Induction of Tumors in Rats by Tissue-Culture Preparations of SE Polyoma Virus

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Summary

Sixty-five newborn Sprague-Dawley rats were inoculated with SE polyoma virus that had been propagated in mouse-embryo tissue cultures. Eighteen of the rats developed renal sarcomas and 7, subcutaneous tumors. A tissue mince of renal sarcoma and spleen from a rat with an induced kidney tumor was used as an initial inoculum for a series of mouse-embryo tissue cultures. The culture fluid from the fourth serial passage induced visceral tumors in hamsters like those produced by previous tissue-culture lines of the SE polyoma virus. The fluid from this culture after 12 serial passages again induced renal sarcomas in rats. The rat is the third mammalian species to develop significant numbers of tumors after receiving, in the newborn period, SE polyoma virus grown in tissue culture.—J. Nat. Cancer Inst. 22: 161-171, 1959.

Recent investigations (1-5) indicate that tissue-culture preparations inoculated with a replicating agent, the SE polyoma virus, induce tumors in (C3Hf × AKR)F1 hybrid mice, randomly bred Swiss mice, and hamsters. Properties which indicate that the replicating agent is a virus are being reported in current publications (6-8). The present report describes the method of inducing neoplasms in randomly bred Sprague-Dawley rats and the recovery of the virus from mouse-embryo tissue cultures inoculated with an extract of tumor tissue from a rat that had received the SE polyoma virus when newborn.

Materials and Methods

The detailed history of the 2 virus cultures used in these studies, lines 3469 and 3919, was given in a previous publication (3). Both lines are related through their common origin from tissues of a single AKR mouse that developed leukemia spontaneously. Tissues from the AKR mouse, which were infiltrated with leukemic cells, were used as an inoculum for a

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monkey-kidney tissue-culture preparation designated 24424. The de-
canted fluid from tissue-culture line 24424, passage 2, induced salivary-
gland tumors in \((C3Hf \times AKR)F_1\) hybrid mice. An extract of the result-
ant salivary-gland tumors of 1 mouse was used to inoculate the first of a
series of monkey-kidney and mouse-embryo tissue cultures. The decanted
fluids derived from these cultures were capable of inducing multiple tumors
in mice and hamsters and were designated 3469. Pooled tissues from 2
\((C3Hf \times AKR)F_1\) hybrid mice that had received fluid from the fourth
passage of tissue-culture line 3469 served as inoculum for a third series of
tissue cultures designated 3672. A fourth series, 3919, was initiated by
inoculating mouse-embryo tissue cultures with the extract of a parotid-
gland tumor induced in a \((C3Hf \times AKR)F_1\) hybrid mouse with fluid from
the third culture passage of line 3672.

The early passages of 3469 were made in monkey-kidney tissue cul-
tures; later passages, including the ones used in this study, were from
Swiss mouse-embryo tissue cultures. Passages were made by trans-
ferring 2 ml. of fluid from a culture that had been incubated at 36° C. for
14 days or longer to a 2-ounce prescription bottle containing a monolayer
of mouse-embryo cells and 8 ml. of medium. The cultures were nourished
with synthetic medium 199 (9) and 1 percent calf serum. Nutrient fluids
were replaced at weekly intervals, but only fluids removed after 2 or more
weeks were transferred to new cultures or used for animal inoculation.

Five litters of rats were inoculated with culture-grown virus preparations
3469 and 3919 (table 1). In experiment 1 a single litter received the
decanted fluid from the thirteenth passage of tissue-culture-grown virus
preparation 3469, and in experiment 2 a litter received decanted fluid
from the nineteenth passage. In experiment 3 a litter of rats was divided;
6 of the litter received the supernatant fluid of centrifuged virus prepara-
tion 3469, culture-passage 20, and 8 received sediment from the same virus
preparation. In experiment 4, 1 litter received the supernatant fluid from
centrifuged virus preparation 3919, culture-passage 10, and a second litter
received the sediment.

Separation of the fractions in experiments 3 and 4 was carried out by
first removing cellular debris in the tissue-culture fluid by centrifugation
in an angle centrifuge at 4000 rpm for 20 minutes. The decanted fluid
was measured and normal rabbit serum was added to a concentration of 1
percent. The mixture was then centrifuged in a #40 rotor in a Spinco
high-speed centrifuge, model L, at 85,780 to 142,900 \(\times g\) for 3 hours.
The top milliliter of fluid in each 10 ml. cup served as the supernatant
inoculums in experiments 3a and 4a. The pellet on the bottom of each
cup was suspended in 1 ml. of Earle's balanced salt solution (10) and
served as the sediment inoculums in experiments 3b and 4b.

A virus tissue-culture line, 632, was initiated by inoculating a 5-day-old
mouse-embryo tissue culture with the minced spleen and tumor tissue from
a rat with an induced renal tumor. The culture was maintained as
previously described, except that horse serum rather than calf serum was
used in the maintenance medium. Fluid from this culture was passed
Tissue-culture-grown virus, in 0.25 ml. amounts, was injected into the subcutaneous tissue of the back of all rats when they were less than 24 hours old. The hamsters received 0.2 ml. of a virus tissue-culture preparation at the same site. The animals were housed in metal, shoe-box cages and given unrestricted quantities of Purina laboratory chow and water. All inoculated animals were allowed to remain with their mothers until at least 21 days of age. They were observed daily for death or signs of disease. Moribund animals were killed. Tumors in either rats or hamsters, unless they occurred subcutaneously, were not noted until the time of autopsy. Selected tissues of rats with macroscopic tumors were fixed in 10 percent formalin, embedded, sectioned, and stained with hematoxylin and eosin.

Results

**Induction of Tumors With Tissue-Culture Passages of Extracts of Mouse Tumors**

The results of inoculating newborn Sprague-Dawley rats with virus tissue-culture preparations are given in table 1. The first four experiments are represented by 54 rats inoculated with tissue-culture passage of lines initiated with extracts of mouse tumors. Eight of the 54 animals died, and these decomposed to such an extent that the animals were unsuitable for adequate *post-mortem* examination. Of the effective total of 46 rats, 4 developed subcutaneous tumors and 12 developed renal tumors. In the 2 experiments designed to concentrate the active principle, 1 of 15 rats inoculated with supernatant fluid from material centrifuged at 85,780 to 142,900 × g developed a renal tumor, while the sediment induced 2 subcutaneous tumors and 9 renal tumors in the 21 animals inoculated. It is important to note that sediment from tissue-culture line 3919, passage 10, was the most potent preparation used, causing 9 of 13 rats to develop renal tumors and accounting for half of all the renal tumors induced. As yet we have no complete explanation for the apparent variations in the potency of a given virus culture preparation. It is obvious that this particular passage of 3919 was more potent than the 3 passages of 3469 which were tested, and that centrifugation is capable of concentrating the tumor-inducing factor.

**Induction of Tumors With Fluid From Tissue Cultures Incubated With an Extract of an Induced Rat Tumor**

Table 2 shows the grossly observed tumors noted in a litter of hamsters that had been inoculated with the fourth passage of culture line 632,
Table 1.—Response of Sprague-Dawley rats inoculated at birth with mouse-embryo tissue-culture preparations of the SE polyoma virus

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Virus line*</th>
<th>Fraction of virus preparation used</th>
<th>Rats inoculated</th>
<th>Rats lost</th>
<th>Effective total</th>
<th>Rats with subcutaneous tumors</th>
<th>Rats with renal sarcomas</th>
<th>Rats surviving without tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3469-13</td>
<td>Decanted fluid</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3469-19</td>
<td>Decanted fluid</td>
<td>12</td>
<td>4</td>
<td>8</td>
<td>1</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>3a</td>
<td>3469-20</td>
<td>Supernatant fluid</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>3b</td>
<td>3469-20</td>
<td>Sediment</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>4a</td>
<td>3919-10</td>
<td>Supernatant fluid</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
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<td>4b</td>
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<td>Sediment</td>
<td>13</td>
<td>2</td>
<td>11</td>
<td>1</td>
<td>9†</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>632-12</td>
<td>Decanted fluid</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>3</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

*The last two numbers designate the particular tissue-culture passage used as the inoculum.
†Tissue from renal sarcoma and spleen of one rat used as inoculum to initiate virus-culture line 632.
RAT TUMORS INDUCED BY SE POLYOMA VIRUS

which had been recovered from a rat with an induced renal sarcoma. The character and distribution of the induced tumors were similar to the tumors induced in hamsters by SE polyoma virus-culture fluid (3). The results for 11 newborn rats that received virus preparation 632 are shown in table 1, experiment 5. Renal sarcomas occurred in 2 rats that died at 56 days of age, in 3 that died within a 2½-month period, and in 1 that died at 4½ months. The remaining 5 rats under observation for 5 months show no sign of disease. Five of the 6 rats with renal sarcomas had bilateral tumors, and 1 showed an early hemangioma of the liver that might have represented a metastasis. Three of the animals also had subcutaneous tumors.

TABLE 2.—Distribution of gross neoplasms in 7 hamsters receiving 4th passage tissue-culture line of 632 at 3 days of age

<table>
<thead>
<tr>
<th>Hamster number</th>
<th>Age at death (days)</th>
<th>Sites of tumors:*</th>
<th>Gastro-intestinal tract</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Less than 4</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Grossly noted tumors in lungs and livers consisted of multiple blood-filled loculi; tumors in the other organs were solid circumscribed masses which occurred on the serosal surface of the gastrointestinal tract and heart, and within the myocardium and renal pyramids. All tumors were macroscopically similar to those described previously by Eddy et al. (9).

Pathology of the Induced Tumors

Selected tissues from 21 rats were studied histologically. The tissues generally included were the heart, lungs, salivary glands, liver, spleen, and kidneys. Four rats each had single subcutaneous tumors. On histologic examination 2 of these tumors had admixtures of stromal and glandular elements typical of the murine fibroadenoma of the breast. The remaining 2 subcutaneous tumors showed evidence of invasive growth and marked anaplasia of connective-tissue cells; 1 of these contained areas of neoplastic fat cells as well as fibrosarcomatous elements. Eighteen rats had tumors originating in the kidneys; in 10 animals the renal tumors were bilateral. The renal tumors were sufficiently alike histologically to be considered as a group. The site of origin of the smaller tumors was near the corticomedullary junction. Growth occurred through extension to the renal papillae and into the pelves of the kidneys. In most cases, even where there was retroperitoneal extension, a thin strip of subcapsular cortex survived. Grossly, the tumors were red due to abundant vascularity. Retroperitoneal hemorrhage from the tumors caused some deaths. In 2 of the animals direct growth of the renal tumor had involved much of the retroperitoneal space and had penetrated the peritoneum where
contiguous growth involved the serosa of the omentum, mesentary, and gastrointestinal tract (fig. 1). The renal tumors of these 2 animals represented the largest primary growths in the group, each measuring over 5 cm. in greatest diameter.

Histologically, all the renal tumors were composed of both solid cell masses and cavernous blood-filled spaces that were lined by tumor cells. The cavernous vascular pattern predominated. The larger spaces were filled with organized thrombi and were lined by multicellular layers of neoplastic cuboidal cells supported by a scant fibrous stroma (fig. 2). Papillary cords of such tissue sometimes separated the vascular spaces into incomplete loculi. At the margins of these areas various neoplastic cell types could be noted. Large fusiform cells were prominent and these areas contained bizarre, sometimes multinucleated, giant cells similar to cells seen in myosarcomas (fig. 3). Myofibrils were apparent in some tumors but striated muscle fibers could not be identified. In other areas, elongated spindle cells with blunt cigar-shaped nuclei formed whorled sheaves and palisading arrangements separated by wavy bundles of collagen—patterns associated with the growth of smooth muscle tumors. Frequently within the same tumor, large areas of closely packed polymorphous cells with large ovoid nuclei, prominent nucleoli, and scant cytoplasm could be found. Such areas frequently contained blood-filled clefts and spaces lined only by tumor cells (fig. 4). In other tumors thin sheets of similar, rapidly growing cells were surrounded by a dense eosinophilic, osteoid-like ground substance which contained areas of mineralization. Mitotic activity in the tumors varied with the degree of anaplasia. The less-differentiated areas showed as many as 3 to 4 mitotic figures per high-power field, while areas of the tumors with well-defined vascular or muscular cells rarely contained mitotic figures.

Three of the 18 rats with renal sarcomas had tumor deposits within pulmonary veins which showed extension into surrounding pulmonary tissue. The pulmonary metastases mimicked the primary renal sarcomas even to the occasional formation of cavernous hemangiomatous spaces (fig. 5). We have tentatively designated the renal tumors as angiosarcomas, but it should be emphasized that the malignant potential of these tumors has not been thoroughly evaluated. The vascular origin is based on the more clearly defined neoplastic vascular elements of the primary tumors and of their metastases; however, not all the cellular changes observed fit into a simple pattern of vascular neoplasia. Several of the tumors showed apparent osteoid formation and calcification. It is quite possible that the vascular pattern represents only one of several lines of neoplastic growth by totipotential cells of the renal blastema. None of the tumors contained identifiable epithelial elements nor did they show any close resemblance to the embryonal adenosarcomas of human kidneys.

Discussion

The significance of the subcutaneous tumors that developed in 7 of the 65 rats is uncertain because such tumors occur occasionally as spontaneous
tumors in our colony. There is little doubt about the significance of the renal sarcomas that developed in 18 of the 65 rats inoculated—an over-all incidence of 28 percent. The experience of Davis et al. (11), with 150 aged female Sprague-Dawley rats, and Simms et al. (12), with over 2,000 Sprague-Dawley rats allowed to live a normal lifespan, indicates, even better than our limited experience, that the incidence of spontaneous renal sarcomas is probably much less than 1 percent. It is apparent that the response of the rat to tumor induction by our technique is not as striking as it is in either the hamster or the mouse. Unequivocally induced primary tumors of the rat were all sarcomas that arose in the kidney, while other tissue-culture passages of lines 3919 and 3469 were capable of inducing primary epithelial and mesenchymal tumors in many sites in mice (5), and primary mesenchymal tumors in the lungs, liver, kidneys, heart, serosal tissues, and subcutaneous tissues of hamsters (3).

There is considerable variation in the response of the hamster and the mouse to various culture passages of the virus. These variations may depend on both the potency of the preparation and the susceptibility of the animal inoculated. Recently, we have shown that the incidence of tumors is dependent on the dosage of the inoculum (6). It is therefore significant that where sedimentation was attempted by high-speed centrifugation, the greatest number of renal tumors was induced by the fraction containing the heavier sedimented particles. This technique may serve as an effective means of concentrating and purifying the agent responsible for tumor induction.

A single attempt to recover a tumor-inducing factor from serially passed tissue cultures inoculated with material from an induced tumor in a rat was successful. This supports our thesis that a replicating agent, the SE polyoma virus, or the product of the interaction of this virus and tissue-culture cells is responsible for the tumors induced.

References


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PLATE 19

Figure 1.—Renal sarcoma in the kidney of a rat with extension of the tumor to peritoneal surfaces of abdominal viscera.

Figure 2.—Rat renal sarcoma showing hemangiomatous pattern of growth. Hematoxylin and eosin. × 210
FIGURE 3.—Rat renal sarcoma showing spindle cells with fasciculated pattern and multinucleated tumor cells with abnormal mitotic figures. Hematoxylin and eosin. × 260

FIGURE 4.—Rat renal sarcoma showing capillary vasculature and fasciculated pattern. Hematoxylin and eosin. × 210

FIGURE 5.—Rat lung showing intravascular metastasis from renal sarcoma. Hematoxylin and eosin. × 210