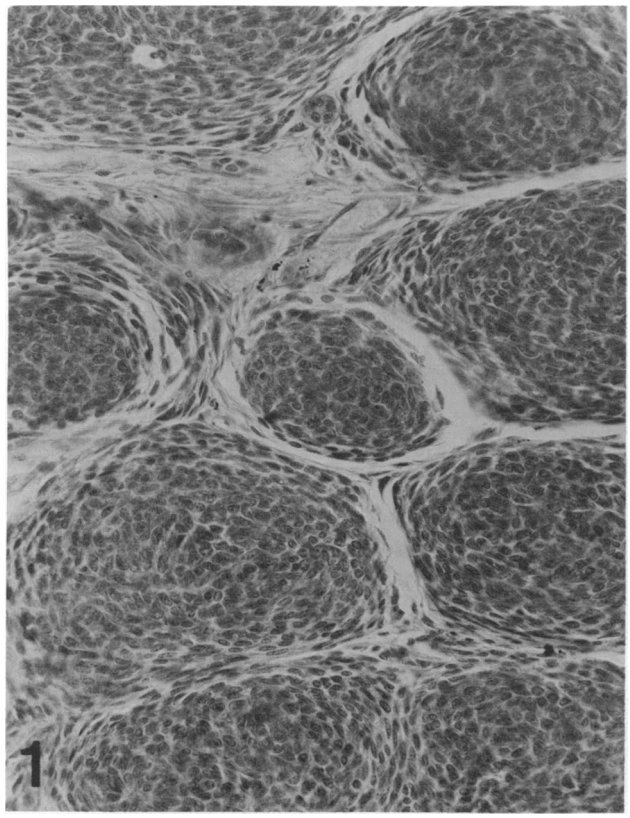
Undifferentiated Blastema

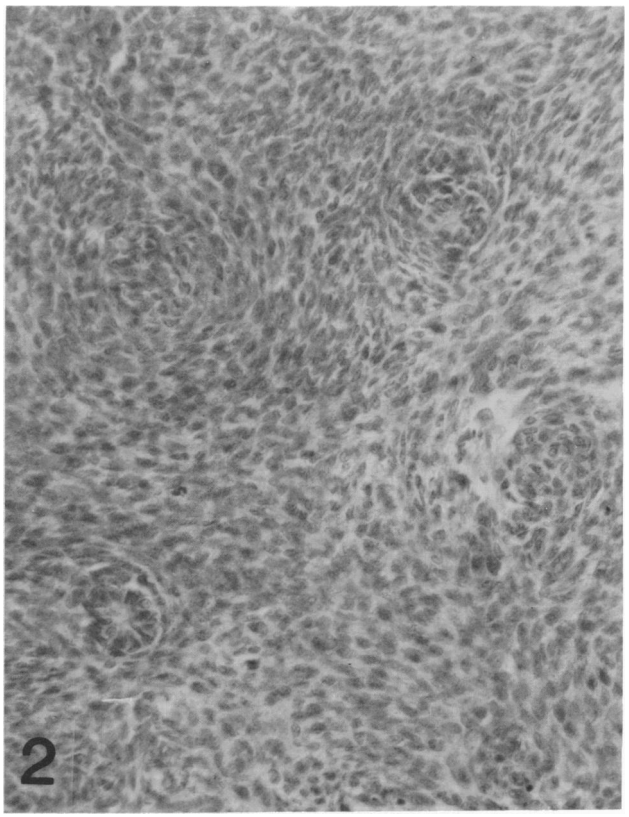
All tumors contained tissue resembling undifferentiated renal blastema as a major component. Blastema consisted of clusters (Figure 1), whorls, and irregular masses or sheets of deeply basophilic cells with scanty cytoplasm and thus a very high nucleus to cytoplasm ratio. The cells were usually so densely crowded as to obscure their individual outline. The nuclei, however, were round to ovoid, conveying the impression of a similar cell shape. Nucleoli were not prominent. Crowding of the cells was greatest at the center of blastemal aggregates, with a tendency for looser arrangement peripherally, in radial or reticular patterns. In sheet form, tumor cells were often less crowded, more fusiform in shape, but invariably associated with aggregations indicative of early organoid differentiation (Figure 2). An occasional tumor consisted almost entirely of blastemal clusters dissected by stroma, with little organoid differentiation, presumably representing the least differentiated variant of the tumor series.

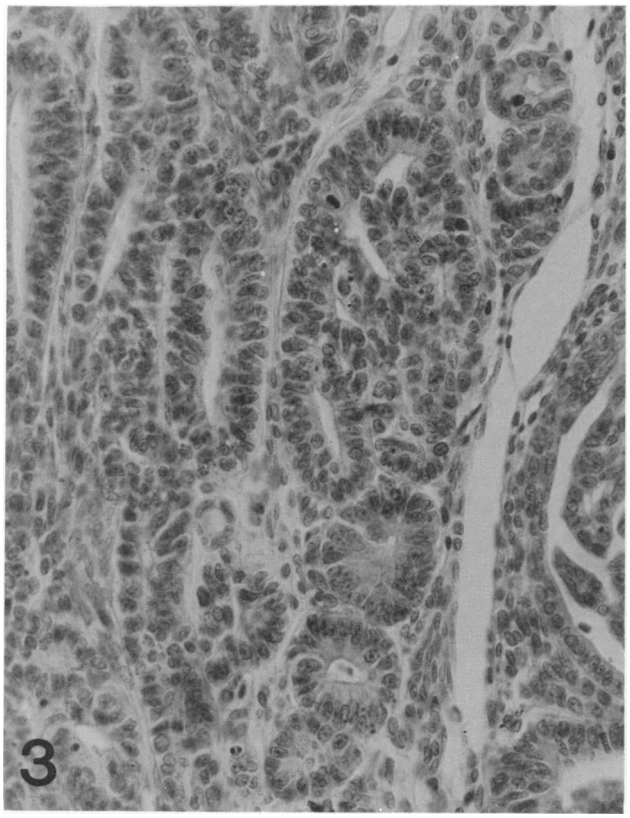
Epithelial Differentiation

Tubular Structures

Tubular differentiation was evident in every tumor. The organoid structures varied from simple rosettes without lumens, through poorly formed multicellular and highly basophilic tubule profiles with lumens (Figure 3), to branching ducts with a papillary, frondlike pattern, lined by cuboidal or columnar epithelium having basal nuclei and eosinophilic cytoplasm (Figure 4). These differentiated elements were scattered through the tumor tissue in association with blastemal clusters. However, two neoplasms consisted almost entirely of serpentine convolutions of highly differentiated tubules lined by regular, cuboidal epithelium (Figure 4). A scarcity of mitotic figures testified further to the relatively benign, highly differentiated form of this almost monomorphous variant. Scattered areas of undifferentiated blastema within these tumors nevertheless confirmed their identity as nephroblastoma rather than tubular adenoma.







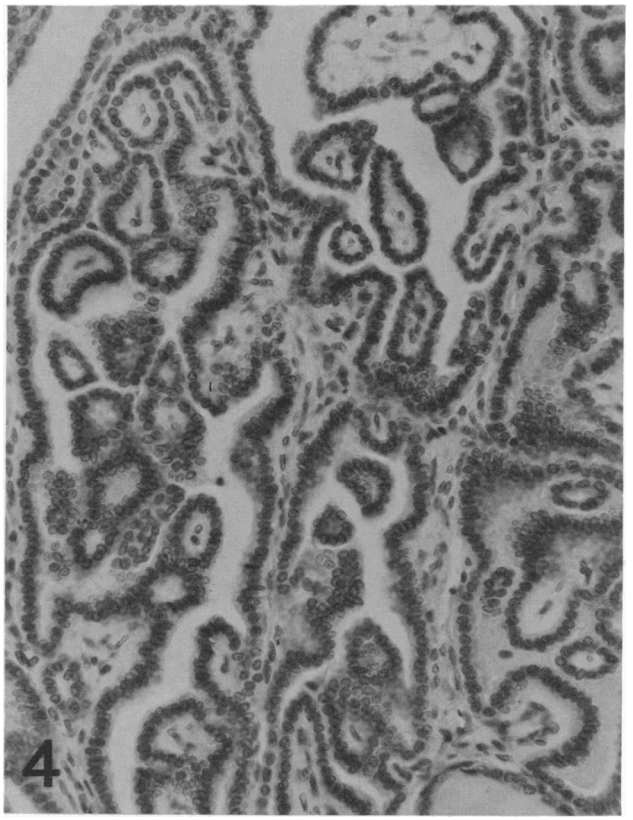


Figure 1 — Repetitive blastemal clusters with peripheral tumor cells radiating into benign stroma. (H&E, ×250) **Figure 2** — Blastemal tumor cells in sheet form with scattered condensations resembling rosettes. (H&E, ×250) **Figure 3** — Tubular differentiation ranging from primitive clusters and rosettes to well-formed, pseudostratified tubule profiles. (H&E, ×320) **Figure 4** — Highly differentiated, branching ducts, with a papillary pattern. (H&E, ×250)

In addition, discrete circumscribed foci of small, highly differentiated tubules, resembling medullary segments of the mature nephron in cross-section, were sometimes seen in more advanced tumors (Figure 5). Such areas were reminiscent of the reniform foci described by Bodian and Rigby.[2] These foci were enhanced by special stains, becoming particularly conspicuous with alcian blue and PAS due to the positive staining of the tubule contents and/or intertubular matrix (Figure 6). In contrast, relatively few of the more primitive tubules scattered throughout the tumor tissue contained luminal mucinous material.

Epithelial Islands

In nearly all neoplasms sampled beyond 7 months, small, solid islands of eosinophilic epithelium with expanded cytoplasm were scattered through the tumor tissue (Figure 6, inset). These foci were compatible with squamous differentiation.

Glomeruloid Structures

Structures believed to represent primitive glomerular differentiation were seen in most of the tumors beyond 4 months. The most convincing of these were simple or segmented invaginations of very small cells with densely basophilic nuclei on stalks of structureless hyaline material, within crescentic spaces lined by cuboidal or flattened epithelial tumor cells resembling Bowman's capsules (Figure 7, inset). Frequently, these bodies were large and of complex configuration, conferring a papillary organization to the structure and, in a discontinuous fashion, to the whole tumor (Figure 7). The simplest attempts at glomerular differentiation appeared to consist merely of narrow clefts in the blastemal background lined by noticeably smaller basophilic cells (Figure 7). A hyaline matrix supporting the invaginations of glomeruloid bodies was a characteristic feature that assisted in the identification of these structures. The connective tissue and basement membrane stains used in this study revealed the hyaline matrix to be distinct from collagen but consistent with basement membrane. In all of these structures the tuft was avascular.

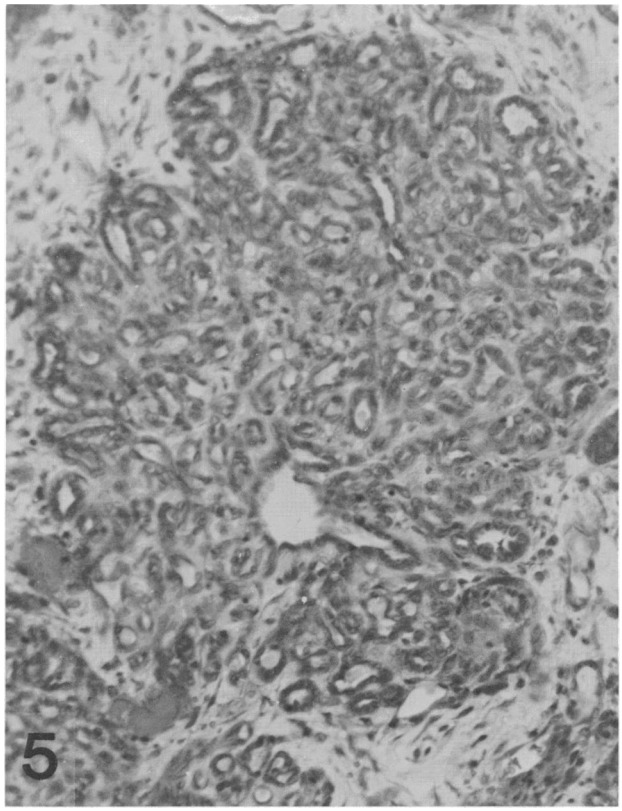
Mesenchymal Differentiation

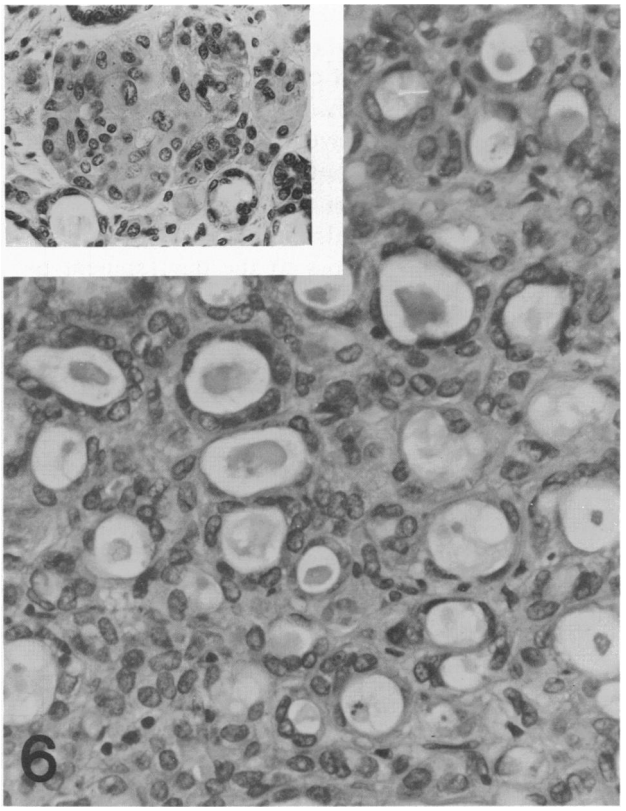
None of the earliest neoplasms displayed tissue patterns that could be identified as representing unequivocal bipotential differentiation into secondary mesenchyme, that is, neoplastic cells of connective tissue type. However, all tumors occurring in animals older than 5 months showed scattered tumor cell disposition which suggested mesenchymal differentiation. In these instances, small aggregates of spindle-shaped tumor cells formed short streaming fascicles in continuity with the periphery of blastemal clusters (Figure 8). The extent of fascicular organization was never sufficient to warrant the designation of fibrosarcoma. Although the general shape of the tumor cells constituting the fascicles was bipolar or fusiform, the cytoplasm, like that of the blastemal tumor cells, was usually scanty or barely discernible. Furthermore, their staining pattern was uncharacteristic for sarcoma, reticulin (and basement membrane) stains featuring an alignment of cells aggregated within fascicles rather than the typical dense meshwork that intimately surrounds individual neoplastic fibroblasts (Figure 9). Frequently the fascicular pattern was augmented by the presence of delicate bundles of collagen, occupying the intervening spaces between tumor cell clumps, which were continuous with a more general collagenous reaction. In fact, the fascicular pattern coincided with an increasing stromal reaction within the tumor and surrounding kidney parenchyma. Careful examination of tissues with various connective tissue stains revealed absolutely no evidence of specialized mesenchymal derivatives, such as smooth muscle, rhabdomyoblasts, striated muscle, adipose tissue, cartilage, or osteoid.

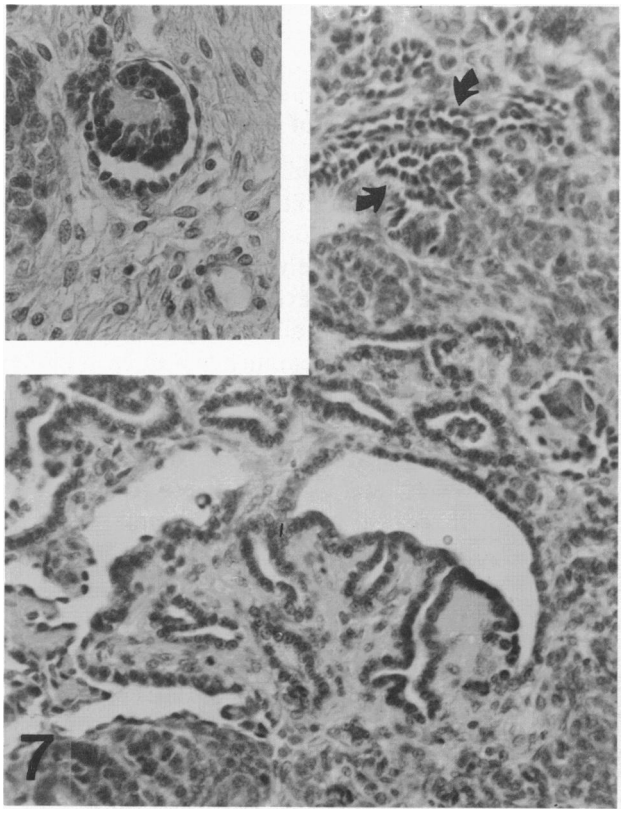
Stroma

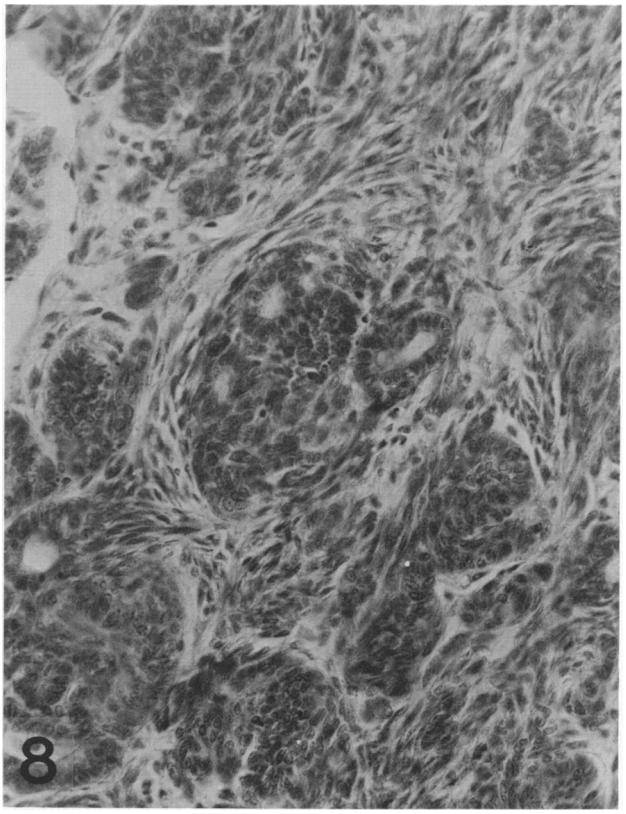
The degree of development of stroma, as represented by a local fibrous and vascular reaction to the neoplastic tissue, was dependent upon tumor stage. Thus stromal development commenced in the earliest tumors, becoming conspicuous by 5 months and massive by 9 months. In the earliest lesions, the

Figure 5—Discrete focus of mature tubule profiles which suggest reniform differentiation. (H&E, ×250) **Figure 6**—Higher magnification of reniform focus showing the hyaline (mucinous) tubule contents and interstitial matrix which make these areas conspicuous with special stains. As elsewhere, the interstitium is amyloid-negative. (Congo red, ×510) **Inset**—Epithelial island resembling squamous differentiation. (H&E, ×250) **Figure 7**—Glomeruloid differentiation. The **inset** depicts a simple glomeruloid body consisting of a tuft of very small, highly basophilic cells supported by acellular hyaline material with staining properties similar to basement membrane, and set within a crescentic space. A complex glomeruloid structure with the same basic components is shown in the main photomicrograph. The narrow clefts lined by small basophilic cells (*arrows*) may represent an early stage in glomerular differentiation. (H&E, ×250; **Inset**, ×320) **Figure 8**—Fascicular, mesenchymelike disposition of tumor cells streaming between and in continuity with blastemal clusters. (H&E, ×250)









stroma formed a thin ramifying network between the aggregates of blastema and tubular structures. It consisted of narrow tracts of collagen, fibroblasts, and normal blood vessels, which were continuous with the mesenchymal connective tissue of the preexisting kidney parenchyma surrounding the tumor nodules (Figure 10). As the tumors expanded in size, the degree of fibrous reaction also increased, leading ultimately in advanced tumors to the development of very large areas of relatively acellular collagenous matrix associated with extensive degeneration of tumor tissue, suggestive of ischemic necrosis (Figure 11). Formation of psammoma bodies and, later, deposits of calcium accompanied the increasing stromal proliferation and necrosis. The degree of development of peripheral fibrous reaction with formation of a pseudocapsule paralleled stromal proliferation within the tumors. Also, the fibrous elements of the peripheral reactive zone were continuous with and identical to the constituents of the internal stroma.

Origin and Progression

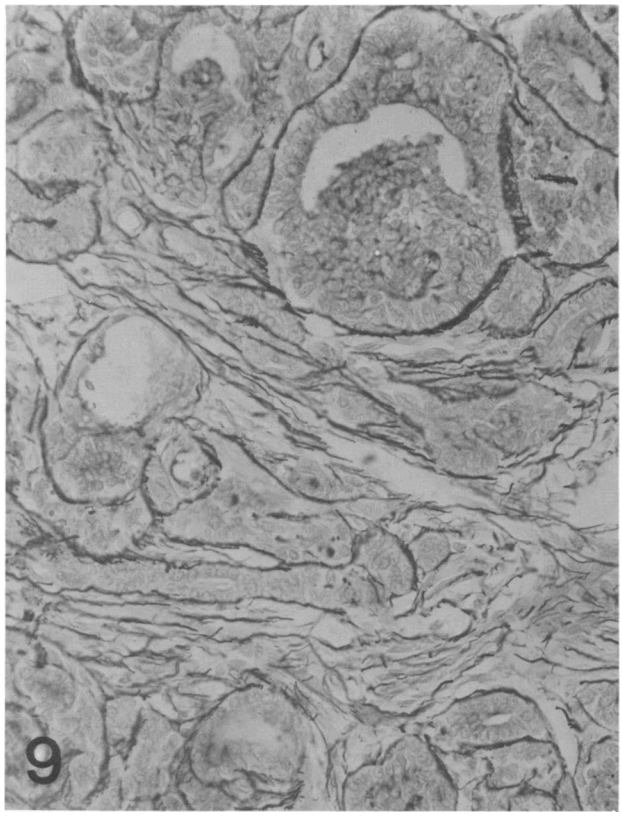
The earliest tumors seen were 1–3-mm nodules in rabbits of 1.25–2 months of age. Several of these lesions appeared to originate within the outer stripe of the outer medulla (Zone 2), but most were located in the cortex (Zone 1). These incipient tumors displayed a particularly constant histologic pattern reminiscent of metanephrogenic blastema. They comprised an admixture of blastemal clumps, primitive tubules, and papillary frondlike formations of more differentiated tubular epithelium (Figure 12); whereas glomeruloid bodies, mesenchymelike fascicular disposition of blast cells, reniform foci, and squamoid islands were absent. Approximately half of the incipient tumors were associated topographically with hereditary cystic tubules (Figure 12). With time, it appeared that the cysts were quickly obliterated by compression from the expanding tumor. None of the early tumors arising in Zone 2 were coupled with cysts, in keeping with the superficial distribution of the latter. The tumors grew by expansion as well as by local invasion beyond the peripheral fibrous reaction, where tumor cell clumps, invariably of blastemal or tubular form, proliferated within the interstitial space or formed occlusions in small blood vessels. Mitotic figures were prominent in almost all of the neoplasms, including the earliest lesions, being most evident in blastemal clusters and developing tubules. Mitoses were not seen in the highly collagenous areas of advanced tumors.

Metastases were found in 4 of the 39 tumor-bearing rabbits, three being visible on gross observation. All four were of the IIIVO/J strain which had been exposed transplacentally to a single, high dose of ENU. The metastatic rate in this particular strain was, therefore, 15% overall, or 25% in response to single dose carcinogenesis. Local invasion of the abdominal wall occurred in 1 rabbit at 5.5 months of age, whereas metastases were found in the lungs of 2 rabbits at 9–10.5 months and, additionally, in the liver and jawbone in a 15-month animal. Metastatic nodules in the first three instances consisted of blastemal aggregates, sometimes with primitive tubular formations. The metastases in the oldest rabbit consisted of an admixture of blastema, primitive and well-differentiated tubules, reniform foci, squamoid islands, fascicles of bipolar tumor cells, and copious amounts of stromal matrix and collagen, a pattern recapitulating that of the primary neoplasm. Glomeruloid differentiation was not seen in any of the metastases.

Independent Alterations of the Renal Parenchyma

Other changes were seen in the normal, nonneoplastic parenchyma, in addition to the hereditary cystic abnormality of the Zone 1 cortical tubules observed in a majority of the younger animals. Venous dilatation, described in previous accounts as vascular bloom, affected almost all kidneys. Detailed examination of the normal parenchyma of each kidney revealed no evidence of nodular renal blastema or nephroblastomatosis, a developmental lesion complex believed to be a precursor of Wilms' tumor in man.[25] However, fetal glomeruli were found in foci of interstitial cortical nephritis in a number of rabbits, and sometimes in association with the hereditary cysts.

Figure 9—Reticulin pattern emphasizing the cluster and linear aggregation of tumor cells in blastemal and fascicular areas, respectivley. An intimate meshwork characteristic of sarcoma is lacking. (Gomori's reticulin, ×250) **Figure 10**—Infiltrating border of an early tumor. The simple, fibrocollagenous stroma dissecting blastemal and tubular clusters is directly continuous with the incipient fibrous reaction, which is beginning to separate the tumor from the normal parenchyma at the right. (H&E, ×250) **Figure 11**—Low magnification of an advanced tumor at 9.0 months, showing extensive development of collagenous reaction, sequestered clumps of basophilic tumor cells, and areas of progressive tumor cell necrosis (*asterisks*). (Masson's trichrome, ×100) **Figure 12**—An incipient tumor at 1.5 months consisting of blastemal clusters and tubular profiles, contiguous with hereditary cortical cysts, which are lined by cuboidal to flattened epithelium. (H&E, ×100)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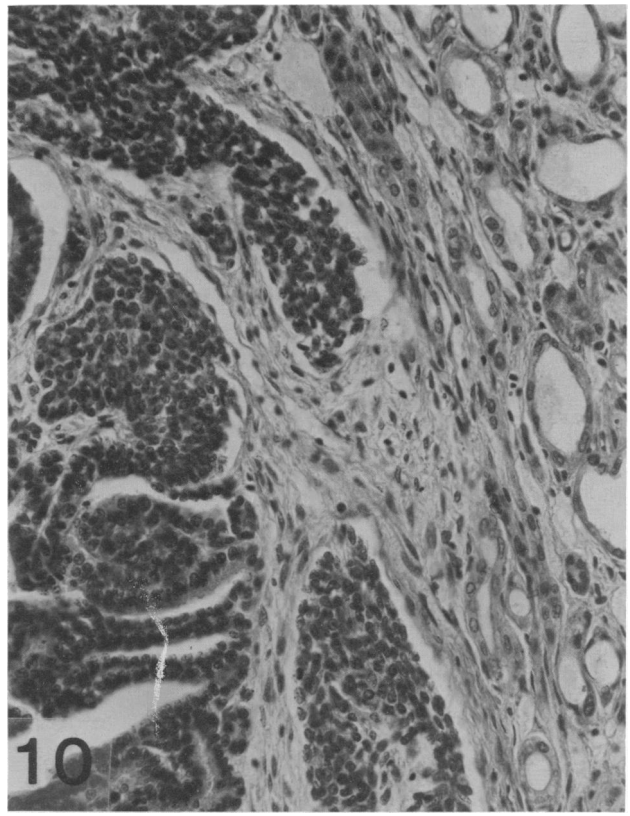
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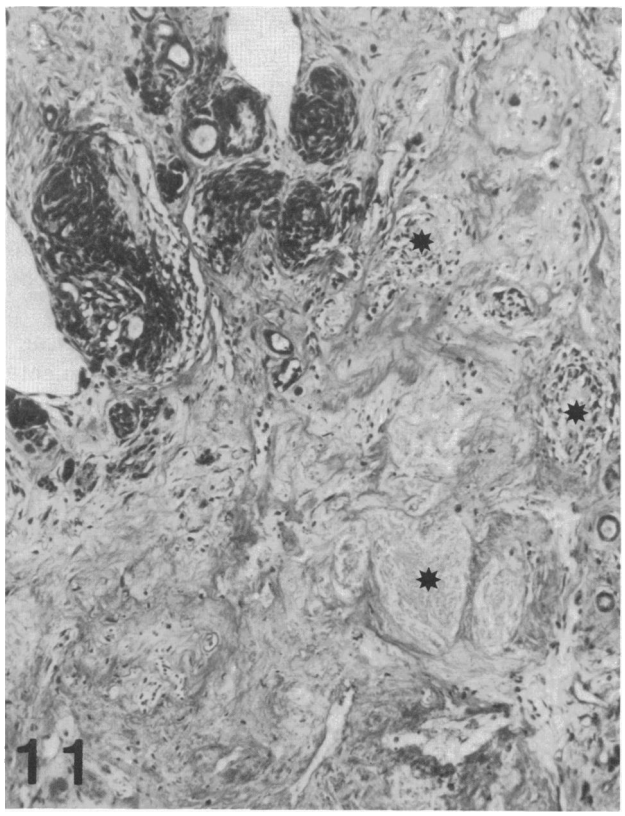
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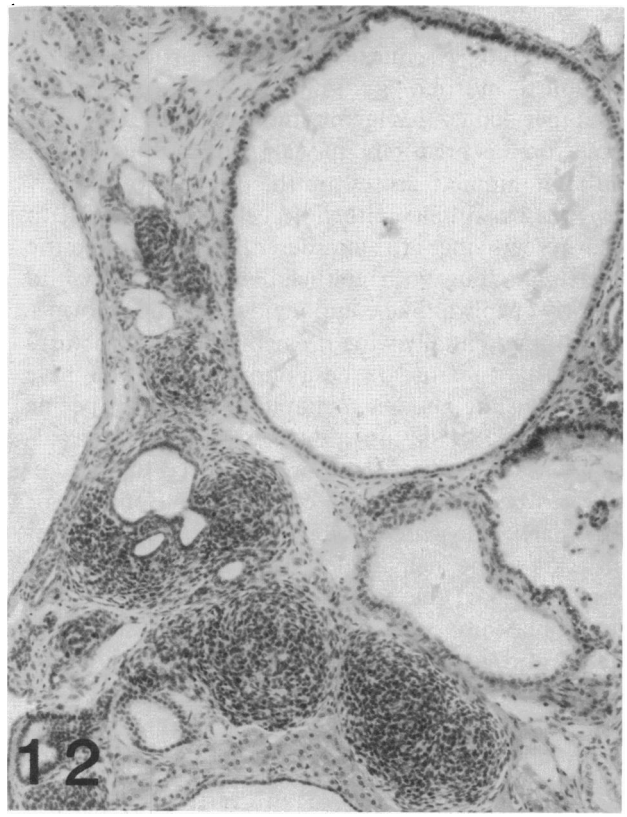
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Discussion

Nephroblastoma in the rabbit has been described variously in the literature as comparatively common (second in frequency to uterine malignancies in this species)[10] or of low spontaneous incidence.[9] A survey by Greene revealed only 9 recorded cases by 1943,[9] a number which had increased to 20 at the time of Weisbroth's review of 1974.[10] In a large, multibreed colony providing 6000 autopsies over a 14-year period, Greene estimated the spontaneous incidence of this tumor to be approximately 0.066% in rabbits. This level of spontaneous occurrence is somewhat higher than that estimated as the overall incidence level of 0.009% in the laboratory rat, but little different from the minimum frequency rate of 0.04% recorded for the inbred Nb rat strain.[8] The figure provides an indication of the natural background incidence of nephroblastoma in the rabbit against which tumor induction by chemical carcinogens can be measured. The spontaneous cases reported to date have been insufficient to establish clear histotypic characteristics for rabbit nephroblastoma. The present collection of 63 ENU-induced renal tumors of diverse age and size, all of which conformed to nephroblastoma, provides the opportunity to define histologic criteria for this rabbit cancer and to observe the chronologic sequence of tumor progression in relation to morphology.

As revealed by this large tumor series, rabbit nephroblastoma is predominantly an epithelial neoplasm. Pathognomonic features are the presence of densely crowded basophilic epithelioid cells with an undeniable resemblance to undifferentiated metanephric blastema, along with epithelial differentiation into primitive nephronlike components. Organoid formation of a potentially wide range of tubular structures is a constant feature of these tumors, while primitive glomeruluslike bodies, characterized by specific morphologic traits, are common. The papillary pattern characteristic of the more advanced tumors appears to be related to the complex development of these particular structures but is due to tubular differentiation in the incipient lesions. The close histologic resemblance of the esssential components of rabbit nephroblastoma, particularly in the early stages, to metanephrogenic blastema, which constitutes the developing kidney at the time of treatment,[26] points to a likely origin in this primordial tissue. In contrast to the transplacental results, these same rabbit strains are not susceptible to renal tumor development if treated postnatally with the carcinogen at 8 weeks of age,[13] when kidney maturation is complete.

In contrast to the overall epithelial emphasis, differentiation into specialized components of secondary mesenchyme, that is, muscle, adipose tissue, cartilage, and osteoid, is notable by its absence, confirming the observations of Greene,[9] Weisbroth,[10] and Hänichen and Stavrou.[21] The stromal fibrous tissue, being of benign character in direct continuity with the pseudocapsule, and following the distribution of normal blood vessels ramifying through the tumor, is clearly a local reaction to the neoplastic tissue. On the other hand, the fascicular disposition of spindle-shaped tumor cells infiltrating into the stromal tracts from the periphery of blastemal clusters was never sufficiently extensive as to warrant the histologic description of fibrosarcoma. Whether this limited mesenchymal disposition of tumor cells represented bipotential differentiation into secondary mesenchyme could not be determined within the constraints of light microscopy. Its delayed acquisition in concert with an increasing stromal reaction suggest that the change in tumor cell shape from epithelial to fusiform may have been conformational rather than a manifestation of differentiation. That is, the presence of so-called Wilms'-like features in rabbit nephroblastoma may be due, at least in part, to the progressive induction of a prominent fibrocollagenous reaction rather than to an expression of bipotential differentiation. In this respect, the rabbit neoplasm is quite different from the variants of human Wilms' tumor in which malignant connective tissue elements such as smooth or striated muscle can occur undisputably in combination with neoplastic blastema.[1,2,27]

With few exceptions, the tumors in this collection conformed in general histologic aspects to the spontaneous rabbit nephroblastomas reviewed and described by Greene[9] and Weisbroth[10] and those induced experimentally with ENU by Stavrou et al.[20] The discordant neoplasms, one in the experimental series of Stavrou et al[20] and one, a spontaneous growth, described by Greene,[9] were almost totally fibrosarcomatous in type and therefore may have represented a different tumor entity. Despite the basic morphologic identity, certain details of difference exist between our observations and the previously described tumor series. These differences pertain in particular to the recognition of increasing stromal reaction as a local response, interpretation of both glomeruloid bodies and the mesenchymal arrangement of tumor cells, rate of tumor development, and the occurrence of metastasis.

The evolution of an increasingly expansive stromal reaction leading to necrosis which can only be revealed by sequential tumor stages has not been ap-

preciated in previous reports. Although pseudoglomerulus structures are described as a common feature of the spontaneous neoplasms,[9,10] Hänichen and Stavrou[21] believed that equivalent bodies within their experimental tumors were not representative of glomeruloid differentiation but simply papillary invaginations within *de novo* tubules. The histologic distinctiveness of the structures in our series, viz. smaller cells supported by a discrete tuft of avascular basement membrane-like material, is strongly indicative of differentiation toward primitive glomerular formation. Electron-microscopic studies in progress confirm this interpretation as correct (Hard et al, manuscript in preparation). The rapid development of palpable nephroblastomas in the preadolescent, Jackson Laboratory rabbits, particularly following a single, high transplacental dose of ENU, contrasts with the maturity or old age of cases recorded previously. Indeed, based on the age distribution, the relatively small size of tumors, and the lack of metastases in all accounts of spontaneous occurrence, it has been accepted that nephroblastoma in the rabbit is a benign neoplasm of slow development.[10] The Jackson Laboratory collection of chemically induced tumors indicates that nephroblastoma can be a neoplasm of the immature rabbit with a reasonably high potential for malignancy, providing the inciting stimulus is adequate. Because the oldest tumor-bearing animal in the series was killed at the relatively early age of 15.25 months, it is likely that the metastatic rate noted in the IIIVO/J strain would have increased with prolonged survival. Furthermore, because one of the metastases in this series was detected at the level of microscopic examination, serial sampling of invasion-prone organs might have revealed a rate of invasion greater than observed. Very recently, various hybrid progeny involving the IIIVO/J strain also have demonstrated an equivalent susceptibility to metastasis from ENU-induced nephroblastoma.[28]

Interspecies comparison underscores some degree of variation in the histologic form of nephroblastoma. In man, these neoplasms represent a range from those consisting entirely of malignant blastema, with or without epithelial organoid differentiation, but dissected by fibrous stroma of benign appearance, to a combination of epithelium with secondary mesenchyme of overt malignancy.[27] In one hospital series, the proportion of epithelial tumors supported by apparently benign stroma, was preponderant over those incorporating malignant secondary mesenchyme as well.[29] The rabbit neoplasms are, therefore, quite similar to a substantial proportion of human Wilms' tumors. One detail of divergence is the sequence of organoid differentiation. Chatten[30] has noted a tendency for epithelial dedifferentiation in human nephroblastomas with increasing age. In rabbits, however, the propensity for expression of maturation in the form of glomeruloid bodies, reniform foci, and squamoid islands is delayed.

In the rat, nephroblastoma is an epithelial neoplasm with no malignant mesenchymal component, but merely a supportive stromal reaction.[7,8,31] Mesenchymal neoplasia in this species is an entity distinct from nephroblastoma, consisting of a spectrum of neoplastic connective tissue types with no malignant epithelial components. Renal mesenchymal tumor, which is frequently induced in rats during experimental studies with nitroso and related compounds,[32] is often confused with nephroblastoma because of the presence of tubules and mature glomerular profiles. Sequential studies using both light and electron microscopy have demonstrated clearly that these epithelial elements are sequestered remnants derived from preexisting parenchyma.[33-36] When directly compared with the rodent neoplasms, rabbit nephroblastoma shows none of the histologic characteristics of rat renal mesenchymal tumor but is identical to rat nephroblastoma by virtue of its epithelial character.

An attempt has been made in this report to define a histologic standard for nephroblastoma in the rabbit. Further characterization of this neoplasm and clarification of some of its histologic uncertainties are being pursued at the ultrastructural level. Classification as nephroblastomas, of all of the rabbit kidney tumors in the series of Jackson Laboratory experiments with ENU or its precursors, emphasizes the value of this high-incidence, single-dose system as a working model for neoplasia of metanephrogenic blastema. The rapid induction during preadolescence and the potential for malignancy indicate that the IIIVO/J strain is an appropriate animal model for the predominant histologic form of human Wilms' tumor.

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