The mechanism of tumor induction by tissue culture preparations infected with polyoma virus has not been determined. There is some evidence that the tumors follow parasitization of host cells by the virus (8, 10), but the oncogenic response may be the result of a very complex interaction between host and virus. In most experiments massive doses of virus-tissue culture inocula have been used that initially permeate nearly all tissues of the immature animal (8). Virus is present in tissues other than those in which tumors form (5, 8, 10), and conversely it is not always possible to recover virus from the induced tumors (9). Thus, available evidence indicates that the parasitized cells could act either directly to stimulate the proliferation of tumor cells or indirectly to initiate a systemic response which in turn is responsible for tumor formation. The latter action would suggest explanations for many puzzling aspects of this phenomenon such as the enhanced capacity of immature animals to form tumors, the occurrence of primary tumors in organs distant from the site of inoculation, and the development of tumors in selected organs. One observation on the distribution of tumors is perhaps pertinent.

The tumors that develop in mice are often found in two or more different tissues that are in apposition (8). Colliding tumors of this type would indicate that proliferation results from a local concentration of an oncogenic factor rather than from a generalized oncogenic stimulus which affects select organs.

The experiments reported here were designed to determine whether the virus-infected tissue culture inoculum could induce tumors exclusively at the site of inoculation. An attempt was made first to induce local tumors by exposing various tissues from newborn mice to fluids from polyoma-infected tissue cultures for 15—80 min. and then implanting the exposed tissues into histocompatible newborn hosts. This unpublished experiment was unsuccessful because, although the implanted tissues survived for many months, no tumors developed at the sites of implantation. Recently, Dawe et al. (2) reported that the epithelial cells of salivary gland tissues of the mouse that were maintained for many days in tissue cultures inoculated with a tumor-inducing agent became hyperplastic and formed intranuclear inclusion bodies similar to those seen in the salivary glands of immature mice inoculated with polyoma virus preparations. But, just as in the case of our experiment, these altered tissues did not grow as tumors when grafted to irradiated adult mice even though tumors developed in organs distant from the site of the graft.
The present experiments concern the successful induction of tumors in the subcutaneous tissues of hamsters at the site of implantation of newborn hamster tissues that had been bathed in fluids from polyoma virus-infected tissue cultures. The experiments differ from the implantation experiments in mice in that the subcutaneous tissues at the site of implantation in the host hamsters as well as the implanted donor hamster tissues are known to be receptive to the development of tumors after polyoma virus inoculation (4, 11).

MATERIALS AND METHODS

The Syrian hamsters (Cricetid aurati) were from randomly bred stock of the Jay-Poiley colony of the National Institutes of Health. Hamsters from this colony that are inoculated during the first month after birth with tissue culture preparations of polyoma virus have consistently developed multiple fibromatosus tumors in the heart, mediastinum, kidneys, and subcutaneous tissues, and cavernous angiomaticus tumors in the lungs and liver (4, 11). Two samples of supernatant fluid from polyoma-infected mouse embryo tissue cultures were obtained from Dr. B. Eddy of the Division of Biologics Standards, National Institutes of Health. The method of preparation and origin of the two culture sublines, designated 5051 and 695, have been reported previously (3). The oncogenic potency of each sample was tested by observing the tumors that developed in hamsters given inoculations within 24 hours after birth of 0.1 ml. of the fluid. Within 60 days, more than 80 per cent of the treated animals developed multiple tumors similar to those noted above and described in previous reports (4). Tests of local tissue response to the samples were then carried out as follows: Blocks of tissue 1 cu. mm. in size from different organs of newborn hamsters were bathed in the infected tissue culture fluids for 5—25 min. at 37°C. and immediately implanted by trocar into the subcutaneous tissues of the axillary region of the right side of either 30-day-old hamsters (Exp. 1) or hamsters less than 48 hours old (Exp. 2). At the same time, blocks of tissue from the same organs were bathed in uninfected tissue culture media for 5—25 min. and implanted into the subcutaneous tissues of the axillary region of the left side of the same animals. To determine the effect of residual fluid carried over to host cells, a third group of eight newborn hamsters received bilateral subcutaneous axillary implants of cotton pledgets measuring 1 cu.mm. The cotton implanted on the right side was bathed in the fluid from infected tissue cultures, and the cotton implanted on the left side was bathed in uninfected culture media. In the first experiment, the weanling hosts were marked according to the type of tissue implanted. The danger of cannibalism prohibited marking the newborns in Experiments 2 and 3; consequently it was possible to identify the specific tissues implanted only in hamsters in which the tissue survived until the animal was killed. The animals were killed at different ages depending on the size of the developing tumors. Twelve of the 27 subcutaneous tumors that developed in the hamsters were tested for their ability to grow as transplants in the subcutaneous tissues of randomly bred hamsters of the Jay-Poiley colony. Histological examination was done routinely on step sections of all subcutaneous lesions and major viscera.

RESULTS

Twenty-one of 39 hamsters with tissue implants and six of eight hamsters with cotton implants developed single or multiple tumors in the subcutaneous tissues. The subcutaneous tumors found in the 27 hamsters were all in the right axillary region, the site of implants that were bathed in the fluids from infected tissue cultures (Table 1). The tissues that were bathed in unaltered tissue culture media and implanted into the left sides could still be recognized histologically in ten animals at death. These tissues had not affected the subcutaneous tissues other than to form small granulomas. None of the implanted tissues bathed in the fluids from polyoma-infected cultures was identified either grossly or in step sections of tissues within or around the subcutaneous tumors. Both the treated and untreated cotton implants in the hamsters of Experiment 3 were easily identified. The polyoma-saturated cotton pledgets consistently demonstrated an interesting relationship to the local subcutaneous tumors. In all six of these hamsters, the residuum of polyoma-saturated cotton in the right axilla was within a radius of 1—10 mm. from the tumors. But none of the cotton pledgets was incorporated into the tumors. Histological study showed that the cotton fibers were infiltrated with fibroblasts and abundant capillaries as well as foreign-body giant cells and a few leukocytes. This granulation tissue was completely confined to the meshworks of the fibers. The tumors, consisting of from one to three circumscribed nodules of compact proliferating spindle cells, in all cases were separated from the cotton granulomas by unaltered areolar tissue (Fig. 1).

Morphology and behavior of the tumors.—The subcutaneous tumors in all of the animals had features indicating origin from mesenchymal cells.
The tumor cells were spindle-shaped with elongated bipolar or multiple cytoplasmic processes, and individual cells were separated by a network of reticular and collagenous fibers. Extensive areas of dense collagen could be found in most tumors. Although all of the tumors had cell types in common, distinct structural patterns predominated in some of the tumors. The most evident variation was seen in the three tumors that are listed in Table 1 as angioid in type. Grossly each of these tumors was a multiloculated hemorrhagic mass that was fixed to the underlying tissues. Histologically, the tumors were composed of blood-filled sacs lined by large, irregularly shaped cells that were often multinucleated and contained atypical mitotic figures (Fig. 3). Invasion of the underlying tissues was seen in many areas.

Two of the three hamsters that had tumors of this type also had multiple tumor nodules in the lung and pulmonary arterioles. The lung deposits in both animals were similar in structure to the angioid subcutaneous tumors at the site of implantation. The most distinctive features of the tumors in both sites were the large, bizarre shaped polygonal cells that tended to form around irregular, blood-filled lakes (Fig. 4).

The other 24 tumors (Table 1) were solid, circumscribed fibromatous nodules that sometimes had a thick collagenous capsule. The cells were more uniform in configuration and had fewer mitotic figures than the angioid tumors. The cells were separated by collagenous fibers or an edematous matrix and were aligned in interlacing fascicles. Unlike the angioid tumors, these fibromatous tumors did not contain cavernous vascular channels lined by tumor cells, but they were often interspersed with numerous minute blood vessels that were lined with multiple layers of endothelial cells (Fig. 2). Some of the tumors were composed predominantly of widely separated stellate cells in an edematous stroma and are listed in Table 1 as mucoid in type (Fig. 7). Others were composed of compact fascicles of fusiform cells that were rich in cytoplasm and had a scant intercellular matrix of collagen fibers. These are listed in Table 1 as fibroid in type (Fig. 5).

### TABLE 1

**Subcutaneous Tumors in Hamsters at the Site of Implantation of Tissues Bathed in Polyoma Virus-infected Culture Fluids**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age at death (months)</th>
<th>Implanted tissue</th>
<th>Right side (virus-bathed implant)</th>
<th>Left side Unexposed implant</th>
<th>Other tumor sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 1: 6 weanlings—implants bathed in polyoma culture 695 (1 hamster had no tumors)</td>
<td>3</td>
<td>Liver</td>
<td>Angioid</td>
<td>32</td>
<td>12th graft</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Kidney</td>
<td>Mucoïd</td>
<td>50</td>
<td>11th graft</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Skin</td>
<td>Mucoïd</td>
<td>50</td>
<td>11th graft</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Bone</td>
<td>Fibroid</td>
<td>15</td>
<td>6th graft</td>
</tr>
<tr>
<td>No. 2: 17 newborns (17 hamsters had no tumors when sacrificed at 9 months)</td>
<td>2</td>
<td>Gut</td>
<td>Mucoïd</td>
<td>45</td>
<td>5th graft</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Bone</td>
<td>Fibroid</td>
<td>20</td>
<td>5th graft</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Skin</td>
<td>Mucoïd</td>
<td>20</td>
<td>5th graft</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>*</td>
<td>Fibroid</td>
<td>45</td>
<td>4th graft</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>*</td>
<td>Fibroid</td>
<td>45</td>
<td>4th graft</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>*</td>
<td>Angioid</td>
<td>45</td>
<td>4th graft</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>*</td>
<td>Fibroid</td>
<td>45</td>
<td>4th graft</td>
</tr>
<tr>
<td>No. 3: 8 newborns—implants bathed in polyoma culture 695 (2 hamsters had no tumors at 9 months)</td>
<td>1</td>
<td>Cotton</td>
<td>Fibroid</td>
<td>10</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Bone</td>
<td>Fibroid</td>
<td>10</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>*</td>
<td>Fibroid</td>
<td>20</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>*</td>
<td>Fibroid</td>
<td>20</td>
<td>Not tested</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>*</td>
<td>Fibroid</td>
<td>20</td>
<td>Not tested</td>
</tr>
</tbody>
</table>

* Implants of either skin, lung, liver, heart, kidney, or vertebra bathed in Polyoma culture 695.
separation of the two types was based on the predominant structure, since most of the tumors had some of the qualities of both types.

Ten of the hamsters that developed solid tumor nodules adjacent to the implants had tumors in other organs (Table 1). Four of this group were hamsters that had received tissue implants shortly after birth. Each of the four had single circumscribed fibromatous nodules in the heart which involved both the endocardium and the myocardium. These tumors had no features which would distinguish them from the subcutaneous tumors at the site of implantation or from the cardiac tumors noted in hamsters inoculated with the virus.

All six of the hamsters that developed tumors adjacent to cotton implants had similar tumors in the heart and mesenchymal tumors in other organs. These tumors were similar to those found in hamsters inoculated with large doses of polyoma-infected culture material both in this experiment and previous experiments (4, 10, 11). The tumors varied in type depending upon the particular organ involved. Tumors of the lung and liver were invariably composed of loculated vascular spaces lined by single layers of endothelial-like cells, whereas tumors in other viscera were composed of solid nodules of spindle cells with finer variations in cytological structure similar to those described in the two types of subcutaneous tumors noted above. None of the visceral tumors had the degree of atypical pleomorphism or mitotic activity noted in the angioid type described above.

Transplantability.—Small tissue samples of twelve of the subcutaneous tumors were grafted by trocar into the subcutaneous tissues of 24 weanling hamsters. Tumors grew at the site of all grafts and were retransplanted into randomly bred weanling hamsters when they reached 30–40 mm in diameter. We have continued to transplant six of these tumors; they are now in their third to twelfth transplant generation. The transplanted tumors have retained their original morphological features and growth characteristics. The two grafted angioid tumors after eleven passages still are composed of cavernous spaces lined by bizarre endothelial cells, and identical metastatic tumor deposits are commonly found in the lungs (Fig. 4). The four grafted solid tumors also have retained the predominant cell structure of the original tumors. Transplants of the mucoid type consistently are composed of nodules of stellate cells in an edematous stroma (Fig. 8), and transplants of the fibroid type are composed of compact fascicles of cells with little intercellular matrix (Fig. 6). Hamsters given grafts of the solid tumors have not developed tumors in other sites even though in some cases the transplants have been allowed to grow as long as 3 months and have exceeded 3 times the weight of their hosts at the time of sacrifice. The rate of growth of three of the six transplanted tumors has increased with succeeding generations, as indicated by the time required for grafts to reach mean diameters of 40 mm. In the two angioid tumors, this period has decreased from 60 days in the early generations to less than 20 days in the eleventh and twelfth generations. Initially all of the solid tumors required over 60 days to reach mean diameters of 40 mm.; the mucoid tumor in the eighth generation now requires less than 40 days to reach this size. Grafts of this tumor have grown in two successive litters of newborn hamsters. The morphology of the tumor has not changed in the newborn animals, nor did the young hosts develop visceral tumors during the 20–60 days that they survived.

![Figure 1](https://cancerres.aacrjournals.org) — Fibromatous tumor (right) adjacent to a granulomatous nodule (left) which surrounds an implanted cotton pledget. The cotton had been soaked in the fluid from a polyoma-infected tissue culture and implanted into the subcutaneous tissues of a newborn hamster 1 month before death. H & E stain, X35.

![Figure 2](https://cancerres.aacrjournals.org) — Detail of a mucoid fibroma that developed in the subcutaneous tissues of a hamster near the site of a kidney tissue implant. Numerous vessels lined by thick layers of endothelium were found in many of the solid tumors. H & E stain, X100.

![Figure 3](https://cancerres.aacrjournals.org) — A subcutaneous tumor of the angiod type that was induced with an implant of tissue soaked in fluid from a polyoma virus culture. The uniform, compact, fascicular arrangement of large spindle cells has persisted through three transplant generations. H & E stain, X100.

![Figure 4](https://cancerres.aacrjournals.org) — A metastatic tumor nodule in the lung of a hamster bearing the seventh transplant generation of the tumor illustrated in Figure 3. H & E stain, X100.
DISCUSSION

The results of the experiment prove that the oncogenic response of hamsters to fluids from polyoma virus-infected cultures can be limited to subcutaneous sites of inoculation. The absence of tumors at the sites of untreated implants in all of the hamsters and in other distant sites in over one-half of the hamsters further indicates that the stimulus for cell proliferation does not result primarily from the effect of the virus (or other elements in the inoculum) on remote systems of the host. It should be emphasized, however, that the local effect of the inoculum does not imply that the tumor cells need be infected by virus. It is conceivable that all of the tumors were derived from local mesenchymal cells of the host, since both the host tissues at the site of implantation and the implanted tissues contained cells capable of forming tumors of the types described. Furthermore, where it was certain that the tumors were derived from host tissues, such as in the case of hamsters implanted with cotton, tumors arose in tissues slightly removed from the cotton pledges even though this infectious nidus became infiltrated with a variety of cells of mesenchymal origin. This observation, although in need of further support, may be related to the previously reported lack of continuity between induced tumors and other types of carcinogens (1, 6, 7). The stimulus for cell proliferation may be a local product of cells altered by the virus rather than the direct effect of viral parasitization.

The problem of whether tumors distant from the site of inoculation represent true cell metastases and thus reflect the potential behavior of some polyoma-induced tumors or invariably represent new tumors as the result of viral dissemination cannot be resolved entirely by morphological studies. However, this experiment would indicate that both processes may occur. The multiple visceral tumors noted in the animals bearing implants of cotton were similar to those noted in hamsters inoculated with large doses of virus culture material. Not all of these mesenchymal tumors could be distinguished from one another, but certainly the simple hemangio-endotheliomatous lesions that occurred in the lungs and liver differed markedly from the fibromatous nodules at the site of implantation. It was also evident that the size and degree of cellular anaplasia in subcutaneous tumors bore no relation to the number of visceral tumors in these animals. On the other hand, the morphological characteristics and local invasive properties of the angiod type of tumor noted at the site of implantation in three hamsters would indicate that tumors of this type could metastasize. Furthermore, hamsters in which this type of tumor was induced and hamsters given grafts of this type of tumor developed tumors of the same type in the lungs and lymph nodes, but did not develop fibromatous tumors characteristic of polyoma virus induction in other organs. Polyoma virus-induced tumors of mice that have the ability to metastasize and show other features of aggressive behavior have been reported (8, 10), but such tumors seem to be relatively rare. It would be most important to learn what relation the virus held to the acquisition of such characteristics by the tumors.

REFERENCES

The Local Oncogenic Response of Hamster Tissues to Polyoma Virus

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