PRODUCTION OF TUMORS OF THE PROSTATE OF THE WHITE RAT WITH 1:2-BENZPYRENE

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Since the development of the pure carcinogenic chemicals by Cook, Kennaway, and their associates (1, 2, 3), the effect of these substances has been studied in a variety of animal species and organs. The greater number of investigations has been concerned with injection into the subcutaneous tissues. Among 53 tumors produced by Haagensen and Krehbiel (4), they identified fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, and squamous-cell carcinoma. The histogenesis of 26 could not be determined with certainty.

Fewer investigators have studied the effect of injections into the viscera. Ilfeld (5) reported the production of squamous-cell carcinoma of the uterus and kidney in mice and of the kidney in rats with 1:2:5:6-dibenzanthracene; also two instances of probable liver-cell carcinoma after intrahepatic injections of the same substance in mice. Oberling, Sannié and Guérin (6) secured negative results with intrahepatic and intrarenal injection of 1:2-benzpyrene in rats. The entire subject is reviewed with a complete bibliography through 1936 by Cook, Haslewood, Hewett, Hieger, Kennaway and Mayneord (7).

The effect of the endocrines on spontaneous and transplantable tumors in animals has been known since the classical observations of Lathrop and Loeb (8) in their study of the influence of ovariectomy on the incidence and age of onset of mammary carcinoma in mice. Ball, Samuels and Simpson (9, 10) noted a depression of the rate of growth and decrease in the incidence of successful grafts of the Walker mammary carcinoma 256 in rats after hypophysectomy. Heiman and Krehbiel (11), in a comprehensive investigation of the effect of both endogenous and exogenous hormones on a transplantable fibroadenoma of the breast of the rat, found changes in the percentage of “takes” and in the histological appearance of the tumor. Of the endogenous factors, castration in the female reduced the successful grafts, but castration in the male greatly increased susceptibility. The direct effect of the testis is further emphasized by their finding that, of the males in which grafts are resorbed, 80 per cent will show takes after castration.

More recently Ball and Samuels (12) have published results of hypophysectomy in rats with 1:2:5:6-dibenzanthracene tumors, identical with those obtained with the Walker 256 carcinoma. It is of interest, however, that the histological criteria of active growth are not correlated with the evident retardation as measured by size. Lacassagne and Nyka (13) secured a similar effect after hypophyseal destruction by radon in rabbits with tumors produced by tar, benzpyrene, and the Shope papilloma virus.

In chickens, the combination of chemical irritation of the testis by zinc chloride and an alteration in the hormonal status, normally or parenterally,
has yielded interesting results in the hands of Michalowsky (14) and Bagg (15). They found that teratoma of the testis in a few animals resulted only if the zinc chloride were injected during the first three months of the year or if the animals were given, in addition, extract of the anterior pituitary gland.

The production of a tumor by repeated injections of a hormone in susceptible animals was first recorded by Lacassagne (16), who reported carcinoma of the breast of male mice from a strain in which the females had a high incidence of mammary cancer, following injections of estrin and similar substances (17). This observation has been amply confirmed by Burrows (18) and Gardner, Smith, Allen and Strong (19). In addition, Lacassagne (20, 21) and Burrows (22) found metaplastic and possibly neoplastic changes in the prostate.

Taylor (23), in a review of the present knowledge on mammary carcinoma, concludes that: "Any attempt to define the stage to which clinical and experimental work has carried the theory of an endocrine cause of breast cancer is difficult and is certain to receive little approval." This summary is probably also applicable to other types of tumors.

In none of the observations discussed above, except those of Michalowsky and Bagg, has the male sex been considered except as a secondary factor in tumors usually seen in females, or as a test animal for the female hormones. In the prostate there is available an organ with epithelial and mesoblastic elements which are under definite hormonal influences of the testis and pituitary. The testicular hormone, which maintains the normal histological appearance and the absence of which results in prompt and conspicuous atrophy, is a crystallizable substance of known structure and composition. The present paper reports a study of the effects of injection of a carcinogenic chemical, 1:2-benzpyrene, on the prostate, and the relation of the testis to the production and course of the tumors produced.

**METHODS**

White rats of unknown genetic constitution but derived from one breeding colony were used. The diet was one known to be adequate in all vitamin and mineral requirements. Animals designated as intact were healthy rats about 150 days of age at the time of the first procedure. Senile animals were over 500 days of age at the start of the experiment. Castration was done by removal of the testes through a single midline incision. Animals used for transplantation were derived from the same colony, but were not necessarily related. They weighed about 100 grams and in some cases were exposed to 500 roentgen units one to three days before transplantation.

The 1:2-benzpyrene (Union Chimique Belge) in 5 per cent concentration was dissolved in lard by heating in a boiling water bath. The lard was never heated above 100° C. A tuberculin syringe was filled with the 5 per cent solution and allowed to cool. The congealed mass could be forced through a 26-gauge needle, which was inserted into the anterior lobe of the prostate exposed by a midline abdominal incision, 0.1 c.c. being injected into each side of the anterior lobe. With this procedure, the injected material was semisolid and did not run out of the prostate when the needle was withdrawn. Lard alone, in the same amount, was injected into control animals.
FIG. 1. **General Appearance of a Cross-section of the Prostate 100 to 200 Days after the Injection of Benzpyrene Dissolved in Lard**

The cystic space above is the result of the injection. Immediately below in order are the bladder neck, the vasa and the ducts of the seminal vesicles.

FIG. 2. **Area of Carcinoma Showing Numerous Mitotic Figures and Anaplasia of the Cells**
The tissues were fixed in Bouin's fluid and paraffin sections stained with hematoxylin and eosin.

**Results**

The general nature of the pathological findings is shown in Fig. 1. The injected material forms a spherical cyst-like space anterior to the bladder or urethra. The cyst is lined by fibrous tissue or squamous epithelium. About the cyst are numerous glands, the epithelium of which shows all types of metaplasia to squamous cells with intercellular bridges. About these acini is a moderate infiltration with lymphocytes and mononuclear cells and a rare polymorphonuclear leukocyte. In some acini there is no sharp separation from the stroma and the epithelial cells form an irregular mass which is free in the connective-tissue fibers (Fig. 2). Mitotic figures are moderately abundant and intercellular bridges can be identified. If the tumor cells are in contact with the capsule, invasion of nerve sheaths is readily demonstrated. The tumors occasionally form masses up to 4 cm. in diameter, as shown in Fig. 3.
Fig. 4. Area of Sarcoma showing the extreme pleomorphism and anaplasia of the cells

Fig. 5. Invasion of a vein by Sarcoma
In the wall of an occasional cyst there are nodules of polymorphous cells with many mitotic figures, manifestly not epithelial in origin (Fig. 4). These cells frequently invade the walls of vessels and partially or completely occlude the lumen (Fig. 5). The nature of these cells will be discussed in connection with histogenesis.

**Intact Animals:** There were a total of 18 rats in which no additional procedure was introduced. These animals died or were killed from 110 to 210 days after a single injection of benzpyrene. The incidence of tumors, as shown in Table I, was 72 per cent carcinoma and 5 per cent sarcoma. The earliest tumor was observed on the 117th day.

<table>
<thead>
<tr>
<th>Duration of experiment in days</th>
<th>Intact</th>
<th>Castrated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number animals</td>
<td>Carcinoma</td>
<td>Sarcoma</td>
</tr>
<tr>
<td>70–109</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>110–150</td>
<td>3</td>
<td>2 (66%)</td>
</tr>
<tr>
<td>151–175</td>
<td>4</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>176–210</td>
<td>11</td>
<td>9 (82%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>18</td>
<td>13 (72%)</td>
</tr>
</tbody>
</table>

**Castrated Animals:** Twenty animals were subjected to castration at the same time that the benzpyrene was injected. The incidence of carcinoma in this group was 65 per cent and of sarcoma 5 per cent, as shown in Table I. The earliest tumor was observed in an animal which died on the 83rd day.

**Senile Animals:** Twelve rats, between 500 and 600 days of age, were injected and observed up to 352 days after the experimental procedure (Table II). In 7, the period of observation was comparable with that in the intact and castrated animals and the incidence of carcinoma was 86 per cent, with no example of sarcoma. The remaining 5 were autopsied after 250 to 352 days; all showed carcinoma, and two sarcoma.

**Castration after Development of the Tumor:** Six intact animals on the 178th day after injection were subjected to laparotomy, a small biopsy specimen of the prostate was taken, and the testes were removed. Thirty-five days later the animals were killed and the character of the tumor in the biopsy and autopsy sections compared. There was no essential difference and no evi-
dence of atrophy of the tumor cells after castration. This observation demonstrates that the prostatic epithelium under the influence of benzpyrene acquires growth characteristics so that it will no longer respond to the hormonal relations to which the cell of origin would react.

_Castration and Replacement by the Male Sex Hormone:_ Four animals were castrated and injected with benzpyrene and after the 86th day given a daily injection of 0.25 mg. of the male sex hormone (Oreton, Schering). One animal died after 48 injections and the other 3 were killed after 62 injections. The total duration of the experiment was 134 days in the first instance and 148 in the others. All the rats showed well developed carcinoma and the 3 which were killed definite sarcoma. The tumors did not differ in

![Fig. 6. Wall of a Cyst](image)

Note the thin layer of epithelium and the various types of metaplasia in the surrounding acini.

their general features from those in the groups described above. Although only four animals were used, this experiment suggests that the male sex hormone may influence the development of a sarcoma. On the other hand, the similar incidence of sarcoma in the intact and castrated rats of the first two series (Table I) does not support this conclusion.

_Control Animals:_ Ten intact and 6 castrated rats, which were injected with lard alone, were examined. In no instance was there any hyperplasia or neoplasia of either the epithelium or stroma. In some a foreign body reaction was present about the cystic spaces filled with lard.

_Transplantation:_ In 8 animals after the development of a tumor, small pieces were transplanted into the subcutaneous tissue of 53 young male rats, 22 of which had been irradiated. Autografts were also made in four. Successful "takes" were obtained from one autograft and four homografts.
Histogenesis

Carcinoma: In the report of Ilfeld (5) it is stated that the squamous-cell tumor of the kidney induced with carcinogenic chemicals is derived from the renal pelvis, but no histological studies are presented to prove this conclusion. In the prostate there is no transitional or squamous epithelium normally present except in the adjacent bladder and urethra. In the sections studied, there is no evidence that these structures participated in formation of the tumors. On the contrary, there is abundant evidence (Fig. 6) that the columnar epithelium undergoes squamous metaplasia in contact with or in the proximity of the benzpyrene cysts. The first change is a decrease in the height of the cells and a loss of the clear space in the peripheral cytoplasm that is characteristic of the normal prostatic epithelium of the rat. The nuclei lose their regular basal arrangement and there is first pseudo-stratification of the cells, and finally true stratification. Later intercellular bridges and small masses of keratohyaline appear. The basal layer of this squamous epithelium is not regular and the cells are taller than the normal malpighian layer of the skin. At several points in the wall of a cyst, the basal layer of the epithelium is absent, and individual cells and groups of cells are free in the adjacent stroma. In most cases this process is observed in cysts that are lined with squamous epithelium, but occasionally the cyst wall is formed of fibrous tissue and the adjacent acini show the changes described. There is no evidence after a tumor develops in one area that invasion into a distant area produces any change in the epithelium or stroma. In other words, the tumor grows by multiplication of its own cells, not by accretion of surrounding tissue.

Sarcoma: These tumors originate in the stroma immediately adjacent to a benzpyrene cyst and usually, but not exclusively, in cysts that are not lined by epithelium. The exact nature of the cell of origin is difficult to determine. With the special stains which differentiate connective tissue and smooth muscle, the cytoplasm of the tumor cells takes the stain for muscle. In many of the spindle-shaped cells there is a suggestion of cross-striations, but Mallory's phosphotungstic acid hematoxylin does not stain any striations similar to those in skeletal muscle. The tumor cells are extremely pleomorphic and multinucleated cells are not uncommon. They never contain vacuoles of fat and there is no histological evidence that they are derived from adipose tissue. Although it cannot be stated with certainty, it therefore seems probable that this tumor is a leiomyosarcoma.

Role of Inflammation: In the stroma adjacent to the benzpyrene cyst in some animals there is a moderate to advanced infiltration with lymphocytes and mononuclear cells. This infiltration is in many, however, entirely absent from the area of neoplastic change and there is no doubt that inflammation is not a constant accompaniment of tumor formation in the prostate.

Association of Carcinoma and Sarcoma: All of the sarcomas occurred in glands that also contained carcinoma. The time intervals shown in Table I indicate that the neoplastic change in the stroma occurs later than the epithelial proliferation. From the morphological standpoint, the stimulation may be different, since the sarcomatous change may occur in areas in which there is no carcinoma.
From these experiments, it is clear that benzpyrene may produce carcinoma in both the normal and the atrophic prostate and that the internal secretion of the testis plays no essential rôle in the process. On the other hand, in spite of the small number of animals, the finding of only one sarcoma in the 17 castrated animals, and an incidence of 75 per cent in the castrated rats that received parenteral hormone, indicates some relationship. A larger series will be necessary to determine with exactness the differences in the two groups and confirm or deny the general conclusion (24) that castration reduces resistance to transplantable tumors. The observation in this series, that the largest tumors occurred in castrated animals, would support that view.

The earliest tumor in this series appeared in a castrated animal that died on the 83rd day after injection, and after 150 days most of the animals had well developed tumors. Since the greater number of animals were killed, the figures cannot be accurately compared with those of other investigators. In the studies of Haagensen and Krehbiel (4) with 1:2:5:6-dibenzanthracene, the first tumors were seen in rats after 214 days, the average being 359 days. Ilfeld (5) saw tumors of the kidney and spleen after 120 days with 1:2:5:6-dibenzanthracene. Burrows (25) observed the earliest tumor following intraperitoneal injection of 1:2:5:6-dibenzanthracene after twenty-three weeks. The tumors of the fowl testis produced with zinc chloride (15) occurred on the 95th and 96th days after injection. With 1:2-benzpyrene, Maisin and Coolen (26) observed a subcutaneous tumor on the 25th day after injection of 1 mg., and Shear (27) was able to transplant tumors on the 47th and 53rd days.

The autonomous growth of the tumors in the prostate is demonstrated by the absence of atrophy after castration. Since the cell from which the tumor is derived would undergo atrophic changes, it follows that the tumor cell has acquired new potentialities of growth and will no longer respond to the normal mechanisms of stimulation and retardation. Metastases have not been observed, but the histologic evidences of anaplasia of the cells and the invasion of blood and lymph vessels leave no doubt of the malignant character of the tumor.

In 12 of the 13 animals with no tumor, fibrosis and lymphocytic infiltration were observed in the area of injection. It is evident, therefore, that the injected material entered the prostate. It is improbable that it leaked into the peritoneal cavity because no tumors of the peritoneum were observed similar to those produced by Burrows (25).

So far as a comparison with the malignant tumors of the human prostate is concerned, the experiments are unsatisfactory. The two types produced are rare in man and apparently have little in common. Sarcoma of the prostate (28) occurs in infants and children, rarely in adults, and may be of several types. Squamous-cell carcinoma (29) is even more infrequent, is observed after the fifth decade, and other than local metastases are uncommon. Squamous-cell carcinoma of the prostate in man and in the rat are both characterized by extensive local growth and invasion, cyst formation, ischemic...
necrosis in the larger tumors, and conspicuous keratinization of the tumor cells.

**SUMMARY**

1. The injection of a 5 per cent solution of 1:2-benzpyrene in lard into the prostate of the rat results in squamous-cell carcinoma in 75 per cent, and sarcoma in 5 per cent of animals after 85 to 150 days.

2. The action of benzpyrene is first to produce squamous metaplasia of the normal columnar epithelium and then malignant proliferation of the metaplastic cells.

3. Castration at the time of injection has no effect on the formation or course of the carcinomas produced. Castration after the appearance of the carcinoma has no effect on further growth. There are no observations on sarcomas.

4. Castrated animals which have received the male sex hormone show both carcinoma and sarcoma, the latter in a higher percentage than in castrated animals not receiving the hormone.

**Bibliography**


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