IN VITRO TRANSFORMATION BY THE ADENOVIRUS-SV40 HYBRID VIRUSES

III. Morphology of Tumors Induced with Transformed Cells

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In the preceding paper, transformation of primary weanling hamster kidney (WHK) monolayer cultures by various adenovirus (adeno)-SV40 hybrid viruses and adeno type 12 and the characteristics of the cell lines which resulted were described (1). This report describes the morphology of the tumors resulting from transplantation of the transformed cell lines to hamsters.

Materials and Methods

Cell Lines.—The origin, nomenclature, passage history, and maintenance conditions of the cell lines have been given in detail (1).

Transplantation to Hamsters.—Cells from various passage levels (see Table I) were dispersed with trypsin, sedimented, suspended in phosphate-buffered saline pH 7.2, counted and inoculated subcutaneously into weanling or adult Golden Syrian hamsters (*Mesocricetus auratus*). The hamsters were observed weekly for the appearance of tumors. When the tumors reached a mean tumor diameter of 3–6 cm (2), the animals were bled, sacrificed, and portions of the tumor were fixed in 10% formol-saline solution or made into 10% suspensions (see accompanying paper). Multiple sections from various portions of each tumor were cut and stained with hematoxylin and eosin in the usual fashion. One tumor from each transformed cell line was transplanted to hamsters; all were transplantable and have been maintained in serial passage.

RESULTS

Tumor Incidence and Latent Period.—In Table I, data on the tumors derived from transplantation to hamsters of cells from the various transformed cell lines are given. All cell lines transplanted to hamsters; however, some, such as the adeno 3–SV40 hybrid–transformed cell lines (Ad. 3^+ HK 1–2, see reference 1) and the Ad. 2^{++} HK-1 cell line, had a distinctly lower malignant potential than the other cell lines. From the one cell line which was transplanted at two passage levels (Ad. 7^+ HK-2), there was some indication that cells at the later passage level were more malignant, as judged by latent period. Generally, tu-

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mors appeared earlier when larger numbers of cells were inoculated; the variation in inoculum size was due to the fact that many of the tumors were taken from "control" animals which were part of transplantation rejection experiments.

Gross Description of Tumors.—Tumors induced with Ad. 7^+ HK cells were lobular, pale gray, firm subcutaneous masses locally invading adjacent fascia and skeletal muscle. There was no gross evidence of metastases. The cut surface was firm, whitish-gray, and rubbery with a whorled appearance. Necrosis and

TABLE I

Tumors Induced with Adeno-SV40 Hybrid Viruses and Adenovirus 12

Cell line	Passage No.	No. cells inoc. (10^6)	No. tumors / No. inoc.	Latent period	Length of observation of negative hamsters	No. tumors studied
				*wk**	*months*	
Ad. 7^+ HK-1	6	1–3	1/10	3	1‡	1
" HK-2	4	1	7/7	4–8		7
" "	11	0.1–2.5	25/25	2–5		17
" HK-3	17	2	5/5	5–8		5
Ad. 2^{+t7} HK-1	3	0.75–1.5	9/10	3–8	5	7
" HK-2	3	0.5–4	10/10	3–11§		9
Ad. 2^{++} HK-1	5	1–3	5/10	8–10	5	5
" HK-2	5	1–3	9/9	3–4		9
Ad. 3^+ HK-1	5	1–3	1/8‖	17	8	1
" HK-2	5	1–3	1/9	16	9	1
Ad. 12 HK-1	4	1–3	10/10	2–4		9
Ad. 12^{+t7} HK-1	3	0.5–3	9/10	2–6	5	9

* Range of time from appearance of first to last tumor.

‡ Experiment had to be terminated at 4 wk due to lymphocytic choriomeningitis virus infection of hamsters.

§ 9 of the 10 tumors had appeared by the 8th wk after inoculation.

‖ Two of eight animals were found missing 8 wk after inoculation.

hemorrhage were variable and tended to be more prominent in the larger tumors. The Ad. 2^{+t7}, Ad. 2^{++}, and Ad. 3^+ HK cell lines gave rise to similar tumors but a greater portion of these tumors were necrotic; often 70–80% of a large tumor (>3 cm mean tumor diameter) consisted of brownish-red necrotic material.

Tumors derived from the Ad. 12 HK and Ad. 12^{+t7} HK lines were alike. Both types were fleshy, lobular, and circumscribed with thin membranous capsules. There was no gross evidence of local invasion or metastases. The reddish-tan cut surface was quite vascular with central liquifaction and hemorrhagic necrosis.

Microscopic Description.—Ad.7$^+$: 30 tumors induced by three lines of Ad. 7^+

HK cells were examined histologically. They were composed of whorls and bands of pleomorphic, elongate spindle cells with varying amounts of intercellular matrix (Fig. 1). Considerable necrosis was present in two-thirds of the tumors. Most of the tumors displayed a prominent anaplastic cellular component characterized by sheets of plump round to polygonal cells with finely granular acidophilic cytoplasm and large ovoid nuclei (Fig. 2). Both the polygonal anaplastic cells and the more differentiated spindle cells had large oval nuclei with sharply defined nuclear membranes, considerable pleomorphism and margination of coarsely clumped chromatin. The vesicular nuclei contained one to three very prominent acidophilic nucleoli that occasionally attained a diameter one-third that of the nuclei (Figs. 3 and 4). Mitoses were frequent and often bizarre. Multinucleated syncytial giant cells with homogeneous acidophilic cytoplasm were present in many of the tumors.

10 of the 30 tumors showed evidence of epithelial differentiation with cells aligned in columns or cords, and in 5 of the tumors there was frank tubule formation (Fig. 5). The epithelial cells were cuboidal with sharp cell margins, densely acidophilic cytoplasm, and small, uniform oval nuclei with uniformly dispersed granular chromatin.

Most of the morphologic features were similar to hamster tumors induced with SV40-transformed WHK cells (3, 4); these tumors were basically pleomorphic fibrosarcomas sometimes containing areas of carcinosarcoma with tubular differentiation. However, certain differences between tumors induced by SV40 and Ad. 7^+ HK cells were noted. The broad sheets of large polygonal anaplastic cells in Ad. 7^+ HK cell–induced tumors did not have a counterpart cell type in tumors induced by SV40-transformed WHK cells, although their nuclei with prominent nucleoli were indistinguishable from nuclei of SV40 tumor cells. 8 of the 30 tumors induced by Ad. 7^+ HK cells contained areas of spindle cell sarcoma with smaller more regular nuclei, more uniformly dispersed chromatin, and more nuclear basophilia than were noted in tumors induced by SV40-transformed cells (Figs. 6 and 7). This nuclear type did not have the prominent nucleoli typical of SV40-induced tumor cells. They more closely resembled nuclei of adenovirus-induced tumors (5, 6), which typically contain uniform small round cells in a trabecular pattern, scanty cytoplasm, and uniform ovoid nuclei (*vide infra*).

Ad. 2^{+t7}, 2^{++}, *and* 3^+: In general, tumors induced by Ad. 2^{+t7}, Ad. 2^{++}, and Ad. 3^+ HK cells resembled those induced by Ad. 7^+ HK cells (Table II); they were pleomorphic spindle cell sarcomas with some areas of myxoid and collagenous stroma. Many of the tumors induced by these hybrid-transformed cell lines had a minor cellular component of large round to polygonal anaplastic cells, but this feature was less prominent than in the tumors induced by Ad. 7^+ HK cells. Epithelial differentiation was present in several of the tumors induced by Ad. 2^{+t7} and Ad. 2^{++} HK cell lines and in one of the two Ad. 3^+ HK cell–induced tumors.

Tumors induced by Ad. 2^{+t7} HK cells tended to be more compact and cellular with less intercellular matrix than tumors induced by either SV40– or Ad. 7^{+}–transformed cells. Of the 16 tumors studied, 12 contained areas of spindle cells with small uniform nuclei which resembled those of adenovirus tumor cells. One tumor induced by Ad. 2^{+t7} HK cells contained two small foci of undifferentiated small round cell tumor surrounded by, and sharply demarcated from, adjacent spindle cell sarcoma (Fig. 8). These small tumor cells had very scanty basophilic cytoplasm and uniform round hyperchromatic nuclei. The two cell nests were very similar to typical adenovirus-induced hamster tumor cells (5).

TABLE II

Summary of Histology of Tumors Induced by Transformed Hamster Kidney Cells

Transforming virus	Tumor cell morphology			Epithelial cord or tubule formation
	SV40*	Ad.‡	Mixed Ad.–SV40§	
Ad. 7^{+}	22/30		8/30	10/30
Ad. 2^{+t7}	4/16		12/16	6/16
Ad. 2^{++}	7/14		7/14	7/14
Ad. 3^{+}	1/2	1/2		1/2
Ad. 12		9/9		0/9
Ad. 12^{+t7}		8/9	1/9	0/9

* Tumors of pleomorphic spindle cell sarcoma type.

‡ Tumors of small cell undifferentiated type.

§ Tumors primarily composed of cells having characteristics intermediate between typical SV40 and adenovirus tumor cells. One tumor induced by Ad. 2^{+t7}–and two by Ad. 2^{++}–transformed cell lines contained small areas resembling adenovirus tumor.

The Ad. 2^{+t7} cell lines were carried for two passages in medium containing a low calcium concentration (0.1 mM) (1, 7), in an attempt to select for cells having the growth characteristics of adenovirus tumor cells. Cells grown in this medium induced tumors with a varied histologic pattern. Some areas of the tumors were composed of spindle cell sarcoma resembling Ad. 7^{+} tumors, and other areas contained undifferentiated cells oriented about vascular spaces in a palisading configuration similar to that of adenovirus tumors (Fig. 9).

Half of the 14 tumors induced by Ad. 2^{++} HK cells contained a spindle cell component with small uniform nuclei similar to those seen in several tumors induced with Ad. 2^{+t7} HK cells. In two of the Ad. 2^{++} HK cell–induced tumors, nodular projections of smaller more uniform spindly cells extended into clefts and vascular spaces (Fig. 10), resembling adenovirus-induced tumors.

One of the two tumors induced by Ad. 3^{+} HK cells was histologically identical to the tumors induced by Ad. 7^{+} HK cells. The other was entirely different, being largely necrotic with a peripheral rim of uniform round to spindly cells

with scanty cytoplasm and medium sized round hyperchromatic nuclei (Fig. 11). This tumor had the appearance of an adenovirus-induced tumor.

Ad. 12: All nine tumors induced by Ad. 12 HK cells were undifferentiated round to spindly cell tumors similar to primary Ad. 12 virus-induced hamster tumors (5, 6). There was considerable necrosis and marked vascularity with surviving islands of tumor cells frequently oriented about capillary channels in a trabecular or palisading arrangement (Fig. 12). Where the palisading configurations were cut in cross-section, rosette formation was evident. The small uniform tumor cells contained regular, ovoid, hyperchromatic to opaque nuclei (Fig. 13). Margination of nuclear chromatin or prominent nucleoli typical of SV40-transformed WHK cells were not noted in these tumors.

Ad. 12^{+t7}: Tumors induced by Ad. 12^{+t7} HK cells resembled the Ad. 12 HK cell–induced tumors (Fig. 14). They were composed of uniform, round, small to medium sized undifferentiated cells with abundant necrosis and prominent perivascular preservation of viable cells. Palisading and rosettes were consistent features. The nuclei of the tumor cells induced by Ad. 12^{+t7} HK cells had the same appearance as those of tumor cells induced by Ad. 12 HK cells with the exception of one tumor. In addition to the typical adenovirus cell type in this tumor, there were other areas of larger cells with more vesicular nuclei and prominent nucleoli indicative of cellular characteristics intermediate between those of the typical SV40- and adenovirus-induced tumor cells (Fig. 15).

Animal Transplantation.—Transformed cell–induced tumors serially transplanted through at least two animal passages generally maintained their morphologic characteristics. Transplanted Ad. 2^{++} HK cell–induced tumors tended to become more cellular and anaplastic, but still retained the characteristic SV40 nuclei. Three Ad. 2^{+t7} HK-1 tumors in the second animal passage had the appearance of undifferentiated large round cell sarcomas; two of these contained typical vesicular SV40 type nuclei and the third contained more uniform adenovirus type nuclei.

DISCUSSION

Hamster tumors induced by several SV40-transformed hamster cell lines have been described as pleomorphic fibrosarcomas, some of which also contained a carcinomatous component with frank tubule formation (3, 4, 8, 9). Tumors induced by Ad. 7^{+}, Ad. 2^{+t7}, Ad. 2^{++}, and Ad. 3^{+} HK cell lines resembled those produced by SV40-transformed WHK cells for the most part and consisted of both sarcomatous and carcinomatous components. Tumors induced by Ad. 12^{+t7} HK cells resembled those induced by Ad. 12–transformed cells with the exception of one tumor with larger cells, vesicular nuclei, and prominent nucleoli more suggestive of SV40 tumor cells. Cytologic examination of these hybrid transformed cell lines in vitro revealed a morphologic picture which was

similar to that of the tumors induced with these cell lines with the exception that tubule formation was not present in the in vitro preparations (1).

Differences between tumors induced by SV40-transformed and Ad. 7^{+}–, Ad. 2^{+t7}–, Ad. 2^{++}–, and Ad. 3^{+}–transformed HK cell lines included the following: the hybrid tumors contained a large polygonal anaplastic cell type not found in the SV40 tumors; within the hybrid tumors there frequently were areas composed of spindle cells with smaller uniform nuclei resembling nuclei of adenovirus tumor cells; several Ad. 7^{+}, 2^{+t7}, and 2^{++} tumors contained two different cell types, one resembling an adenovirus and the other an SV40 tumor cell; one Ad. 3^{+} tumor had the histologic appearance of an adenovirus tumor. Thus, features were present in some tumors induced with all the hybrid-transformed cell lines which suggested the phenotypic expression of both adenovirus and SV40 genomes in the transformed cells.

Huebner et. al. (10) reported that subcutaneous inoculation of adeno 7–SV40 hybrid virus into newborn hamsters could result in tumors histologically similar to either SV40– or Ad. 7–induced tumors, and that one tumor was clearly dimorphic. Rabson et. al. (11) recently reported that hamster tumors induced with adeno 7–SV40 hybrid–transformed hamster kidney cells resembled Ad. 12 virus-induced tumors with small areas resembling fibrosarcoma. Kirschstein et. al. (12) found that intracerebral inoculation of the adeno 7–SV40 hybrid into newborn hamsters produced ependymomas identical to those induced by SV40. These reports, together with the data presented here, indicate that both or either viral genomes in the hybrid particle may be phenotypically expressed. The factors that determine which portion of the hybrid genome will be expressed are not known at present. In the Ad. 12^{+t7}–transformed cell the Ad. 12 genome determines the phenotypic expression although both T antigens are present in the transformed cells. In the Ad. 7^{+}–, Ad. 2^{+t7}–, and Ad. 2^{++}–transformed cells the SV40 genome predominates with respect to morphology and antigen content but preliminary evidence suggests that adeno antigens are present in these cells as well (1).

The Ad. 7^{+} and 3^{+} hybrids were derived from weakly oncogenic strains of adenovirus 7 and 3 respectively. Therefore, the determinants of adenovirus characteristics in tumor cells derived from cells transformed by these hybrids were from the DNA's of known, although moderately, oncogenic adenoviruses. This may also be true with Ad. 2^{+t7} hybrid–transformed cells, since some adenovirus 7 DNA is present within this hybrid (13). The only adenovirus DNA, however, present in the Ad. 2^{++} hybrid preparation is adenovirus 2 DNA (14), and the fact that some transformed cell–induced tumors had adenovirus characteristics suggests that a portion(s) of the adenovirus 2 genome is operative in at least some transformed cells. Presumably, the presence of the SV40 genome enhanced the integration of a portion(s) of the genome of an adenovirus which has not been shown to be oncogenic by itself.

The presence of epithelial differentiation with tubule formation in the carcinosarcomatous tumors induced by the Ad. 7^+, 3^+, and 2^{+t7} hybrids indicates that sufficient SV40 genome is present in these hybrids to duplicate all the histologic variations present in tumors induced with SV40-transformed hamster cells. Whether the genetic information which is responsible for this histology is identical to that which codes for the T (15) and transplantation rejection antigens (16), the only other known markers of the SV40 DNA present in the hybrid population, is not known at present.

It is noteworthy that the target cell for the SV40 and adenovirus-SV40 hybrid viruses in WHK may be the same cell since the transformations by these viruses may result in morphologically similar tumors. An analysis of SV40-transformed WHK cells revealed that clones derived from single cells produced mixed tumors in hamsters. It was postulated that the target cell involved in this transformation was an undifferentiated metanephric blastema cell (4). This same cell may be involved in the hybrid transformation and would, therefore, be susceptible to the adenovirus capsid, as well as SV40, since all the particles in the 7^+, 2^{++}, 2^{+t7}, and 12^{+t7} populations are contained in adenovirus capsids. If one cell type is involved, the fact that SV40-like tumors result from cells transformed by the 7^+, 2^{++}, and 2^{+t7} hybrids and adeno-like tumors from Ad. 12– and Ad. 12^{+t7}–transformed cells would indicate that different genomes in the same cell result in different morphologic types. At present one cannot rule out the possibility that two different cell types were infected by some of the hybrid preparations, one leading to an SV40 and the other to an adeno type morphology. The fact that some tumors from all hybrid-transformed cells contained cells with a morphology intermediate between adenovirus and SV40 tumor cells suggest that both genomes were operative in at least these cells. Studies are in progress to determine whether transformation by these various viruses of a cell population derived from a single cell results in the same or different morphological expressions of the viral genomes.

SUMMARY

Tumors induced with hamster kidney cells transformed by the adeno 2–, adeno 3–, adeno 7–, and adeno 12–SV40 hybrid viruses, and by adenovirus type 12, were examined histologically. The tumors induced with adeno 2–, adeno 3–, and adeno 7–SV40–transformed cells were similar to tumors induced with SV40–transformed hamster kidney cells but contained cells intermediate in morphology between SV40 and adenovirus tumor cells and occasionally contained nests of adenovirus-like cells. Cells transformed by the adeno 12–SV40 hybrid and by adenovirus type 12 gave rise to morphologically similar tumors.

The results suggest that both viral genomes are operative in hybrid-transformed cells but that one genome is apparently responsible for the predominant

morphology of the tumor. Evidence that the morphology of a single transformed target cell is determined by the transforming genome was discussed.

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EXPLANATION OF PLATES

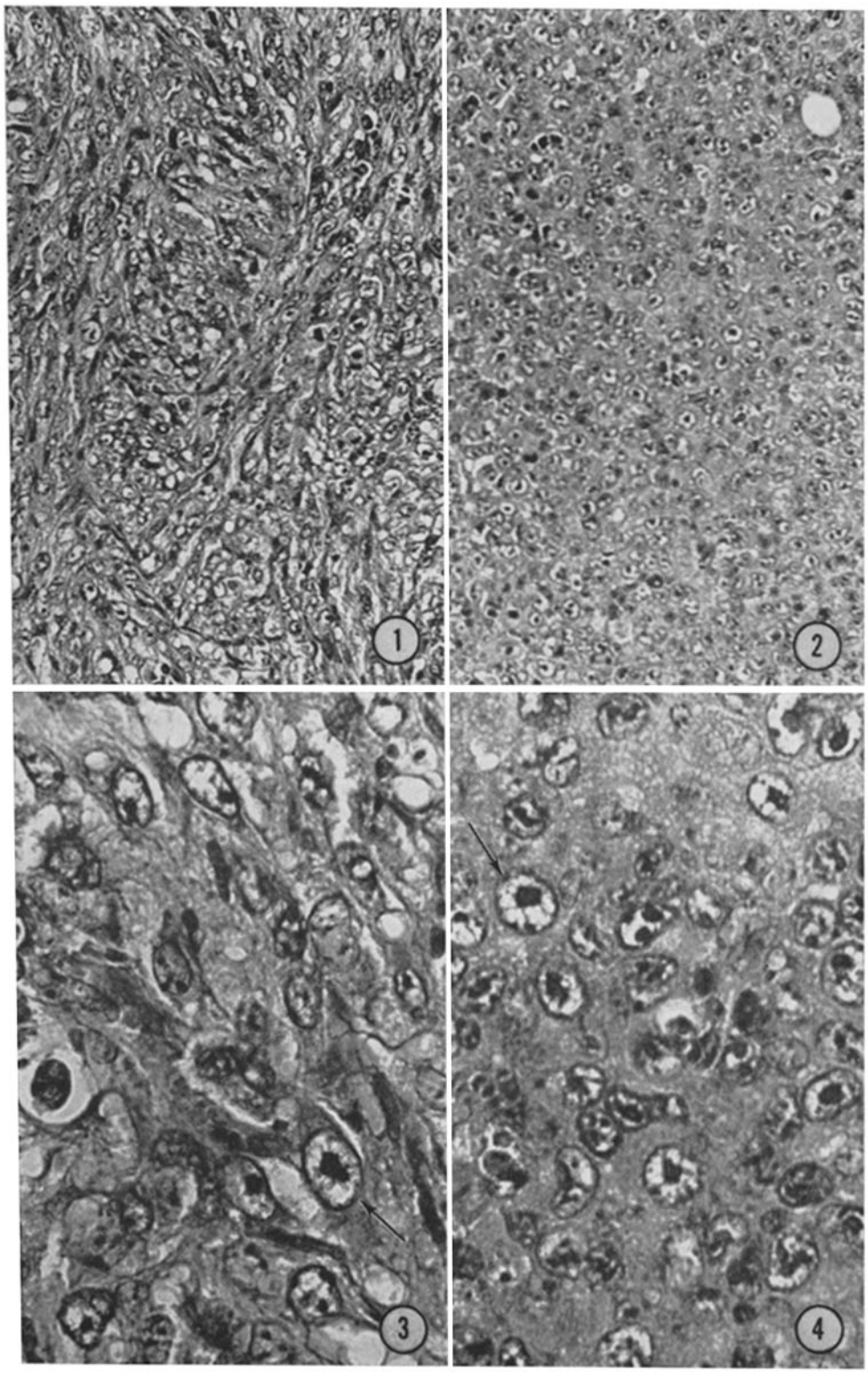
PLATE 77

FIG. 1. Pleomorphic spindle cell sarcoma induced by adeno 7–SV40 transformed cells. This pattern is typical of SV40 viral-induced hamster tumors and the sarcomatous component of tumors induced by SV40-transformed WHK cells. × 130.

FIG. 2. Anaplastic cellular component in tumor induced by adeno 7–SV40–transformed cells. The cells are large, round to polygonal, with no organoid pattern. × 130.

FIG. 3. Higher magnification of Fig. 1, showing large ovoid, vesicular nuclei with prominent nucleoli (arrow). These nuclear characteristics are typical of SV40 tumors. × 400.

FIG. 4. Higher magnification of anaplastic component of adeno 7–SV40 HK cell–induced tumor demonstrating nuclear similarities (arrow) to the spindle cell component (see Fig. 3). × 400.



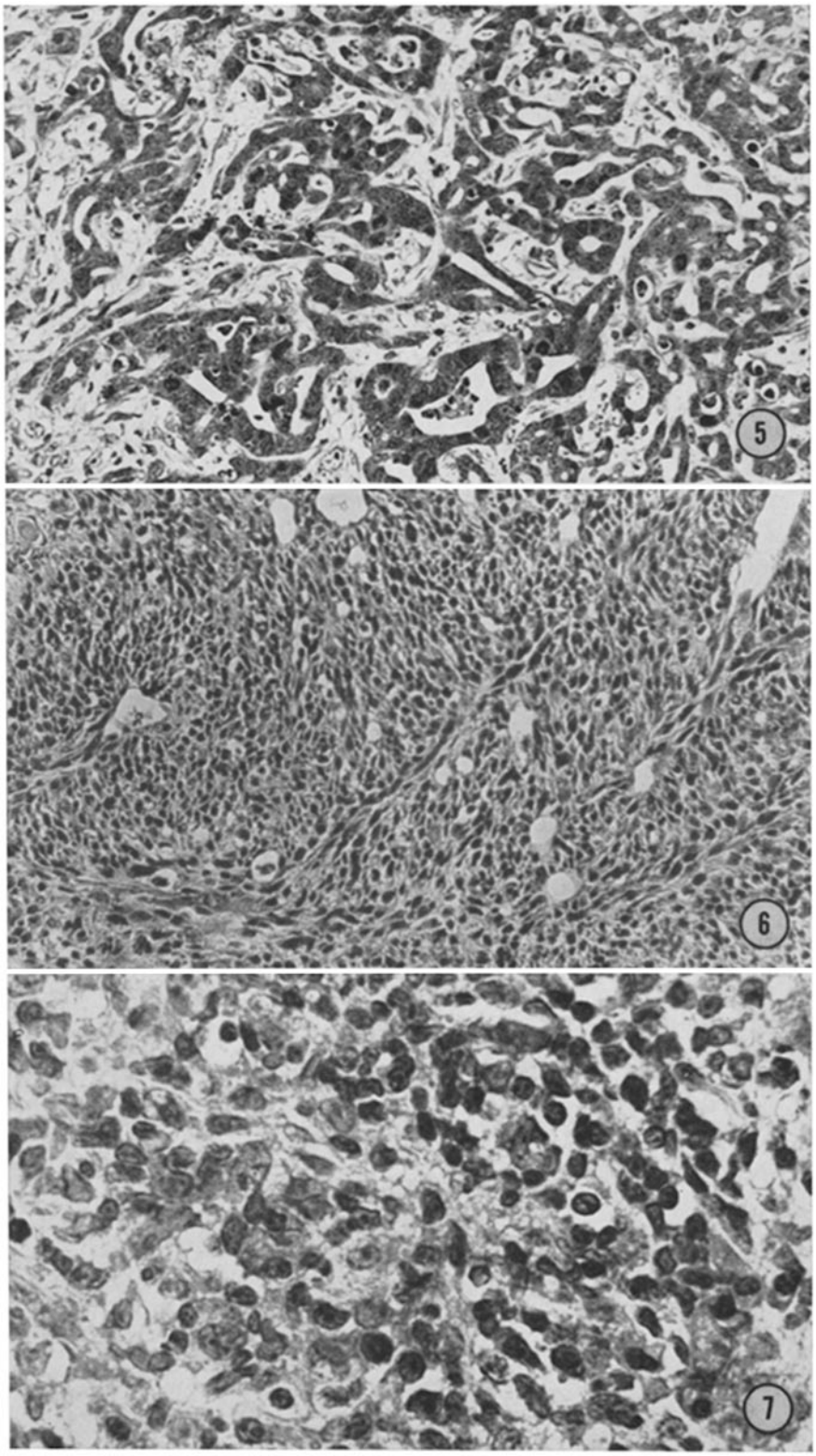
(Igel and Black: Adenovirus-SV40 hybrid viruses. III)

PLATE 78

FIG. 5. Epithelial differentiation with tubule formation in tumors induced by adeno 7–SV40–transformed HK cells. The cuboidal epithelial cells are sharply demarcated from the adjacent sarcomatous tissue. Such differentiation is typical of tumors induced by SV40–transformed hamster kidney cells. × 130.

FIG. 6. Small spindle cell component in tumors induced with adeno 7–SV40–transformed cells with partially developed perivascular polarity and palisading suggestive of adenovirus-induced tumor. × 130.

FIG. 7. Higher magnification of Fig. 6, showing smaller hyperchromatic nuclei more closely resembling those of adenovirus–transformed HK cell–induced tumors (compare with Fig. 13). × 400.



(Igel and Black: Adenovirus-SV40 hybrid viruses. III)

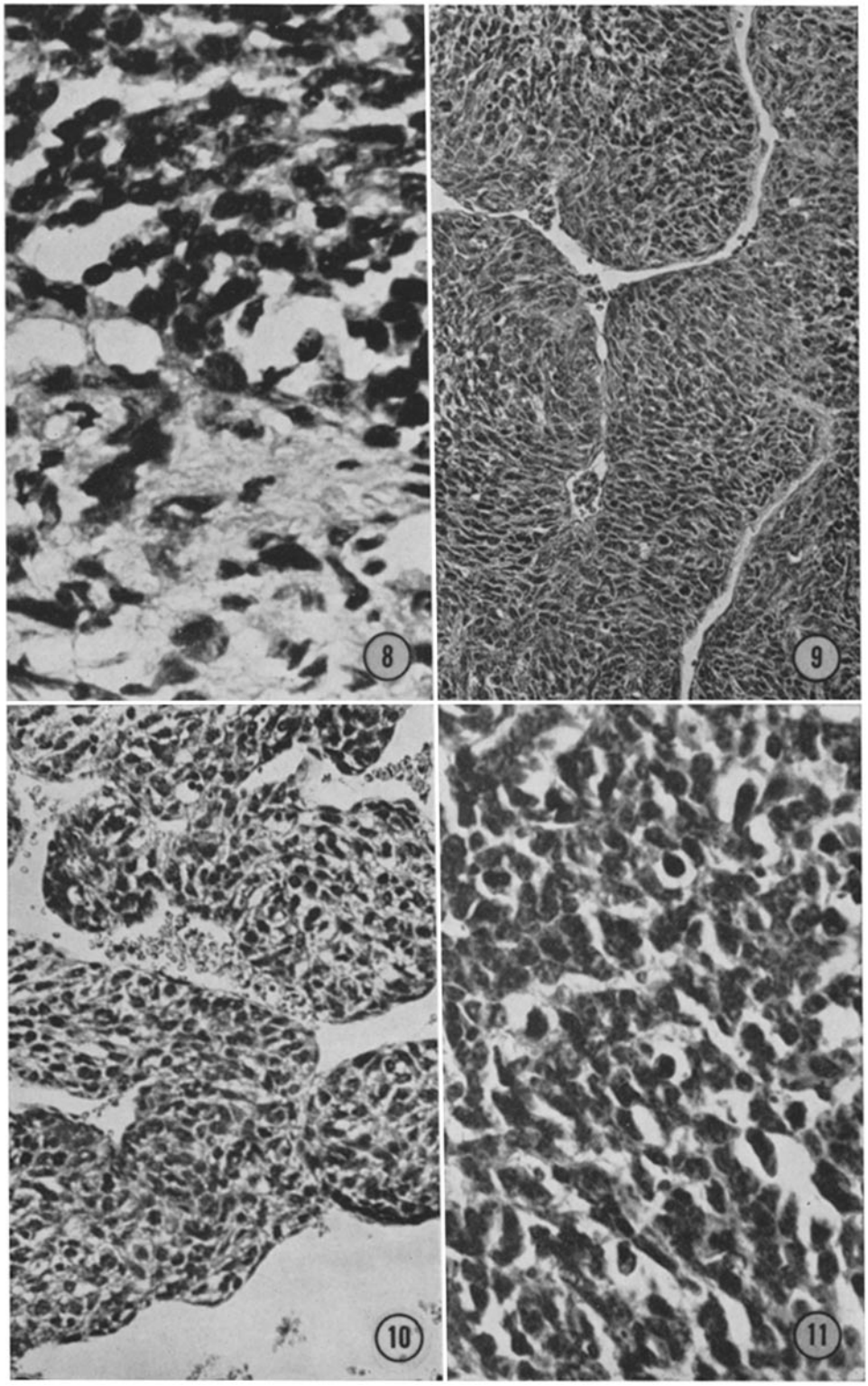
PLATE 79

FIG. 8. Dimorphic pattern in tumors induced by Ad. 2^{+t7}–transformed cells. Small cells resembling adenovirus–induced tumor are sharply demarcated from the adjacent pleomorphic spindle cell sarcoma. × 400.

FIG. 9. Tumor induced by Ad. 2^{+t7}–transformed cells carried in culture in the presence of low calcium medium. The fairly uniform cells oriented about numerous vascular channels are similar to adenovirus–induced tumor cells. × 130.

FIG. 10. Nodular projections of fairly uniform cells into vascular spaces of a tumor induced by Ad. 2^{++} HK cells. This configuration is more suggestive of adenovirus than SV40–induced tumor morphology. Note similarity to Figs. 6 and 12. × 130.

FIG. 11. Peripheral rim of viable tumor induced by adeno 3–SV40–transformed cells. The cells containing hyperchromatic nuclei and scanty cytoplasm are quite similar to those of tumors induced by Ad. 12–transformed cells in Figs. 12 and 13. × 400.



(Igel and Black: Adenovirus-SV40 hybrid viruses. III)

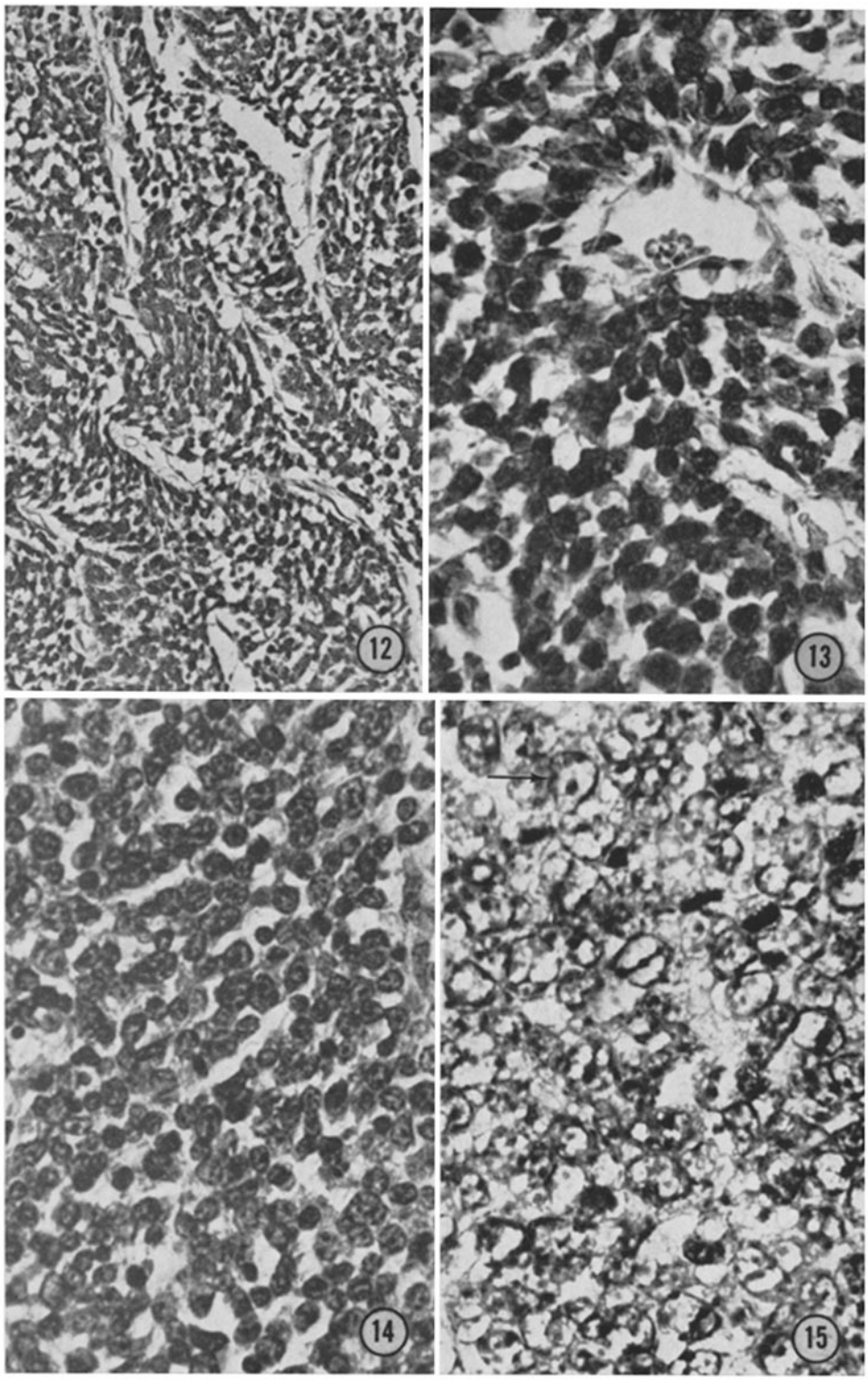
PLATE 80

FIG. 12. Morphology of tumors induced by adenovirus type 12–transformed cells. Note compact uniform cell type with prominent vascularity. This pattern is also typical of adeno 12 virus–induced tumors. × 130.

FIG. 13. Higher magnification of Fig. 12 illustrating the small uniform undifferentiated adeno type cell with uniform round hyperchromatic nucleus. × 400.

FIG. 14. Uniform small round cells of tumor induced by Ad. 12^{+t7} HK cells almost identical to those noted in tumors induced by Ad. 12–transformed cells (Fig. 13). × 400.

FIG. 15. Large anaplastic cell type found in one of the tumors induced by Ad. 12^{+t7}–transformed cells. These cells have pleomorphic vesicular nuclei, prominent nucleoli, and margination of nuclear chromatin (arrow) resembling nuclei of tumors induced by SV40–transformed HK cells. × 400.



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