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Morphological and Biological Characteristics of X-Ray Induced Transplantable Ovarian Tumors*

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The observation that total body irradiation in female mice is frequently if not inevitably followed by the development of ovarian tumor (8) has been confirmed and extended by several investigators (13, 15, 17, 19). The induction period of the tumors is very long. While regenerative changes begin after 4 to 6 months (5), tumor-like growth does not appear before about 7 months; large tumors are infrequent and metastases are rare.

Six years ago experiments were begun to ascertain by transplantation studies the neoplastic character of the hyperplastic nodules that occur following irradiation and to secure pure lines of neoplastic cells of different sorts. Transplantations are suitable to study the morphogenic potencies of the ovarian tumor cells of different types, their interrelationship and the secondary changes they produce. Thus, 16 ovarian tumor strains have been studied, of which a luteoma has already been described *in extenso* (9). The successful transplantation of granulosa tumors and their striking secondary changes have been recorded in preliminary reports (6).

It is the purpose of this paper to survey the characteristics of the 16 strains of transplantable ovarian tumors and to find out what they contribute toward the understanding of ovarian tumor problems. Fifteen attempts to transplant x-ray induced tumors were unsuccessful in the first passage. Most of these were done under unfavorable conditions and in a small number of mice, and will not be reviewed.

Tumors arising in irradiated ovaries are complex. Most transplanted neoplasms are of the granulosa cell type; a smaller number are luteomas and tubular adenomas. All of these types are frequently present in the same x-rayed ovary, and the type of tumor particles used for transplantation, the growth rate of the different elements, and probably other factors residing in host tissue and donor determine which and how many types of transplantable neoplasms are isolated from a single irradiated ovary. Less commonly encountered neoplasms are hemangiomas and endotheliomas, (two of which resembled chorio-epitheliomas) and sarcomas. Only two types of cells thus far transplanted were found to be associated with hormone production: granulosa cell tumors cause morphological changes indicative of the production of estrogens, and luteomas of progestins.

Increase of blood volume goes invariably with granulosa tumor (11, 20) and for the hypothetical substance responsible for this, the name plethorin will be used.

MATERIALS AND METHODS

The ovarian tumors were cut up in Tyrode's solution and implanted through various routes, including the spleen, liver, and the anterior chamber of the eye. Since the tumors were induced in F_1 (Ak/Rf) hybrids, the transfers were made routinely in young (2 to 3 months old) mice of this stock. The organs studied were fixed in Zenker-formol or formol solution, embedded in paraffin or cut in the frozen state, stained routinely by hematoxylin and eosin and occasionally with Mallory's

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anilin blue, Masson's trichrome, Sudan III, and other special procedures as will be named. The irradiation factors and induction of the tumors have been described (7).

Strain I: Granulosa Type

The animal from which this strain originated received 175 r when 40 days old, was painted with methylcholanthrene twice a week during 4 weeks (beginning one day after irradiation), and was killed 12 months after irradiation. The right ovary was replaced by a white-yellow-brown tumor, 18 × 13 mm. in the two greatest diameters. The left ovary appeared normal. A metastatic node was found in the liver.

The ovarian tumor was of the granulosa cell type. In places the cells formed amorphous masses, in places cords and follicle-like structures. The cytoplasm was scanty and the nuclei showed a coarse stippled chromatin pattern (Fig. 1). In some masses and follicles, the cells liquefied into an eosinophilic transparent substance. There were many edematous and necrotic areas.

In the first passage, the same structures were found in the tumors (Fig. 2); but the follicular formations of Graafian type were more abundant (Figs. 3 and 4).

In the second subpassage, deeply stained fibroblast-like cells appeared. In parts granulosa cells with elongated nuclei showed a bundle-like arrangement, some were detached, resembling plasma cells.

In the third subpassage antrum-like structures occurred in the solid masses (Fig. 4). They contained desquamated cells, or a transparent eosinophilic liquid, or red blood corpuscles. The tubules were lined with cuboidal cells (Fig. 5) some with papillary formations. In some tumors, the low cylindrical cells formed rows along the capillaries.

In the fourth subpassage, the structures remained the same. Areas of degeneration (Fig. 6), fibrosis and necrosis were present in almost all tumors. The histological picture did not change in the subsequent passages and the ability of the tumor cell to form follicles was retained after a year of subpassages.

Transplantation and biological behavior:—The results of all transplantations are summarized in Table 1. The per cent of takes after subcutaneous injection of normal animals fluctuated after an increase in the first and second subpassages; it dropped and rose again in the fifth subpassage. The males seemed to be slightly more susceptible (55 per cent) than the females (42 per cent). Intrasplenic injections were successful in all of 8 normal mice of both sexes.

The latent period of the tumors did not change in a constant direction. There was an increase until the fourth subpassage, followed by a decrease. The average was 30 days with a range of 7 to 85 days. The rate of growth did not seem to parallel the latent period.

The secondary changes produced by this strain as well as by other granulosa strains are of at least two kinds: a) estrogenic; b) a dilatation of sinusoidal system with blood volume rise, hereafter referred to as plethoric. These effects have been mentioned in previous papers (11, 20) and are being further investigated currently (14).

The estrogenic influence was manifest from the first subpassage on. Previous irradiation or gonadectomy of the host did not materially alter them.

All routes of introduction save the intrasplenic had these effects. The latter does not usually go with hormonal changes except when the tumor adheres to structures adjacent to the spleen. The congestive changes became manifest during the second subpassage and persisted until the final subpassage. They were independent of the site of injection and in the females a slightly greater tendency of manifesting this change was recorded.

Intraabdominal hemorrhages occurred in 12 cases, due mainly to the rupture of the exceedingly congested liver, spleen, or adrenal. The tumors exhibited malignant properties: subcutaneous tumors penetrated in the peritoneal cavity and metastasized to the liver (10 cases); lungs (15 cases); kidneys (1 case), and ovary (1 case). Only in two cases was the tumor the source of the hemorrhage. Hemorrhage into the thorax was also seen. In one case pleural hemorrhage from pulmonary metastasis was the cause of death.

In addition to these two main changes (estrogenic and plethoric) most animals had a high degree of relative anemia and splenomegaly with marked extramedullary hemopoiesis.

Strain II: Granulosa Type

The original animal was irradiated with 175 r when she was 40 days old. She was painted with methylcholanthrene for 5 weeks, twice weekly (7) and was killed 7 months after irradiation. The right ovary was replaced by a hemorrhagic yellow-grey tumor of 15 × 13 × 9 mm. The uterus was about 3 mm. thick. The tumor was composed of granulosa-sarcoma cells with coarse and fine stippled nuclei, forming masses, and follicular structures (Fig. 7). In places, the tumor cells regressed leaving a stroma rich in capillaries.

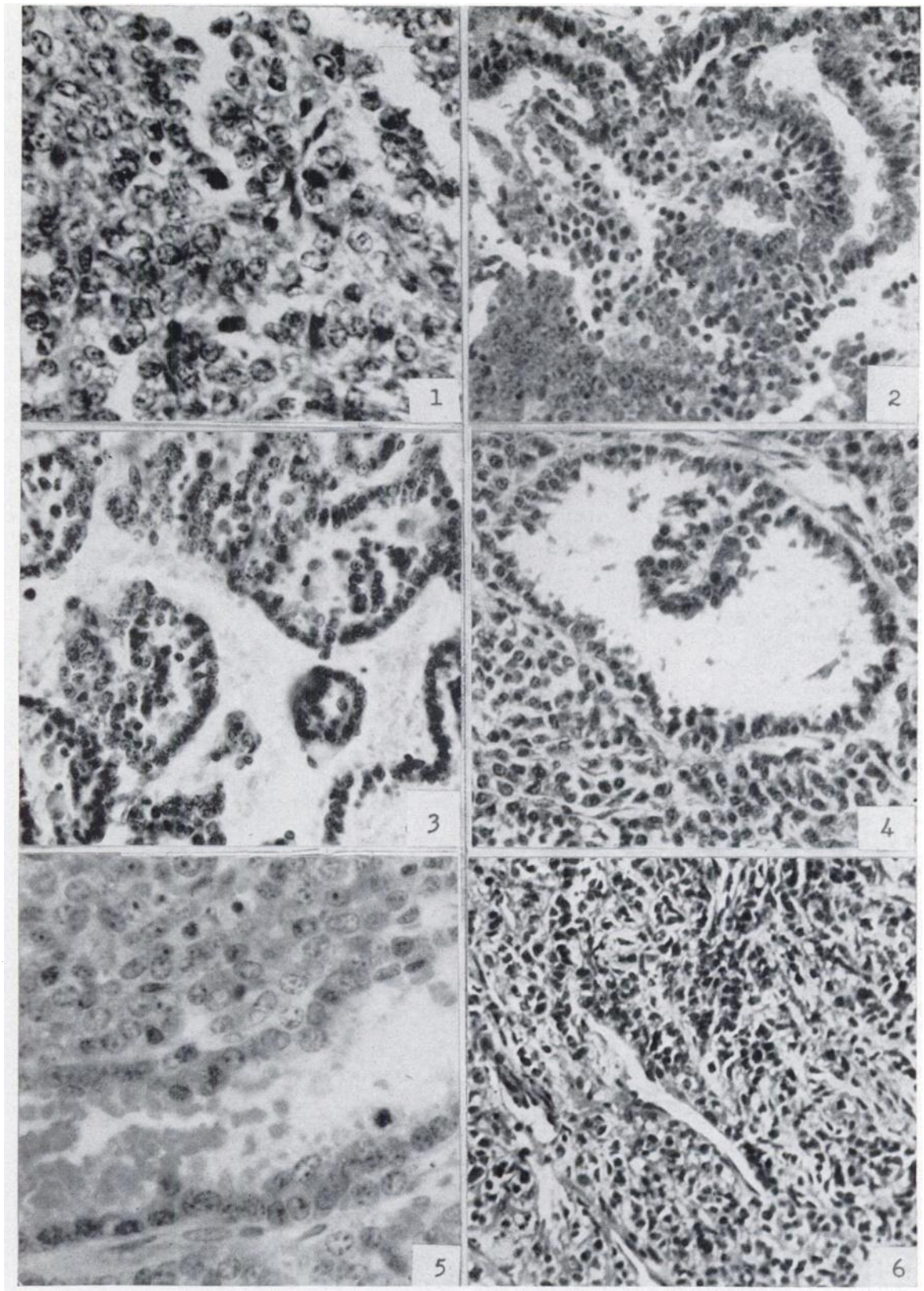
In the first passage the tumors were of pure granulosa cell type, forming solid masses and cords. Areas of fibrosis were present. In the second passage, sarcomatous forms appeared. There were abundant necrotic areas, many of which were calcified, some ossified. The microscopic picture did not change essentially in the course of subpassages, although in some tumors, cords in others masses or follicular arrangements were predominant. The sharpness of stippling of nuclei was variable; it was usually present (Figs. 8 and 9). A cavernous hemangioma developed in one of the recipients of the fourth subpassage.

Figures 10 to 12 show metastatic lesions in lung, kidney, and liver respectively. Sudanophile fat was abundant in granulosa cells in areas of degeneration and scant if any in "healthy" cells. Acid-fast granules (ceroid) appeared in granulosa cells in areas of degeneration (10).

Tubular necrosis in the kidney as illustrated in Figure 22 was a frequent finding.

Transplantation and biological behavior:—The average per cent of takes was 47 per cent in both sexes. No significant change occurred in the course of the subpassages.

The latent period of the tumors increased from an initial 17 days to 55 days in the fourth subpas-



FIGS. 1 to 6.—Strain I.

FIG. 1.—Solid masses of granulosa cells. Original tumor. Mag. × 800.

FIG. 2.—Solid masses, rows and pyknotic granulosa cells. First subpassage. × 350.

FIG. 3.—Follicle-like structures. Sixth subpassage. × 280.

FIG. 4.—An antrum follicle with a cumulus oophorus-like formation. Fourth subpassage. × 280.

FIG. 5.—Solid masses and rows of granulosa cells. Fifth subpassage. × 400.

FIG. 6.—Replacement of degenerating tumor by connective tissue. Fourth subpassage. × 220.

sage. In the fifth and sixth subpassages it dropped back to 17 days. The shortest latent period was 5 days, the longest 172 days. The growth rate of the tumors dropped in the second passage, suddenly increased in the fourth subpassage.

Estrogenic effects on the tumor-bearing hosts were recorded in 11 of 27 males and 6 of 27 females. Congestive changes were often encountered. Both estrogenic and congestive changes were manifest also in irradiated recipients. The site of the tumor had no influence on the congestive changes.

The transplanted tumors were malignant. Two penetrated into the abdominal cavity, 5 metastasized to liver, 8 to lung, 1 to kidney, and 1 to adrenal.

Abdominal hemorrhage occurred in 7 cases. This was due either to an intraabdominal tumor mass or to the rupture of the highly congested liver, spleen, or adrenal.

Strain III: Granulosa, Luteoma, Ademona, and Sarcoma Lines

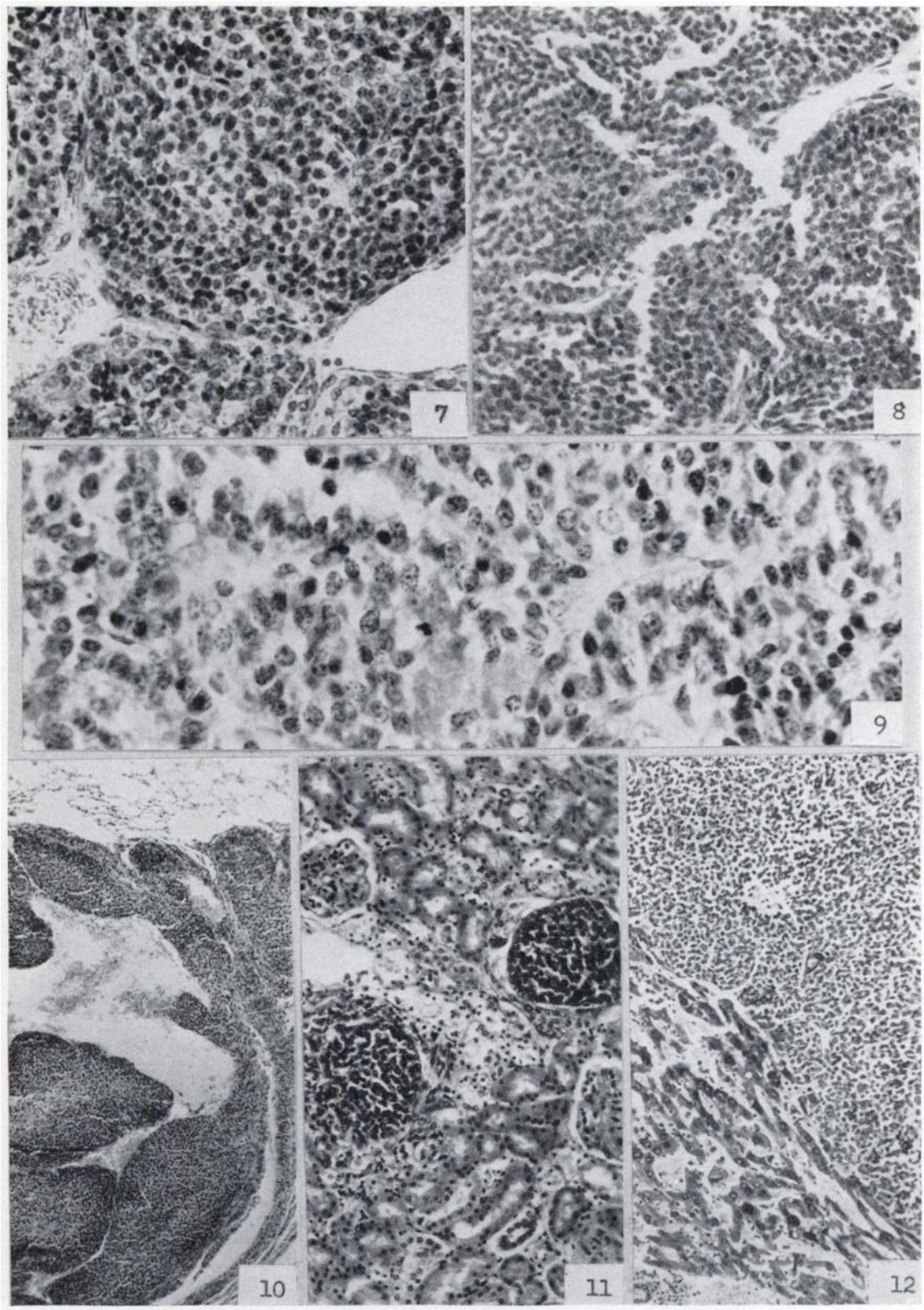
The original animal was irradiated with 175 r at 47 days of age and painted with methylcholanthrene twice during one week. Ten months later the left ovary was replaced by a yellow-

TABLE 1

TRANSPLANTATION DATA ON OVARIAN TUMORS INDUCED BY X-RAYS

Strain	Type of tumor	Number of Passages	Number of Subpassages	Sex	Normal		Gonadectomized			Pre-irradiated
					Subcutaneous	Intrasplenic	Subcutaneous	Intrasplenic	Intrahepatic	Subcutaneous
I	Granulosa			F	47/112 (42%)	3/3 (100%)	6/15 (40%)	3/3 (100%)	1/3 (33%)	
		34	10	M	119/218(55%)	5/5 (100%)	8/16 (50%)	2/3 (66%)	3/4 (75%)	4/7 (57%)
II	Granulosa			F	14/30 (46%)		2/10 (20%)			25/69(36%)
		15	6	M	8/17 (47%)					26/62(42%)
III	Granulosa			F	52/108 (49%)		11/29(38%)	0/5		9/13 (69%)
		27	9	M	51/114 (45%)		9/9 (100%)	0/1		0/4
	Luteoma			F	12/13 (92%)					
		3	1	M	9/10 (90%)					
	Fibroscarcoma	1	1	F	7/9 (78%)					
	Granulosa			F	27/64 (42%)		7/12 (58%)			7/19 (37%)
		16	4	M	26/68 (38%)					0/3
IV	Adenoma			F	1/9 (11%)		2/3 (66%)			
		4	2	M	1/2		0/3			
V	Granulosa			F	99/323 (31%)	3/13 (23%)	19/78(24%)	11/18(66%)	11/14(78%)	6/19 (31%)
		80	15	M	104/267(39%)	6/12 (50%)	26/55(47%)	18/33(55%)	4/7 (57%)	0/2
	Granulosa			F	0/10		1/8 (13%)			
		4	1	M	0/7		4/19 (21%)			
VI	Adenoma-granulosa			F	0/31		11/14(78%)			3/12 (25%)
		13	4	M	2/25 (8%)		4/4 (100%)			4/7 (57%)
	Fibrosarcoma	1	1	F	1/6 (17%)					
VII	Adenoma			F	0/10		13/24(54%)	0/2		1/4 (25%)
		13	3	M	1/21 (5%)		6/14 (43%)	0/2		
VIII	Adenoma			F			2/12 (17%)			2/5 (40%)
		5	2	M	2/11 (18%)					
IX	Luteoma			F	103/205(50%)	2/2 (100%)	12/18(66%)	1/4 (25%)		6/8 (75%)
		39	10	M	75/169 (44%)	1/2 (50%)	9/23 (39%)	1/4 (25%)		2/6
X	Adenoma			F	0/13		6/10 (60%)			0/2
		5	2	M	2/4					
XI	Luteoma-granulosa	17	2	F	20/81 (25%)					
				M	24/57 (42%)					
XIII	Adenoma-granulosa	5	2	F	4/14 (29%)					
				M	7/18 (39%)					
	Fibrosarcoma	9	3	F	11/44 (25%)					
				M	9/28 (32%)					
XIV	Granulosa	15	3	F	5/47 (11%)	0/3	10/11(91%)			
				M	9/68 (13%)	1/2				
XV	Adenoma	8	2	F	1/26 (4%)	2/2				
				M	5/30 (17%)	2/2				
XVI	Adenoma	5	2	F	0/7					
				M	4/25 (16%)					
XVII	Adenoma	3	1	F	0/11					
				M	5/19 (26%)					
	Leuteoma	6	1	F	1/14 (7%)					
				M	7/26 (27%)					
XVIII	Adenoma	2	1	F	1/5 (20%)		3/5 (60%)			
				M	0/2					

The ratios indicate the number of positives over the total number of mice injected.
Subcutaneous implantation of Strain I to 20 Ak mice and intravenous and intracardiac injections to 10 homologous Rf/Ak mice were not successful.
Intraperitoneal injections made to 20 mice of Strain II were successful in only one and were not successful in 4 mice of Strain III.
With Strain I, intratesticular injections were successful in 2 of 8 mice, intramuscular in 3 of 4, and intravenous in 1 of 3.
Intraocular implantations of Strain V were successful in 21 of 30 mice of the homologous stock, in 13 of 17 mice of the heterologous Rf stock, and in 1 of 13 rats. Intraocular implantations of Strain IX were successful in 2 of 6 mice of the heterologous Rf stock. Intraocular implantations of Strain XIII were unsuccessful in 6 mice of the homologous stock.



FIGS. 7 to 12.—Strain II.

FIG. 7.—Solid follicle-like structures. The tumor is made up of nodules of granulosa cells. Original tumor. × 200.

FIG. 8.—Solid growth of similar cells without follicle formation. Fourth subpassage. × 200.

FIG. 9.—Higher power of Figure 8, from the proximity of a "cleft." Fourth subpassage. × 300.

FIG. 10.—Metastasis to lung. Sixth subpassage. × 50.

FIG. 11.—Metastasis to kidney. Fourth subpassage. × 150.

FIG. 12.—Metastasis to liver. Fourth subpassage. × 100.

grey partially hemorrhagic and necrotic tumor, measuring 2 × 5 × 1.5 cm. The right ovary was yellow and 1 × 2 mm. in size. The uterus was 3 mm. thick.

The left ovarian tumor used for transfer was composed of granulosa cells, imitating Graafian follicles. Calcium and other crystalloid deposits and foreign body giant cells were seen in the margin of necrotic areas. The tumor cells stained with Gomori's technique were acid phosphatase negative and slightly positive for alkaline phosphatase.

In the first passage granulosa cells formed masses and follicular structures; there were large areas of necrosis and edema. Hortega stains showed a network of reticulum fibers, in places coarse, in places fine-meshed. Sudanophilic fat was present in cells in the edematous areas.

The accumulation of some fluid material separating widely the tumor cells (Fig. 13) and some degenerative changes were characteristic of this strain. When connective tissue growth was massive the microscopic picture resembled scirrhous carcinoma (Fig. 14). Calcification and ossification of stroma was common in older tumors (Fig. 15). In one animal of the fifth passage a well differentiated fibrosarcoma replaced the tumor (Fig. 16). This was readily transplantable.

One tumor in the first passage showed a typical luteoma and upon subpassages a pure luteoma line was secured (Fig. 17). A third line yielded a fibrotic tubular adenoma. Thus, this strain was divided in three lines of which the granulosa and luteoma lines were carried for several generations; the adenoma line was lost.

Some secondary changes in granulosa tumor-bearing mice included: massive atrophy of testes (Fig. 18), of ovary (Fig. 19), feminization of the kidney (Fig. 21), and of the submaxillary gland (Fig. 20). Sections of kidneys frequently showed an advanced necrosis of proximal tubules.

Transplantation and biological behavior:—The granulosa line of this strain showed a fluctuation in the per cent of takes. The highest ratio of takes (around 65 per cent) was in the fourth, fifth, and sixth subpassages and the average somewhat below 50 per cent in both sexes. The pre-irradiation had no significant effect. All of the gonadectomized males but only 38 per cent of the females developed tumors (Table 1).

The luteoma line had a higher percentage of takes than the granulosa line, (about 91 per cent) and was equal in males and females.

The latent period of the granulosa line decreased during the subpassages from 81 days to 17 days, the longest was 138 days. The growth rate did not significantly change during the subpassages; the tumors reached a dimension of about 33 × 20 mm. in 2½ months.

The granulosa tumors were malignant. Twelve lung, 5 liver, 6 intraperitoneal, 1 splenic, and 1 ovarian metastases were noted. Two subcutaneous tumors penetrated into the abdominal cavity. Massive hemorrhages were common. Thus 7 cases of intraperitoneal, 1 intrathoracic, 1 intrasplenic, 1 intrahepatic, and 1 intraadrenal hemorrhages were recorded. Four mice had generalized edema, one with ascites. Anemia and splenomegaly were present in most of the granulosa tumor-bearing animals.

The luteoma line had an average latent period of 42 days, the shortest being 16, the longest 74 days.

Estrogenic changes were recorded in only 15 of 33 granulosa tumor-bearing females and in 2 of 6 gonadectomized females. Of 25 normal males, 19 were recorded as exhibiting estrogenic changes while 2 gonadectomized males did not show them.

Congestive changes were recorded in 19 of 25 normal and in 5 of 6 gonadectomized females. The ratio of congestive changes was 18/25 in normal and 2/2 in gonadectomized animals. Thus, there was no marked difference between males and females and normal and castrated recipients.

Strain IV: Granulosa, Adenoma, and Endothelioma Lines

The original animal received 300 r when 41 days old. Sixteen months later a laparotomy was performed and a piece from each ovarian tumor was transplanted separately.

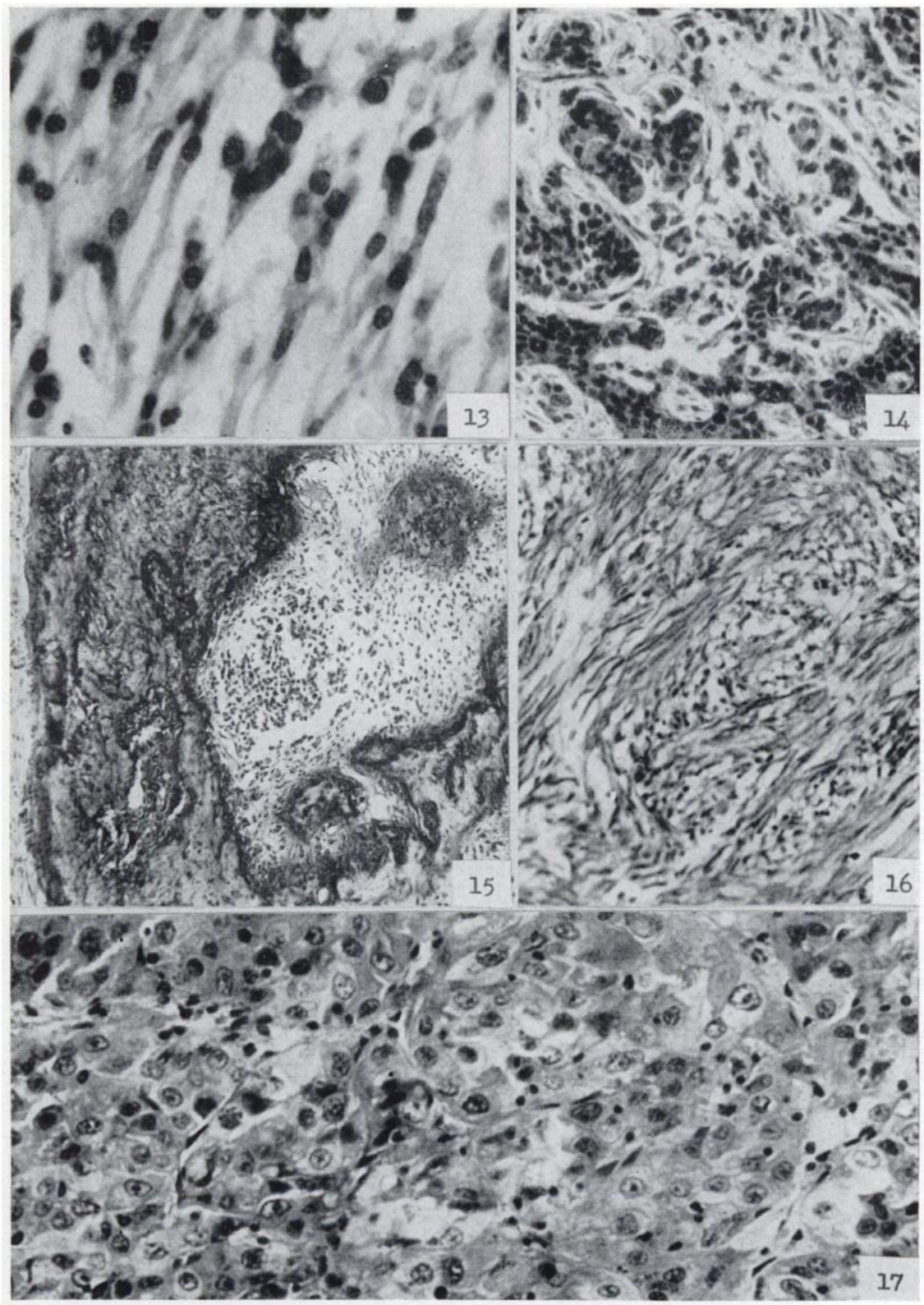
The left ovarian tumor was of the granulosa cell type with areas of massive type of growth, cords (Fig. 23), and follicle-like structures surrounded by "oat-shaped" cells. In a narrow area germinal epithelial tubules were seen with a stroma of spindle-shaped cells. The right ovary had a tubular adenoma with few granulosa cells among the tubules.

During the subpassages of this tumor, granulosa cells gained an upper hand in one line. Granulosa cells were seen inside the basement membrane of tubules as well as in spaces among them as shown in Figure 44. These differentiated into "anovular" follicles (Fig. 28) or the secondary proliferation products of germinal epithelium of Brambell and Parkes (5). Transfers from this line were unsuccessful.

The passages from the left ovarian tumor invariably gave granulosa cell tumors with areas of trabecular and follicular arrangements. Mixed with typical granulosa cells were encountered elongated cells with scanty cytoplasm and an "oat-shaped" nucleus, rich in chromatin (Fig. 26). These are frequently referred to as theca cells. They formed bundles along vessels and around follicular structures. In places they outgrew the spherical granulosa cells. The presence of the two types of cells with carcino-sarcoma-like appearance was characteristic of this line. Accumulations of fatty material with formation of lutein-like cells are shown in Figures 24 and 25. A tubular adenoma line as illustrated in Figure 27 soon vanished in the course of subpassages.

Several tumors had capillaries with cavernous dilatation. The tumor cells among them appeared to vanish. Thrombi had formed in these capillaries. In some tumors connective tissue overgrew the tumor cells among the capillaries; in others an active proliferation of endothelial cells appeared to take place. This was carried on as a pure line believed to be either a chorio-epithelioma or hemangio-endothelioma (Fig. 29). Accepting the majority opinion of specialists and because of lack of hormonal production, we are considering it as an endothelioma. It will be described separately with other endotheliomas (1).

Transplantation and biological behavior:—The granulosa line of this strain had at first a higher per cent of takes: in the first passage 57 per cent in males, 83 per cent in females; in the next passage 91 per cent in males and 100 per cent in females.



FIGS. 13 to 17.—Strain III.

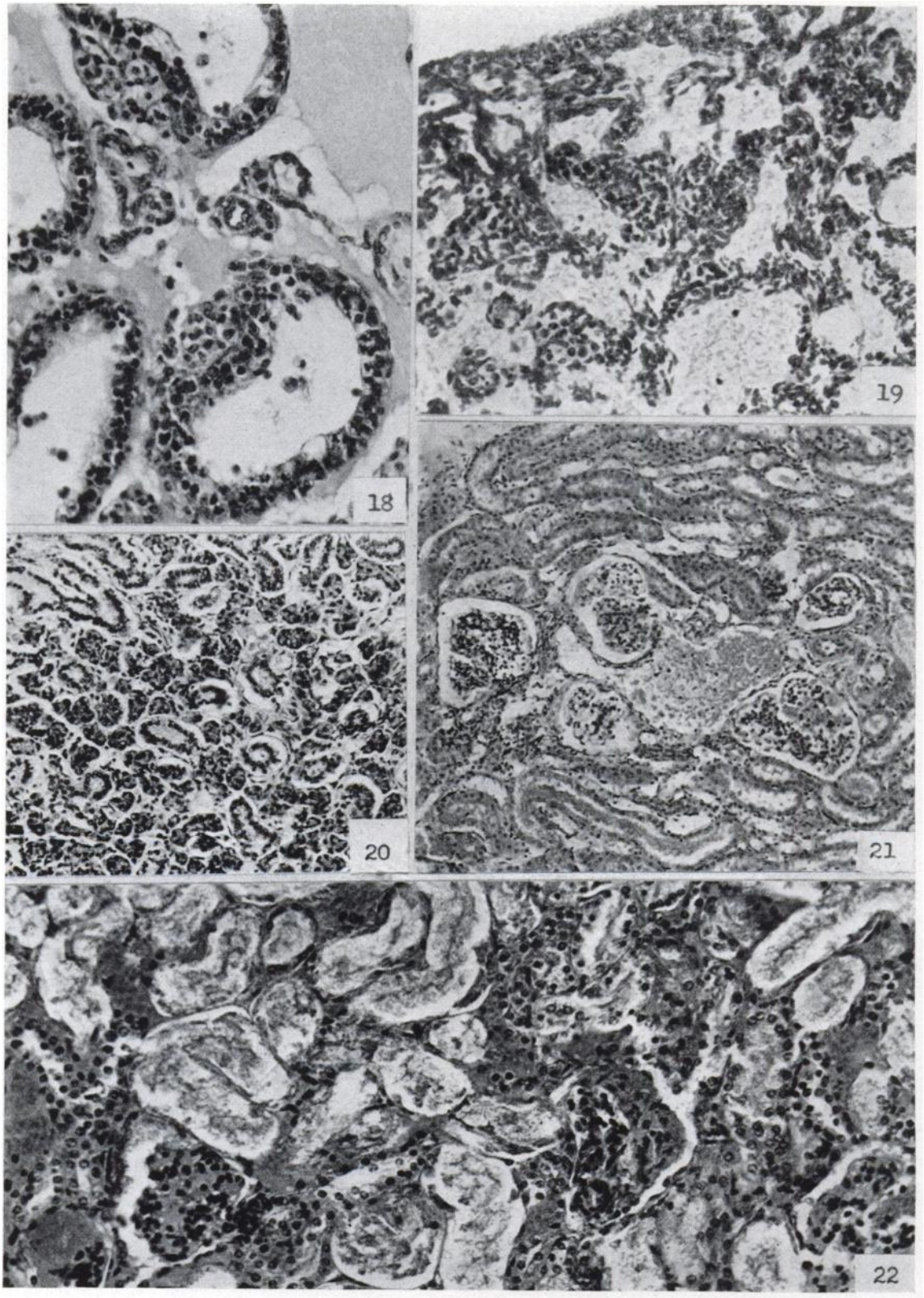
FIG. 13.—Degenerative changes with elongation and separation of cells probably by some secretion product. Sixth subpassage. × 400.

FIG. 14.—Connective tissue overgrowths; resemblance to scirrhous carcinoma. Third subpassage. × 220.

FIG. 15.—Beginning ossification of stroma. Fifth subpassage. × 80.

FIG. 16.—A well-differentiated fibrosarcoma replacing the tumor. Fifth subpassage. × 280.

FIG. 17.—Luteoma line appearing in the first subpassage. × 500.



FIGS. 18 to 22.—Strain III. Secondary changes in tumor-bearing mice.

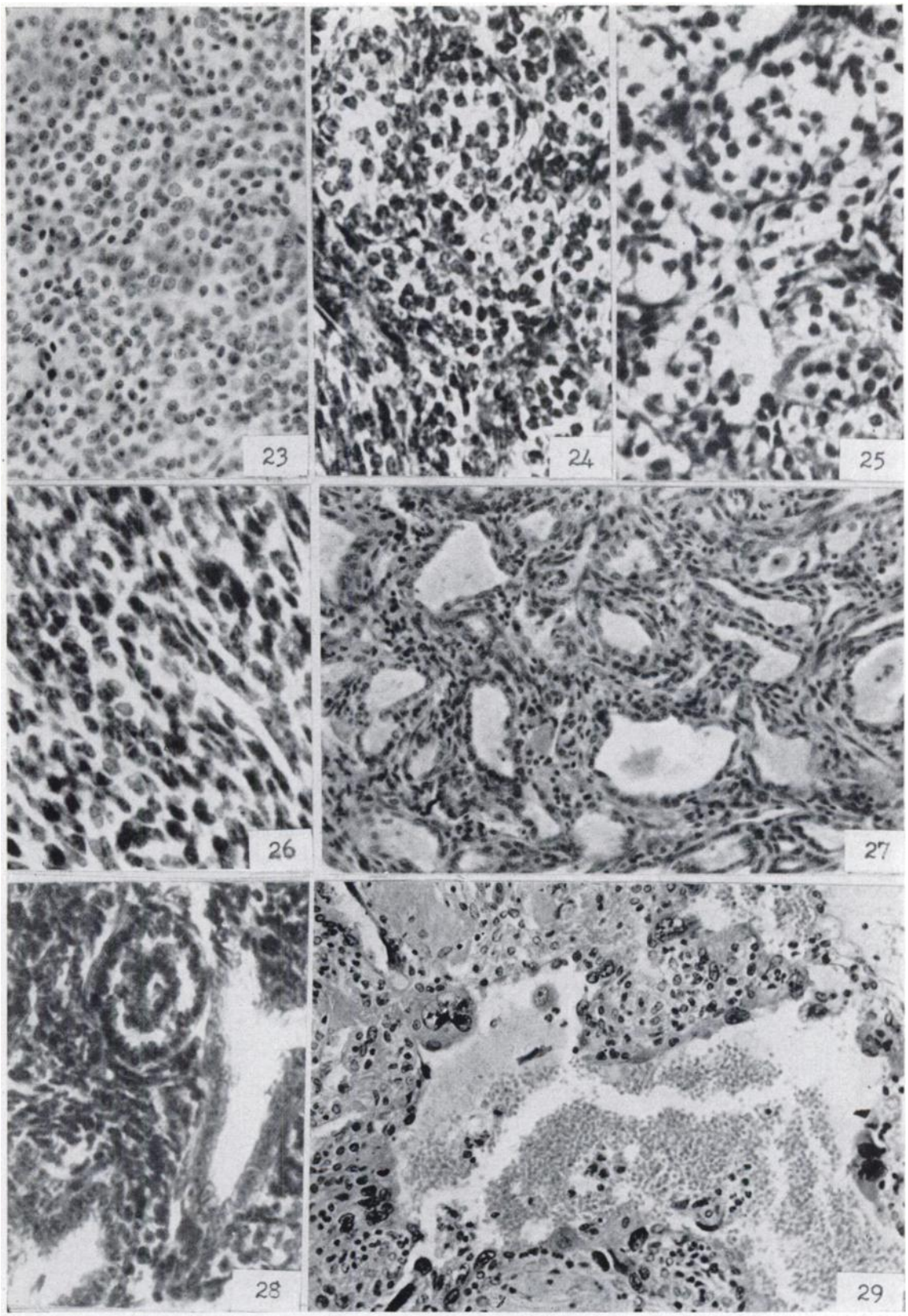
FIG. 18.—Marked atrophy of testis. Sixth subpassage. × 360.

FIG. 19.—Marked atrophy of ovary. Seventh subpassage. × 250.

FIG. 20.—Effect on submaxillary gland of a male mouse; collapsed tubules with expansion of acini. Sixth subpassage. × 80.

FIG. 21.—Feminization of parietal layer of Bowman's capsule of a male mouse. Fourth subpassage. × 150.

FIG. 22.—Necrosis of proximal tubules, male mouse. Fifth subpassage. × 400.



FIGS. 23 to 29.—Strain IV.

FIG. 23.—Solid granulosa growth. Original tumor. × 280.

FIG. 24.—"Fattened" granulosa cells; suggestive of early partial luteinization. Second subpassage. × 300.

FIG. 25.—Luteinization of granulosa cells. Fourth subpassage. × 300.

FIG. 26.—Sarcoma-like forms of granulosa cells. Third subpassage. × 400.

FIG. 27.—Tubular adenoma line. Second subpassage. × 150.

FIG. 28.—Complex anovular granulosa follicle, tubular adenoma, and spindle cells. First subpassage. × 300.

FIG. 29.—Endothelioma line with chorio-epithelioma-like areas. Third subpassage. × 150.

This dropped in the subsequent subpassage to 30 per cent for males and 22 per cent for females.

Pre-irradiation did not significantly influence the success of inoculations. The tubular adenoma grafts were successful in but a small per cent of animals.

The latent period in the granulosa line was about 66 days. The growth rate of granulosa tumors was very slow, the tumors reaching about 3 × 2 mm. in 1 month. Nevertheless, they were malignant: 2 penetrated into the abdominal cavity, 1 metastasized to lung, and 9 from spleen to liver. Several tumors regressed in the third subpassage. The growth rate of the tubular adenoma was also slow, the tumors reaching about 9 × 6 mm. in 11 months.

The hormonal activity of this strain was slight, only two gonadectomized females exhibiting estrogenic changes. No congestive changes were observed.

Strain V: Granulosa Type

The original animal was irradiated with 300 r when 41 days old. Sixteen months later a grey tumor 6 mm. in diameter replaced the right ovary. This was transplanted. The left ovary was 2 mm. in diameter and yellow. No hyperplasia of uterine horns was observed. The mammary glands were atrophic.

The original tumor showed a mixed composition, the major parts being of granulosa type, the remaining a tubular adenoma. The granulosa cells formed follicles and tubules, in some of the latter the lining cells were partly granulosa and partly germinal epithelium.

The left ovary was a tubular adenoma with ciliated epithelium, pigmented (ceroid) cells, and a small number of granulosa cells or ovariocytes (21) among the tubules. The uterine horn showed a slight cystic hyperplasia.

In the first subpassage the tumor showed a massive type of granulosa growth (Fig. 31). In many places the cells formed rows, in others follicular structures (Fig. 30), with an eosinophilic substance in some. Ossification was seen in the stroma (Fig. 34).

In the subsequent subpassages the chromatin arrangement in nuclei changed from granular chromatin structure into a more homogeneous chromatin arrangement with slight condensation in the juxta-membranous parts of the nucleus (Fig. 33). Cavernous dilatation of the capillaries was characteristic. In the third subpassage granulosa cells of a sarcomatous type were seen in some recipients. In others, the insular form of Varangot (22), namely large islands of tumor cells surrounded by connective tissue, have been observed. In many tumors the nuclei resembled those of the germinal epithelium (Fig. 31). In the sixth subpassage, microfollicular formations (Varangot) and spaces resembling Call-Exner bodies were seen. In the tenth and later subpassages "oat-shaped" cells were numerous. In some recipients of the twelfth subpassage some tumor cells were detached and resembled histiocytes and plasma cells.

In a second granulosa cell line of this strain, the granulosa cells formed trabecular structures separated with wide connective tissue septa. The cells tended to form rows as in the "moire-silk" pattern of Varangot (Fig. 32) and papillary proliferations of somewhat cylindrical cells. Extensive bone formations were seen in the stroma of some recipients (Fig. 34). These tumors were stone hard and it was surprising to note on microscopic examination "healthy" tumor cells side by side with "healthy" bone (Fig. 34).

In the sections stained with Sudan III, the cells in fibrotic areas and those with pyknotic nuclei were heavily laden with fat. The "healthy" cells did not contain fat, not even discrete droplets. In the line with "moire-silk" pattern, some recipients showed a luteinization of the granulosa cells.

Estrogenic changes were the rule. Some of these as illustrated in Figures 18 to 22, have already been mentioned. Figure 35 shows a marked atrophy of the ovary with hyaline masses marking the site of the ova.

Transplantation and biological behavior:—The males were slightly more susceptible than the females (39 per cent and 31 per cent). Gonadectomy seemed to increase the "takes" in males (47 per cent) and decrease them in females (24 per cent).

The latent periods fluctuated between 66 days and 8 days and the growth rates roughly paralleled the latent periods. These are extreme values: one tumor reached 16 × 12 mm. in 5 months and another 32 × 13 mm. in 2 weeks. The average growth rate of intrasplenic tumors was about 18 × 17 mm. in 1 month. The intraocular tumors filled the anterior chamber in 2 or 3 weeks. The intrahepatic tumors had a growth rate of about 21 × 16 mm. in 6 weeks.

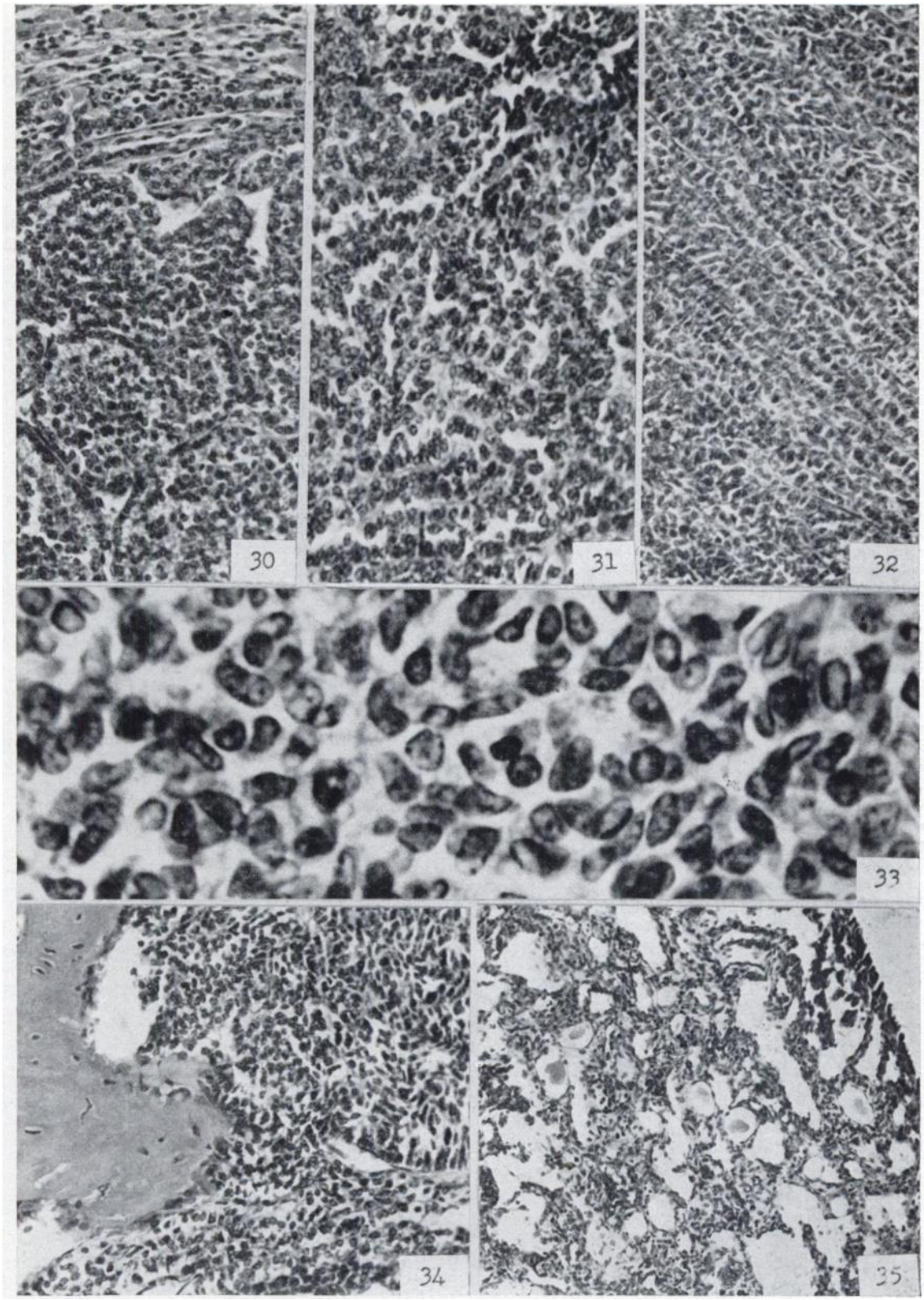
The tumors were malignant. Two were recorded to have penetrated the abdominal wall, 8 to have lung metastases (one from an intrahepatic, the others from subcutaneous tumors). In 27 mice, liver metastases were seen, all of which had come from splenic grafts. In 18, abdominal hemorrhages were seen, 11 of which had intrasplenic grafts and 3 intrahepatic tumors.

In 4 mice, the tumor particles were injected intrasplenically but at death no tumors were found in the spleen, instead, large tumor masses were present in the liver.

Estrogenic effect was recorded in 19 per cent of the 102 positive normal females and in 32 per cent of positive gonadectomized females; in 31 per cent of normal males and 6 per cent of gonadectomized males. The intrasplenic tumors had estrogenic changes only in the presence of perisplenic adhesions or liver metastases with few exceptions, while mice with splenic tumors usually had congestive changes.

Congestive changes were noted in 25 per cent of the positive normal females, in 17 per cent of 41 gonadectomized females and in 23 of the normal males and 19 per cent of gonadectomized males. Intraocular tumors did not have any secondary manifestations.

The following routes of transplantation proved more successful than the conventional subcutaneous route. Intraocular grafts gave 89 per cent takes in females, 45 per cent in males. Intrahepatic



FIGS. 30 to 35.—Strain V.

FIG. 30.—Pulmonary metastasis reproducing the folliculoid pattern. Third subpassage. × 300.

FIG. 31.—Solid, poorly differentiated growth but still having the characteristic granulosa pattern. Third subpassage. × 400.

FIG. 32.—Formation of rows, "moire-silk" pattern. First subpassage. × 250.

FIG. 33.—Solid growth; nuclear pattern resembles that of the germinal epithelium and does not show the chromatin arrangement of typical granulosa cells. Third subpassage. × 850.

FIG. 34.—Bone formation in the stroma. Third subpassage. × 250.

FIG. 35.—Atrophy of the ovary with hyaline remnants of the ova. Fifth subpassage. × 150.

and intrasplenic routes (55 to 78 per cent, Table 1). Very recently intramuscular grafts made in association with Dr. R. G. Gottschalk have yielded a high per cent of takes. This is the oldest strain carried in our laboratory. Now after 4 years of passages it grows rapidly and its appearance in sections is that of an anaplastic carcinoma, in places suggestive of reticulum-cell sarcoma. It grows rapidly, reaching 1 to 2 cm. in about 2 weeks, yet it produces both estrogenic and congestive changes.

Strain VI: Granulosa, Adenoma, Fibrosarcoma Lines

The original animal was irradiated with 175 r when 46 days old. Seventeen months later a yellow-grey firm spherical tumor 6 mm. in diameter was found at the site of one ovary. This was composed of closely packed germinal epithelial tubules; the lining epithelium had a large lightly stained nucleus with very fine chromatin granules and one or two nucleoli (Figs. 36 and 38). This type of nucleus is characteristic of the nuclei of active germinal epithelium seen in the mouse embryo. Among the tubules, small clumps of granulosa cells were seen, which in some places formed small follicles (Fig. 39).

In the first passage, some recipients showed an increment of granulosa cells which formed cords and follicular structures (Fig. 37). Luteinization was observed in some granulosa cells (Fig. 36). Connective tissue increment was observed in some tumors. In the passages some recipients developed a massive pure granulosa cell tumor while the others had the complex tumors with mere increment in granulosa cells, fattening and luteinization of these cells, and a slight increment in "oat-shaped" cells.

In the 2 recipients of this first subpassage, a sarcoma developed in the stroma (Figs. 39 and 40). The connective tissue stroma was marked in the donor of one of these sarcomas and it is probable that this had an inapparent sarcoma. In the second subpassage, a granulosa cell line continued as a pure line. In some sections numerous variations were observed in the germinal epithelial tubules such as ramifying forms and papillary proliferations; the lining cells varied from tall to flat endothelium-like cells.

Transplantation and biological behavior:—The subcutaneous transplantation of granulosa cell tumors was unsuccessful in normal mice. Gonadectomy enhanced transplantability of both granulosa and tubular adenoma lines. The latter was unsuccessful in normal females and successful in only 8 per cent of normal males, while it gave 79 per cent to 100 per cent takes in gonadectomized animals. Irradiation also enhanced susceptibility (Table 1).

The incubation period averaged 83 days in the first passage. This increased to an average of 105 days in the tubular adenoma line and decreased to 20 days in the granulosa line.

The tumors gave no evidence of malignancy. Six of the 11 gonadectomized females showed hormonal changes; these had subcutaneous tubular adenomas overgrown by granulosa cells. Congestive changes were noted in 2 of 5 gonadectomized males with granulosa cells in the tumors.

Strain VII: Adenoma Type

The original animal was irradiated with 175 r when 35 days old. Seventeen months later a laparotomy was performed. A grey-yellow tumor replacing the left ovary was removed and implanted subcutaneously in the host and other animals. The uterus and breasts of the host were hyperplastic. Two months later the animal died, and a tumor was found at the site of implantation. This was a tubular adenoma with markedly proliferated stroma and fattened cells in the intertubular spaces as in Figure 41. The first passage gave tubular adenomas with an abundance of granulosa cells forming nest and tubular structures which in places resembled very closely testis tubules (Fig. 42). The granulosa cells showed fattening in many tumors (Fig. 41). In this passage only the subcutaneous tumor in the donor was of the granulosa type. Many cells underwent a "brown degeneration" which is due to deposits of some acid-fast lipoid (ceroid) in the cytoplasm (10).

In subsequent passages the granulosa cells were more abundant. They formed nests, cords, and follicular structures, many fattened and luteinized.

Transplantation and biological behavior:—The subcutaneous injections remarkably failed in 31 normal mice with one exception but were fairly successful in gonadectomized mice. The latent period ranged between 109 and 312 days. The growth rate was also slow, tumors reaching about 13 × 9 mm. in 17 months in the first and second subpassage.

Four gonadectomized females had secondary hormonal changes and in none were congestive changes observed.

Strain VIII: Predominantly Adenoma Type

The original animal received 175 r at 33 days of age. Seventeen months later the ovaries were yellow, 5 × 3 mm. each. The left ovary had a small cyst; the uterine horns were atrophic.

The first passage gave a tubular adenoma partly overgrown by granulosa cells. In subpassages the granulosa cell masses among the tubules differentiated into cords and follicles in some recipients while in others they showed fattening and degenerative changes. Some had areas of fibrosis.

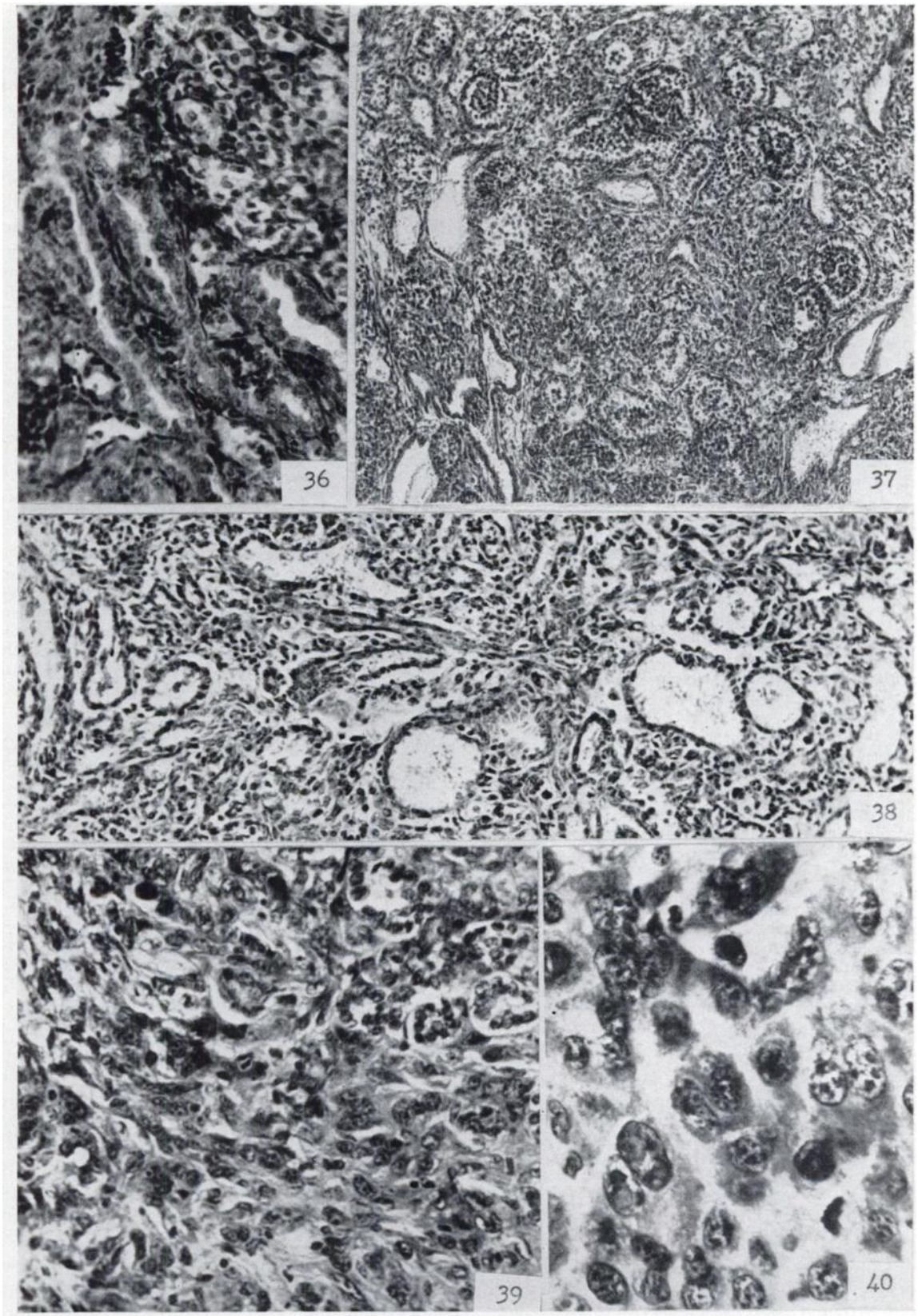
Transplantations were moderately successful (Table 1). The latent period was about 2 months. The growth rate was very slow, the tumors reaching about 10 × 3 mm. in 24 months in the first passage and 6 × 5 mm. in 19 months in the next passage. No invasion or metastasis was seen. Only one gonadectomized female showed hormonal changes. This had a tubular adenoma overgrown by granulosa cells. Congestive changes were absent.

Strain IX: Luteoma Type

A pure luteoma has been described (9). It is still being kept in the laboratory. Its hormonal effects are not as marked as years ago. A report on its ability to raise the blood volume very slightly and increase considerably the red cell level will be published (14).

Strain X: Adenoma-Granulosa Type

The original animal was irradiated with 175 r at 40 days of age. Seventeen months later a laparotomy was performed. The small tumor of the right ovary used for transfer was a tubular



FIGS. 36 to 40.—Strain VI.

FIG. 36.—Tubular adenoma with partially luteinized stroma cells. First subpassage. × 250.

FIG. 37.—Complex tumor with granulosa cells predominating. Anovular follicles. First subpassage. × 100.

FIG. 38.—A field, representative of this section, predominantly a tubular adenoma. Third subpassage. × 180.

FIG. 39.—The granulosa tumor is invaded by fibrosarcoma arising in the stroma. First subpassage. × 250.

FIG. 40.—Higher magnification of a sarcomatous area in the above tumor. Third subpassage. × 800.

adenoma with few granulosa follicles and many ceroid cells among them (Fig. 45). The uterus was hyperplastic.

In the first passage one recipient showed a preponderance of granulosa cells while others showed predominantly tubules of germinal epithelium type. The subpassage made from the latter gave tumors in which the granulosa cells formed the main structure. A second subpassage was unsuccessful. Figure 44 shows convincingly the origin of granulosa cells in tubules of the germinal epithelium.

Transplantation and biological behavior:—This was a poorly transplantable strain, successful in normal males only. After ovariectomy, however, subcutaneous transplantations were highly successful in females.

The long latent periods, 111 days in the first passage, 630 days in the subpassage, are noteworthy. The growth rate paralleled roughly the latent period. No invasiveness or metastasis was seen. Three gonadectomized females showed the hormonal changes but all three had adrenal cortical nodules (23). All three had tubular adenomas overgrown by granulosa cells. No congestive changes were seen.

Strain XI: Luteinizing Granulosa Type

The original animal was irradiated with 175 r when 47 days old, painted twice with methylcholanthrene, and killed 14 months after irradiation. The right ovary was replaced by a yellow tumor with grey and red areas measuring 25 × 12 × 10 mm. The left ovary was 2 × 3 mm. The uterine horns were slightly thickened.

The complex tumor was composed predominantly of lutein cells with finely vacuolated cytoplasm; among large islands of lutein cells an arborization of capillaries was seen. Germinal epithelial tubules and strands of fibroblasts were along the vessels. In the center of the lutein islands were masses of granulosa cells. The border between granulosa and luteoma areas was indistinct; in this zone of transition the granulosa cells acquired a larger and finely vacuolar cytoplasm, while retaining at first the characteristic coarse stippled nuclei. In the adjacent zone the cells acquired the characteristic vesicular nucleus of the luteoma cell with finely dispersed chromatin particles and one nucleolus.

The uterus and the vagina showed effects of luteinization: elongation of uterine horn with hyperplastic narrow glands, and mucification of vagina.

The small ovary of this host showed a tubular adenoma with many granulosa cells and masses of ceroid cells among them.

In the first passage most tumors had both granulosa and luteoma cells (Fig. 47). In subpassages some of the tumors were of pure granulosa cell type, others showed exclusively lutein cells; in others granulosa cells were "oat-shaped" and cytoplasm scanty (Fig. 46). They exhibited ability to form follicular structures.

In the second subpassage a hemangioma developed in one of the recipients which is still being carried as an independent line (1). Mixed types of tumors also persisted in some of the subpassages.

Transplantation and biological behavior:—The mixed luteoma and granulosa lines were more readily transplantable in males than in females. The latent period increased from 62 days in the first passage to 167 days in the subpassage and later declined slightly. The growth rate paralleled roughly the latent period. Two tumors penetrated into the abdominal cavity. Ten of 20 normal females and 6 of 29 normal males showed hormonal changes. Only the original animal had the secondary changes of both luteoma and ganulosa cells, the others showed only the lutein cell influence such as adrenal cortical atrophy and obesity (9). The pure granulosa cell tumors did not show hormonal changes.

Strain XIII: Adenoma, Granulosa, Fibrosarcoma Lines

The original animal received 175 r when 40 days old and was painted twice with methylcholanthrene (7). When killed 16 months later a 1 cm. tumor replaced the right ovary; the left ovary was atrophic. The uterine horn was hyperplastic with a polypoid mass in one horn. The mammary gland showed hyperplasia of the ducts.

The small left ovary contained a tubular adenoma with intertubular granulosa cells that were fattened in some areas. There were also areas of luteinized and ceroid cells.

The right ovarian tumor had a follicular arrangement of granulosa cells with a basement membrane and dense papillary ingrowths (Fig. 48). In the first passage of this tumor the tendency to papillary formations was even more conspicuous. In some recipients there was cystic enlargement of the granulosa nodules, thus taking the shape of a mature follicle. In the first subpassage such formations closely resembling Graafian follicles were numerous. In some recipients, the granulosa cells formed a reticular structure, in others they closely resembled tubular adenoma, but retained the coarse stippled nuclei of granulosa cells. A metastatic well-differentiated folliculoma is shown in Figure 49.

In still other cell recipients a sarcoma developed. Although they were discovered in seemingly pure granulosa cell tumors, we believe the latter contained sarcoma cells. At first the sarcoma had an irregular structure with large hyperchromatic nuclei and many giant and undifferentiated round cells (Fig. 50). In later passages the cells assumed the form of a more mature fibrosarcoma.

Transplantation and biological behavior:—There was no marked difference in susceptibility between males and females in the different lines (25 to 39 per cent). Intraocular implantations were unsuccessful. The latent period was 260 days in the first and 143 days in the subpassage. With the appearance of the sarcoma in the second subpassage the latent period dropped to 37 days and to 7 days in the third subpassage, in which the tumors were purely sarcomatous. The growth rate paralleled the latent period. The tumor measured about 8 × 7 mm. in 12 months in the first passage and 20 × 18 mm. in 1 month in the subpassage, and it did not change thereafter.

The tumors of specialized ovarian parenchyma showed but little invasiveness while the sarcomas had a great tendency to penetrate into the abdominal cavity.

Of 4 females that had tubular adenoma combined with granulosa tumors, one showed second-

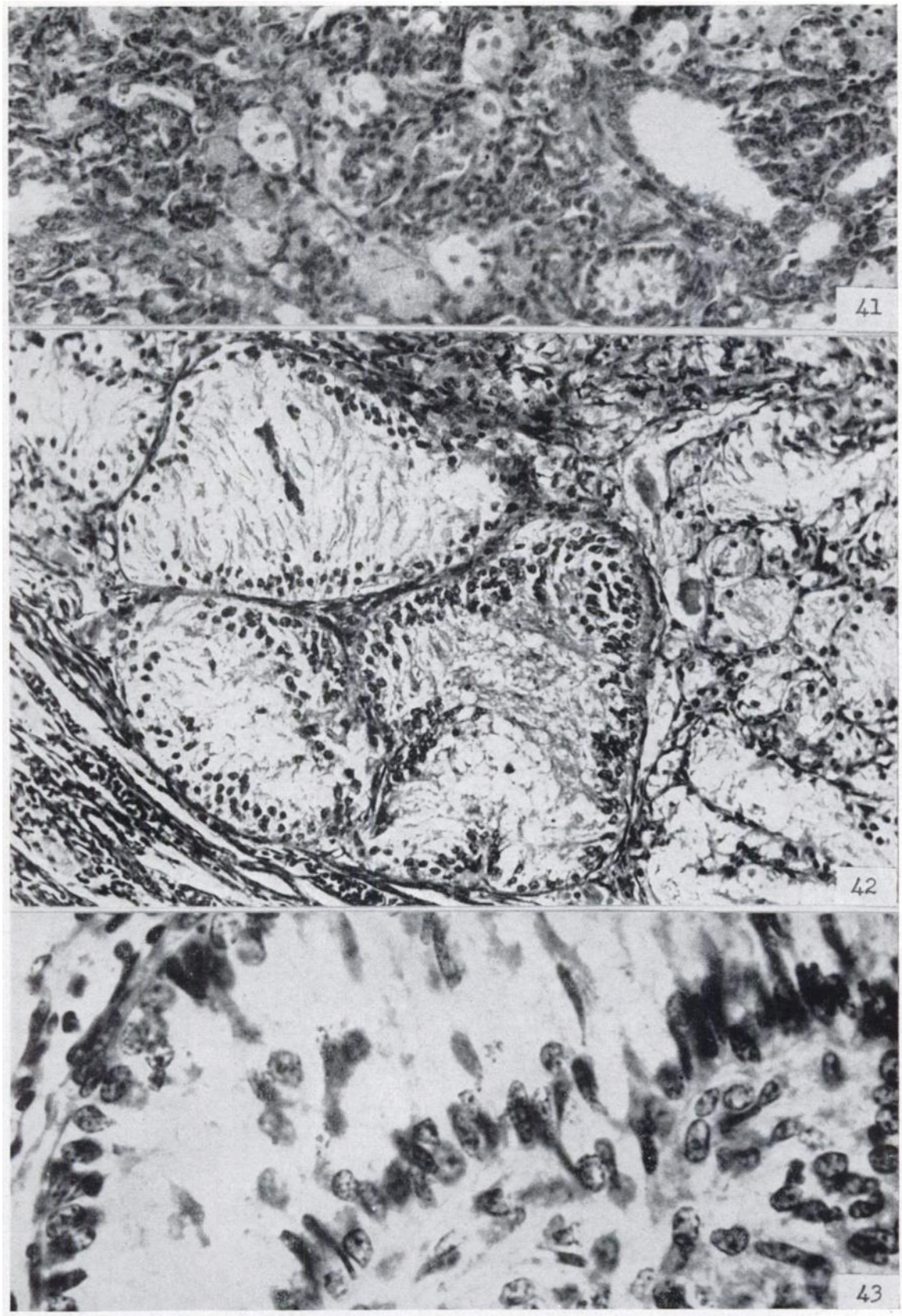
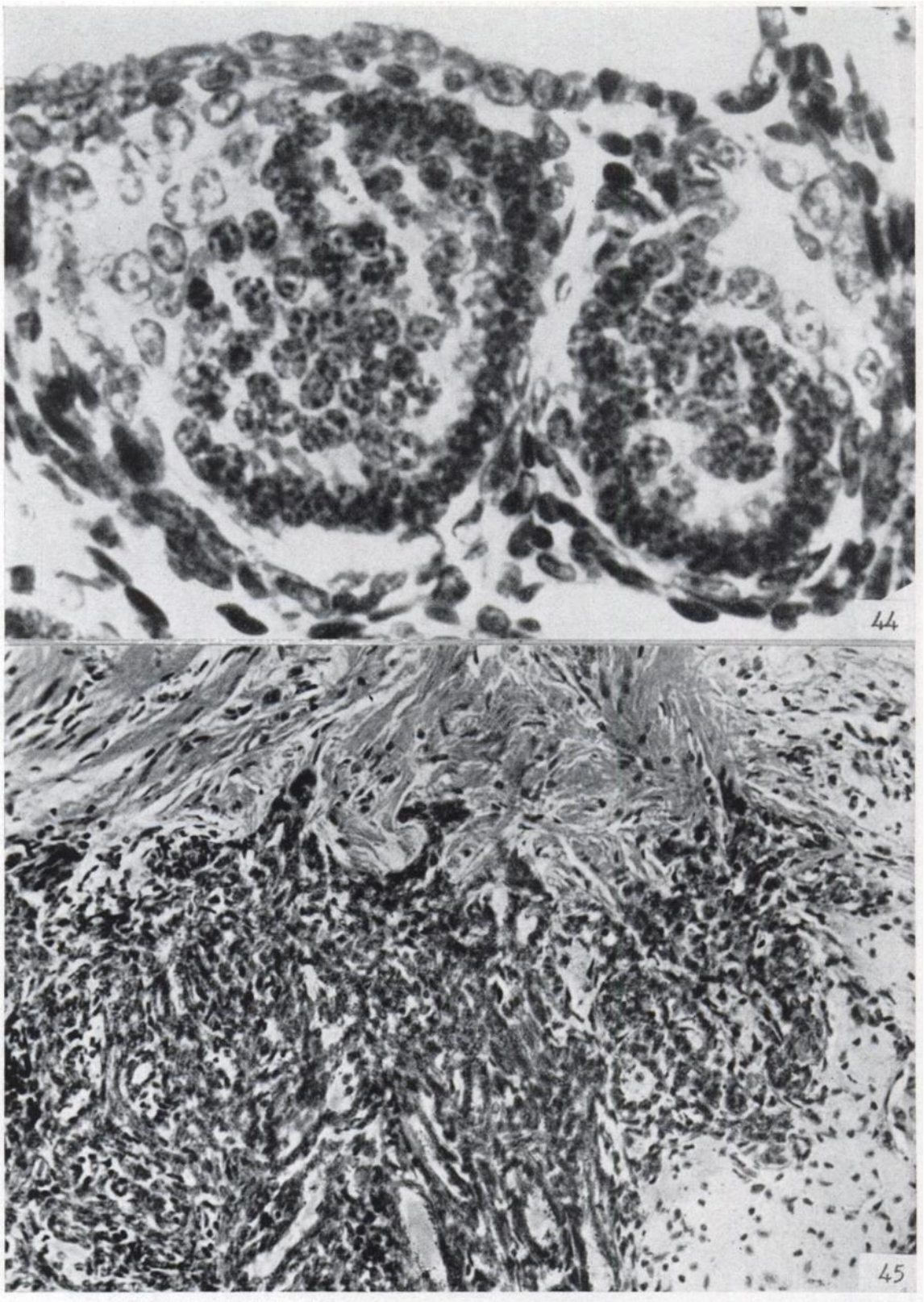


FIG. 41.—(Strain VII) Complex tumor. Few adenomatous tubules; small follicles of granulosa cells; many are "fattened," others changed into ceroid cells. First subpassage. × 250.

FIG. 42.—Tubular structures reminiscent of male (Wolffian) structures in a complex tumor of Strain VII. × 250.

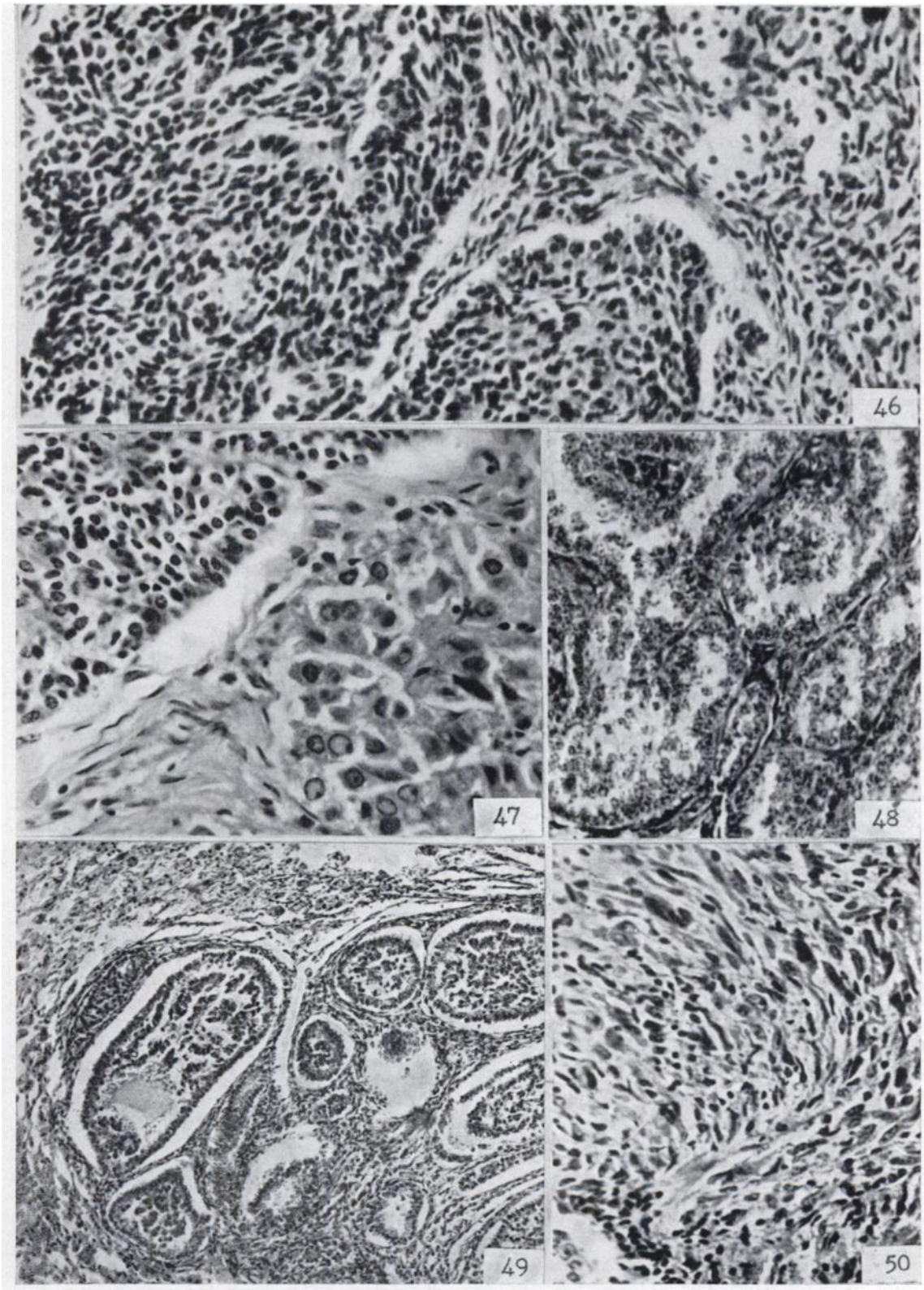
FIG. 43.—Tubule formation in a granulosa tumor of Strain XIV. The cytoplasm is fibrillary, those of two rows of cells appear connected; the structure resembles testis tubules. First subpassage. × 660.



FIGS. 44 and 45.—Strain X.

FIG. 44.—Two convoluted tubules. Apparent differentiation of germinal epithelial tubules into granulosa follicle, both within the same basal membrane. First subpassage. × 850.

FIG. 45.—Predominantly tubular adenoma. Ceroid cells in right lower corner. The muscular wall of the uterine horn on the upper part of the field is invaded by the adenoma. Original tumor. × 200.



FIGS. 46 and 47.—Strain XI.

FIGS. 48 to 50.—Strain XIII.

FIG. 46.—Granulosa tumor with "oat-shaped" cells. These resemble those of the stroma but unlike the latter tend to form circumscribed areas some of which are reminiscent of follicles; the stroma cells form bundles but no circumscribed masses. First subpassage. × 200.

FIG. 47.—Junction line between large areas of well-differentiated luteoma and granulosa growths. Second subpassage. × 250.

FIG. 48.—Well-differentiated follicular growth of the original tumor XIII. × 250.

FIG. 49.—Pulmonary metastasis with reproduction of the follicular pattern. First subpassage. × 100.

FIG. 50.—An undifferentiated sarcoma in the third passage of a granulosa line. × 250.

ary hormonal changes; none had congestive changes.

Strain XIV: Granulosa Type

The original animal was irradiated with 87 r when 47 days old. When killed 29 months later a 12 mm. sized grey-pink spherical tumor was found to replace the right ovary and the left ovary was occupied by a yellow-grey growth 6 mm. across. The uterus was 4 mm. thick and cystic. The animal had also a generalized myeloid leukemia. The breast was hyperplastic and of lactating type.

In the first passage pure granulosa cell tumors were obtained with few unusual formations. The cells formed undifferentiated masses, follicular and occasionally tubular structures. The latter were lined with a single layer of cylindrical cells; some of these cells had a tall cytoplasm with a fibrillary structure toward the lumen (Fig. 43). Some tumors showed an abundant stroma which in some areas contained an intercellular substance with characteristics of mucin. In some sections the theca externa type of cells were numerous. In the second subpassage the same forms persisted and in addition "moire-silk" pattern was seen in some masses. These forms persisted in the subsequent passages one or the other structure being predominant.

Transplantation and biological behavior:—Gonadectomy increased transplantability. No significant difference was observed between normal females and males. The latent periods showed but slight differences. The growth rate fluctuated slightly, the tumor reaching about 22 × 14 mm. in 1½ months. In one line the growth rate tended to increase; in another to decrease. No invasion or metastasis were observed.

Estrogenic changes were recorded in 7 of 10 gonadectomized, and in all 5 normal females, and in 8 of 9 normal males. One of 5 normal females, and 3 of 10 gonadectomized females, and 2 of 9 normal males showed congestive changes.

Strain XV: Predominantly Tubular Adenoma Type

The original animal received 175 r when 40 days old and was killed 23 months later. She had bilateral ovarian tumors and the pituitary was about 10 times the normal size. The right ovarian tumor was composed of germinal epithelial tubules containing papillary proliferations with a moderate number of granulosa cells. In the left ovarian tumor granulosa cells had overgrown the tubules.

In the first passage the derivatives of the left ovarian tumor resembled the original tumor. One tumor was locally invasive. Derivatives of the right ovarian tumor were granulosa cells with few tubules. The intrasplenic tumors had the same histological appearance as the subcutaneous tumors.

Transplantation and biological behavior:—The subcutaneous injections were more successful in normal males (17 per cent) than in females (4 per cent). Intrasplenic injections were positive in all of 4 males and females. The latent period was 144 days in the first passage and 229 in the subpassage. The growth rate was constant at about 14 × 11 mm. in 4 months. The tumors were not invasive and did not metastasize. Only one of the subcutaneously injected normal females with granulosa tumor showed hormonal manifestations. No congestive changes were seen. The intrasplenic tumors showed neither hormonal nor congestive changes.

Strain XVI: Adenoma Type

The original animal received 350 r when 40 days old. Twenty-three months later a laparotomy was performed and a 25 × 18 mm. sized yellow tumor replacing the right ovary was removed. The uterine horn was normal. When killed the animal had several hepatomas. The ovarian tumor was a tubular adenoma of germinal epithelium type. Strands of similar cells were also seen along some tubules with suggestive changes into granulosa type. In some areas granulosa cells formed follicles. Large cysts full of blood were present. In the first passage the granulosa cells overgrew the tubular adenoma.

Transplantation and biological behavior:—The implantations were moderately successful in normal males (16 per cent) and unsuccessful in females. The latent period was 51 days in the first passage and 73 days in the subpassage. The tumors were not invasive or metastasizing. In the first subpassage one of 4 positive males had both hormonal and congestive changes.

Strain XVII: Adenoma and Luteoma Lines

The original animal received 87 r when 33 days old. Twenty-nine months later the right ovary was replaced by a spongy tumor 2.5 × 2 × 1.8 cm. in size, and the left ovary by a grey firm tumor. The uterine horns were elongated, thickened, and cystic. Both tumors were transferred separately.

The left ovary had a tubular adenoma with papillary growths in the lumina and many cells with fine stippled nuclei among the tubules. The right ovarian tumor was composed of lutein cells with extremely dilated capillaries. The uterine horn had long and tortuous glands with scanty stroma.

In passages the tumors originating from the left ovary retained the original structure. The papillary growths and granulosa cells were slightly more numerous in some tumors; in others the granulosa cells were fattened.

The tumors originating from the right ovarian tumor were exclusively luteomas rich in mast cells.

The tubular adenoma line of this strain had a fair ratio of takes in males (26 per cent) and none in females. The luteoma line could also be transplanted with greater success in males than in females.

The latent period did not change significantly through the passages (about 113 to 125 days). The growth rate dropped from 22 × 12.7 mm. in 7 months in the first passage, to 5 × 5 mm. in 9 months in the first subpassage.

The tumors did not show any invasiveness and had no metastasis. No hormonal or congestive changes were observed.

Strain XVIII: Adenoma, Hemangioma, Reticulum Cell Sarcoma Lines

The original animal was irradiated with 350 r at 33 days of age and killed 17 months later. The right ovary was replaced by a yellow tumor 10 × 5 mm. which was used for transfer. The part sectioned showed a tubular adenoma with granulosa cells forming masses and cords of ceroid cells among them. A growth at the site of the left ovary was composed mainly of reticulum cells.

The first passage gave a reticulum cell sarcoma in one recipient and a tubular adenoma in the other. In the latter granulosa cells among the tubules formed follicles. A third tumor in this passage was a hemangioma.

Transplantation and biological behavior:—Transplantations were fairly successful only in ovariectomized mice. The latent period decreased from 204 days in the first passage to 94 days in the subpassage. Several tumors regressed in the subpassages. No invasion or metastases were noted. One of the 3 gonadectomized females with an adenoma rich in granulosa cells showed estrogenic changes; none had congestive changes.

DISCUSSION

Multiplicity of ovarian neoplasms induced by x-rays:—The first fact that emerges from these studies is the multiplicity of neoplasms that develop in the irradiated ovaries and the constancy with which they develop (7). Although serial sections were not made and the bulk of the tumors was often used for transfers, there is ample evidence indicating that most ovarian growths are complex containing at least three elements: tubular adenoma, granulosa, and luteoma of which sooner or later one type tends to overgrow the other. Using grossly separable pure fragments for transfer these neoplasms can be established as pure types.

In addition, sarcomas and endotheliomas may develop in the stroma (1). Many angiomas have doubtless been dismissed as mere telangiectasias because our interest was focused on hormone-producing cells.

Thus, five types of neoplasms seem to develop in x-rayed ovaries: 1) Tubular adenoma, a product of the germinal epithelium; 2) Granulosa cell tumors which we believe to be their derivatives; 3) Luteomas, the derivatives of granulosa cells; 4) Sarcomas; and 5) Angiomas and endotheliomas. Transformation of 1) into 2) and of 2) into 3), once established, seems irreversible.

Transplantations as performed require masses of cells and are influenced by so many variables that they do not directly indicate which of the cell masses transplanted are neoplastic and if so of what grade, conditionally neoplastic or normal. But those that were transplanted in series and grew progressively in normal hosts can be regarded as neoplastic.

Origin and interrelationship of the different induced neoplasms:—After a few passages the granulosa cell tumors and luteomas became pure lines and did not change in the course of many subpassages. In morphological appearance the granulosa cell tumors manifested about all variations described in human tumors (3, 22). The various strains possessed individual features as is the rule with neoplasms. The luteomas on the contrary were monotonously alike exhibiting only minor morphologic variations. They were characteristically rich in mast cells (2).

The tubular adenomas resulted from downgrowth of germinal epithelium and there were many intermediate forms between germinal epithelium and tubule formations. Morphological observations suggest that the germinal epithelial cells lining the tubules and cell masses among them can turn into granulosa cells. Consequently tubular adenomas are frequently overgrown by functioning granulosa cells. Nevertheless, tubular adenomas were transplantable as such and none carried in subpassages ended up as a pure, readily graftable granulosa tumor. This requires clarification.

No morphological evidence has been encountered, suggesting that the granulosa or lutein cells originate from the ovarian stroma cell (ovariocyte). In the irradiated ovaries, the ovarian stroma does not seem to give rise to secreting neoplasms. The character of the theca cell and the concept of embryonal multipotent character of the ovarian stroma cell requires reconsideration. In the histogenesis of ovarian tumors they appear to play a minor role, but the subject is highly controversial (13, 18, 21, 22).

The transformation of one cell type into another with acquisition of hormone production (germinal epithelium into granulosa) or change of type of hormone produced (progestin in place of estrogen) is a profound modification that appears irreversible, thus differing from common metaplasia. While transitional forms are abundant in the material here analyzed, once a tumor differentiated it remained true to type.

The lutein cell transformation is frequently confused with mere deposition of fatty material in the cytoplasm of granulosa cells also called luteinization. Granulosa tumors so affected are more correctly named "folliculome lipidique." The nuclei of these cells have the typical granulosa pattern and their cytoplasms are not those of acidophilic, sudan negative lutein cells. The cytogenetics and histochemistry of this transformation deserves further study. Evidence strongly suggestive of transformation of perhaps already neoplastic granulosa cells into lutein cells was seen only in Strain XI.

All strains that began as tubular adenomas were sooner or later overgrown by granulosa cells. Although the tubules may have been subjected to pressure by the surrounding granulosa cells they showed no degenerative changes. It is possible that undifferentiated stromal ovariocytes have differentiated into granulosa cells or the granulosa cells may have overgrown the tubular epithelium, or

the tubular cells may have been transformed into granulosa cells. Actually there were masses and anovular follicles of granulosa cells between the epithelial cells within the basement membrane of the tubules as illustrated. Some lining epithelial cells of such tubules were of the granulosa type; others were of germinal epithelium type. This could be explained as invasion of the tubules by granulosa cells. However cells of the granulosa type could be found around small germinal epithelium formations outside the ovarian tissue, where an ovarian origin is unlikely. Conversion of the tubular adenoma into a granulosa cell tumor was also noted by Li and Gardner (18). This is a field in which morphologists studying the same situations have arrived at opposing view-points, and clarification awaits further work with newer techniques.

Fibrosarcomas originated in one granulosa and in two mixed (tubular adenoma and granulosa) strains. Two of the fibrosarcomas appeared in the second generation and one in the sixth generation. Hemangiomas occurred in two granulosa, one luteoma, and one in a complex strain. One of the hemangiomas appeared in the first passage, the others in the fifth, third, and second passages. After their appearance these tumors rapidly replaced the original structures. Two hemangioma strains became increasingly malignant and anaplastic (1). The sarcomas on the other hand, although equally malignant, developed more mature features.

The available evidence suggests that these tumors were already present in the original growth in minute foci. Going back to a section of the original tumor we actually saw them in one case. We believe that these "secondary" tumors originated from the stroma of irradiated ovaries and that the respective cells carried with them potency of neoplastic proliferations through a few subpassages of the carrier tumors as Cohnheim nests, until favorable conditions enabled them to form neoplasms of their own. The possibility that stroma cells of transplanted tumors may undergo malignant transformation should be considered.

Malignancy of induced tumors:—The x-ray induced tumors have been compared with those that arise in grafts of ovaries in spleen of castrated mice (4, 12, 11, 16). Both seem to contain cells with a wide range in character varying from those non-neoplastic to those highly malignant. On the basis of transplantability to normal hosts most x-ray induced growths appear to be neoplastic in contrast to those induced by the Biskind procedure, most of which appear to be mere hyperplasias (10).

Many granulosa tumors and some luteomas manifested malignant properties such as invasiveness and ability to metastasize. Metastasizing granulosa cell tumors are rare in man. Some mouse strains are more likely to metastasize than others. Some had metastases in the original animal while others manifested this potency only after a few passages. Pre-irradiation and gonadectomy of the host did not seem to influence the formation of metastases. In microscopic appearance the metastases were similar to the original tumor. The sites of metastases of the subcutaneous tumors were (in order of frequency): lung, liver, kidney, and lymph nodes. The latter were not often examined microscopically.

Metastases of intrasplenic tumors to the liver were a common finding. In several instances hepatic metastases appeared without a detectable primary growth in the spleen. The impression is gained that granulosa tumors metastasize by way of the blood stream rather than the lymphatics. Although all but one of the metastasizing tumors were large, there is no direct evidence suggesting that metastases are proportional to the size of the tumor; neither do they seem to depend on the growth rate or the duration of the tumors.

Factors influencing the success of transplantation:—Difficulties at transplantation may be due in part to genetic, in part to other factors that deserve a thorough analysis since they may disclose forces that influence the genesis and the growth of spontaneous tumors in general. Although both donors and recipients were first generation hybrids of Rf/Ak mice, we are uncertain as to the genetic homogeneity of donors and recipients. The experiments lasted many years during which several sublines were established and donors and recipients came from varied sublines. The genetic composition of both tumors and animals often change with successive generations; in spite of this, several important factors were identified.

Sex and hormonal influence.—Transplantation of granulosa cell tumors is slightly more successful in males than in females. The sum of transplantation results of seven granulosa strains is as follows:

		Normal		Gonadectomized			Pre-irradiated
Recipients		Subcut.	Intrasplenic	Subcut.	Intrasplenic	Intrahepatic	Subcut.
Males	No. of mice	759	19	99	37	11	78
	Takes, per cent	42	63	48	54	64	38
Females	No. of mice	694	19	163	26	17	120
	Takes, per cent	35	32	34	54	71	39

More recent transplantations with Strain V made in association with Dr. R. G. Gottschalk have confirmed this trend.

The following summary of transplantation of three luteoma strains shows a trend to some extent the reverse of that of the granulosa tumors:

Recipients		Normal		Gonadectomized		Pre-irradiated
		Subcut.	Intra-splenic	Subcut.	Intra-splenic	Subcut.
Males	No. of mice	205	2	23	4	6
	Takes, per cent	44	50	39	25	33
Females	No. of mice	116	2	18	9	8
	Takes, per cent	50	100	67	25	75

An unexpected observation is the marked increase in the per cent of takes in grafting tubular adenomas in gonadectomized mice as shown by the following tabulation of nine strains in which the cells were predominantly of this type:

Recipients		Subcutaneous grafts		
		Normal	Gonadectomized	Irradiated
Males	No. of mice	157	21	12
	Takes, per cent	18.5	48	25
Females	No. of mice	126	63	18
	Takes, per cent	5.6	54	39

This is a combined tabulation of nine strains (Table 1). Several of these strains could be grafted in males only and gonadectomy greatly raised the per cent of takes particularly in females (Table 1). This observation suggests that these adenomas may be inhibited by hormones of the gonads, particularly in females, or stimulated by excessive amount of pituitary gonadotrophins. This working hypothesis brings the adenomatous tumors of the ovary in the group of those whose endocrine genesis and control may be possible.

The endothelioma strain that resembled a (non-secreting) chorio-epithelioma likewise grew better in gonadectomized mice, while a typical endothelioma did not:

Endothelioma strain		Subcutaneous grafts			Intrasplenic
		Normal	Gonadect.	Pre-irrad.	Gonadect.
IV. Chorio-epith.-like	No. of mice	104	21	9	9
	Takes, per cent	31	71	33	100
XI. Hemangioma type tumor	No. of mice	108		8	14
	Takes, per cent	31		75	0

It is possible though unlikely that strain IV was actually a chorio-epithelioma and is therefore influenced by the endocrine state of the host. It is of practical importance to know factors that markedly influence the per cent of takes, such as gonadectomy, irradiation, sex, and sites of implantation. But for theoretical interpretation more data are needed such as the effect of hypophysectomy and of hypophyseal and other hormones, alone and combined, on the transplantability of ovarian tumors. Furthermore, data are needed on the specific relation of these factors to tumors of different types.

Latent period and the growth rate:—Enormous variations were encountered. The latent periods were longest with tubular adenomas, averaging 207 days, while those of granulosa cell tumors were 80 days, and of luteomas 68 days. The longest latent period was also encountered among the tubular adenomas (about 2 years) while that in granulosa lines was 260 days and in luteoma lines 167 days. The growth rates were also greater in the granulosa and luteoma tumors than in tubular adenoma.

Hormonal activity.—The observations concerning hormonal activity were merely incidental to this work and require a special study. All granulosa tumors and luteomas that have been studied for some time have given evidence of stimulation by estrogens or progestins respectively, while the tubular adenomas appear inactive.

A unique feature of the granulosa tumors is their ability to produce congestive changes with rise in the blood volume. This is due to a hypothetical substance, now named plethorin, which is directly or indirectly related to granulosa cells.

The question arises whether or not the two types of activities of granulosa cell tumors (estrogenic and congestive) are bound to the same substance. Estrogenic activity of the tumors begins earlier than the congestive change (Table 2). The granulosa cells that have overgrown the tubular adenomas had only estrogenic activity. The two can occur independently (Table 3). The estrogenic changes seem to come to a standstill when the tu-

TABLE 2

THE RELATION BETWEEN TUMOR SIZE AND INTERNAL SECRETORY ACTIVITY OF GRANULOSA TUMORS

		Tumor size			
	Strain	1+*	2+	3+	4+
I	No. studied	9	10	21	58
	No. with estrogen production	0	0	6	21
	No. with plethorin production	1	2	11	40
II	No. studied	1	10	12	24
	No. with estrogen production	0	2	4	10
	No. with plethorin production	0	2	2	11
III	No. studied	1	3	20	37
	No. with estrogen production	0	2	13	21
	No. with plethorin production	0	0	17	29
IV	No. studied	12	12	6	7
	No. with estrogen production	1	0	0	2
	No. with plethorin production	0	0	0	0
V	No. studied	13	15	22	45
	No. with estrogen production	1	6	12	24
	No. with plethorin production	0	4	10	24
VI	No. studied	0	4	0	0
	No. with estrogen production	0	1	0	0
	No. with plethorin production	0	2	0	0
VIII	No. studied	5	0	2	0
	No. with estrogen production	0	0	0	0
	No. with plethorin production	0	0	0	0
XIV	No. studied	0	8	7	2
	No. with estrogen production	0	7	7	1
	No. with plethorin production	0	1	3	1
Total no. studied		41	62	90	173
No. positive with estrogen production		2	18	42	79
No. positive with plenthorin production		1	11	43	105
Per cent estrogen production		4.8	29.0	46.6	45.7
Per cent plethorin production		2.4	17.7	47	60.7

* Tumor Size: 1+ = The two greater diameters less than 10 mm.
2+ = The two greater diameters each 10 to 20 mm.
3+ = One of the greater diameters is less, the other greater than 20 mm.
4+ = Both diameters greater than 20 mm.

The figures in Tables 2 and 3 include normal, gonadectomized and irradiated hosts. The organs studied for estrogenic effects were: vagina, uterus, ovary, submaxillary gland; testes and seminal vesicles. For localization of congestive changes see text.

Intrasplenic tumors are included in Table 2 but not in Table 3. Intraocular tumors are not included.

The two mice of Strain VI that showed congestive changes only, were among the five mice with granulosa tumors that have overgrown tubular adenomas, as was one mouse of Strain VII.

TABLE 3

RELATION BETWEEN ESTROGENIC AND CONGESTIVE CHANGES

		Number of cases *Changes*			
Strain	Number studied	Estrogenic only	Congestive only	Both	Neither
I	101	5	31	22	43
II	47	8	8	7	24
III	61	5	22	25	9
IV	63	12	5	24	42
V	100	17	14	38	31
VI	4	1	2	0	1
VII	1	1	0	0	0
XIII	7	0	0	0	7
XIV	22	14	0	6	2
Total	406	63	82	102	159
Per cent		15.5	20.2	23.1	36.7

mors reach about 2 cm. in diameter whereas the congestive changes progress. These findings suggest that the two types of activities are independent of each other.

Another observation pointing in this direction is that the estrogenic substance is inactivated in the liver, whereas the congestive substance does not seem to be affected by the liver:

SECRETORY ACTIVITY AFTER INTRASPLENIC GRAFTS

	With liver metastasis		No liver metastasis	
Granulosa strains	Estrogen	Plethorin	Estrogen	Plethorin
I	1/4	*2/4	0/3	2/3
II			1/2	*2/2
V	5/25	**5/25	4/34	**6/34
Total	6/29	***7/29	5/39	***10/39
Per cent	20.7	24.1	12.8	25.6

Fractions indicate number of positive mice over the number studied. Each asterisk designates an animal that also exhibited estrogenic stimulation.

The above data suggest that estrogen and plethorin are different substances. It has been suggested that plethorin originates in the liver (cf. 20).

It is known that the blood volume rise precedes that of recognizable congestive changes. Thus many more animals had an increased blood volume than indicated in the tables. This is probably true also for estrogenic stimulations. The precise relation of the two remains to be analyzed. However, there is ample evidence to conclude that plethorin is specifically related to granulosa cells.

Pathogenesis.—Hormonal imbalance has recently been stressed as the most important factor in the pathogenesis of ovarian tumors (12, 18) and the now classical Biskind procedure (4) and the many observations of Gardner and his associates (12), indicate that it doubtless is a major factor. However, there is no evidence indicating that the growths induced by the Biskind procedure and the nodules frequently found in endocrines are truly neoplastic, but some can turn into true cancers.

We are impressed as are Van Eck-Vermande and Freud (21) by the double effect of irradiation: an immediate which is destructive and a delayed, which is an anomaly in development, "comparable to mutations obtained by x-rays where an invisible immediate effect entails a new trend in later development" (21). The immediate effect is a disorganization of the ovary; this is followed by a disturbed regeneration; some cells stimulated by the hypophysis proliferate and secrete and these in turn influence other cells of this organ and the pituitary itself. The magnitude of the delayed x-ray effect on cells of the ovary, the role of disorganization, and of the varied hormonal influences, all basic problems of tumor genesis, remain to be analyzed.

SUMMARY AND CONCLUSIONS

Exposure of ovaries to x-rays causes the development of tumors of different sorts. Five types have been transplanted in series: granulosa tumors, luteomas, tubular adenomas, sarcomas, and angioendotheliomas.

The granulosa tumors occur in such a wide range of morphological forms and simulate so many different types of neoplasms that their identification on a morphological basis alone is often not possible.

Common to all granulosa growths is the ability to produce or initiate production of estrogens and plethorins (a substance that raises blood volume) although not all tumor-bearing hosts show effects of these substances.

Manifestations of estrogen and plethorin stimulations can occur independently, although both are related to activities of granulosa cells.

No evidence was found to indicate that any cell other than a variant of the granulosa cells secretes estrogens.

Well-developed granulosa tumors did not change into luteomas. After a few transplantations each strain had certain morphological features that remained true through numerous subpassages.

All transplantable luteomas studied produced secondary changes indicative of progestin production.

The tubular adenomas are derivatives of the germinal epithelium. Those transplanted were benign and of exceedingly slow growth. In the course of subpassages sooner or later most of them either changed into or were overgrown by granulosa cells.

Male hosts are more susceptible to grafts of tubular adenoma and of granulosa tumors than females. This sex influence does not seem to be due to a gonadal hormone since it is usually magnified by gonadectomy.

Spleen and liver are better soils for granulosa grafts than the subcutaneous tissue; the success of splenic grafts is not necessarily due to inactivation of estrogens in the liver.

In the genesis of these tumors two major forces

are postulated; direct and delayed x-ray effect, and a hormonal "imbalance." Both require a more precise analysis to unravel the complex sequence of events initiated by irradiation of ovaries. The exposure to x-rays may last only for seconds but the chain of events which follows covers the entire life span of the animal.

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ADDENDUM

Discussions at the American Association for Cancer Research meeting on April 16 and 17 disclosed that there are no established criteria to differentiate by bioassay, androgens from different organs (testiculoids, progestins, and corticoids). Masculinization of submaxillary gland is produced by testiculoids only according to unpublished observations of A. Kirschbaum and M. J. Frantz.

In the present study no major role is attributed to interstitial cells: the luteomas are related to granulosa cells and none of the hormone-secreting tumors are derived from interstitial cells. These observations are contrary to those reported by others and should lead to further study but not to generalizations.

REFERENCES

1. Bali, T., and Furth, J. Hemangioma. An Experimental and Morphological Study. To be published.
2. Bali, T., and Furth, J. Mast Cells in Neoplasms. Am. J. Path. 1949. In press.
3. Barzilai, G. Atlas of Ovarian Tumors, Grune and Stratton: New York, 1943.
4. Biskind, M. S., and Biskind, G. R. Development of Tumors in Rat Ovary After Transplantation into the Spleen. Proc. Soc. Exper. Biol. and Med., **55**:176–179, 1944.
5. Brambell, F. W. R., and Parkes, A. S. Changes in the Ovary of the Mouse Following Exposure to X-rays. Part III. Irradiation of the Non-Parous Adult. Proc. Roy. Soc., London, s. B **101**:316–328, 1927.
6. Furth, J. Transplantability of Induced Granulosa Cell Tumors and of Luteoma in Mice. Secondary Effects of These Growths. Proc. Soc. Exper. Biol. and Med., **61**:212–214, 1946.
7. Furth, J., and Boon, M. C. Induction of Ovarian Tumors in Mice by X-rays. Cancer Research, **7**:241–246, 1947.
8. Furth, J., and Furth, O. B. Neoplastic Diseases Produced in Mice by General Irradiation with X-rays. Am. J. Cancer, **28**:54–65, 1936.
9. Furth, J., and Sobel, H. Transplantable Luteoma in Mice and Associated Changes. Cancer Research, **7**:244–260, 1947.
10. Furth, J., and Sobel, H. Neoplastic Transformation of Granulosa Cells in Grafts of Normal Ovaries into Spleens of Gonadectomized Mice. J. Nat. Cancer Inst., **8**:7–16, 1947.
11. Furth, J., and Sobel, H. Hypervolemia Secondary to Grafted Granulosa Cell Tumors. J. Nat. Cancer Inst., **7**:103–113, 1946.
12. Gardner, W. U. Hormonal Imbalances in Tumorigenesis. Cancer Research, **8**:397–411, 1948.
13. Geist, S. H., Gaines, J. A., and Pollack, A. D. Experimental Biologically Active Ovarian Tumors in Mice. Histogenesis and Relationship to Similar Human Ovarian Tumors. Am. J. Obst. and Gynec., **38**:786–797, 1939.
14. Gottschalk, R. G., and Furth, J. Data to be published.
15. Kaplan, H. S. Observations on Radiation Induced Lymphoid Tumors of Mice. Cancer Research, **7**:141–147, 1947.
16. Li, M. H. Malignant Granulosa Cell Tumor in an Intrasplenic Ovarian Graft in a Castrated Male Mouse. Am. J. Obst. and Gynec., **55**:316–320, 1948.
17. Li, M. H., Gardner, W. U., and Kaplan, H. S. Effects of X-ray Irradiation on the Development of Ovarian Tumors in Intrasplenic Grafts in Castrated Mice. J. Nat. Cancer Inst., **8**:91–98, 1947.
18. Li, M. H., and Gardner, W. U. Further Studies on the Pathogenesis of Ovarian Tumors in Mice. Cancer Research, **9**:35–41, 1949.
19. Lorenz, E., Heston, W. E., Eschenbrenner, A. B., and Deringer, M. K. Biological Studies in the Tolerance Range. Radiology, **49**:274–285, 1947.
20. Sobel, H., and Furth, J. Hypervolemia in Mice Bearing Granulosa Cell Growths; Time of Onset and Some Associated Physiological and Chemical Changes. Endocrinology, **42**:436–447, 1948.
21. Van Eck-Vermande, G. J., and Freud, J. Structure and Function of Mouse Ovaries after X-raying. Arch. internat. de pharmacodyn. et de thérap., **78**:49–62, 1949.
22. Varangot, J. Les tumeurs de la granulosa (folliculomes de l'ovaire). L. Arnette: Paris, 1937.
23. Woolley, G. W., and Little, C. C. The Incidence of Adrenal Cortical Carcinoma in Gonadectomized Female Mice of the Extreme Dilution Strain. Cancer Research, **5**:193–202, 1945.