

Malignant Renal Tumors in Male Hamsters (*Cricetus auratus*) Treated with Estrogen*

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In 1944 Vasquez-Lopez (1) reported certain observations made upon hamsters treated with estrogen. His material included a male which had carried a subcutaneously implanted pellet of diethylstilbestrol for 299 days, starting on the 42nd day

lowing: (a) 20-mg. pellets of diethylstilbestrol,¹ implanted subcutaneously on the 49th or the 68th day of life, with the animals killed 90, 120, 150, 170, 180, 217, 240, 270 or 300 days later; (b) the same, except the pellets contained diethylstilbestrol and cholesterol in a ratio of 1:4 and were implanted on the 53rd day of life; (c) 0.6 mg. of diethylstilbestrol microcrystals suspended in 0.2 cc. of saline solution or dissolved in the same amount of sesame oil, injected subcutaneously every other day, from about the 50th day of life, and continued for total periods of 90, 120, 150, 190, 210, 240 and 270 days, or from 322 to 497 days as indicated in Table 1. Kidneys from untreated male and female hamsters, ranging from 93 to 865 days in age, were examined also.

TABLE 1

OCCURRENCE OF METASTASES IN RENAL TUMOR-BEARING MALE HAMSTERS*

The age of the animals on the first day of treatment varied from 93 to 76 days.

LENGTH OF TREATMENT			Total length of treatment days	NUMBER OF ANIMALS WITH TUMORS	NUMBER OF ANIMALS SHOWING METASTASES
Subcutaneous injection of 0.6 mg. of diethylstilbestrol every second day in 0.1 cc. of sesame oil days	Subcutaneously implanted pellets of diethylstilbestrol† in 0.1 cc. of 0.9 per cent NaCl sol. days	Subcutaneously implanted pellets of diethylstilbestrol† days			
		217	217	2	0
		270	270	2	0
		300	300	2	2
107	215		322	1	0
	331		331	2	1
	360		360	2	1
107	259		366	1	0
157	215		372	2	1
107	277		384	1	0
205	215		420	1	1
214	236		450	1	0
157	340		497	1	1

* All males treated for 250 days or longer developed renal tumors.

† The average daily absorption was 0.090 mg.

of life. This animal was referred to in a table as showing "a large secondary deposit macroscopically visible in the left kidney." Aside from our own preliminary reports (2-5), as far as we know, this is the only reference in the literature to what might be an estrogen-induced renal tumor.

In our own work we have examined the kidneys of male and female hamsters treated with the fol-

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Renal tumors (Fig. 1) were found in all males treated with estrogen for 250 days or longer (Table 1), except those in which diethylstilbestrol-cholesterol pellets were implanted; in these, no renal tumors have been detected. In kidneys from two males carrying pellets of pure diethylstilbestrol for 217 days, numerous, small, adenomatous nodules were observed microscopically, but not grossly. No renal tumors have been observed in any female, in any untreated male, in any treated, castrated male, or in any control treated with sesame oil or with saline solution.

The tumors have always occurred bilaterally. They are usually adenomatous in character and always cortical in location. Tumor masses frequently appear as well defined but never encapsulated, more or less rounded, solid nodules of varying size (Fig. 2). They are often cystic and sometimes hemorrhagic (Fig. 3); they have not been observed to ulcerate. As a rule, the epithelium consists of small more or less cuboidal cells, rather embryonal in appearance, arranged in branching and anastomosing cords.

The tumor cells contain a sudanophilic, singly refractive lipid; they frequently resemble small

¹ The estrogen used in this study was generously supplied by Dr. E. Oppenheimer of the Ciba Pharmaceutical Products.

signet rings. There are spindle-celled areas which are sarcoma-like in appearance. "Ciliated" cells, while rare in most of the tumors, sometimes occur in large numbers, both in primary and in metastatic growths. They vary from low columnar to high cuboidal in shape and are smaller and more acidophilic than are the nonciliated tumor cells. They are sometimes arranged in the form of tubules; in most cases, however, they comprise peripheral bands of simple epithelium, partially surrounding cords of stratified cuboidal nonciliated epithelium, as seen in section (Fig. 4). Superficially, such arrangements simulate, but are not homologous with developing renal corpuscles. Rosette-like arrangements of epithelial cells are common, with the cells frequently radiating outward from a central clear space lined by a single layer of flattened cells.

No lymph- nor blood-borne metastases have been observed. Metastasis occurs by implantation after mechanical transport through the peritoneal cavity. In advanced cases, however, metastatic growths are found from diaphragm to scrotum, frequently invading the parenchyma of abdominal viscera. While studies to be reported later by Dr. A. C. Griffin show that the tumors resemble true neoplastic growths in regard to their content of riboflavin, desoxyribonucleic acid, etc., they are not considered highly malignant. For the time being, we are calling them malignant adenomas rather than adenocarcinomas or embryomas.

Some of the tumor cells appear to arise from proximal convoluted tubules (Fig. 5). It is possible that other segments of the nephron, or even interstitial tissue, may contribute; but so far this has not been clearly demonstrated. As suggested earlier (2), it is believed that a significant contribution may be made by a localized band of sudanophilic epithelial cells in the lining of the renal pelvis (Fig. 6). However, indications of such an origin have been observed only in kidneys bearing well developed tumors; i.e., no early stages in the development of tumors from pelvic epithelium have been seen. A possibility that the tumors may be myoepitheliomas is considered improbable, but has not been completely ruled out.

While it is possible that the "ciliated" cells may be derived from a Müllerian duct rest, we have observed no other indication of the existence of such a rest in the kidney. It is considered more likely that the "cilia" represent a considerably modified brush border. It is hoped that cytological material, now being prepared, will provide the answer to this question. In material fixed according to Bouin and Rossman and stained by the periodic acid-Schiff method of MacManus (6), "cilia" are negative, while basal granules and brush borders are distinctly positive. "Ciliated" borders are much thicker than brush borders, and "cilia" show no indications of being embedded in a ground substance.

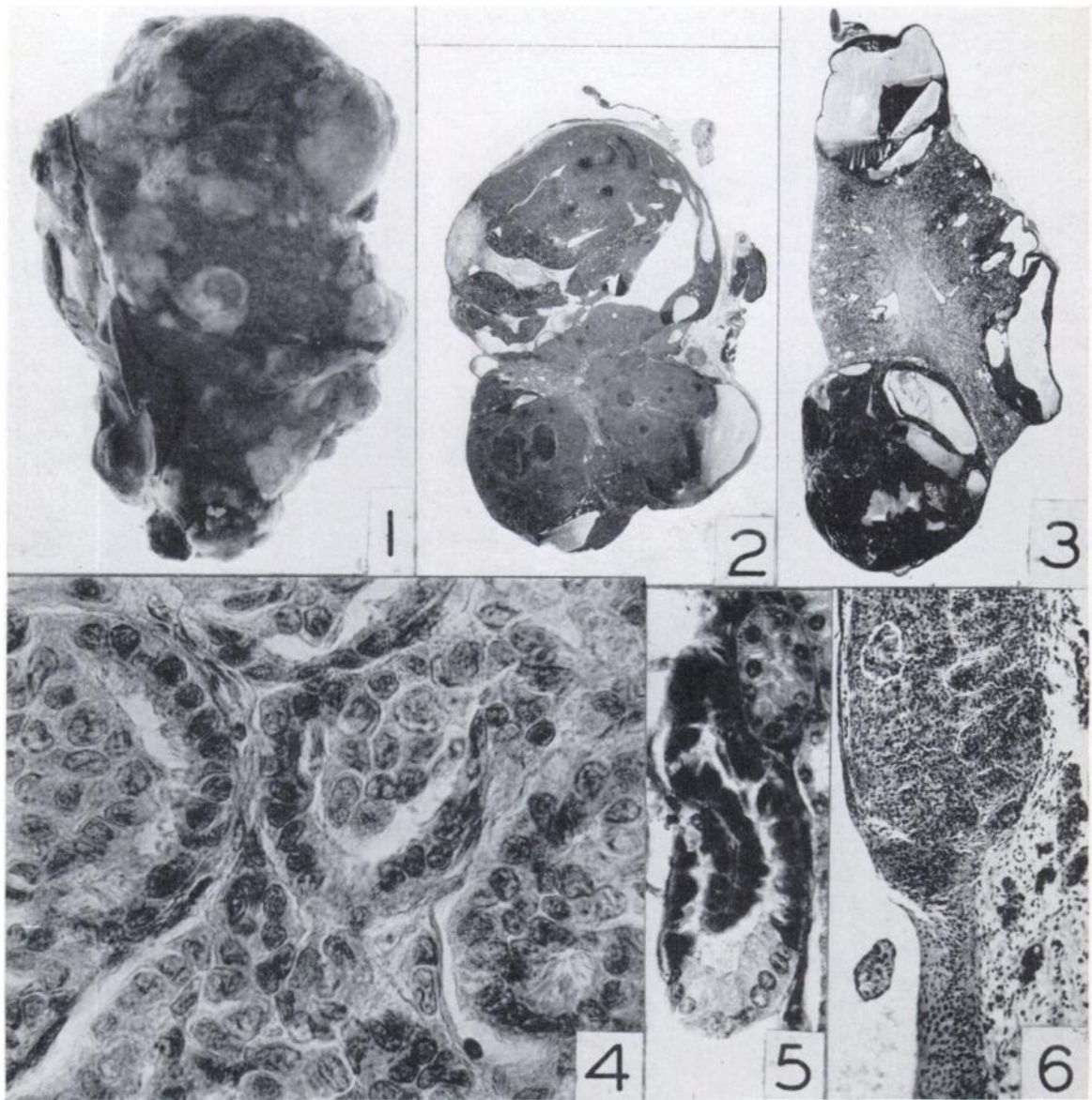
SUMMARY

In summary, it has been found that chronic treatment of male hamsters with diethylstilbestrol is followed by the appearance of multiple cortical tumors in both kidneys. The exact origin or origins of the tumor cells is still under investigation. Ultimately, the tumors become malignant, but metastases have not been observed outside the abdominal cavity.

A detailed description of the tumors will appear later, as will accounts of experimental work now in progress.

REFERENCES

1. VASQUEZ-LOPEZ, E. The Reaction of the Pituitary Gland and Related Hypothalamic Centers in the Hamster to Prolonged Treatment with Estrogens. *J. Path. & Bact.*, **56**:1-13, 1944.
2. MATTHEWS, V. S.; KIRKMAN, H.; and BACON, R. L. Kidney Damage in the Golden Hamster Following Chronic Administration of Diethylstilbestrol and Sesame Oil. *Proc. Soc. Exper. Biol. & Med.*, **66**:195-96, 1947.
3. KIRKMAN, H., and BACON, R. L. Some of the Effects of the Chronic Administration of Diethylstilbestrol in Golden Hamsters. *Anat. Rec. (Suppl.)*, **100**:135-36, 1948.
4. ———. Renal Adenomas and Carcinomas in Diethylstilbestrol Treated Male Golden Hamsters. *Ibid.*, **103**:59-60, 1949.
5. BACON, R. L., and KIRKMAN, H. Some Effects of Diethylstilbestrol on the Reproductive Tract in Golden Hamsters. *Anat. Rec. (Suppl.)*, **103**:5, 1949.
6. MACMANUS, J. F. A. Histological and Histochemical Uses of Periodic Acid. *Stain Technol.*, **23**:99-108, 1948.



Figures 1, 2, 3 and 6 are of kidneys from male hamsters (*Cricetus auratus*) injected subcutaneously, every other day, with 0.6 mg. of diethylstilbestrol, Figs. 1, 2 and 6 for 360 days, Fig. 3 for 372 days. Fig. 4 is from a scrotal metastasis in a male hamster treated for 331 days. Fig. 5 is from a kidney of a male hamster implanted with a 20-mg. pellet of diethylstilbestrol on the 68th day of life; the animal was sacrificed 217 days later,

after having received 0.5 cc. of a freshly prepared 0.5 per cent solution of trypan blue in 0.9 per cent NaCl solution per day per 100 grams of body weight for 8 days. Upon recovery, the pellet weighed 2.5 mg. Magnifications are as follows: Figs. 1, 2 and 3 are 2.4 \times , Fig. 4 is 680 \times , Fig. 5 is 370 \times , and Fig. 6 is 80 \times .