

Induction of Kidney Tumors in Mice by the Use of 20-Methylcholanthrene-impregnated Strings*

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SUMMARY

Cotton strings impregnated with 20-methylcholanthrene were placed in the right kidney of 34 male mice. Nine renal carcinomas and nine spindle-cell sarcomas were produced. Sixteen animals showed no tumor.

Whereas the transitional epithelium of the mouse kidney seems responsive to induction of neoplasia by 20-methylcholanthrene, the renal parenchyma appears resistant.

The method herein presented is recommended as a useful tool for the study of renal carcinogenesis.

Attempts to induce neoplasms of the kidney in rats and mice with chemical carcinogens have met with little success. Table 1 lists those experiments which have been relatively successful. Of the various chemical carcinogens which have been applied directly to the kidneys of these animals, 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, and 20-methylcholanthrene have yielded the highest percentage of tumors. In view of the potent carcinogenic property 20-methylcholanthrene has for other areas, its carcinogenic effect on the kidneys of rats and mice has been comparatively slight. This report concerns our attempt to induce carcinomas of the kidney in mice by the use of strings impregnated with 20-methylcholanthrene.

MATERIALS AND METHODS

The looped ends of 3-0 cotton sutures were dipped into pure 20-methylcholanthrene kept just above the melting point of 180° C. This resulted in the absorption of approximately 0.8 mg. of carcinogen per string. Thirty-four young mature male C57BL6 mice were anesthetized with Pentobarbital injected intraperitoneally. An abdominal incision was made and the right kidney exposed. With a fine, straight needle the sutures impregnated with the carcinogen were inserted into the kidney. The excess string was cut off evenly with the kidney surface. The incision was then closed in two layers with 3-0 silk suture. Only clean

* Supported by Grant C 3125 from the National Cancer Institute.

Received for publication April 28, 1962.

technic was used. With a little practice the method proved simple. The loss of animals was minimal and occurred only at the time of surgery. The animals were kept four per cage in steel cages and fed Purina Laboratory Chow and tap water ad libitum. A group of twenty control mice was kept under similar conditions.

RESULTS

The first tumor was found after 5 months in an obviously sick mouse. This tumor proved to be a renal-cell adenocarcinoma. A carcinoma of the renal pelvis was also found in this animal. Thereafter the animals were killed when they looked ill or when masses were palpable in the right upper abdomen. The tumors appeared intermittently, most of them between the 5th and 7th months of the experiment, and the remaining mice were sacrificed at the end of 1 year. None of the animals killed after 1 year had developed a tumor.

At autopsy the string could be identified in all the animals. When a tumor was present the string was within it; when no tumor was present the string was encapsulated by dense, fibrous tissue.

In the 34 kidneys seven squamous-cell carcinomas were found, two of which had metastasized to the lung. Most of the tumors were locally invasive and usually involved the liver. They often reached twice the size of the kidney in which they developed (Fig. 1). On microscopic examination they were found to be composed of masses of rather well differentiated large squamous cells. In some areas abundant keratin production was observed

(Fig. 2). The metastatic tumors found in the lung were multiple small nodules scattered throughout the lungs. Histologically, they resembled the squamous tumors but were less differentiated.

One transitional-cell carcinoma, one adenocarcinoma, and one adrenal-cell carcinoma were also observed. The transitional-cell tumor reached about the same size as the kidney in which it developed. The representative cells were small and possessed a spheroid or ovoid dark nucleus. Most cells were grouped in trabecular fashion, with each trabecula composed of a single or double string of cells (Fig. 3). The adenocarcinoma, which developed on the upper pole of the kidney, reached about twice the size of the kidney. The tumor was expansive rather than infiltrative and had a well defined margin. Microscopically, abortive tubule-like structures could be observed, surrounded by irregularly shaped cells with large pale nuclei (Fig.

4). The adrenal-cell tumor was a mass equal in size to the kidney and attached to the upper pole of the kidney; it was firm and yellow-gray. The cells composing this tumor were fairly uniform in size and shape. The nuclei were round and dark, with a rim of pink cytoplasm. They formed medullary masses separated by scanty stroma (Fig. 5).

Nine of the tumors were spindle-cell sarcomas. These tumors grew to 3 or 4 times the size of the kidney in which they arose and often nearly encased the kidney; they were pink-gray and firm. Microscopically, the tumors were composed of slender cells which formed interlacing bundles. The cell nuclei were fusiform, large, and vesicular and often contained two to three nucleoli (Fig. 6). Occasionally large, round cells with abundant eosinophilic cytoplasm were seen. No cross-striations were observed, and the cells assumed a reddish-gray color with Masson's trichrome stain.

TABLE 1
EXPERIMENTAL INDUCTION OF KIDNEY TUMORS IN RATS AND MICE

Reference	Year	Compound	Route administered	Animals	No. tumors	Type of tumors
Ilfeld (5)	1936	Dibenzanthracene-cholesterol	Implant into kidney	36 Mice	10	Epidermoid carcinomas
Ilfeld	1936	Dibenzanthracene-cholesterol	Implant into kidney	4 Rats	2	Epidermoid carcinomas
Esmarch (2)	1942	20-Methylcholanthrene	Direct injection	10 Rats	1 6	Squamoepithelioma Sarcomas
Gagni <i>et al.</i> (3)	1954	Benzpyrene	Injection into pelvis	30 Mice	5	Squamous-cell carcinomas
Gagni <i>et al.</i>	1954	Benzpyrene	Injection into pelvis	31 Rats	4	Squamous-cell carcinomas
Hendry <i>et al.</i> (4)	1955	4'-Fluoro-4-amino-diphenyl	S.C. in arachis oil	24 Rats	20	Benign tumors of tubular origin
Morris <i>et al.</i> (7)	1957	N-4(4'Fluoro)bi-phenylacetamide	P.O.	16 Rats	7	Renal epithelial tumors
Magee and Barnes (6)	1959	Dimethylnitrosamine	P.O.	20 Rats	14	Some adenocarcinomas
Zak <i>et al.</i> (8)	1960	Dimethylnitrosamine	P.O.	78 Rats	19	Similar tumors as Magee and Barnes
Argus and Hoch-Ligeti (1)	1961	Dimethylnitrosamine	P.O.	22 Rats	12	1 Sarcoma 11 Adenocarcinomas

FIG. 1.—Gross picture of large renal tumor produced by 20-methylcholanthrene-impregnated string in C57 mouse.

FIG. 2.—Squamous-cell carcinoma developing in kidney pelvis. H. & E., $\times 400$.

FIG. 3.—Transitional-cell carcinoma developing in kidney pelvis. H. & E., $\times 400$.

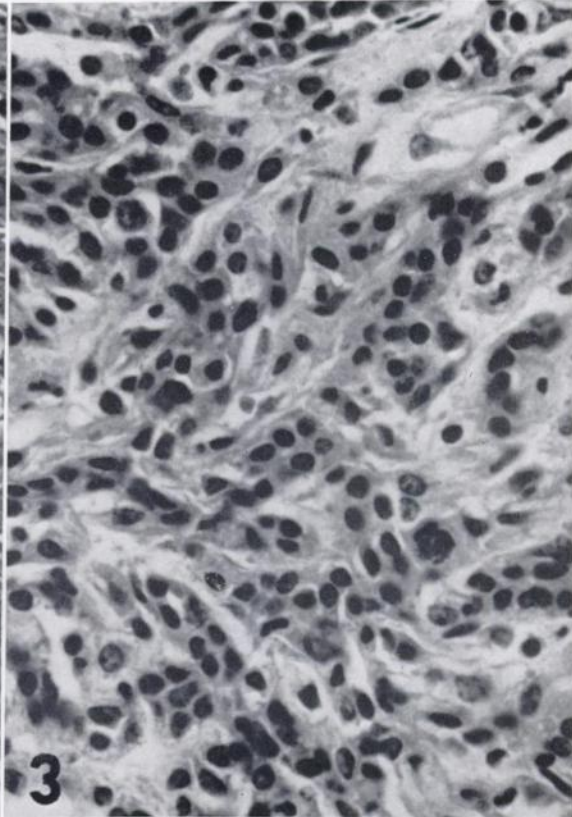
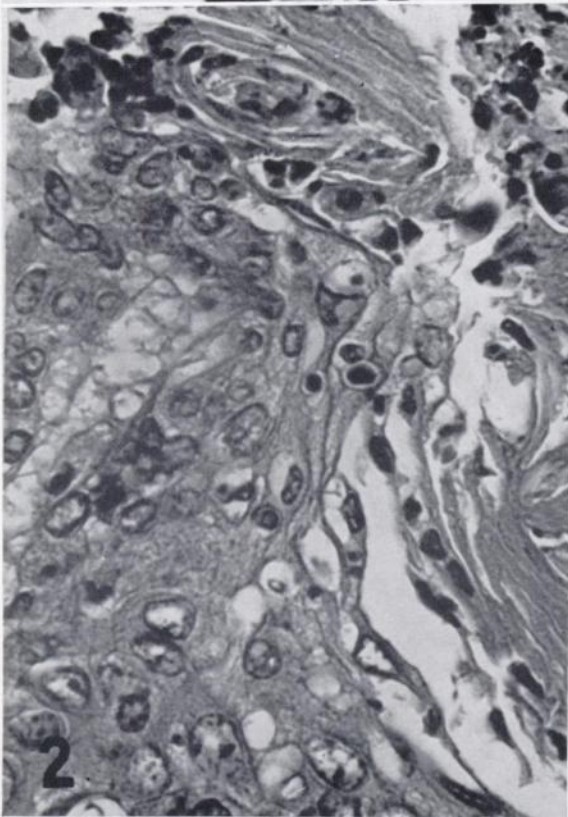
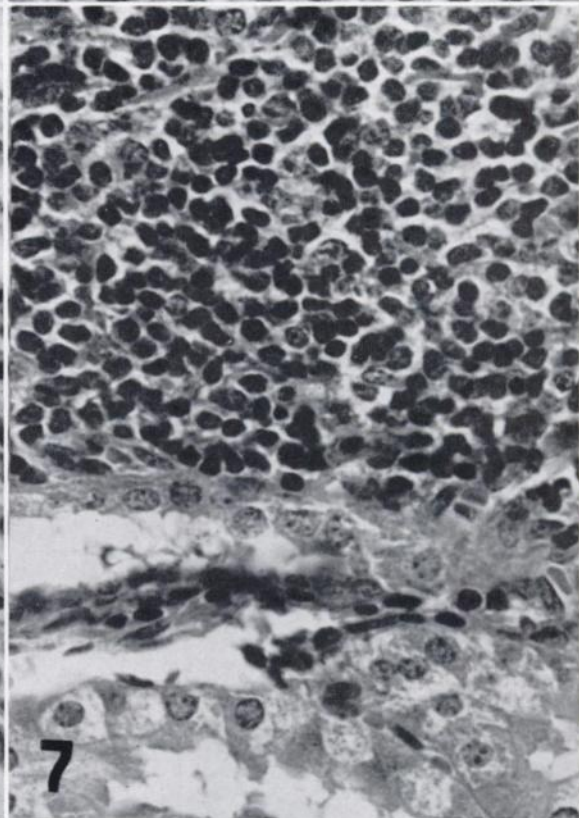
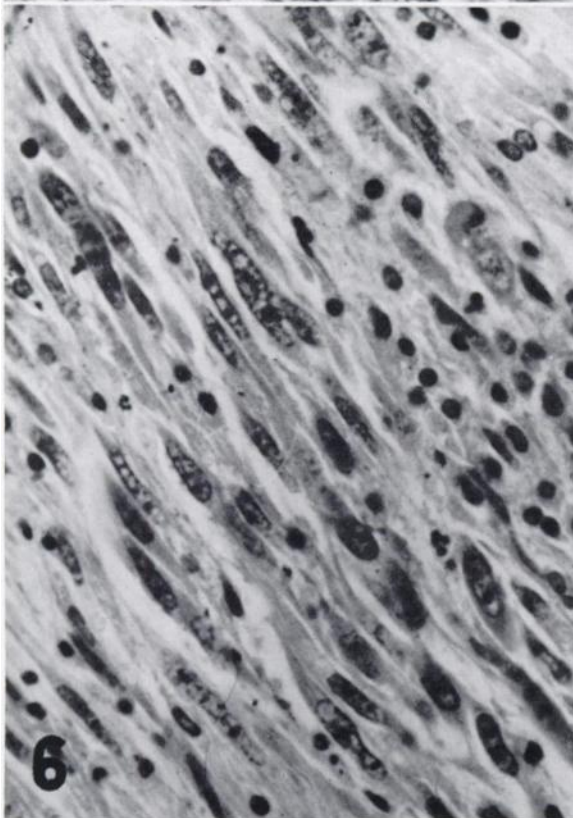
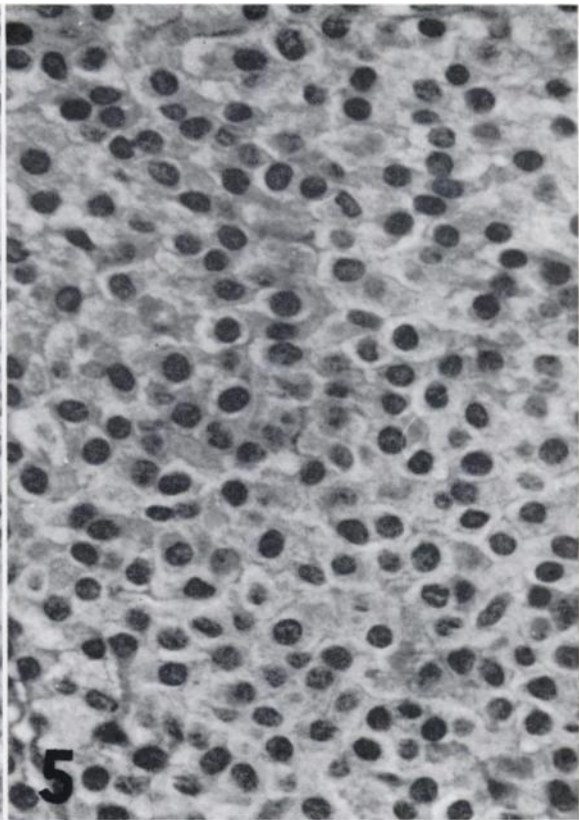
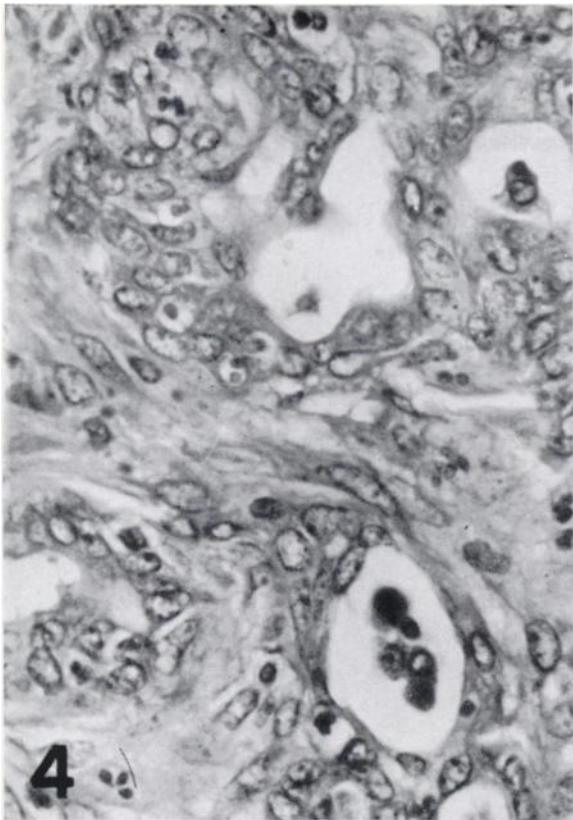


FIG. 4.—Adenocarcinoma developing in renal parenchyma. H. & E., ×400.

FIG. 5.—Adrenal-cell carcinoma produced in adrenal gland by 20-methylcholanthrene-impregnated string. H. & E., ×400.

FIG. 6.—Spindle-cell sarcoma (fibrosarcoma) developing in kidney. H. & E., ×400.

FIG. 7.—Neoplastic-like proliferation of reticulum cells in renal parenchyma. H. & E., ×400.



They did not form any collagen. Since the site of origin of these cells remained unknown, the descriptive term "spindle-cell sarcoma" was used.

In the group which we considered as tumor-free animals, every treated kidney showed a multifocal accumulation of mononuclear cells, which were interpreted as reticulum cells. This suggested an early lymphoma, but since no large tumor masses were observed we withheld this diagnosis (Fig. 7). Also in this group clusters of foamy macrophages laden with small yellow particles could be observed, probably representing particles of phagocytosed 20-methylcholanthrene around the encapsulated string.

DISCUSSION

The renal parenchyma of rats and mice is apparently somewhat resistant to the induction of neoplasia by chemical carcinogens. The epithelium of the renal pelvis seems more susceptible to neoplasia, but little use has been made of it as a biological tool.

Although our over-all tumor yield was nearly 50 per cent, our carcinoma yield was approximately 25 per cent. In each mouse kidney where we

could be certain that the string had been placed through the kidney pelvis a carcinoma developed.

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Cancer Res 1962;22:1177-1179.

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