Summary. 1. Administration of Δ^1-cortisone and 9α-fluorohydrocortisone changes normal urinary excretion of steroid metabolites, indicating adrenal depression or altered steroid metabolism. 2. Only a small proportion of administered 9α-fluorohydrocortisone is excreted as α-ketolic metabolites; Δ^1-cortisone also is converted to a great extent to non-α-ketolic elements. 3. Elemental sulfur has been found to give a positive reaction with blue tetrazolium. This interferes with the quantitative determination of α-ketols in extracts of cat urines. 4. The α-ketols and sulfur can be separated by chromatography and each determined quantitatively by the blue tetrazolium reaction, using a modified method. 5. Administration of Δ^1E and 9α-fluorohydrocortisone causes an increase in urinary excretion of sulfur-containing compounds in cats, which leads us to conclude that these steroids alter sulfur metabolism.

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Prostatic Neoplasms of the Wistar Rat Induced with 20 - Methylcholanthrene. (22787)

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Prostatic tumors have never been reported to occur spontaneously in the rat, in contrast to man(1) and dog(2,3). McCoy(4) examined nearly 100,000 wild rats finding 103 primary tumors, none of which originated in the prostate. Moore and Melchionna(5) were first to induce with 1:2 benzpyrene in lard squamous cell carcinoma and sarcoma of the anterior lobe of the rat prostate but they did not report metastases. Dunning, Curtis and Segaloff(6) produced metastasizing squamous cell carcinoma in rat prostate with methylcholanthrene but the designation of the prostatic lobe where this carcinoma was induced was not mentioned. Neither groups reported occurrence of adenocarcinoma of the rat prostate.

The purpose of this paper is to report the induction of three types of experimental neoplasms particularly adenocarcinomas in the ventral lobe of the prostate glands of Wistar rats using methylcholanthrene crystals.

Material and methods. 122 male inbred Wistar rats, 3 months of age, were kept in individual cages and given Purina Laboratory chow and water *ad libitum*. Under nembutal or ether anesthesia, the ventral prostate was exposed through a posterior midline incision. Using aseptic technic, a pocket was made in the ventral prostate, and crystals of 20-methylcholanthrene were carefully deposited. The pocket was then closed by suturing with surgical silk. Extreme care was taken to avoid scattering of methylcholanthrene. The technical success of the intraprostatic injections was evidenced by presence of a small yellow bleb. Following the operation, the rats were examined routinely for presence of palpable prostatic masses; biopsies were repeatedly performed to study the character of the growth. This method was used to establish the latent period of the tumor. 23 animals were excluded from evaluation because they died shortly after the operation. The remainder of operated rats, 99 in number, were kept until they showed signs of impending death. These were sacrificed and autopsied. Wherever possible, small fragments of prostatic tumors were removed and immedi-

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TABLE I. Induced Prostatic Neoplasms in 99 Intact Wistar Rats.

Tumor latent period (days)	Adeno-carcinoma	Squamous cell carcinoma	Leiomyo-sarcoma
100-150		1	
151-200	2	19	
201-250		4	
251-300		2	
301-350		4	3*
Total	2 (2%)	30 (30.3%)	3 (3%)

* 2 rats having leiomyosarcoma had associated squamous cell carcinoma.

ately inoculated subcutaneously into rats of the same strain. The tissue for histologic study was fixed in formaldehyde or Bouin's fluid, embedded in paraffin and sections stained with Harris' hematoxylin and eosin.

Results A. Tumor incidence, latent period and growth behavior of prostatic neoplasms. Table I shows the incidence and latent period of various prostatic neoplasms. Fig. 1 shows a section of a normal mature 6-month-old rat prostate and Fig. 2-5 illustrate prostatic tumors observed in experimental rats. The earliest squamous cell carcinoma was seen on 118th day. Adenocarcinomas appeared on 180th and 198th day. Leiomyosarcoma of the prostate occurred after 301 days, much later than any of the other neoplasms. In a different series 24 rats that had their ventral prostates sutured only with surgical silk had not developed neoplasms. The average dimensions of the tumors were 3.5 x 4 x 2.5 cm. The squamous cell carcinoma grew the fastest. The average survival time beyond the latent period of rats bearing these tumors was 63 days for squamous cell carcinomas, 92 days for leiomyosarcomas, and 63 days for adenocarcinomas. Death was due to obstruction and/or metastases produced by the tumor. Hydronephrosis, hydroureter, bladder obstruction, anemia and hematuria were complications often seen in rats in which the prostatic masses grew to 3 x 2.5 x 1.5 cm or more. Near death the rats became very irritable, and external and internal bleeding was evident.

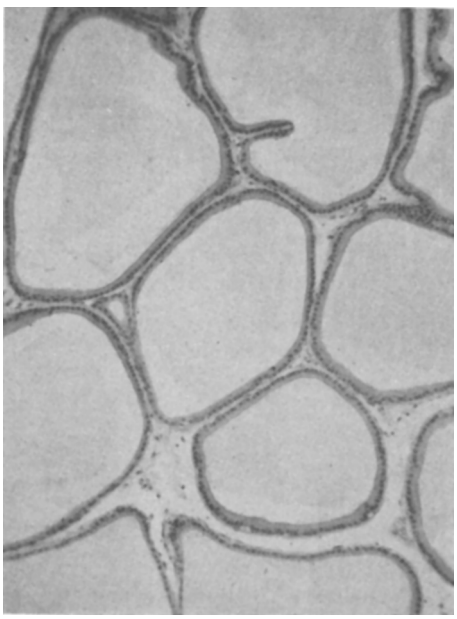

FIG. 1. Section through normal mature 6 mo old rat prostate, ventral lobe. Mag. 150× H & E.

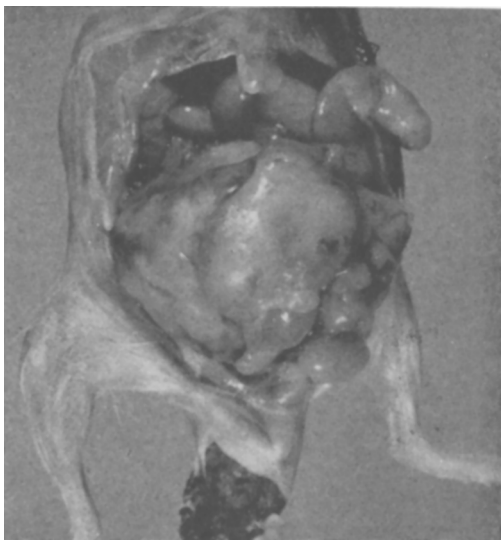

FIG. 2. Photograph of Wistar rat bearing an *in situ* adenocarcinoma of prostate gland. Tumor was 2.8 × 3.2 × 2.5 cm and metastasized to lungs and ribs. Tumor latent period 180 days.

Fragments of 21 of the squamous cell carcinomas were transplanted into rats of the same strain. Table III summarizes the growth behavior of 4 of these tumors. Unfortunately, the adenocarcinomas and leiomyosarcomas were not transplanted. Detailed data in Table III on the growth behavior of these transplantable tumors at various generations are advanced to illustrate

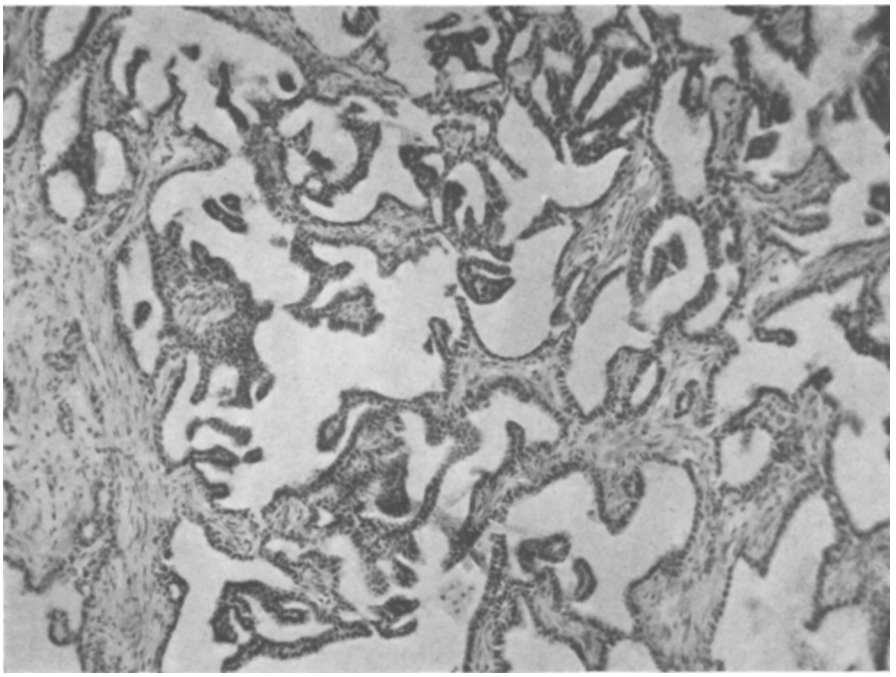

FIG. 3. Section through adenocarcinoma of rat prostate. Sections through tumor mass revealed that there was diffuse infiltration by adenocarcinoma. Carcinomatous acini show characteristic lack of orientation. In some area there was a lack of basement membrane encircling each gland, and irregularity of glandular epithelium. No presence of secretion in the gland was seen. Mag. 150× H & E.

similarities and differences that exist among the 4 tumors. Some of the prostatic tumors that metastasized grew very poorly or not at all in inbred female rats, although the reverse was true when transplanted to male rats.

Histological Findings B. — Of the 35 tumors examined histologically on the basis of cellular structure of the tumor and its staining properties, two were judged to be adenocarcinomas, 30 squamous cell carcinomas, 3 leiomyosarcomas (Fig. 2-5). Invasion and/or metastases of these tumors are summarized in Table II. In most instances, prior to appearance of neoplasms, considerable inflammatory reactions, squamous cell metaplasia and abscess formation were observed, and these reactions were frequently seen in rats that developed squamous cell carcinoma and leiomyosarcoma, but not in rats developing adenocarcinoma of the prostate.

In the 2 adenocarcinomas examined, the tumor cells were arranged in glandular formation around irregular spaces, or trabeculae and the stroma was composed of a small

TABLE II. Frequency Distribution of Metastases and/or Invasion of Induced Prostatic Neoplasms in Wistar Rats. Leiomyosarcoma did not metastasize.

Organ	Squamous cell carcinoma	Adenocarcinoma
Lung	10	1
Thymus	1	0
Heart	1	0
Liver	3	0
Spleen	1	0
Pancreas	1	0
Adrenal	3	0
Kidney	4	0
Lymph nodes	8	0
Intestines	5	0
Seminal vesicle	12	0
Coagulating gland	12	0
Dorsal prostate	14	0
Testes	2	0
Epididymis	6	0
Ribs	2	1
Pelvic bone	0	0
Bladder	11	1
Diaphragm	3	0

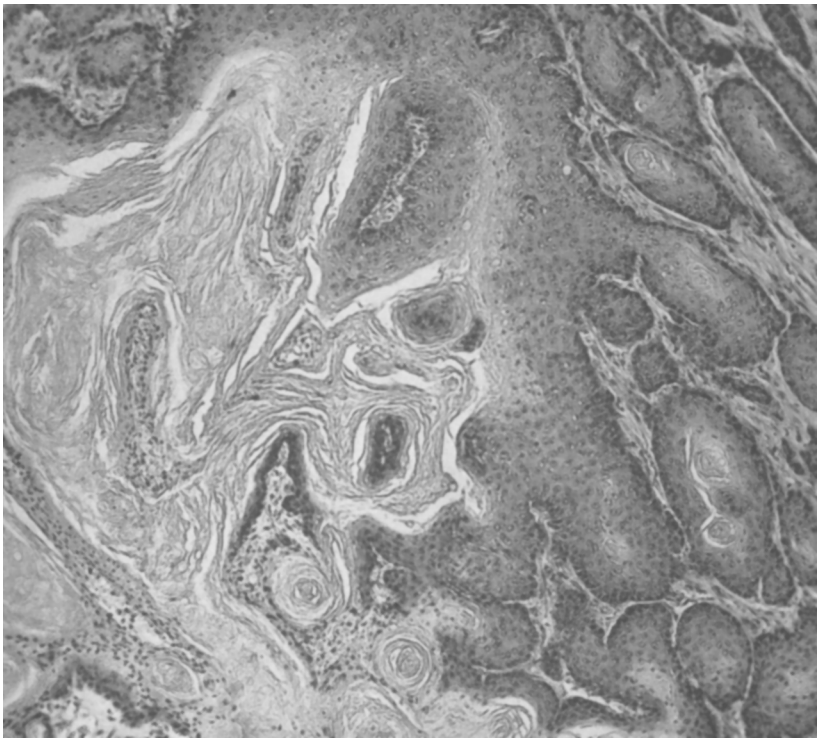

FIG. 4. Section through squamous cell carcinoma of rat prostate. Tumor measured was 3.9 × 4.8 × 2.2 cm and metastasized to lung, rib, kidney, mesentery, and omentum. Mag. 150× H & E.

amount of connective tissue accompanied by slender capillaries. The tumor cells had round or oval hyperchromatic nuclei of varying size situated in the basal part of the cells. The cytoplasm appeared more swollen and foamy or vacuolated and the nucleus became pyknotic. No evidence of secretion was seen in the gland with diffuse infiltration by adenocarcinoma.

The leiomyosarcomas consisted of cells that were large and spindle-shaped with abundant eosinophilic cytoplasm; among these were many giant cells. In some areas of the tumors, the cells showed pleomorphism with round or oblong nuclei as the predominant form and many mitotic figures were common. The cells of this tumor often tended to form interlacing bundle running in different directions. Two of the three rats with leiomyosarcomas that had a latent period of 301 and 308 days respectively had associated with it areas of squamous cell carcinoma. Although the leiomyosarcomas did not metastasize, the general histological appearance supported the belief that the tumors were malignant.

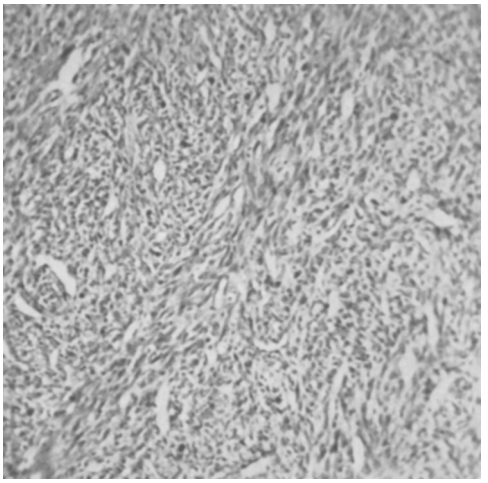

FIG. 5. Section through rat prostate with leiomyosarcoma. Tumor measured 2.8 × 2.2 × 3.0 cm, grew slowly and did not metastasize. Glandular nature of prostate was completely absent. Mag. 125× H & E.

TABLE III. Results of Homologous Transplantation of Several Prostatic Squamous Cell Carcinomas into Wistar Rats.

Designation No. of tumor	Response of tumor at generation	No. of rats transplanted into	Site in host inoculated	Growth characteristics: Takes, %	Latent periods, days	Kill host, days	Infiltration and metastases	Special features
1	12	12 ♂	Subcut.	90	8–10	30–40	Local invasion, lung	No regression, ulceration
2	18	12 ♂	"	60	14–21	30–60	Local invasion	Regression after ulceration (15%)
		12 ♀	"	70	12–18	25–52	*Idem*	" " " (20%)
3	16	12 ♂	"	85	7–14	25–30	Lung, lymphnodes	Hemorrhage, necrosis, no regression
		12 ♀	"	20	14–22	40–56		Hemorrhage, necrosis, regression
		12 ♂	Intraper.	56		44–54	Lung, ribs, local invasion	Necrosis, hemorrhage, no regression
		12 ♀	"	0				
		12 ♂	Pleura cavity	36		18–23	Lungs, ribs, adrenal diaphragm	Necrosis (less extensive), hemorrhage, no regression
		12 ♀	*Idem*	0				
4	10	12 ♂	Subcut.	25	14–28	50–60	Lung, occasional local invasion	Regression rare, necrosis (less extensive)
		12 ♀	"	10	24–28			Regression

Histologically most squamous cell carcinomas were composed of irregular masses or sheets of more or less differentiated basal and columnar cells with an inconspicuous stroma. In many of the tumors, the cancerous cells formed epithelial pearls or small cystic areas with mucoid material in the center. In some tumors the epithelial elements which differentiated and formed keratin, also had areas that appeared glandular. It seemed as if the "neoplastic" change did not go on to full squamous cell differentiation.

Discussion. It has been shown that prostatic neoplasms, similar to the 3 types that occur in man, can be induced with methylcholanthrene in the ventral prostate of Wistar rats, squamous cell carcinoma being more frequent in the rat than in man. Adenocarcinoma of the prostate gland in the rat showed great similarity to that of man. Moreover, these neoplasms with the exception of leiomyosarcoma not only exhibited extensive local growth and invasion, but also distant metastases. Judging by distribution of metastases in various organs, the tumors were autonomous, and spread was through blood and/or lymphatic pathways.

The fact that this non-specific, poorly soluble carcinogen induced so high a proportion of prostatic squamous cell carcinomas proved not to be out of keeping with the results of Moore *et al.*(5) and Dunning *et al.*(6). On the other hand, our results differ from the above investigators by the induction of adenocarcinomas and leiomyosarcomas, by a more extensive distribution of invasion and/or metastases of prostatic tumors to various organs, and by a more detailed account on the growth characteristics of transplantable prostatic tumors, some of which are in their 18th transplantable generation. Moore *et al.* and Dunning *et al.* used a different strain or subline of rats in their respective studies. It is noteworthy to point out that different species and sometimes even different strains of the same species vary markedly in the relative sensitiveness of their various tissues to induced and spontaneous tumor development. Moreover, the nutritional, hormonal and metabolic status of the gland are important

factors too, that can greatly affect the tumorgenic response of a gland to a carcinogen.

It is becoming increasingly important that when working with the prostatic gland of the rat that the designation of the prostatic lobes be carefully indicated despite having a common origin arising as buds of the urogenital sinus. The observation that the prostatic lobes in an animal is not a homogenous structure as judged by its response to estrogen, enzyme concentration, chemical constituents (7) and zinc[65] uptake(8,9) has been late in discovery notwithstanding the fact that histological procedures do not reveal any differences among the lobes of the prostate gland. It is therefore reasonable to assume that lobes of the rat prostate gland can react differently to methylcholanthrene or to other carcinogens. Preliminary studies using different carcinogens to various prostatic lobes seem to support this concept.

Summary. (1) Intraprostatic deposits of 20-methylcholanthrene crystals induced three types of prostatic neoplasms in the ventral lobes of the Wistar rat's prostate gland: adenocarcinoma (2%), leiomyosarcomas (3%) and squamous cell carcinomas (30.3%). The squamous cell carcinomas and adenocarcinomas metastasized. (2) A detailed account of the distribution of invasion and/or metastases of these prostatic tumors to various organs are presented as well as an account on the growth characteristics of transplantable squamous cell carcinomas.

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Nitrogen Sparing Action of Neutral Fat and Fatty Acids in the Phlorhizinized Rat.* (22788)

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In a recent review devoted to the relationship of protein metabolism to that of fat and carbohydrate, Munro summarized the extensive data indicating that carbohydrate is a protein sparer, regardless of whether the animal is starving or is receiving a protein free or protein containing diet(1). By contrast, Munro noted that the results of studies dealing with the ability of fat to prevent excessive nitrogen loss are in conflict. Thus, some reports indicate that when fat is the sole source of calories, the nitrogen output of an animal is as great as during total starvation(2,3,4,5); on the other hand, data has been presented which demonstrates that fat is a protein sparer, even in the absence of protein in the diet, if minimal caloric requirements are satisfied(6,7). In view of the divergent results, and because it seemed to us that fat, under

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