

Further Studies on the Pathogenesis of Ovarian Tumors in Mice*

MIN HSIN LI, PH.D.,† AND W. U. GARDNER, PH.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven, Connecticut)

The formation of granulosa-cell tumors and luteomas in intrasplenic ovarian grafts in castrated rats (1) and in intrasplenic and intrapancreatic ovarian grafts in castrated male and female mice has been reported previously (6-9). These experiments were based on two principles: (a) the capability of the liver to inactivate ovarian hormones when the hormones circulate through the hepatic portal system and (b) the increase of pituitary

subcutaneous tissues or testes of intact and castrated mice did not readily become tumorous.

The present experiments are concerned with the influence of gonadal and gonadotrophic hormones in the development of tumors in intrasplenic ovarian grafts in castrated mice. The purpose of the investigation was to obtain additional evidence on the possible role of gonadotrophic hormones in the genesis of ovarian tumors.

TABLE 1
EFFECTS OF GONADAL AND GONADOTROPHIC HORMONES ON TUMORIGENESIS
IN INTRASPLENIC OVARIAN GRAFTS IN MICE

GROUP	HOSTS	No. OF MICE	TREATMENT	TYPE OF OVARIAN TUMOR AND AGE RANGE OF THE GRAFTS			
				Granulosa- cell	Lute- oma	Mixed	Number and age of nontumorous grafts
A	♂	13	None	10 (207-278)	0	2 (239, 261)	1 (187)
	♀	9	None	5 (246-296)	4 (212)	0	0
B	♂*	4	None	0	0	0	4 (138-276)
	♀*	6	None	0	0	0	6 (185-282)
C	♂	8	Estradiol 16 µgm/week	0	0	0	8 (225-255)
	♀	5	Estradiol 16 µgm/week	0	0	0	5 (160-265)
D	♂	8	Testosterone 1.25 mg/week	0	0	0	8 (192-243)
	♀	6	Testosterone 1.25 mg/week	0	0	0	6 (220-267)
E	♂	8	Progesterone 1 mg/week	5 (213-254)	0	2 (242, 253)	1 (193)
	♀	5	Progesterone 1 mg/week	3 (243-257)	1 (284)	1 (273)	0
F	♂	5	Gonadotrophin (PMS) 25 I.U./day	1 (236)	0	2 (205, 232)	2 (222, 268)
	♀	5	Gonadotrophin (PMS) 25 I.U./day	0	0	3 (205-278)	2 (264, 284)

* Unilateral castrates.

gonadotrophins subsequent to castration. It was assumed that the prolonged stimulation by augmented amounts of gonadotrophic hormones, to which the ovaries were subjected in these sites, was responsible for the neoplastic growths. Ovarian tumors were not observed in intrasplenic or intrapancreatic ovarian grafts with vascularized adhesions that permitted drainage through other than the hepatic portal system, or in female hosts that showed regular estrous cycles. Ovarian grafts in

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† Anna Fuller Fund Fellow in anatomy. Now at the National University of Peking, Peiping, China.

MATERIALS AND METHODS

Male and female mice of the A, C₃H, and C₅₇ strains, and several groups of hybrid mice (A × C₃H and CBA × C₅₇) were used.¹ They were kept in an air-conditioned room and were fed with Purina Fox Chow and water. Gonadectomy and autoplasmic or homoplasmic grafting of an ovary into the spleen were performed in one-stage operations. Most animals were 1 to 3 months old at the time of grafting. Six groups of experiments were undertaken (Table 1). The steroid hormones were administered subcutaneously in oily solutions and the gonadotrophic hormones were injected daily in aqueous solution. All injections were started in mice bearing grafts that were 92 to 138 days old.

¹ Dr. L. C. Strong supplied some of these mice.

noted. On the other hand, the ovarian follicles and interstitial cells formed the main structures of the ovarian grafts taken from the male hosts. Little lutein tissue was encountered. Ingrowths of the germinal epithelium were observed in small areas in the oldest ovarian graft in a male mouse.

The average uterine weight of the unilaterally castrated female mice was 105 mg. The intact testes and accessory genital organs in the males were maintained in a normal condition.

C. OVARIAN GRAFTS IN ESTROGEN-TREATED CASTRATED MICE

Castrated males.—No tumors were noted in intrasplenic ovarian grafts in 8 castrated males treated with an estrogen. The treatment was started 103 to 138 days subsequent to the grafting, and most of these hosts had received more than 10 weekly injections (Fig. 5).

The ovarian grafts were 3 to 4 mm. in diameter. They contained few to many small and medium-sized follicles. The stroma was composed of interstitial cells and, frequently, luteinized interstitial cells. Proliferation and tubular ingrowths of the

germinal epithelium were observed in some areas in seven grafts, and some of the tubular structures were lined by simple cuboidal or columnar cells, as described previously (8). A few atretic and hemorrhagic follicles and corpora lutea were present in three grafts.

The urinary bladders of the male hosts were distended with urine. Effects of the estrogenic hormone were also indicated by the histological features of the seminal vesicles, prostates, kidneys, and submaxillary glands. The x-zones of the adrenal glands were absent, and groups of deeply stained small cells were noted in the subcapsular regions of the cortex of almost all adrenal glands examined.

Castrated females.—The intrasplenic ovarian grafts in 5 castrated females treated with an estrogen were nontumorous. The treatment was started 103 to 133 days subsequent to the grafting; 5 to 10 weekly injections were administered (Fig. 5).

Ovarian follicles and corpora lutea made up the greater part of three grafts that showed ingrowths of the germinal epithelial cells. A few hemorrhagic follicles were present in one of these grafts. Regres-

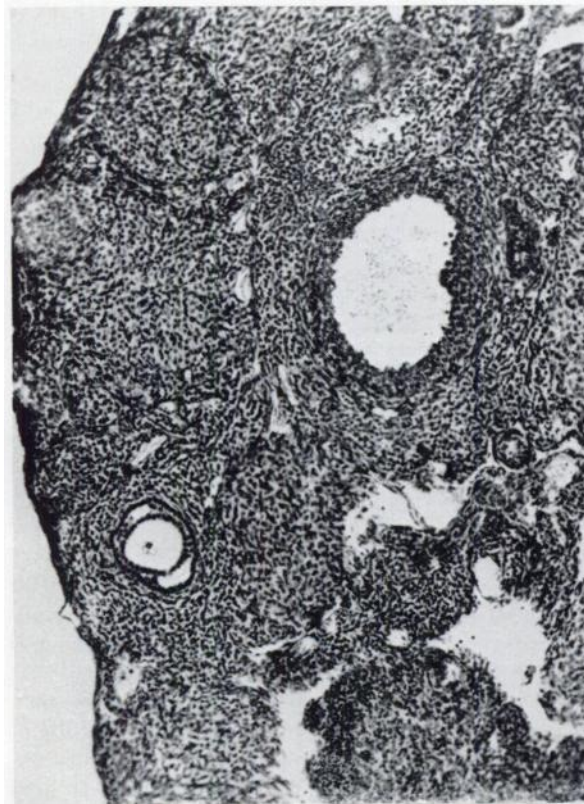


FIG. 3.—Photomicrograph of a large part of an intrasplenic ovarian graft that had persisted 246 days in a unilaterally ovariectomized mouse. Several small and medium-sized follicles are present. $\times 105$.

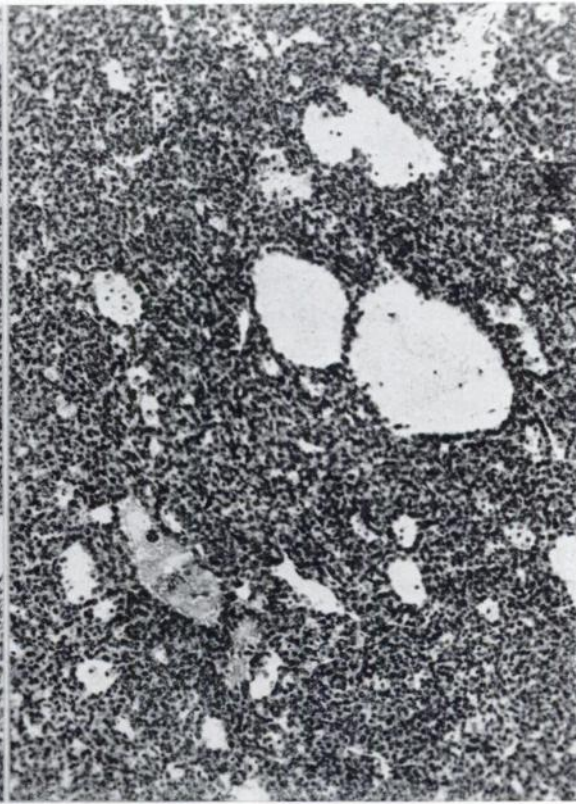


FIG. 4.—A small area of a large granulosa-cell tumor that had arisen in an intrasplenic ovarian graft in an ovariectomized mouse. No follicles were present in this graft. $\times 105$.

sive changes were observed in the two other grafts, although they contained ovarian stroma and few primary follicles.

The urinary bladders were also distended in the female hosts. Occasional cornified vaginal smears were obtained from one female mouse before estrogen treatment was started. The average uterine weight was 149 mg. The histological appearance of the adrenal glands resembled that of the males.

D. OVARIAN GRAFTS IN ANDROGEN-TREATED CASTRATED MICE

Castrated males.—No tumors developed in intrasplenic ovarian grafts in 8 castrated male mice that had been treated with testosterone propionate.

Gonadectomized Mice with Intrasplenic Ovarian Grafts 16 μ gm. α -Estradiol Benzoate Injected per Week

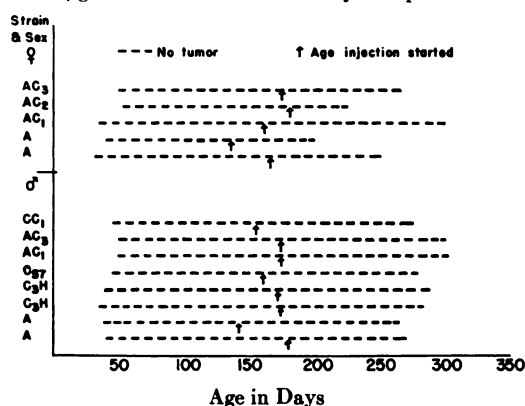


FIG. 5.—For meaning of symbols refer to Fig. 1

The treatment was started 96 to 130 days subsequent to the grafting, and 9 to 17 weekly injections were administered (Fig. 6).

The ovarian grafts ranged from 2 to 6 mm. in greatest diameter. A unique histological feature of these grafts was the increase of fibrous connective tissue in, and vascularity of, the stroma. With the exception of two degenerate grafts, they consisted largely of ovarian follicles and a few atretic and hemorrhagic follicles. No corpora lutea were noted. Tubular and cystic ingrowths of the germinal epithelium were observed in three grafts, and most of these structures contained coagulated fluid and macrophages.

The seminal vesicles and prostates of the androgen-treated male hosts were large, and their average weight was 0.35 gm. No x-zones were noted in the adrenal glands, and groups of small subcapsular cells were present in some areas. The kidneys and submaxillary glands were of the male type.

Castrated females.—Six castrated female mice, bearing intrasplenic ovarian grafts, received 13 to

17 weekly injections of testosterone propionate, starting 101 to 128 days subsequent to the grafting. The ovarian grafts were small and nontumorous (Fig. 6). Their histological structures were similar to those in the male hosts, although more luteinized interstitial cells were present in the stroma. In two grafts, germinal epithelial cells formed papillary ingrowths. Large cysts or tubular cavities were derived from ingrowths of the same type of cell. Extensive degeneration was observed in one of the six grafts.

An estrous vaginal smear was obtained in one female host before the injection of testosterone propionate was started. The average uterine weight of this group was 133 mg. Other organs exhibited evidence of androgenic effects, as in the male hosts.

E. OVARIAN TUMORS IN PROGESTERONE-TREATED CASTRATED MICE

Castrated males.—Five granulosa-cell tumors and two mixed tumors—both granulosa and luteoma cells—arose in the intrasplenic ovarian grafts in 8 castrated males treated with progesterone.

Gonadectomized Mice with Intrasplenic Ovarian Grafts 1.25 mg. Testosterone Propionate Injected per Week

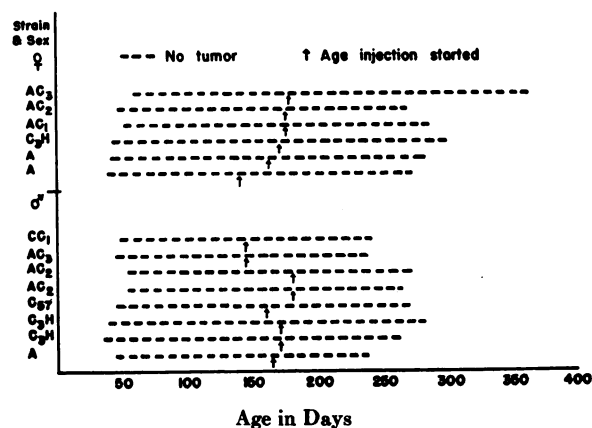


FIG. 6.—For meaning of symbols refer to Fig. 1

The treatment was started 115 to 138 days after the grafting, and 9 to 19 weekly injections were administered (Fig. 7). The largest tumor measured $14.5 \times 15 \times 21$ mm.

In general, the granulosa-cell tumors were partially encapsulated and exhibited diffuse, folliculoid, or trabecular arrangements. Numerous mitotic figures indicated rapid growth. Some folliculoid structures were filled with blood, others with coagulated material containing a few degenerating cells. Luteinized interstitial cells were noted adjacent to small edematous or necrotic areas. Prolifer-

ation and tubular ingrowths of the germinal epithelium were present in four of these tumors. A spicule of bone developed at the periphery of the largest granulosa-cell tumor, and the tumor had metastasized to the liver. This tumor was transplanted subcutaneously into other mice of the same strain (6).

The mixed tumors were composed of masses of granulosa and luteoma cells, in addition to areas of luteinized interstitial cells and ingrowths of the germinal epithelium. The one nontumorous graft was 139 days old and consisted primarily of luteinized interstitial cell stroma and ovarian follicles. Some of the follicles were filled with blood. Four other intrasplenic ovarian grafts with vascularized adhesions were not included in this paper.

The seminal vesicles and prostates of the male hosts were atrophic. The submaxillary glands and the kidneys resembled those of castrated animals.

Castrated females.—Three granulosa-cell tumors, one luteoma, and one mixed tumor developed in intrasplenic ovarian grafts in 5 castrated females treated with progesterone. The treatment was started 127 to 134 days after the grafting; 14 to 19

the other females was 41 mg. Hyperplasia of the small subcapsular cells was noted in three adrenal glands.

F. OVARIAN TUMORS IN GONADOTROPHIN-TREATED CASTRATED MICE

Castrated males.—One granulosa-cell tumor and two mixed tumors were found in intrasplenic ovarian grafts in 5 castrated males treated with daily injections of PMS, starting 100 to 119 days after the grafting (Fig. 8). The largest tumor measured $4 \times 4 \times 6.5$ mm. (Fig. 9). One nontumorous ovarian graft was attached to the peritoneum of the body wall and the other to the small intestines.

The seminal vesicles and prostates of the male hosts were atrophic. The adrenals showed degeneration of the x-zones. The histological structure of

Gonadectomized Mice with Intrasplenic Ovarian Grafts
1 mg. Progesterone Injected per Week

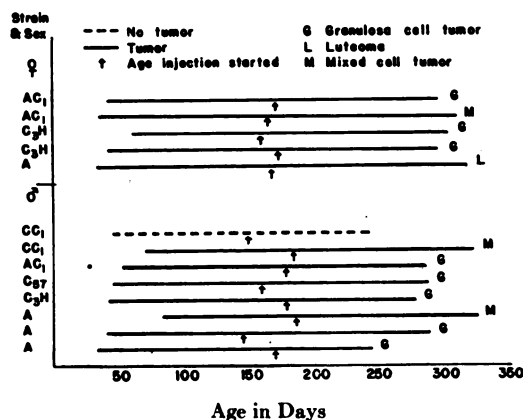


FIG. 7.—For meaning of symbols refer to Fig. 1

weekly injections were administered (Fig. 7). The largest tumor measured $12 \times 15 \times 17$ mm.

Histologically, the granulosa-cell and mixed tumors resembled those in the male hosts, with the exception of more extensive areas of luteinized tissue and necrosis. No metastasis was noted from these tumors.

An estrous vaginal smear was observed once, before the progesterone injections were started, in a female mouse of the C₃H strain that had the largest granulosa-cell tumor, whose uterus weighed 150 mg. at autopsy. The average uterine weight of

Gonadectomized Mice with Intrasplenic Ovarian Grafts
25 I.U. Gonadotrophin (PMS) Injected per Day

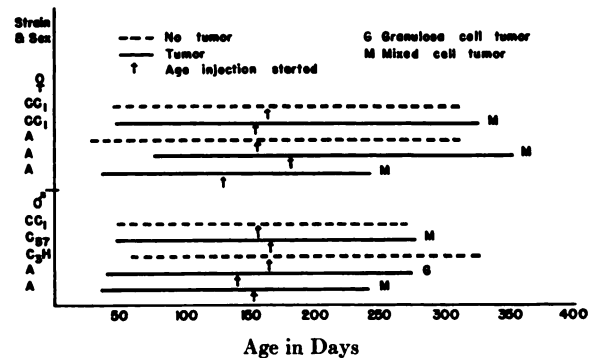


FIG. 8.—For meaning of symbols refer to Fig. 1

the kidneys and submaxillary glands was similar to that of the gonadectomized male mice.

Castrated females.—Three mixed tumors developed in intrasplenic ovarian grafts in 5 castrated females treated with daily injections of PMS, starting 92 to 127 days after the grafting (Fig. 8). The largest tumor measured $5 \times 7 \times 8$ mm.

The histological appearance of the mixed tumors was similar to that of the male hosts similarly treated. In one tumor, spicules of bone were observed in a necrotic area adjacent to the granulosa-tumor cells. Numerous osteoblasts lined the surfaces of the bone matrix, and fibroblasts and macrophages were scattered around the osseous tissue. The two nontumorous grafts were small and consisted of a few ovarian follicles, corpora lutea, and degenerative connective tissue stroma.

Irregular estrous vaginal smears were obtained in one female before the daily injections of PMS were started; no cornified estrous smears were noted in the female hosts following treatment. The

average uterine weight was 42 mg. The x-zone of the adrenal glands was absent. As in the castrated males, the parietal layer of the renal corpuscles were usually composed of low epithelial cells. The terminal tubules of the submaxillary glands in two animals bearing tumorous grafts, however, consisted of columnar cells.

DISCUSSION

The present experiments provide further evidence that pituitary gonadotrophic hormones are involved in ovarian tumorigenesis, at least in the

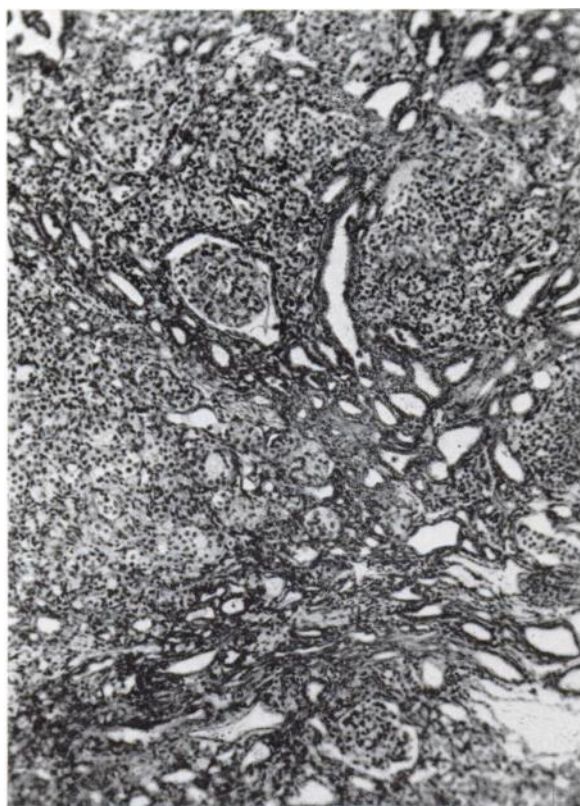


FIG. 9.—A small section of a tumorous graft that had been in the spleen of a castrate male mouse for 268 days. This mouse had received gonadotrophin (pregnant mare's serum). The area of the graft photographed shows epithelial-lined spaces surrounded by partially luteinized cells. The epithelial cords are continuous with the germinal epithelium that partially surrounds all well-developed grafts. Some of the tubules contain cells similar to those in the intertubular areas. $\times 105$.

experimental ovarian tumorigenesis in castrated mice bearing intrasplenic ovarian transplants. The failure of ovarian tumors to appear in the intrasplenic ovarian grafts in unilaterally castrated male and female mice shows that the intact gonads inhibit tumor formation in the transplants. These observations support those made previously that

intrasplenic grafts with extensive adhesions to the body wall or uterus—areas drained by other than the hepatic portal system—did not become tumorous (6, 7). Both the intact gonad as well as the transplanted gonads, if connected with the caval venous system, inhibit tumorigenesis.

Whether the steroid hormones usually presumed to be produced by the gonads—namely, estradiol and testosterone—or some other or unknown substances inhibited tumorigenesis of the transplants was not revealed by experiments of the type referred to above. The failure of ovarian tumors to appear in intrasplenic ovarian grafts in castrated mice that were given injections of estradiol benzoate or testosterone propionate indicates that the endocrine products presumably produced by the normal gonads inhibit the formation of granulosa-cell tumors and luteomas. Progesterone in the amounts used and with the weekly schedule of injection did not alter the incidence of tumors. Larger amounts might be effective. Burrows and Hoch-Ligeti (3) failed to alter the incidence of mammary tumors in mice of the C_3H strain given weekly doses of 1 mg. of progesterone.

The observations on the group of mice that received the gonadotrophic preparation (PMS—Anteron) were inconclusive. Such preparations produce a marked luteinization of the ovaries (12, 14), and the ovaries so treated produce substances that have an androgenic effect (11, 12). At the present time it is assumed that the pituitary stimulation responsible for tumorigenesis in intrasplenic grafts in castrated mice is primarily the follicle-stimulating hormone. It is well known that the urine of postmenopausal women contains increased amounts of a substance that is primarily follicle-stimulating. The occurrence of mixed granulosa-cell tumors and luteomas in the castrate male mice indicates that the gonadotrophin did have some effect, as such tumors are noted rarely among untreated, graft-bearing male mice.

Whether or not the tumors arising in intrasplenic transplants are comparable to those arising in x-rayed mice has been questioned (4). Certainly, they possess a capacity to metastasize to the liver (4, 6), and several of them have been transplanted successfully into other and untreated hosts of the same strains. Intrasplenic ovarian grafts when irradiated did not become tumorous more rapidly than when they were not irradiated (10). The period subsequent to irradiation at which tumors appear is longer than that subsequent to transplantation into the spleens of castrate mice. If the theory that ovarian tumors are induced in irradiated mice by attainment of a hormonal imbalance proves to be correct, then the hormonal factors attained sub-

sequent to irradiation must be less favorable. Irradiated ovaries do produce periodic vaginal cornification in mice; Brambell, Parkes, and Fielding (2) described estrous cycles in mice made anovular by roentgen irradiation. A low level of estrogen production may reduce the increased production of gonadotrophin and hence increase the length of the period of stimulation required.

Unlike tumors of some types, the ovarian tumors appear in mice of all strains that have been studied up to this time. Similar tumors were originally described in rats (1).

The tumors produce estrogen, as the uteri of the hosts bearing them are larger than those of castrated animals. The estrogen must be produced in amounts sufficiently large to permit some of it to pass through the liver or to be of a type not inactivated by the liver. Several animals showed hepatic damage, and one showing the most extensive indications of damage had a large uterus weighing 135 mg.

Adrenal tumors have been described in mice castrated at birth (15, 16) or at later ages (5). Strain differences in the tendency for such tumors to appear have also been described (13, 16). None of the mice in the present study had such tumors. The period of survival was not sufficiently long for them to have been expected. They have been noted, however, in x-irradiated mice (unpublished).

SUMMARY AND CONCLUSIONS

1. Granulosa-cell tumors, luteomas, and mixed tumors (granulosa and luteoma cells) developed in intrasplenic ovarian grafts in castrated male and female mice. There is apparently no strain limitation in the formation of these tumors in mice.

2. No ovarian tumors were observed in the intrasplenic grafts of ovaries in (a) unilaterally gonadectomized mice, (b) gonadectomized mice that received estradiol benzoate or testosterone propionate, or (c) gonadectomized mice with vascularized adhesion that permitted ovarian hormones to by-pass the hepatic portal circulation. The gonadal hormones are assumed to act indirectly by inhibiting the production and secretion of the pituitary gonadotrophic hormones.

3. Weekly treatment with 1 mg. of progesterone did not prevent tumor formation in intrasplenic ovarian grafts.

4. Daily injections of a gonadotrophin from the

pregnant mare's serum (PMS) exerted luteinizing influence on the ovarian tumors.

5. The malignancy of the induced granulosa-cell tumors is shown by the ability to metastasize and the transplantability into new hosts.

6. These experimental results appear to substantiate further the assumption that prolonged stimulation by increased amounts of gonadotrophic hormones is responsible for the genesis of ovarian tumors.

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