Spontaneous Ovarian Tumors in Han:NMRI Mice: Histologic Classification, Incidence, and Influence of Food Restriction[1]

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ABSTRACT—In a life-span study with female Han:NMRI virgin mice (300 of a fat subline, 300 of a lean subline, and 300 controls), a total of 424 mice developed 673 ovarian tumors. Half of the mice in each group were fed ad libitum, and for the other half food was restricted. Most prevalent were tubular adenomas followed by granulosa and Sertoli cell tumors. Altogether, 42 neoplasms were classified as tubular adenocarcinomas, and 21, as luteomas. The general incidence of tumors increased sharply beyond the 18th month of age. Granulosa cell tumors arose relatively early, and tubular adenocarcinomas occurred very late in life. The occurrence of ovarian tumors depended mainly on life expectancy. All animals subjected to food restriction lived longer and developed more ovarian neoplasms than those fed ad libitum.—JNCI 1984; 72:1383-1395.

Reports on spontaneous ovarian tumors in mice with incidences above 10% are rare (*1-3*). It is probably for this reason that only a few attempts have been made (*3*) to classify ovarian neoplasms of the mouse according to the World Health Organization classification for tumors of domestic animals (*4*). The high incidence of ovarian tumors in the present study with Han:NMRI mice gave the opportunity to investigate the extent to which this classification is applicable, whether a selection for high or low body fat influences tumor incidence or range, and whether the known effect of tumor suppression by caloric restriction (*5, 6*) is also true for ovarian tumors.

MATERIALS AND METHODS

Animals and diets.—A total of 900 female virgin 8-week-old Han:NMRI mice were divided into 3 groups: 300 mice of a fat subline (18.0% relative body fat), 300 mice of a lean subline (11.3% relative body fat), and 300 control animals (16.7% relative body fat). The sublines (12th and 13th generation) were obtained from a running selection experiment from the Department of Population Genetics and Data Processing at our Institute (*7*).

The mice were kept in one room of a barrier-type animal house with 22±1°C room temperature, 55±5% relative humidity, +15 mm H_2O atmospheric pressure, 12 hours light-12 hours dark circadian sequence, and an air change of 20 times per hour. They were housed in groups of 5 in polycarbonate cages on autoclaved soft wood granules until the natural end of their lives. Half of each group was fed an autoclaved commercial cereal-based diet ad libitum, and the other half was fed a daily ratio of 80% of the level consumed ad libitum. Acidified and pasteurized tap water was always available. We checked the animals daily, and at natural death or killing we performed a necropsy. Histologic examination was done of all pathological alterations and of various organs including both ovaries on 5-μm thick sections stained with H & E after fixation in 10% Formalin and embedding in paraffin. Selected slides of ovarian tumors were stained by the following methods: Azan, according to Heidenhain, Sudan III, and a combined Alcian blue-PAS. Semithin sections, 0.5-1-μm thick, were obtained from representative tumors embedded in methacrylate and stained with toluidine blue and silver methenamine (*8*). We omitted 33 mice from the results because we could not obtain histologic diagnosis on both ovaries for reasons of autolysis or cannibalism. Statistical evaluations were done by chi-square analysis and by Student's *t*-test.

RESULTS

Of a total of 867 mice, 424 developed ovarian tumors. Of these, 198 animals showed neoplasms in both ovaries. Table 1 shows the incidence of unilateral and bilateral tumors for each animal group within 4 age periods. Of the ad-libitum-fed animals, only 3 survived beyond the age of 30 months and therefore were not considered as a separate age group in further tables and figures. Only a few ovarian neoplasms were found in animals younger than 19 months of age. Generally, the number of mice with bilateral ovarian tumors increased with age, whereas the incidence of unilateral tumors decreased after the age of 24 months (text-fig. 1). This development was more obvious in food-restricted animals since they lived longer ($P \leq .001$) and showed a higher number of bilateral tumors than animals fed ad libitum ($P \leq .05$). The general incidence of ovarian neoplasms was higher in the control group than in the sublines (58 vs. 44%; $P \leq .05$); however, no difference existed between the sublines. Food restriction had no influence on neoplasm incidence within the sublines, whereas among the control mice 13% more were affected ($P \leq .01$) in proportion to their longer life-span when fed a limited amount of food.

Ovarian tumors were classified according to the World Health Organization scheme for domestic ani-

ABBREVIATIONS USED: H & E=hematoxylin and eosin; PAS=periodic acid-Schiff.

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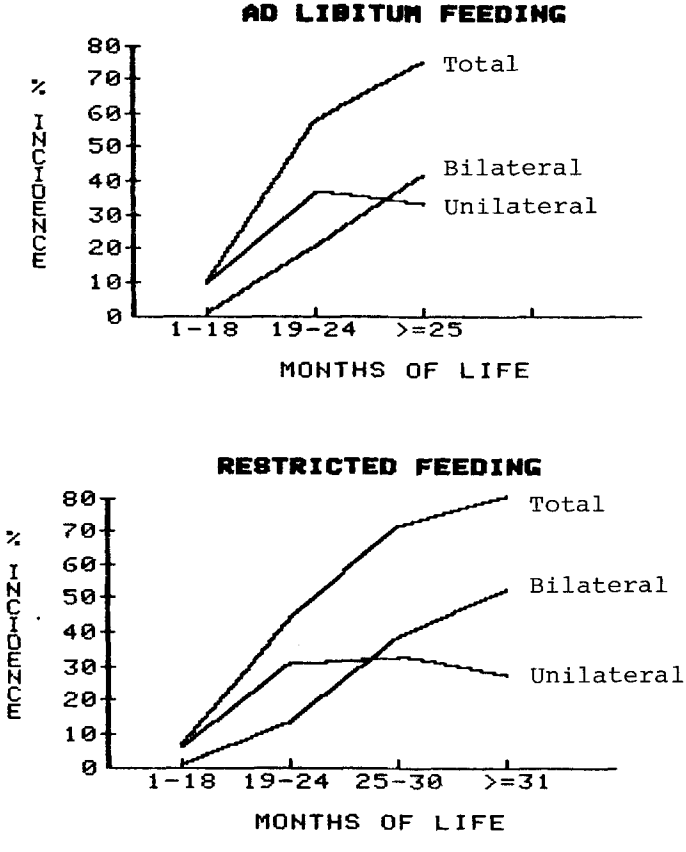
TABLE 1.—*Incidence of unilateral or bilateral ovarian tumors in Han:NMRI mice in relation to age*

Animal group	Tumor incidence within life-span intervals, mo[a,b,c]																							
	1–18					19–24					25–30					≥31					Total				
	No.			%		No.			%		No.			%		No.			%		No.			%	
	U	B		U	B	U	B		U	B	U	B		U	B	U	B		U	B	U	B		U	B
Ad libitum feeding																									
Control	2	1	(26)	8	4	30	10	(73)	41	14	18	11	(42)	43	26	1	1	(2)	50	50	51	23	(143)	36	16
Fat	1	1	(53)	2	2	23	11	(62)	37	18	6	17	(30)	20	57	—	—	(0)	—	—	30	29	(145)	21	20
Lean	9	—	(61)	15	—	18	19	(57)	32	33	8	14	(27)	30	52	1	—	(1)	100	—	36	33	(146)	25	22
Total	12	2	(140)	9	1	71	40	(192)	37	21	32	42	(99)	32	42	2	1	(3)	67	33	117	85	(434)	27	20
Restricted feeding																									
Control	1	1	(17)	6	6	12	5	(29)	41	17	27	16	(59)	46	27	15	16	(39)	38	41	55	38	(144)	38	26
Fat	1	—	(28)	4	—	13	3	(46)	28	7	11	16	(43)	26	37	5	13	(24)	21	54	30	32	(141)	21	23
Lean	4	—	(53)	8	—	11	8	(42)	26	19	8	23	(39)	21	59	1	12	(14)	7	86	24	43	(148)	16	29
Total	6	1	(98)	6	1	36	16	(117)	31	14	46	55	(141)	33	39	21	41	(77)	27	53	109	113	(433)	25	26

[a] *Numbers in parentheses* are the numbers of mice evaluated.
[b] U=animals with tumors in one ovary; B=animals with tumors in both ovaries.
[c] —=tumor not observed.

mals (*4*) and the modified version for mice (*3*). For the calculation of the absolute incidence of various tumor types (table 2), bilateral tumors of the same classification were taken into account only once, whereas all tumors were considered in the relative incidence (tables 3, 4). Ten neoplasms not classifiable due to autolysis were neglected.



TEXT-FIGURE 1.—Incidence of total, unilateral, and bilateral ovarian tumors in Han:NMRI mice in relation to age.

Tubular Adenoma

Tubular adenomas were the most common tumors found in Han:NMRI mice; they arose bilaterally in 35%. Their sizes ranged between 0.2 and 3 cm in diameter. We found three histologic growth patterns: papillary, solid, and cystic adenomas. Of all ovarian tumor types, the tubular adenomas revealed the widest morphologic range (figs. 1-4). Their most characteristic feature was tubules lined by simple cuboidal, columnar, or attenuated epithelium with a narrow rim of acid mucopolysaccharides, revealed by the Alcian blue stain (fig. 1). This was also seen on sections of normal germinal epithelium. Between the tubules varying amounts of luteinized (fig. 2) and other stromal cells displayed a spectrum of patterns. The stromal cells were pleomorphic, often indistinguishable from granulosa cells, and sometimes resembling thecal cells. Many of the larger tubular adenomas revealed insular structures, bordered by small amounts of connective tissue and filled with rounded (fig. 3) or elongated cells (fig. 4). The elongated cells resembled Sertoli cells of the testes, and quite often areas of well-differentiated Sertoli cell tubules were found interspersed in tubular adenomas. The few cystic tubular adenomas measured 5-10 mm in diameter and were composed of numerous variably sized dilated tubules and sparse stromal elements consisting of luteinized cells. In general, mitotic activity of the tubular adenomas was rare. In some instances, tubules were found growing out into the fat of the surrounding bursa. Occasional concentrically laminated foci of mineralization resembling psammoma bodies were present. Of 63 tumors available for Sudan III staining, 51% totally lacked lipids, 35% contained a slight amount, and 14% contained a moderate amount

TABLE 2.—*Absolute incidence of ovarian tumors in Han:NMRI mice in relation to age*

Age intervals, mo	No. of animals	Tumor incidence[a]															
		Tubular adenoma		Tubular adeno-carcinoma		Teratoma		Granulosa cell tumor		Luteoma		Sertoli cell tumor		Mesenchymal tumor		Autolytic tumor	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Ad libitum feeding																	
1–18	140	4	(3)	—				11	(8)	—		1	(1)	—		—	
19–24	192	66	(34)	3	(2)	1	(0.5)	37	(19)	6	(3)	19	(10)	1[b]	(0.5)	3	(2)
≥25	102	58	(57)	4	(4)			18	(18)	9	(9)	19	(18)	—		2	(2)
Total	434	128	(29)	7	(2)	1	(0.2)	66	(15)	15	(3)	39	(9)	1	(0.2)	5	(1)
Restricted feeding																	
1–18	98	4	(4)	—		1	(1)	2	(2)	—		—		—		—	
19–24	117	28	(24)	4	(3)	—		22	(19)	2	(2)	7	(6)	1[c]	(1)	1	(1)
25–30	141	69	(49)	17	(12)	—		25	(18)	3	(2)	19	(13)	—		4	(3)
≥31	77	50	(65)	13	(17)	—		12	(16)	1	(1)	10	(13)	1[d]	(1)	—	
Total	433	151	(35)	34	(8)	1	(0.2)	61	(14)	6	(1)	36	(8)	2	(0.5)	5	(1)

[a] —=no tumors of the indicated type present in the respective age interval.
[b] Angiosarcoma.
[c] Angioma.
[d] Fibrosarcoma.

TABLE 3.—*Relative incidence of ovarian tumors in Han:NMRI mice*

Animal group	No. of tumors	Tumor incidence[a]															
		Tubular adenoma		Tubular adenocarcinoma			Teratoma		Granulosa cell tumor			Luteoma		Sertoli cell tumor		Mesenchymal tumor	
		No.	(%)	No.	(%)	%[b]	No.	(%)	No.	(%)	%[b]	No.	(%)	No.	(%)	No.	(%)
Ad libitum feeding																	
Control	103	57	(55)	—			1	(1)	26	(25)	4	5	(5)	14	(14)	—	
Fat	89	48	(54)	1	(1)	100	—		21	(24)	—	6	(6)	13	(15)	—	
Lean	115	63	(55)	6	(5)	67	—		22	(19)	18	4	(3)	19	(17)	1[c]	(1)
Total	307	168	(55)	7	(2)	71	1	(0.3)	69	(22.4)	7	15	(5)	46	(15)	1	(0.3)
Restricted feeding																	
Control	139	71	(51)	12	(9)	33	—		36	(26)	—	5	(3)	14	(10)	1[d]	(1)
Fat	99	61	(62)	10	(10)	50	—		12	(12)	—	1	(1)	14	(14)	1[e]	(1)
Lean	118	78	(66)	13	(11)	62	1	(1)	13	(11)	15	—		13	(11)	—	
Total	356	210	(59)	35	(10)	49	1	(0.3)	61	(17.1)	7	6	(2)	41	(11)	2	(0.6)

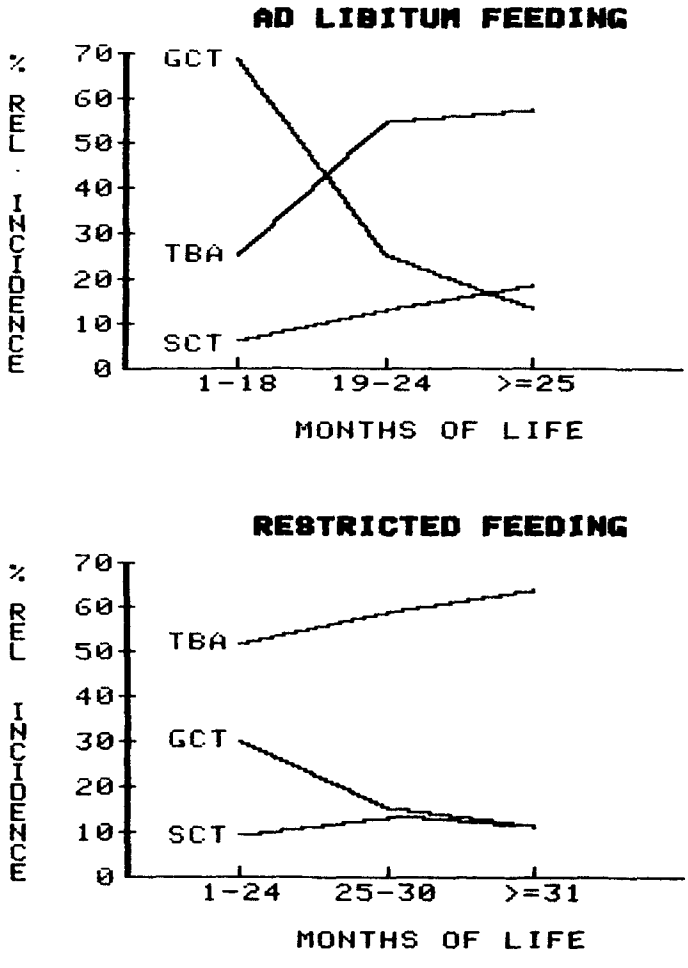
[a] —=no tumors of the indicated type present in the respective animal group.
[b] Percentage of metastasized tumors.
[c] Angiosarcoma.
[d] Angioma.
[e] Fibrosarcoma.

TABLE 4.—*Relative incidence of tubular adenoma in relation to growth pattern and size*

Tumor	No. of tumors	Tumor type						Tumor size, mm					
		Papillary		Solid		Cystic		≤2		>2–≤5		>5	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Ad libitum feeding													
Tubular adenoma	168	70	(41)	95	(57)	3	(2)	68	(40)	77	(46)	23	(14)
Ovarian tumors	307	70	(23)	95	(31)	3	(1)	68	(22)	77	(25)	23	(8)
Restricted feeding													
Tubular adenoma	210	67	(32)	135	(64)	8	(4)	83	(39)	94	(45)	33	(16)
Ovarian tumors	356	67	(19)	135	(38)	8	(2)	83	(23)	94	(26)	33	(9)

of lipid-laden cells that were in most cases luteinized cells. However, many other stromal cells also possessed fine, cytoplasmic lipid granules.

Irrespective of the feeding schedule, the absolute and relative incidence of tubular adenomas increased with age (table 2 and text-fig. 2). At each time point the percentage of animals bearing tubular adenomas in the food-restricted group was lower and reached higher total values only after 31 months. The total number of adenomas (table 3) was higher in food-restricted animals, although this was statistically significant only in the control group ($P \leq .05$). No statistically significant differences in tumor incidence existed between control mice and the sublines within feeding groups. Table 4 shows that the ad-libitum-fed mice with a shorter life-span developed more papillary tumors but fewer solid tumors ($P \leq .05$) compared to tumor development in food-restricted mice, because tumor size and growth pattern depended on the age of the tumor-bearing animals; i.e., with increasing age, the neoplasms enlarged and changed from papillary to solid and cystic patterns (table 5A).



TEXT-FIGURE 2.—Relative incidence of the most prevalent ovarian tumors in Han:NMRI mice in relation to age.

Tubular Adenocarcinomas

These tumors measured 0.5-3 cm in diameter and were observed in 41 animals. Bilateral tubular adenocarcinomas were found only in one instance in a food-restricted 27-month-old lean mouse. Tubular adenocarcinomas were characterized by a few or numerous cysts (fig. 5) and by the presence of blood and thrombi. In some cases remnants of tubular adenomas were found. Sparse stromal cells consisted of luteinized cells. Size and thickness of the cysts varied considerably even within a single tumor, ranging from one to six layers of epithelial cells with round or oval nuclei (fig. 6). Highly attenuated and elongated cells were present regularly, often forming the outer lining of cysts or constituting single- or double-layered cysts themselves. The smallest configuration found within the tubular adenocarcinomas were rosettes: 5-20 cells with round basal nuclei and eosinophilic cytoplasm arranged in a circle, frequently forming a central lumen and an outer border of a few attenuated cells (fig. 7). Mitotic activity was extremely rare. Acid mucopolysaccharides were seen mainly in residual areas of tubular adenomas, and lipids were limited to the few luteinized stromal cells. Metastases in lungs developed in 50% of the afflicted animals. Grossly, they appeared as tiny red spots up to 1 mm in diameter, diffusely distributed over the lung. Histologic examination revealed blood-filled cysts with a network of flat, elongated cells. In addition, the above-described rosette formations were found in arteries, capillaries, and in the network of the blood-filled cysts.

Tubular adenocarcinomas were relatively rare, were never seen in the ad-libitum-fed control group, and occurred more often ($P \leq .01$) in food-restricted animals (8%) than in mice with constant access to feed (2%). This finding was partly related to survival time, since these neoplasms were found for the first time in animals aged 24 months, and on the average between the 27th and 29th month (table 5A).

Teratomas

Two mice at the ages of 6 and 20 months developed teratomas 3 and 0.5 cm in diameter, respectively. The larger tumor caused the death of the animal; it consisted of keratinizing squamous epithelium, glandular and connective tissues, as well as focal areas of cartilage and bone. The smaller teratoma appeared in conjunction with a granulosa cell tumor and showed extensive keratinizing squamous epithelium together with tissue that resembled intestinal mucosa.

Granulosa Cell Tumors

From a total of 130 granulosa cell tumors, 28 could be found only by microscopic examination. The largest tumors of this type reached a diameter of 4 cm, mainly due to high accumulations of blood. Three animals (2 of the fat mice and 1 of the control group fed ad libitum) developed bilateral tumors (table 6) at the age

TABLE 5A.—*Average age of Han:NMRI mice at the occurrence of ovarian tumors*

Animal group	Average life-span, mo ± SD	Age of tumor occurrence, mo ± SD[a]						
		Tubular adenoma						Tubular adenocarcinoma
		Papillary	Solid	Cystic	≤2 mm	>2–≤5 mm	>5 mm	
Ad libitum feeding								
Control	22.0±5.0	24.2±3.3	24.9±3.7	23[b]	24.9±3.8	24.3±3.3	24.8±3.9	—
Fat	19.8±5.1	23.6±2.9	25.0±2.8	—	23.7±3.0	24.1±2.5	26.3±1.9	30[b]
Lean	19.4±5.8	22.2±2.8	24.4±3.7	27.5±3.5	23.0±4.3	23.6±3.0	25.8±4.0	27.2±3.9
Total	20.4±5.3	23.3±3.0	24.7±3.5	26.0±3.6	23.9±3.7	23.7±2.9	26.0±3.4	27.6±3.7
Restricted feeding								
Control	26.0±6.7	25.9±5.9	30.5±4.0	30.0±4.0	28.5±5.5	29.2±4.9	31.4±3.3	29.3±2.7
Fat	23.5±6.6	26.5±5.2	28.6±4.0	30.5±6.4	26.4±4.8	29.1±3.6	31.4±3.3	29.5±3.7
Lean	21.5±7.4	27.9±4.5	27.6±4.1	—	26.1±4.7	28.0±3.4	30.1±4.4	29.2±4.8
Total	23.7±6.9	26.9±5.1	28.9±4.2	30.2±5.0	26.9±5.0	28.5±4.1	30.8±3.7	29.3±3.8

[a] —=no tumors of the indicated type present in the respective animal group.
[b] 1 tumor.

TABLE 5B.—*Average age of Han:NMRI mice at the occurrence of ovarian tumors*

Animal group	Average life-span, mo ± SD	Age of tumor occurrence, mo ± SD[a]					
		Teratoma	Granulosa cell tumor		Luteoma	Sertoli cell tumor	Mesenchymal tumor
			Microscopic	Macroscopic			
Ad libitum feeding							
Control	22.0±5.0	20[b]	21.0±4.1	22.6±3.5	26.4±2.2	25.5±2.6	—
Fat	19.8±5.1	—	22.5±3.0	21.5±4.1	25.3±3.2	24.4±2.9	—
Lean	19.4±5.8	—	22.0±2.8	21.2±5.8	24.3±2.1	25.1±4.3	22[b,c]
Total	20.4±5.3	20	21.9±3.2	21.8±4.6	25.4±2.6	25.0±3.5	22
Restricted feeding							
Control	26.0±6.7	—	26.6±5.6	26.4±4.6	28.4±3.3	28.3±5.3	33[b,d]
Fat	23.5±6.6	—	21.6±1.8	29.1±2.5	22[b]	29.0±3.7	19[b,e]
Lean	21.5±7.4	6[b]	22[b]	25.1±6.2	—	28.0±4.7	—
Total	23.7±6.9	6	24.3±4.8	26.5±4.9	27.3±3.9	28.4±4.5	26±7.0

[a] —=no tumors of the indicated type present in the respective animal group.
[b] 1 tumor.
[c] Angiosarcoma.
[d] Angioma.
[e] Fibrosarcoma.

TABLE 6.—*Incidence of bilateral ovarian tumors in Han:NMRI mice*

Tumor type	Combined tumors		Animals with bilateral tumors of the indicated type[b]	
	$n1/n2$[a]	Percent of bilateral tumors	$n3/n4$[c]	Percent of mice bearing bilateral tumors
Tubular adenoma	378/275	73	279/99	35
Tubular adenocarcinoma	42/31	74	41/1	2
Granulosa cell tumor	130/49	38	127/3	2
Luteoma	21/8	38	21/—	—
Sertoli cell tumor	87/66	76	75/12	16

[a] $n1/n2$=No. of tumors of the indicated type/No. of tumors occurring in combination with any tumor in the contralateral ovary.
[b] —=no bilateral tumors of the indicated type present.
[c] $n3/n4$=No. of animals bearing the indicated type of tumor/No. of animals bearing bilateral tumors of the indicated type.

of 19 months. The histologic picture varied depending on the amount of blood (lacking in the small tumors), connective tissue, and fat.

Larger granulosa cell tumors were characterized by formations similar to those found in the normal developing follicle: In the midst of diffusely arranged granulosa cells, there were empty areas or areas containing a few cells, often heavily laden with fat (fig. 8). Generally, granulosa cell tumors showed a relatively homogeneous cell type with round nuclei containing

coarsely clumped chromatin and scanty cytoplasm with no distinct borders. In some instances, cells were grouped together by delicate strands of connective tissue giving the tumor a trabecular or insular pattern. The macroscopically visible neoplasms frequently revealed necrotic areas with secondary mineralization, cholestorol clefts, and thrombi. Almost regularly lipofuscin pigment (positive PAS reaction) was present. Of 30 granulosa cell tumors, 70% possessed a moderate and 7% possessed a high amount of lipids, located in granulosa cells, necrotic areas, and luteinized cells in the form of coarse granules. Approximately 50% of all granulosa cell tumors showed moderate to high mitotic activity. Metastases, however, were rare, mainly seen in the lungs and regional lymph nodes. In one instance we found severe metastases also in the spleen and liver.

In general, the absolute incidence of granulosa cell tumors was 15% (table 2). Control mice and sublines showed similar frequencies under ad libitum conditions. With food restriction, however, there was an increase of these neoplasms in the control group and a reduction in the sublines, resulting in a significant difference ($P \leq .01$) between the sublines and the control group. Granulosa cell tumors developed relatively early; the absolute incidence increased up to age 24 months and reached a plateau thereafter (table 2). Compared to other ovarian neoplasms, the percentage of granulosa cell tumors steadily declined with age (text-fig. 2). On the average the tumors occurred in the ad-libitum-fed mice at 22 months of age and at 26 months in food-restricted animals (table 5B). Only in food-restricted mice did the sublines reveal an age difference between animals bearing small and large tumors.

Luteomas

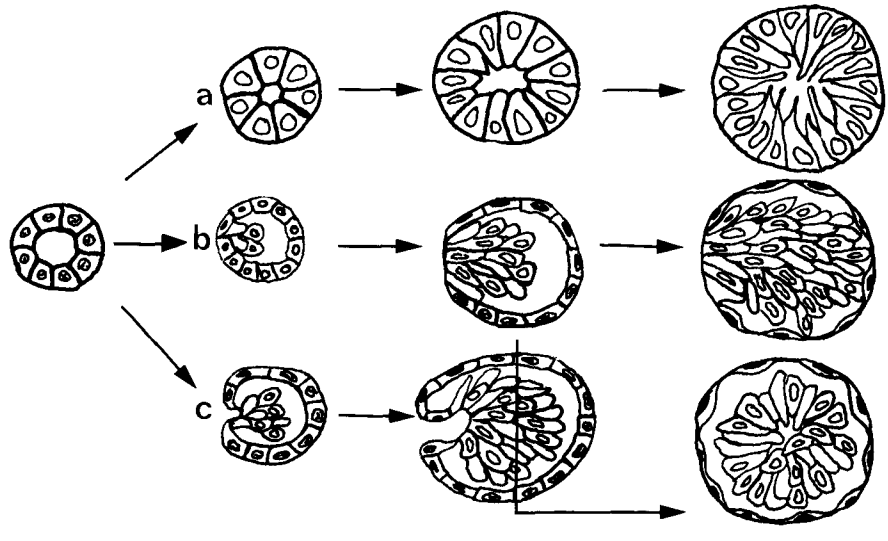
Luteomas were rare and were found solely in unilateral position in Han:NMRI mice varying in size between 2 and 10 mm. Microscopically, these tumors were characterized by closely packed large round or pleomorphic cells with abundant eosinophilic cytoplasm, regularly containing lipid-laden vacuoles. Pale, centrally located nuclei with single nucleoli occasionally revealed hyaline inclusions. Mitotic activity was low.

The highest absolute incidence (3%) of luteomas was found in the ad-libitum-fed mice. In control mice food restriction had no influence on luteoma frequency, whereas in the sublines only 1 animal from the fat group developed a luteoma.

Sertoli Cell Tumors

With a total of 87, Sertoli cell tumors were the third most often diagnosed ovarian neoplasms in this study; 16% arose bilaterally. Besides a few microscopic tumors, Sertoli cell tumors usually reached sizes between 0.5 and 2 cm in diameter. The morphologic picture depended on the extent of differentiation, which varied even within a single tumor. Sertoli cell tumors were characterized by closely packed seminiferous-like tubules, clearly separated from each other by fibrovascular stroma. Well-differentiated round tubules were formed by one to three layers of pyramidal epithelium with basally oriented, pale, oval nuclei and abundant, faintly eosinophilic cytoplasm reaching out into a lumen (fig. 9). In less well-differentiated areas, the tubules were elongated or distended and filled with polymorphic cells.

As shown in figure 10, 3 different types of tubules were found, suggesting different types of development (text-fig. 3). In some, the elongated cells were situated directly on a basement membrane giving the impression that they had continuously developed from cuboidal cells by hypertrophy and hyperplasia. In other tubules, Sertoli-like cells were separated from the surrounding basement membrane by a layer of flat or cuboidal cells that stained positive for acid mucopolysaccharides



TEXT-FIGURE 3.—Sertoli cell tumor development from tubular elements: a) diffuse hypertrophy and proliferation; b) and c) focal hypertrophy and proliferation without (b) or with (c) invagination of the surrounding basement membrane.

similar to tubular adenomas. These tubules seemed to have been formed by invaginations from which cells proliferated, distending the tubules. The pyramidal or pleomorphic Sertoli-like cells did not stain positive with Alcian blue. A third formation was observed suggestive of a focal hyperplasia of the tubular epithelium, since a central stalk of invaginated basement membrane and stromal tissue was missing. Mitotic figures were rare, and 25% of the Sertoli cell tumors exhibited mineralizations reminiscent of psammoma bodies. We observed 4 animals with exceptionally undifferentiated Sertoli cell tumors, classified malignant for histologic reasons. These tumors were 4 cm in diameter, very firm, and greyish-white in color. Stromal components were sparse, and 2 of the neoplasms possessed scattered necrotic areas. Only occasionally could we see distended tubular structures as described above (fig. 11). The cells were pleomorphic or elongated and densely packed with a reduced amount of cytoplasm (fig. 12). Mitotic figures were frequent.

Of 25 tumors investigated, 40% possessed no lipids, 32% revealed little, and 28% revealed a moderate content of fat. These lipids were located in a few luteinized cells, in the cytoplasm of the Sertoli cells, and in the lumina of the tubules.

The absolute and relative incidence of Sertoli cell tumors increased steadily with ad libitum feeding. In food-restricted animals these tumors arose later in life and their frequency did not increase further beyond the 30th month of age (table 2 and text-fig. 2). However, no statistically significant differences in incidence could be noted between animal groups and feeding schemes.

Mesenchymal Tumors

We found 3 mesenchymal tumors: 1 angioma, 1 angiosarcoma, and 1 fibrosarcoma. The angioma consisted of large blood-filled cavities lined by mostly elongated endothelial cells. A single thrombus was noted in an angioma found in conjunction with a tubular adenoma in the same ovary.

The angiosarcoma showed diffusely arranged pleomorphic endothelial cells with erythrocytes and thrombi scattered among them.

The fibrosarcoma consisted of closely packed elongated cells arranged in interwoven bundles and whorls, enclosing islands of ovarian stromal cells. The tumor possessed a moderate amount of collagen fibers.

Metastatic Tumors

A single 25-month-old, ad-libitum-fed mouse of the fat group was found with metastases from a mammary adenocarcinoma type B (*9*) in one ovary with a tubular adenoma. In various types of generalized hematopoietic tumors (*10*), the following incidences were seen in the ovaries: Lymphosarcoma, 132 (33%); reticulum cell sarcoma type B, 61 (13%); reticulum cell sarcoma type A, 33 (18%); and nonclassifiable hematopoietic tumors, 41 (17%).

Multiple Tumors

Multiple tumors in one ovary were only taken into account when a sufficient amount of tissue was present and a distinct border existed between the neoplastic regions. Forty-five ovaries showed 2, one showed 3, and another one showed 4 different neoplastic areas in the single organ. Only 9 animals bearing these tumors had no neoplasm in the contralateral ovary. The most frequent combination (23 cases) was a tubular adenoma in conjunction with a Sertoli cell tumor followed by 9 cases of a tubular adenoma with a granulosa cell tumor.

Bilateral Ovarian Tumors

Table 6 shows the general incidence of bilaterally occurring ovarian tumors and the incidence of animals bearing neoplasms of the same histologic type in both ovaries. Over 70% of the Sertoli cell tumors, tubular adenomas, and adenocarcinomas were associated with a neoplasm in the contralateral ovary, whereas only half as many were observed in the case of granulosa cell tumors and luteomas.

Hormone Activity

No specific relationship was found between the type of ovarian tumor or tumor-free state and the vaginal cycle, hyperplasia of the mammary gland, and the endometrium. Eight mice, however, possessed salivary glands exhibiting a male morphologic pattern with tubules containing large quantities of intracytoplasmic secretory granules. Besides tubular adenomas, 5 of the animals had Sertoli cell tumors, 1 had a granulosa cell tumor, and 1 had a luteoma. An age-corrected comparison of the ad libitum group (animals surviving beyond 18 mo) showed that pituitary gland tumors were diagnosed more often ($P \leq .01$) in mice with ovarian neoplasms. However, this finding was not confirmed in the food-restricted group, and therefore it is not possible to establish a definite relationship between ovarian and pituitary tumors.

DISCUSSION

Carcinogenicity studies were conducted in mice at least up to the age of 18 months (*11, 12*). At this age the incidence of spontaneous tumors rises, and therefore knowledge on strain-specific frequency as well as type of spontaneous neoplasm is of great value.

A high incidence of ovarian neoplasms similar to that in the present study is reported for the inbred mouse strains C3HeB (*1*), RIII/J, and C3HeB/Fe (*13*) developing mainly tubular adenomas and granulosa cell tumors. Consecutive breeding experiments proved a strong genetic influence on the frequency of ovarian tumors (*14*). Various experiments that involved the depletion of the ovary of estrogen-secreting follicles were followed by the development of tubular adenomas and sex cord stromal tumors induced by prolonged

stimulation by increased amounts of gonadotrophic hormones (*15–22*). Spontaneous ovarian tumors in normal untreated mice are therefore only due to arise after ovarian activity ceases at an age of 12–16 months followed by an increase in gonadotrophins (*23*). In our study not only the number of afflicted animals increased with age but also the number of involved ovaries, i.e., bilaterality. The most prevalent tumor in our mice was tubular adenoma or mesothelioma (*3*), histogenetically derived from downgrowths of the germinal epithelium. The morphology of this neoplasm varied considerably due to the extent of tubular growth and differentiation and the number of stromal cells. Therefore, these tumors also have been referred to as "complex tubular adenomas" (*20, 24*). It was reported that granulosa cell tumors developed from transplants of these complex neoplasms (*20*). The Alcian blue stain allowed a distinction between highly complex tumors and granulosa cell tumors with trabecular or insular formations. The low mitotic index suggested slow growth, beginning as a small papillary process according to the infoldings of the downgrowing epithelium. With increasing age, the growth of tubular and stromal elements lead to large and solid neoplasms. Rare cystic forms are probably comparable to described serous cystadenomas (*3*), although ciliated cells were not present. The origin of the stromal cells in tubular adenomas is yet unclear. They are probably derived from thecal or granulosa cells and from the tubular epithelium (*22, 24*). The tubular epithelium seemed to develop into insular structures and elongated cells similar to those found in the tubules of the Sertoli cell tumors. Tubular adenomas were found most often in conjunction with a Sertoli cell tumor in the same ovary, and both showed similar incidences of bilaterality. In view of the above described stromal patterns, we have problems with classifying tubular adenomas as epithelial tumors (*3*). Other investigators (*25*) have therefore classified them along with the sex cord stromal tumors (Notman J: Personal communiction). From our observations they seem to range in an intermediate position from which pure epithelial forms, such as cystic tubular adenoma and carcinomas or variants of the sex cord stromal tumors, can develop.

Food restriction significantly increases the life-span of laboratory rats and mice (*5, 6*) and generally is also associated with a significant reduction of tumors (*26*). However, this finding did not prove to be true for most of the present ovarian neoplasms, even though the secretion of gonadotropins is impaired under limited feeding conditions (*27*). A possible early ovarian atrophy caused by restricted feed intake (*28, 29*) could be responsible for a premature deficiency of estrogen-secreting follicles. Since tubular adenomas seldom caused the death of the animal, they were likely to remain latent over a long period, mimicking a postponed occurrence when found at death in food-restricted animals.

We did not find any reports on tumors comparable to the tubular adenocarcinomas of the Han:NMRI mice. Diagnostic criteria for these tumors developing from tubular adenomas were drawn from residual structures found in the carcinomas and from tubular adenomas with evidence of focal transformation. The number of stromal cells was reduced, leaving spaces between tubules into which blood emerged for unknown reasons. In contrast to the histologic benign features (rare mitotic figures, lack of invasiveness), 50% of tubular adenocarcinomas developed metastases; therefore, we classified them as malignant neoplasms. The rosettes found in the tumors and lungs showed great similarity to anovular follicles, derived from the surface epithelium in aged ovaries (*30*). It cannot be decided by light microscopy whether the highly attenuated cells lining the blood-filled cysts in the lungs originate from those lining the cysts in the tumor or whether they represent endothelial cells from arteries and capillaries congested by tiny rosettes.

Because littermates were distributed evenly between the 2 feeding groups, the higher incidence of adenocarcinomas found in food-restricted animals was mainly related to their greater life-span leading to an enhanced probability of malignant transformation. A genetically determined difference of susceptibility could be responsible for the lean subline, with the shortest average life-span, developing most of the tubular carcinomas.

Teratomas are generally, as in Han:NMRI mice, rare neoplasms that develop early in life and originate from parthenogenetically activated oocytes (*31*). Granulosa cell tumors frequently have been described in mice (*2, 32*), and as in the present study, their incidence is the second highest after that of tubular adenomas (*3*). Thecomas, often grouped with granulosa cell tumors, were not encountered in their pure form. Granulosa cell tumors can develop from persisting follicular cells, thecal tissue, or anovular follicles of the germinal epithelium (*20, 24, 33*). The high mitotic rate and necrosis indicate rapid growth and are histologic criteria for malignancy. Metastases, however, were observed only in 7 cases. These findings agree with reports on the biological behavior of granulosa cell tumors found in mice and humans (*34*). In contrast to the suppression of induced granulosa cell tumors by inanition (*35*), food restriction in the present study did not significantly affect the incidence statistically.

Luteomas, not considered to be true neoplasms in humans (*36*), were rare tumors in the Han:NMRI mice and found only in unilateral position relatively late in life. These findings differ to those of others (*3*), possibly due to strain-specific variations. Ultrastructural investigations (*37*) showed that luteomas develop from granulosa cell tumors; i.e., they represent a spectrum of differentiation of the same cell type. The biological behavior of luteomas in the present study confirmed these findings, since they occurred significantly later than granulosa cell tumors; both luteomas and granulosa cell tumors showed a comparable incidence of bilaterality and tended to accumulate lipids, and the influence of food restriction on the frequency of their occurrence was similar.

Reports on induced or spontaneous Sertoli cell tumors in mice are very rare. It has been suggested that Sertoli cell tumors are derived from the rete ovarii, epoophoron, or tubular downgrowths of the germinal epithelium (*24*). We support these suggestions by observations of the present study described in the discussion of tubular adenomas. Furthermore, Sertoli cell tumors occurred later in life than most of the tubular adenomas. Reports on malignant Sertoli cell tumors of the ovary were found only for humans (*38*).

Immunohistologic studies in humans (*39*) revealed that sex cord stromal tumors are pluripotent, secreting testosterone as well as estradiol and progesterone, affecting corresponding target organs. Further evidence for hormonal activity can be drawn from the presence of lipids that are considered to be precursors of steroids (*39*). It was difficult to assess the effects on endometrium and mammary gland in Han:NMRI mice because of a simultaneous influence from pituitary gland neoplasms that secreted prolactin (*40, 41*). Possible androgenic effects were found by a male morphology of the submandibular salivary glands (*42*). Since pituitary gland tumors were readily induced by exogenous estrogens (*40*), ovarian neoplasms secreting estrogen could have been responsible for an endogenous induction of pituitary gland tumors (*33, 43*). The present observations, however, without the application of specific techniques, do not give sufficient evidence for hormonal activity.

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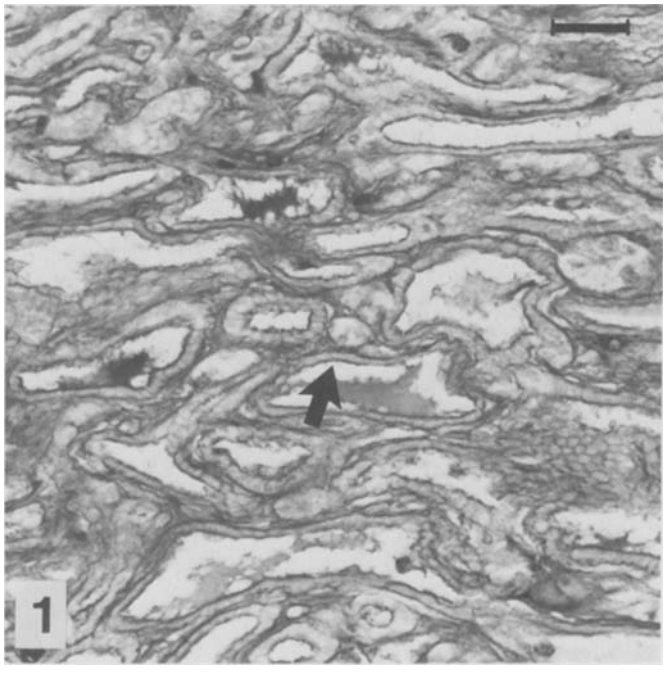


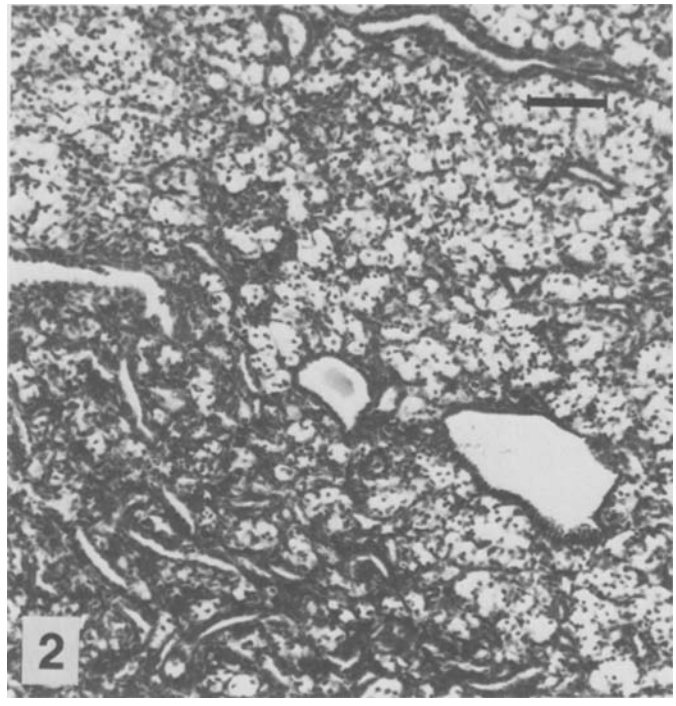
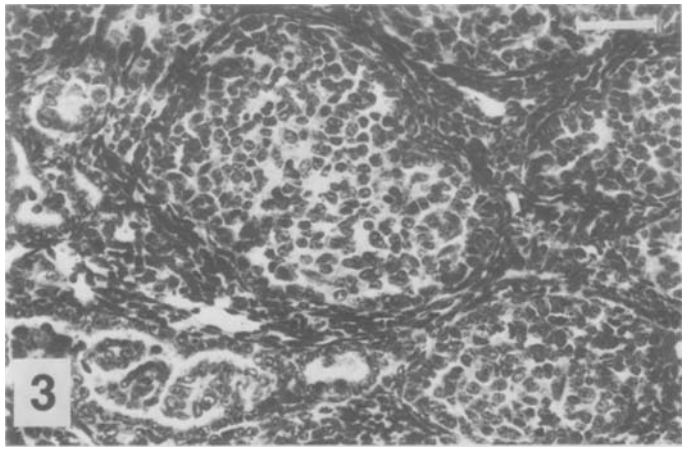
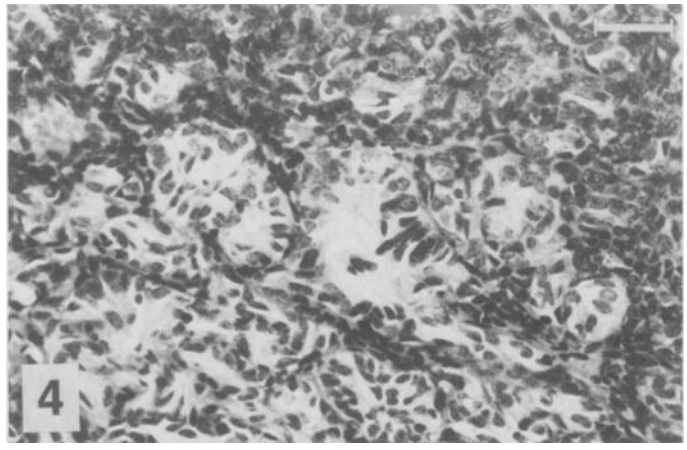
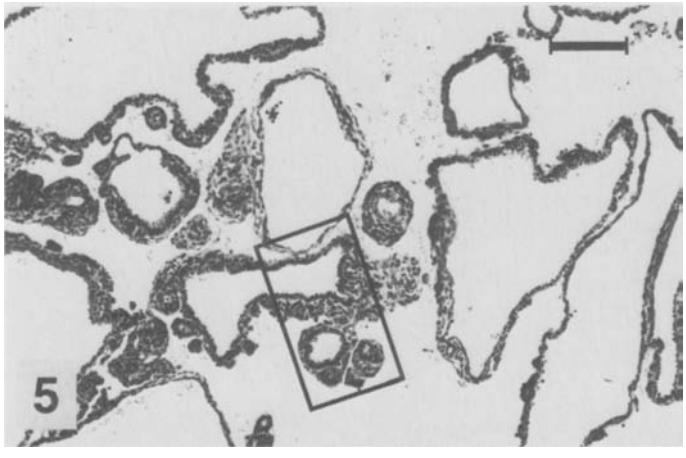
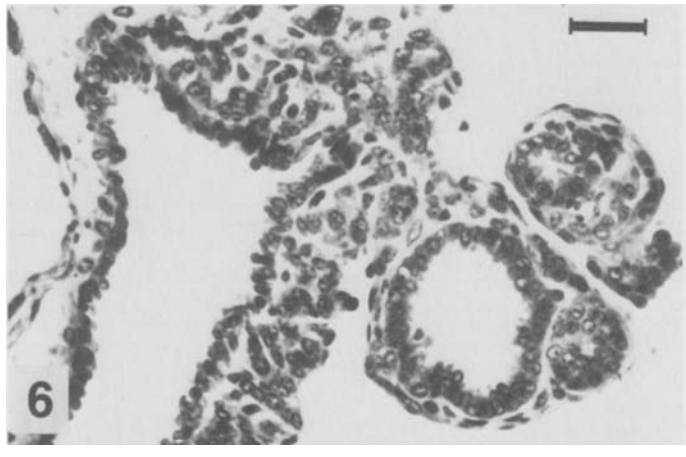


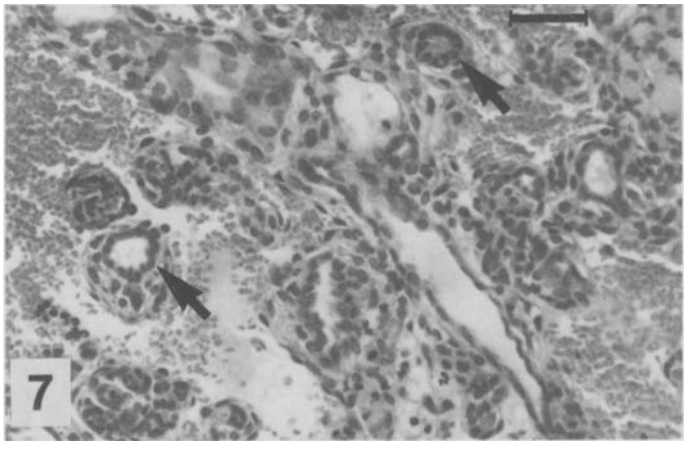
FIGURE 1.—Cystic tubular adenoma. Dilated tubules lined by acid mucopolysaccharides (*arrow*). Alcian blue, PAS. *Bar*=41 μm.
FIGURE 2.—Tubular adenoma showing prominent luteinization. H & E. *Bar*=91 μm.











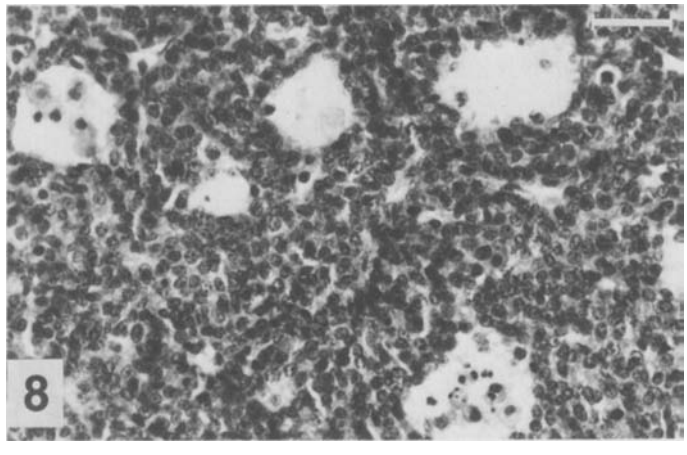
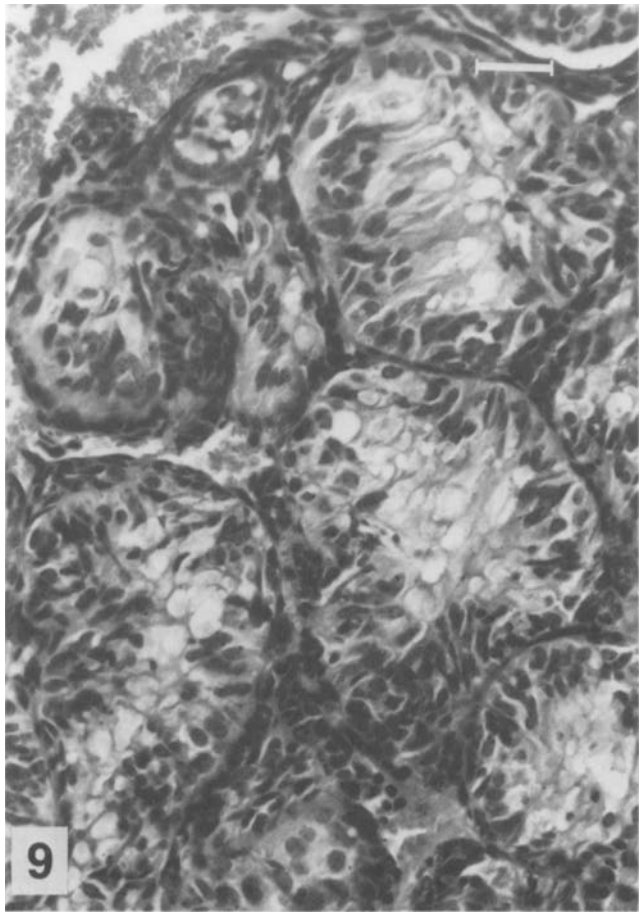


FIGURE 3.–Tubular adenoma revealing insular structures filled with round cells. H & E. *Bar*=52 μm.
FIGURE 4.–Tubular adenoma with Sertoli cell-like tubular differentiation. H & E. *Bar*=47 μm.
FIGURE 5.–Tubular adenocarcinoma with variably sized cysts. H & E. *Bar*=122 μm.
FIGURE 6.–Tubular adenocarcinoma. Higher magnification of area indicated in fig. 6. H & E. *Bar*=35 μm.
FIGURE 7.–Tubular adenocarcinoma showing rosette formation (*arrows*). H & E. *Bar*=52 μm.
FIGURE 8.–Granulosa cell tumor with multiple follicular structures. H & E. *Bar*=51 μm.



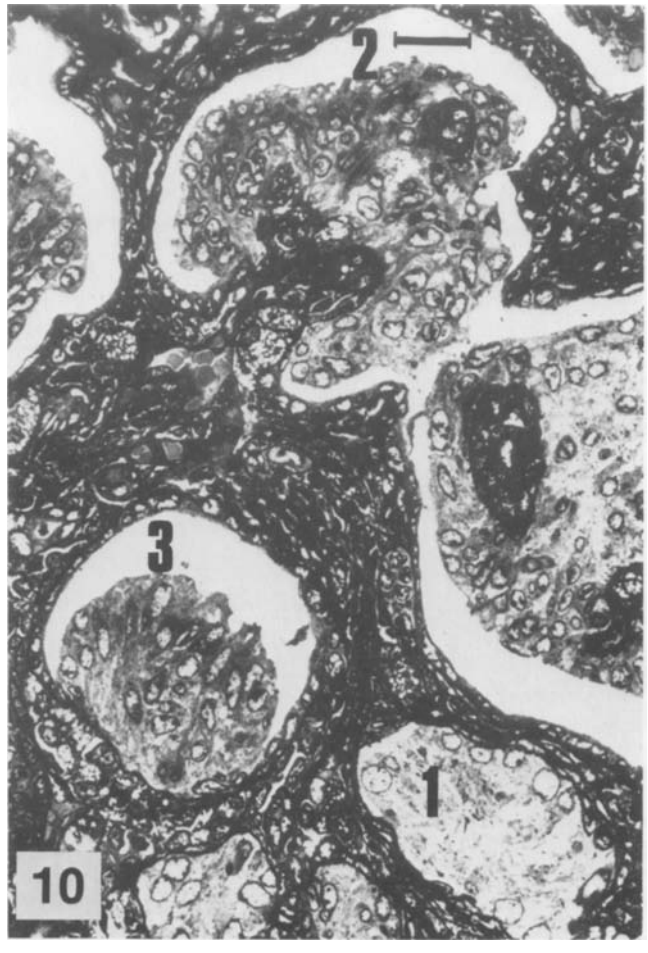
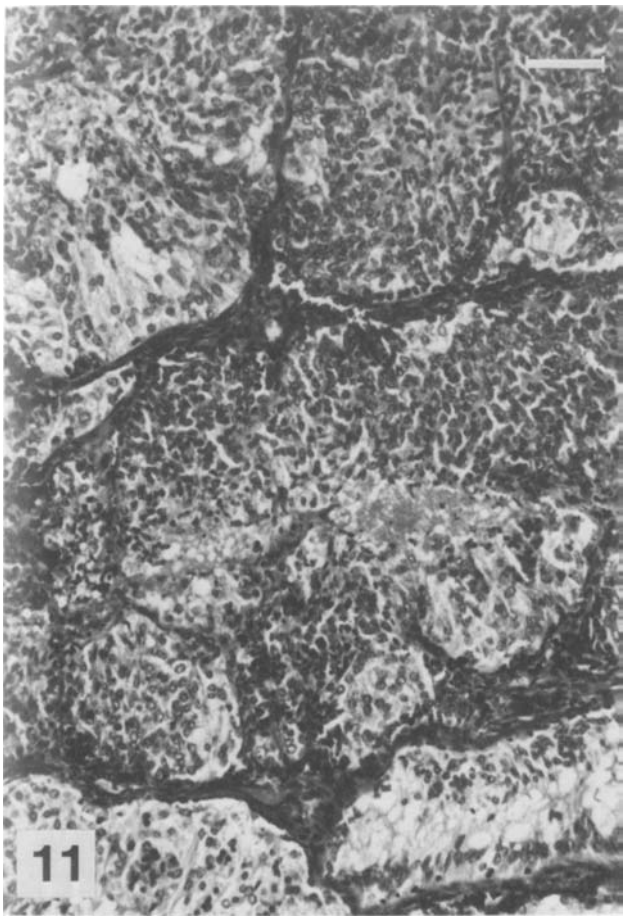


FIGURE 9.—Sertoli cell tumor. Well-differentiated seminiferous-like tubules. H & E. *Bar*=39 μm.

FIGURE 10.—Sertoli cell tumor. Diffuse hypertrophy (1), focal hyperplasia with invagination (2) and without invagination (3). Methenamine silver. *Bar*=26 μm.



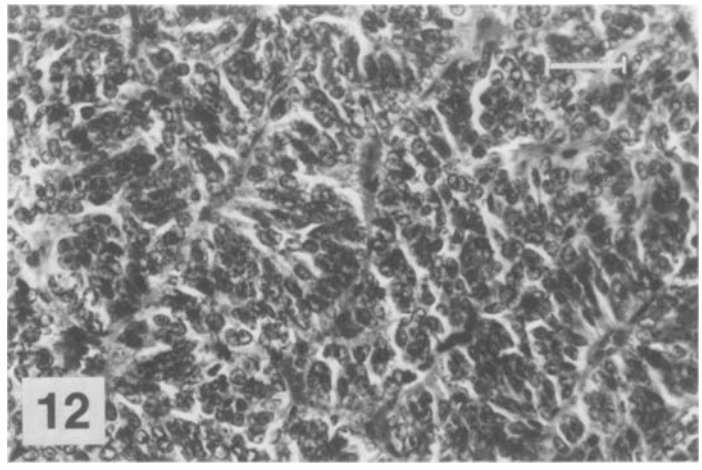


FIGURE 11.—Malignant Sertoli cell tumor. Irregular, distended tubules filled with pleomorphic cells. H & E. *Bar*=77 μm.
FIGURE 12.—Malignant Sertoli cell tumor composed of elongated cells with sparse cytoplasm. H & E. *Bar*=50 μm.