THE EXPERIMENTAL PRODUCTION OF VISCERAL TUMORS WITH HYDROCARBONS

FREDERIC W. ILFELD, M.D.

(From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.)

The activity of 1:2:5:6-dibenzanthracene, methylcholanthrene, and 1:2-benzpyrene in inducing malignant growths by cutaneous or subcutaneous application in experimental animals has by now been well established (Barry, Cook, Haslewood, Hewett, Hieger, and Kennaway, 1). The tumors thus produced have been either epitheliomas of the skin or subcutaneous sarcomas. In only rare instances has the effect of a hydrocarbon been tried on the internal organs of animals. Burrows (2), in 1932, produced "spindle-celled tumors" in mice and rats by intraperitoneal injection of dibenzanthracene in olive oil. Lacassagne (3), in 1933, injected a mixture of lard and this same hydrocarbon into the breast, knee joint, marginal vein of the ear, and testicle of rabbits. He was able to produce a neoplasm only in the testicle. This growth was thought to be derived from remnants of the adrenal cortex which may occur normally in the rabbit's testicle. Yoshida (4), in 1934, reported the production of cancer of the liver and bile ducts by feeding a chemical compound, o-amido-azo-toluol, to rats. Furthermore, visceral cancer has been experimentally obtained with tar in the renal pelvis (Lateri, 5), bladder (Watanabe, 6), pleura (Shû Uno, 7), and stomach (Bonne, 8, Voronoff and Alexandrescu, 9). It has been known for many years that workers with aniline dyes are likely to develop cancer of the bladder (10). These facts suggested that tumors of some of the visceral organs might be produced experimentally with carcinogenic chemicals.

TECHNIC

For these experiments the hydrocarbons were administered in cholesterol pellets prepared as described by Shear (11). Pellets of two sizes were used. The smaller were 20 gauge (English standard wire gauge), or 0.9 mm. in diameter, and the larger 14 gauge, or 2 mm. in diameter. These were made by heating a 5 per cent mixture of hydrocarbon in cholesterol in a test tube over a Bunsen flame, care being taken to avoid heating much above the melting point. The mixture was then allowed to cool and harden. This process of heating and cooling was repeated six times to insure thorough mixing. The solution was then drawn up into a suitable glass capillary tube which had previously been coated with mineral oil. When cooled the mixture forms a solid pellet, which is expressed from the glass capillary with a metal plunger or copper wire and stored in Petri dishes. Inhalation of fumes was avoided by working under a hood, and rubber gloves were worn to protect the fingers. In this manner pellets were made each of which contained 5 per cent of dibenzanthracene, of methylcholanthrene, or of benzpyrene.
The animals were anesthetized with pentobarbital or ether, the part to be treated was surgically exposed, and a section of a pellet, about 0.5 cm. in length, was inserted into the kidney, spleen, liver, uterus, testicle, bone marrow, subperiosteum, stomach, or brain. In one rat two pellets were placed, one in the kidney and one in the spleen; and in one dog pellets were injected both into the subperiosteum and the cancellous bone. Otherwise, pellets were implanted in only one organ of each animal. The pellets were easily inserted by pushing the tip of a hypodermic needle into the organ and then expelling the pellet from the needle with a metal plunger or copper wire. It was found that the pellet remained in situ and was neither dissolved nor extruded. In this way the hydrocarbon was brought into direct contact with the cells of the organs. Pellets containing cholesterol alone were not injected into the organs, since Shear (11) has already shown that such pellets placed under the skin in susceptible animals cause no growths after twelve months.

RESULTS

Kidney: Table I shows the effect of dibenzanthracene and of methylcholanthrene on the kidneys of 51 pure strain mice, 1 stock mouse, and 9 rats. Eighteen of the 61 animals died of intercurrent disease, usually pneumonia; 12, or 41.3 per cent of the surviving animals, developed renal tumors. All of the renal tumors (Table II) were epidermoid carcinomas. Thirteen mice and one rat were killed for histologic study before tumors were palpable. Seventeen animals are still alive. The three animals receiving methylcholanthrene show no tumors after six months.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Animal</th>
<th>Number Died of Intercurrent Disease</th>
<th>Killed for Study</th>
<th>Number Living</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibenzanthracene</td>
<td>Strain A</td>
<td>21</td>
<td>5</td>
<td>7</td>
<td>4 cancers</td>
</tr>
<tr>
<td></td>
<td>Strain C3H</td>
<td>23</td>
<td>3</td>
<td>6</td>
<td>9 cancers</td>
</tr>
<tr>
<td></td>
<td>Strain M</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>0 Negative</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>1 Negative after six months</td>
</tr>
<tr>
<td>Methylcholanthrene</td>
<td>Stock mouse</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1 Negative after six months</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2 Negative after six months</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>61</td>
<td>18</td>
<td>14</td>
<td>17 12 renal cancers</td>
</tr>
</tbody>
</table>

Pieces of tumor tissue from cases 3, 4, 5, and 6 were transplanted into mice of the same pure strain. The transplant from Case 3 was carried through four generations of animals. In the first generation small fragments of tumor were placed under the skin of 5 Strain A mice. All of the fragments

1 The solution contained 1 1/2 grains of pentobarbital to 15 cubic centimeters of water; 0.25 cubic centimeters injected intraperitoneally into mice caused anesthesia.
2 Pure strain mice came from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. There were three strains: A, C3H, and M. Stock mice were albino mice obtained from a dealer and were not pure strain animals.
of tumor grew. Pieces from one of these first generation transplants were then implanted into 6 Strain A mice. All of these mice developed large subcutaneous tumors. Pieces from one of the tumors of the second generation transplant were again transplanted subcutaneously into 6 Strain A mice, again with six takes. The tumor of the third generation was passed once more into 6 mice with positive takes in all cases. In one animal of the fourth generation the transplanted tumor invaded the peritoneal cavity, spread over the peritoneum, and eroded a blood vessel, causing death by intraperitoneal hemorrhage. Transplants from cases 4, 5, and 6 were carried through one generation. In cases 4 and 6 transplants were made in each instance into 4 Strain A mice, with positive takes in all the animals. Transplants from case 5 into 4 similar mice resulted in only 3 positive takes. Autopsies of the mice showed that the transplanted tumor did not metastasize to distant organs but spread by direct extension.

The interval between injection of the pellet and palpability of the tumor, i.e. the tumor incubation period, varied from six to ten and one-half months, the average being nine months. Neoplasms appearing within six months were obtained with the larger pellets, which were 0.5 cm. in length and contained about 0.6 mg. of dibenzanthracene. The 20 gauge pellets, containing from 0.12 to 0.19 mg. of dibenzanthracene, caused tumors in eight to ten and one-half months. In case 1 the tumor was removed by nephrectomy. Autopsy was done four months after the operation. There was no recurrence of the tumor and there were no metastases.

The twelve renal tumors were composed of gray, granular, cellular tissue with a few small scattered areas of hemorrhage and necrosis. There was no capsule. The renal parenchyma was invaded by strands of tumor cells. In many instances the tumor invaded a large portion of the organ (Fig. 1). In such cases the kidney was several times its normal size (Fig. 2). Adhesions
<table>
<thead>
<tr>
<th>Case</th>
<th>Animal</th>
<th>Length of Pellet</th>
<th>Estimated Amount of Dibenzanthracene</th>
<th>Date of Insertion of Pellet</th>
<th>Palpability of Tumor</th>
<th>Histology</th>
<th>Size</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rat</td>
<td>0.5 cm. (14 gauge)</td>
<td>0.6 mg.</td>
<td>11-28-34</td>
<td>6 months</td>
<td>Epidermoid carcinoma</td>
<td>Small nodule</td>
<td>Nephrectomy: Autopsy 4 mos. later showed no recurrence or metastases</td>
</tr>
<tr>
<td>2</td>
<td>Rat</td>
<td>0.3 cm. (14 gauge)</td>
<td>0.3 mg.</td>
<td>11-28-34</td>
<td>10½ months</td>
<td>Epidermoid carcinoma</td>
<td>Small nodule</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mouse</td>
<td>0.5 cm. (14 gauge)</td>
<td>0.6 mg.</td>
<td>12-7-34</td>
<td>6½ months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td>Subcutaneous transplants carried through four generations</td>
</tr>
<tr>
<td>4</td>
<td>Mouse</td>
<td>0.6 cm. (20 gauge)</td>
<td>0.19 mg.</td>
<td>12-8-34</td>
<td>8 months</td>
<td>Epidermoid carcinoma</td>
<td>All of kidney involved</td>
<td>Subcutaneous transplants made</td>
</tr>
<tr>
<td>5</td>
<td>Mouse</td>
<td>0.4 cm. (20 gauge)</td>
<td>0.12 mg.</td>
<td>12-7-34</td>
<td>8 months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td>Subcutaneous transplants made</td>
</tr>
<tr>
<td>6</td>
<td>Mouse</td>
<td>0.5 cm. (20 gauge)</td>
<td>0.16 mg.</td>
<td>12-8-34</td>
<td>8 months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td>Subcutaneous transplants made</td>
</tr>
<tr>
<td>7</td>
<td>Mouse</td>
<td>0.4 cm. (20 gauge)</td>
<td>0.12 mg.</td>
<td>11-21-34</td>
<td>10½ months</td>
<td>Epidermoid carcinoma</td>
<td>Small tumor surrounding</td>
<td>Small tumor surrounding pellet</td>
</tr>
<tr>
<td>8</td>
<td>Mouse</td>
<td>0.5 cm. (20 gauge)</td>
<td>0.16 mg.</td>
<td>12-22-34</td>
<td>9½ months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Mouse</td>
<td>0.3 cm. plus several small pieces (20 gauge)</td>
<td>—</td>
<td>12-31-34</td>
<td>8 months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td>Hydro nephrotic kidney found at operation, 3-25-35. Fluid removed and injected subcutaneously in another mouse with negative results after 8 months</td>
</tr>
<tr>
<td>10</td>
<td>Mouse</td>
<td>0.5 cm. (20 gauge)</td>
<td>0.16 mg.</td>
<td>12-22-34</td>
<td>8½ months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Mouse</td>
<td>0.6 cm. (20 gauge)</td>
<td>0.19 mg.</td>
<td>12-31-34</td>
<td>8½ months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Mouse</td>
<td>0.5 cm. (20 gauge)</td>
<td>0.16 mg.</td>
<td>1-5-35</td>
<td>10 months</td>
<td>Epidermoid carcinoma</td>
<td>Most of kidney involved</td>
<td></td>
</tr>
</tbody>
</table>
from the omentum or intestines to the kidney were occasionally found. In several tumors unilocular or multilocular cysts were present. These cysts were 0.2 to 1.0 cm. in diameter and were filled with a soft substance resembling keratin. Smears stained by Gram's method and broth cultures from two of these cysts were negative for bacteria. The original pellets were recovered still intact in nearly every tumor. No metastases were observed in any of the cases.

Microscopic sections of the renal tumors showed groups of epithelial cells irregularly arranged, invading the renal parenchyma, and frequently forming epithelial pearls (Fig. 3). The tumor cells resembled renal pelvic rather than glandular epithelium with basal cells supporting flat cells. In some instances the tumor cells tended to take a glandular appearance, which was apparently due to their growth along the renal reticulum surrounding the tubules. Following the disappearance of the tubule cells, a clear area was left surrounded by tumor cells. Mitoses were common. In places cavities were found filled with flattened, desquamated, keratinized cells. Infiltration of polymorphonuclear leukocytes, monocytes, and lymphocytes was often observed.

The transplanted tumors resembled the original renal epidermoid carcinomas. After three weeks' growth the tumors measured about 1.0 by 1.5 by 1.0 cm. They increased in size so that after a period of six weeks to several months large cysts filled with soft gray material were present. In some animals the tumor ulcerated through the skin and formed an irregular granular surface. Microscopic sections of the seven groups of transplanted

**Fig. 2. Case 10: Tumor of Left Kidney, Indicated by Arrows**
tumors showed the same histology as that of the mother tumor. The epithe-
lium grew in an aberrant and irregular manner with pearl and cyst forma-
tion from the accumulation of keratinized cells and invaded regional fat and
muscle. The definite epidermoid character of the transplanted tumors was
another indication of the origin of the tumor from renal pelvic rather than
glandular epithelium.

Fourteen animals which had been operated on six to ten months previously
were killed to study the histologic changes produced by the pellets before
tumors had developed. In some of the animals the microscopic sections
showed an inflammatory foreign body reaction around the pellet. This con-
sisted of fibrosis (Fig. 4), multinucleated giant cells, and regional infiltration

![Image](https://example.com/image)

**Fig. 3.** Case 4: Kidney Parenchyma, on the Right, Being Invaded by Tumor, on the Left

of monocytes and polymorphonuclear leukocytes. In other animals the slides
showed epithelial cells partially surrounding the pellet and supported in most
places by a layer of dense hyalin fibrillated tissue in which occasional leuko-
cytes were imbedded (Fig. 5). The epithelial cells next to the pellet occurred
in some areas in single layers, in others they were stratified two to ten cells
deep. From these cells surrounding the pellet small down-growths of epithe-
lial cells, frequently showing mitotic figures, invaded the subjacent paren-
chyma. These epithelial down-growths probably represent the area of change
from benign to malignant cells. In some areas near the pellet there was
necrosis of tubules with small clusters of lymphocytes.

*Spleen:* Twenty gauge pellets about 0.5 cm. long and containing 5 per cent
of dibenzanthracene were injected into the spleens of 50 mice and 6 rats.
After eleven months the results were negative.

A 14 gauge pellet, about 0.6 cm. long, containing 5 per cent of methyl-
cholanthrene was inserted into the spleen of one rat and of one stock mouse.
FIG. 4. CROSS-SECTION OF RAT'S KIDNEY AND PELLET
The clear area represents the pellet, which is surrounded by a narrow band of connective tissue.

FIG. 5. GIANT CELLS ABOVE NEAR EDGE OF THE PELLET; STRATIFIED EPITHELIAL CELLS BELOW SUPPORTED BY CONNECTIVE TISSUE
Four and one-half months later a mass was palpable in the mouse. Autopsy disclosed a grayish-white tumor intimately connected with the spleen, extending out from the hilum and invading the pancreas. The pellet was found imbedded in the tumor. The histology was that of a rapidly growing unencapsulated fibrosarcoma.

Liver: Twenty gauge dibenzanthracene pellets were placed in the livers of 22 Strain A, 20 Strain C3H, and 14 Strain M mice and 5 rats; a benzpyrene pellet in the liver of 1 rat; and methylcholanthrene pellets in the liver of 1 rat and of 1 stock albino mouse. These experiments have so far been negative except in 2 instances. A pure strain C3H mouse was found dead from pneumonia and peritonitis. Two pellets, each 0.3 cm. in length and together containing about 0.16 mg. of dibenzanthracene, had been injected nine months previously. Autopsy disclosed a tumor in the liver measuring $1 \times 0.8 \times 0.6$ cm. Though careful search was made, the pellets were not found. Because of post-mortem changes, the histology of this tumor was indefinite. Another mouse of the same strain was found dead ten months after the implantation of two pellets, each 0.3 cm. long and together containing about 0.16 mg. of dibenzanthracene. There was a mass $1 \times 0.9 \times 0.7$ cm. in the anterior part of the liver. This mass was composed of grayish-white tissue with small areas of hemorrhage and necrosis. The pellets were found in the center of the tumor. Histologic examination of the tumor showed pleomorphic epithelial cells arranged in an irregular manner, invading the hepatic parenchyma (Fig. 6). The nuclei of the tumor cells were large, oval, and contained one to three nucleoli. The cytoplasm was abundant and eosinophilic. The histology was consistent with but not diagnostic of a primary liver-cell carcinoma.

Uterus: A small incision was made in one uterine horn of each of 21 mice. A piece of 20 gauge dibenzanthracene pellet, 0.3 to 0.6 cm. in length, was then inserted through the incision and fixed in position with number A silk sutures. Nine months after this procedure had been carried out, an albino mouse which had received a pellet 0.3 cm. long, containing about 0.08 mg. of the hydrocarbon, was discovered sick. Examination disclosed a large mass filling about half of the peritoneal cavity. At autopsy a grayish-white tumor connected with the uterus was found. The tumor measured $1.7 \times 1.7 \times 1.0$ cm. The pellet was found in its center. Microscopic examination of the tumor showed an epidermoid carcinoma. No attempt to transplant the tumor was made because of bacterial contamination.

Other Organs: Pellets containing carcinogenic hydrocarbons were also implanted into other organs, but at the present time no positive results have been recorded. These experiments are still in progress. Small dibenzanthracene pellets were placed in one testicle of each of 19 mice. No tumors were noted after nine months. Small pellets of dibenzanthracene were inserted through a drill hole into the cancellous bone of the femur of 11 rats. After twelve months this experiment is also negative. Dibenzanthracene pellets were placed under the periosteum and into the cancellous bone of the femur of a dog. The result is negative after one year. Dibenzanthracene pellets were implanted in the wall of the stomach of a dog; and dibenzanthracene and benzpyrene pellets were placed under the gastric serosa of 6 ferrets. The results are negative after twelve and four months respectively. Through
experimental production of visceral tumors

an opening in the skull small pellets of dibenzanthracene were inserted into the cortex of the brain of 2 stock mice, with negative results after eight months.

Discussion

These experiments show that cancer can be induced in some of the visceral organs with certain carcinogenic hydrocarbons. All 12 of the renal tumors were caused by 1:2:5:6-dibenzanthracene and were epidermoid carcinomas.\(^3\) One renal tumor was transplanted through four generations of mice. The tumors seem to have originated from renal pelvic epithelium. There was a

![Fig. 6. Liver Parenchyma, in Lower Portion of Picture, Being Invaded by Tumor Cells](image)

definite incubation period for the renal tumor, \textit{i.e.} until it was large enough to be felt, of at least six months, the average being nine months. In discussing the development of x-ray cancer in man Wolbach (12) stressed the observation "that the acquisition of malignant properties is not a sudden one, that it is gradually acquired in the course of years." In this experiment the malignant change in mice and rats required a period of months.

Examination of the 14 kidneys containing dibenzanthracene pellets and having no tumor showed a moderate inflammatory reaction around the pellet with infiltration of polymorphonuclear leukocytes, lymphocytes, and monocytes. In some slides renal pelvic epithelium partially or completely sur-

\(^3\) Since this paper was submitted 5 additional renal tumors have developed, 4 of which are epidermoid carcinomas and 1 is a rapidly growing fibrosarcoma of the kidney.
rounded the pellet either in a single or in a stratified layer. This first reaction of the epithelium seemed to be a protective mechanism. The epithelial cells in most instances were supported by dense collagenous connective tissue. After coming into contact with the hydrocarbon the epithelial cells became stratified and later assumed malignant characteristics, as shown by invasive down-growths.

The prolonged tumor incubation period together with the presence of a moderate amount of inflammation and the absence of healing might perhaps be considered as pointing to chronic irritation as the causative factor rather than chemical change. However, the evidence is not sufficient to warrant a definite conclusion. It may be pointed out, also, that in the case of the renal tumors the animals which had received the largest doses of dibenzanthracene developed tumors the earliest. It is worth noting, however, that while the amount of dibenzanthracene in the pellet varied from 0.08 to 0.6 mg., because of the solid nature of the pellet the amount of dibenzanthracene actually in contact with the cells was only that present in the surface of the pellet. Compared with the total amount of dibenzanthracene present in the pellet, this must have been a very small quantity.

One fibrosarcoma was produced in the experiment. In this instance a pellet containing methylcholanthrene had been implanted in the spleen of a stock mouse. This tumor originated either from the connective tissue of the spleen or from the stroma of the pancreas, probably the former. In the two tumors of the liver caused by dibenzanthracene the histology is indefinite, because of post-mortem changes. The histology of the second tumor is more definite than that of the first and is consistent with that of a primary liver-cell carcinoma. The uterine tumor caused by dibenzanthracene is an epidermoid carcinoma originating from the endometrium. The results of inserting pellets into the other organs so far have been negative.

**Summary**

1. Cholesterol pellets containing 5 per cent of either 1:2:5:6-dibenzanthracene, methylcholanthrene, or 1:2-benzpyrene were implanted into the kidney, spleen, liver, uterus, testicle, bone marrow, subperiosteum, stomach, or brain, of 244 experimental animals.

2. Twelve renal epidermoid carcinomas were produced with pellets containing dibenzanthracene. Ten of these cancers were in mice and two in rats; these corresponded to 41.3 per cent of the surviving animals. The tumor incubation period varied from six to ten and one-half months.

3. The renal tumors were proved malignant by gross and histologic examination in all cases and by subcutaneous transplantation in four instances. One tumor was transplanted through four generations. The transplanted cancers had the same histology as the parent tumors.

4. The histology of the renal cancers indicated their origin from renal pelvic epithelium.

5. The renal histologic changes produced by pellets before tumors had developed are described.
(6) The production of one fibrosarcoma by methylcholanthrene and presumably arising from the spleen, one uterine epidermoid carcinoma by dibenzanthracene, and two tumors of the liver by dibenzanthracene, is also described.

This work was done in cooperation with the staff of the Office of Cancer Investigations, United States Public Health Service, Harvard Medical School, who furnished the chemicals, the pure strain animals, and their care and subsistence. I am especially indebted to Drs. J. W. Schereschewsky, M. J. Shear, and H. B. Andervont for their help, which made this experiment possible. I also wish to express my indebtedness to Dr. M. J. Rosenau, and to thank Dr. S. B. Wolbach for the microscopic sections and Dr. E. C. Cutler for the dogs.

BIBLIOGRAPHY