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Ovarian Tumors of the Hen

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Present available information regarding ovarian tumors in hens is incomplete in most aspects, and this lack of knowledge hampers use of hens as models for study of ovarian cancer. A study of 466 hens ranging from 2 to 7 years of age and covering a period of more than 3 years has provided much needed information relative to reproductive tract neoplasia. On the basis of this study, it is apparent that hens have a high rate of ovarian tumors, but that such tumors are uncommon in hens less than 2 years of age. Adenocarcinomas with a high degree of morphologic variability are the most common ovarian tumors in hens. Hormonal imbalance does not appear to be a factor in the development of these adenocarcinomas. Steroidogenic and morphologically distinctive granulosa cell tumors originating from follicles in atrophic ovaries represent another common ovarian tumor type. Unique to the hen are oviductal adenocarcinomas. These tumors arise from the albumin-secreting glands of the oviduct, occur with relatively high frequency, and must be differentiated from ovarian adenocarcinomas.

Introduction

There is a general consensus among poultry pathologists that ovarian tumors occur at a high rate among chickens. The use of the hen as a model for studies has been suggested (*1–3*). A review of the information available on the subject indicates that our knowledge of avian ovarian tumors is very incomplete. Much work must be done before we have sound data on tumor incidence, differential diagnosis and the histopathogenesis of different types of ovarian tumors, effects of steroid hormones and fertility on tumor development, and possible causal or promoting factors associated with husbandry practices or with genetic influences. Finally, there has been an almost total lack of work to exploit the hen as an experimental animal in the area of ovarian tumorigenesis. A brief review of the literature pertaining to tumor incidence, diagnosis, and histopathogenesis illustrates some of these points.

Incidence of Ovarian Tumors in Chickens

As with domestic animals in general, it is impossible to get any solid data on the incidence of tumors in chickens. Additional complicating factors, adding to the incompleteness of the data, are that oviductal and ovarian tumors are generally not differentiated, and genital tumors occur mainly in hens older than the ages at which most are slaughtered. In commercial poultry operations, hens are usually killed after their first year of lay, from 22 to 24 months of age. Occasionally they are kept for a second year of lay, and it is among these hens that most field cases are found. Another problem with accepting known incidence rates is that early cases could easily escape detection, creating a bias toward underreporting.

Reports on the incidence of ovarian tumors in birds have come from three main sources: descriptions of field cases submitted to diagnostic laboratories (*4–6*), reports of unusual flock problems (*7–9*), and slaughterhouse condemnation statistics (*10*). Reports from poultry diagnostic laboratories generally give a figure of around 1% of all cases submitted as involving the genital tract. Extremely high rates of genital tumors have been reported in flocks from the United Kingdom, with figures as high as 83% of all birds; however, these were outbreaks of oviductal rather than ovarian cancer (*8–9*). It is doubtful if similar high rates for ovarian cancer in chickens have occurred. Condemnation reports for mature fowl of about 2 years of age may give the best idea of how prevalent genital tumors are in the general poultry population. Causes listed for condemnation of mature fowl by the USDA Inspection Service include 38% from neoplastic disease (*10*), of which the overwhelming majority were of the genital tract (K. Langheinrich, personal communication).

As mentioned, distinguishing between ovarian and oviductal tumors is difficult. Oviductal tumors arise from the albumin-secreting cells of the oviduct, and in well-differentiated cases, the characteristic cytoplasmic granules of ovalbumin are easily identified, but in less differentiated cases of oviductal adenocarcinoma these granules are missing. In addition, both oviductal and ovarian tumors can implant widely throughout the abdominal cavity, and the primary site may be impossible to identify. Electron microscopy (*11*) and identification of ovalbumin (*12*) have been used for specific diagnosis of oviductal tumors.

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Histopathogenesis of Ovarian Tumors

Most descriptions of avian ovarian tumors have been of a few cases seen in diagnostic laboratories (*13–22*). The most extensive review of avian ovarian tumors has been that of Campbell (*23*). Many of his diagnostic criteria were taken from histopathologic descriptions of human tumors. Such comparisons are somewhat tenuous in that there are substantial differences between the histologic structure, as well as the physiology, of avian and mammalian ovaries. In addition, field cases that have provided almost all the material for histopathogenesis studies have been in advanced stages, obscuring earlier, more definitive lesions. The area of study receiving most attention is in the ovarian lesions observed in hens which have undergone sex reversal (*24–30*).

It appears then that the currently available information on avian ovarian tumors is incomplete in every aspect. At the present time, this incompleteness hampers use of hens as a possible model system. More complete studies are needed to provide some basic information, but this requires considerable effort because hens free from common poultry pathogens, including oncogenic viruses, must be observed for a long period of time since tumors occur only in aged birds. It appears, although no real data exist, that there is some genetic predisposition for genital tumors. Therefore, a known susceptible strain of chickens must be studied. Additionally, many birds must be followed to find possible predisposing factors, such as steroid hormone imbalances, because genital tumors occur sporadically in a flock. And finally, environmental conditions must be kept constant.

These considerations were the framework for a study conducted at the University of Connecticut on 466 hens followed for the development of tumors over a period of 3.5 years. Because hormonal factors were thought to be most likely associated with these tumors, a fairly large number of measurements of estrogen, progesterone, and testosterone plasma levels were made. Fertility of individual hens was periodically checked, and finally an effort was made to define the histopathogenesis of ovarian tumors, particularly those in early stages of development.

Materials and Methods

Chickens

White Leghorn hens from three groups were used for this study. The groups were designated as Flocks 29, 30, and 31, and at the start of the study they were 4 to 7, 3, and 2 years of age, respectively. Flock 29 was derived from specific pathogen-free (SPF) stock that had been maintained at the University of Connecticut since 1955. The stock was reproduced by random breeding within a small group of about 50 birds each year.

Flock 30 differed from this original stock in two ways. The original Flock 30 was killed in an accidental flooding, but it was possible to partially maintain the original line by obtaining, from an outside source, F_1 hybrids of females from the original White Leghorn stock and males from another isolated flock of White Leghorns. The hens of Flock 30 were raised for their first year in another location before being housed at the University of Connecticut. Flock 31 was derived by random mating of Flock 30 males and females.

All flocks were free of infection with avian leukosis virus on the basis of COFAL test; however, they were infected with an extremely mild, nontumorigenic strain of Marek's disease virus, which was endemic on the premises. During the entire 3.5 years of the study, no cases of lymphoid leukosis or Marek's disease were observed, nor were any cases of other viral diseases observed. No vaccinations were administered, and the degree of isolation under which the birds were maintained was sufficient to prevent adventitious infection with pathogen microorganisms. All bird were raised in floor pens, given a commercial layer mash and water *ad libitum*, and kept on a 14-hr on, 10-hr off lighting schedule. They were examined daily, and any birds showing tumor development were identified by evidence of ascites or palpable abdominal mass.

Diagnostic Procedures

All hens killed or dying during the experimental period of 3.5 years were necropsied. Tissues, fixed in 10% formalin, were sectioned, stained with hematoxylin and eosin, and examined microscopically. In some cases, tissues were fixed in 2% glutaraldehyde, postfixed in osmium tetroxide, embedded after alcohol dehydration in Epon, sectioned, stained with lead citrate, and viewed by a Phillips 300 electron microscope. Some birds killed during or at the end of the study were classified as having neoplastic disease, although some cases were in very early stages of development and distinguishable only by histologic examination. A number of hens died with nonneoplastic diseases diagnosed at necropsy and verified by histopathologic examination. Ovarian function was judged on the basis of large follicles filled with yolk material, as contrasted with ovaries that had either shrunken and discolored follicles that had undergone involution or atretic follicles that appeared small and pearl-like.

Hormone Assays

Radioimmunoassays for estrogen, progesterone, and testosterone were performed as described elsewhere (*31*). Plasma samples were obtained either from the cubital or jugular vein with a syringe containing 0.25 mL heparin solution (1000 U/mL; Upjohn, Kalamazoo, MI). A total of 5 mL of blood was usually collected, and almost all bleedings were done between 9:00 a.m. and noon. Plasma was stored at $-70°$ C prior to the hormone assays. The time when samples were collected did give a bias for increased progesterone levels for hens that

had oviposited that morning because the progesterone peak accompanying ovulation occurred at about 4:00 a.m. Thus, there was a residual peak for progesterone, which heightened progesterone values of laying hens. Estrogen and testosterone values were not affected by time of sampling. Progesterone and estrogen receptors were measured according to methods described elsewhere (*32*).

Estimates of Egg Production

The total number of eggs laid daily was recorded for each flock throughout the entire study. In addition, during part of each year, the number of eggs laid by each hen was recorded by trapping the hens as they entered nests. Hens were released within about 15 min after oviposition, and their wing tag numbers were recorded. Trap nests were used for 14 months during the observation period of 3.5 years. Observation periods were during July, August, October, and November of the first year, April through November the second year, and March and April of the third year. Birds laying two or more eggs during a trapping period were considered to be fertile.

Biopsy Procedure

Hens were anesthetized with ether and an incision was made on the left side of the abdomen just caudad to and parallel with the last rib. This incision afforded a good view of the ovary and oviduct. In some cases, suspicious-looking follicles were excised from the ovary for histologic examination. The incision was closed with gut suture, and almost all hens had an uneventful recovery. Estimates of the stage of tumor development were based on the following criteria: Stage 1: detectable formation of solid tumors in the ovary; Stage 2: extensive tumors but still restricted to the ovary; Stage 3: extensive ovarian tumors with early abdominal seeding; and Stage 4: late abdominal seeding with extensive tumors in the mesentery and pancreas with ascites. Granulosa cell tumors never went beyond Stage 2, with two exceptions that went to Stage 3. Oviductal tumors, because they initially grew into the lumen, were not grossly visible until they had grown through the oviductal wall, and by this time, almost all of these tumors were at Stage 3 or Stage 4.

Results

Tumor Incidence

Of the 466 hens in this study, 149, or 32%, developed ovarian tumors (Table 1). The number with oviductal tumors was 39, or 8%. In addition, 22, or 5%, had benign leiomyomas of the suspending ligament of the oviduct. Thus, 45% of all hens had tumors associated with the reproductive tract. The majority of these tumors were malignant ovarian adenocarcinomas (24% of all hens). A rather remarkable finding was the extremely low incidence of epithelial tumors from sites other than the reproductive tract. Also, hematopoietic tumors, known to be caused by oncogenic viruses, were not seen. Nonneoplastic diseases occurred in 89, or 19%, and consisted mainly of salpingitis and visceral gout. "Salpingitis" is the diagnostic term used to describe the filling and distention of the oviduct with impacted ova. "Visceral gout" refers to a renal disease in which the tubules have become filled with urate crystals. The other 36% of the hens which were killed had no gross or microscopically discernible lesions. The cumulative diagnostic data are shown for each flock according to age (Fig. 1*A*) and are also broken down into the three main categories of genital tumors (Fig. 1*B*). It is obvious that Flock 29 had a considerably higher incidence of genital tumors than Flock 31; Flock 30 had an intermediate incidence. Tumor incidence continued to increase with age in all three flocks.

Fertility and Development of Genital Tumors

Fertility decreases with age among hens because the period between clutches of eggs increases. This general rule did not apply to all hens: 18 laid up to 7 years of age, one to its 8th year, and another to its 9th. From these observations it appears that there is no finite span of fertility in the hen, and ovarian function appears to be maintained by some hens for less time than others. This was particularly true in this study among hens of Flock 31, most of which went out of lay at 3 years of age. This sudden cessation was possibly associated with a high incidence of salpingitis (Table 1). Hens in Flock 31 did not develop many genital tumors. Comparisons between tumor incidence and fertility are therefore somewhat contradictory.

Hens that did not develop genital tumors were used as the control group for each flock. Comparing the number of eggs laid by hens that did or did not develop genital tumors (Table 2), it appears that fertility had no effect on tumor development. A comparison of flocks shows the disparity between Flocks 29 and 31. At 4 years of age, hens in Flock 29 laid about three times as many eggs as those in Flock 31, with Flock 30 somewhat intermediate. The figures for Flock 31 indicate that many hens were nonlayers throughout all observation periods, and in one trapping period, none of the birds in the flock laid any eggs. In contrast, the continued fertility of many hens in Flock 29 was more or less sustained throughout all six trapping periods.

The average number of eggs laid by hens that went on to develop genital tumors was very similar to those that did not develop tumors. While these data give some idea of fertility among groups of hens within the same flock, they do not indicate the number of hens that laid continuously or over a restricted period of time. This can be estimated by grouping the egg-laying data according to the number of trapping periods in which two or more eggs were laid. These data, grouped for all hens

Table 1. Diagnoses for all hens dying or killed during the 3.5-year study period.

Diagnosis	Flock 29		Flock 30		Flock 31	
	Number of hens[a]	Age[b]	Number of hens	Age	Number of hens	Age
Ovarian adenocarcinoma	79 (39)	6.1	23 (19)	4.2	10 (9)	3.9
Oviductal adenocarcinoma	26 (11)	6.0	9 (8)	4.2	4 (3)	3.8
Granulosa cell tumor	30 (13)	7.0	3 (3)	4.7	0	—
Sertoli cell tumor	4 (2)	6.3	0	—	0	—
Other tumors	5 (2)	7.2	2 (2)	4.0	0	—
Leiomyoma	9 (4)	6.4	10 (9)	4.5	3 (3)	3.7
Salpingitis	13 (6)	6.0	8 (7)	3.4	18 (15)	3.6
Visceral gout	15 (7)	6.6	9 (8)	4.0	3 (2)	3.3
Peritonitis	0	—	4 (3)	4.0	2 (2)	2.5
Trauma	2 (1)	5.0	4 (3)	3.5	2 (2)	3.0
No lesions	47 (20)	6.6	46 (39)	4.2	76 (65)	4.0
Total number of hens	230		118		118	

[a]Number of cases diagnosed; some were diagnosed only microscopically. Numbers in parentheses are the percentage of total number of hens in each flock.

[b]Average age, in years, at the time of death or sacrifice.

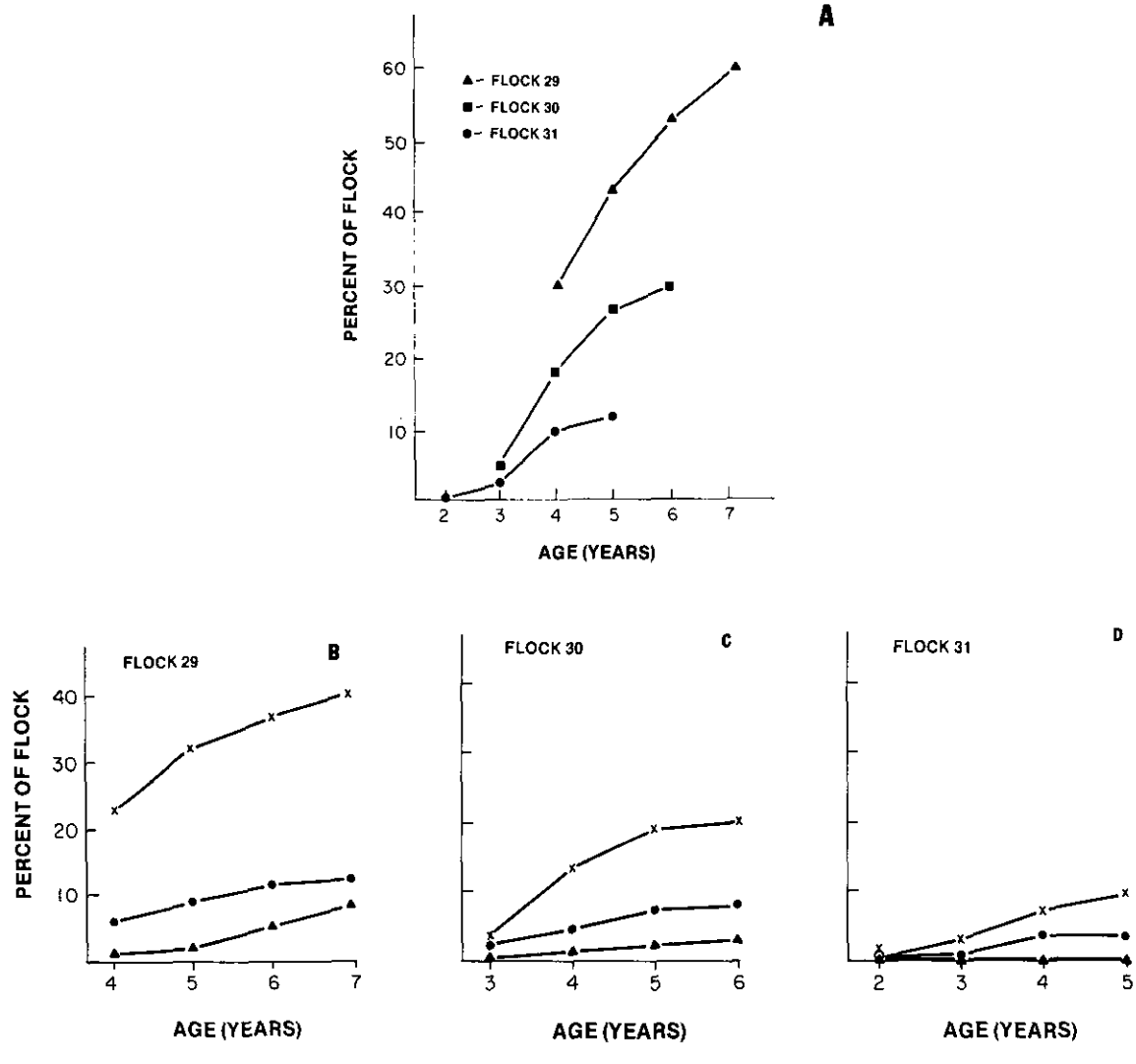

FIGURE 1. (*A*) Cumulative diagnoses of genital tumors in Flocks 29, 30, and 31 according to age in years. Diagnostic records were incomplete prior to year 4 for Flock 29, and a few hens were older than 7 years when the study was concluded. Flocks were killed at the termination of the study during the last year designated for each flock. Tumors were diagnosed in early stages for some birds killed during the study. (*B,C,D*) Individual flock diagnoses for ovarian adenocarcinoma (×); oviductal adenocarcinoma (●); and granulosa cell tumors (▲).

Table 2. Comparison of the average number of eggs laid during each trapping period by hens that did (+) or did not (−) develop genital tumors.

	Flock 29			Flock 30			Flock 31		
	Age,	Tumors		Age,	Tumors		Age,	Tumors	
Trapping period	years	+	−	years	+	−	years	+	−
1	4	18.4	19.1	3	12.4	14.0	2	13.7	11.8
2	4	9.3	9.4	3	10.5	9.9	2	12.8	12.6
3	5	11.4	12.9	4	12.8	13.1	3	6.9	7.2
4	5	14.0	13.7	4	11.3	11.8	3	0.0	0.0
5	6	14.7	15.1	5	9.3	10.3	4	5.0	7.4
6	6	5.2	5.8	5	4.5	4.4	4	0.0	2.5

for which sufficient trapping data were available (Table 3), also show that fertility has little effect on tumor development with one exception, which is not brought out in these data: sex cord tumors almost invariably developed in nonfunctional ovaries.

Relationship Between Hormonal Levels and Genital Cancer

Estrogen and progesterone plasma levels varied considerably within diagnostic groups (Table 4). The only appreciable difference among groups was in estrogen levels for granulosa cell tumors, with a mean about twice that for other diagnoses (Table 4). The extremely high standard error of the mean is mainly because of the positive relationship between tumor mass and plasma estrogen levels. Thus, granulosa cell tumors diagnosed only microscopically did not have high levels of estrogen. Estrogen levels in oviductal adenocarcinoma were moderately depressed. Within diagnostic groups there was also a lot of variation in progesterone levels (Table 4), but cases of leiomyoma and salpingitis were higher in diagnostic groups as compared with controls. Ovarian adenocarcinomas were not associated with abnormal levels of either hormone.

Comparing levels of estrogen, progesterone, and testosterone in hens having ovarian tumors with fertile or infertile nontumorous hens, two differences are evident (Table 5). Estrogen levels are high for hens with granulosa cell tumors, as was progesterone for laying hens. The estrogen levels for the granulosa cell tumors are higher than for the same group in Table 4 because they represent those hens with advanced tumor size. High progesterone levels would be expected for laying hens because of the preovulatory surges of this hormone (*31*). It appears that age is another reason for such variability among groups. As hens grow older, hormone levels decrease. Since multiple plasma samples were taken for many hens, some idea can be gained of hormonal levels prior to tumor diagnosis. An example of a hen with ovarian adenocarcinoma that was sampled 11 times over a period of 8 months, prior to dying from a Stage 3 ovarian adenocarcinoma, is given in Table 6. This sort of profile is fairly typical of hens that developed ovarian adenocarcinoma and had relatively average to low levels of both estrogen and progesterone. This profile differs from that of a hen with a large granulosa cell tumor (Table 7). It is unclear whether the high levels of estrogen seen in hens with large granulosa cell tumors were preceded by severe aberrations from normal, because no hen developing a large granulosa cell tumor was followed for a sufficiently long period of time. Several hens that developed small granulosa tumors were followed for up to 2 years, but they only had microscopic tumors when they were sacrificed. These hens had low to moderately elevated levels of estrogen and progesterone compared to hens without lesions.

Table 3. Comparison of the number of fertile trapping periods and tumor development for hens observed over all trapping periods.

Number of fertile trapping periods[a]	Number of hens[b]	
	Tumorous	Nontumorous
0	14 (22)	29 (15)
1	9 (14)	25 (13)
2	2 (3)	19 (10)
3	17 (27)	45 (23)
4	14 (22)	57 (29)
5	5 (8)	14 (7)
6	2 (3)	10 (5)
Total	63	199

[a]A hen was judged fertile if it laid two or more eggs during a trapping period.
[b]Numbers in parentheses are the percentage of total.

Table 4. Plasma estrogen and progesterone levels according to diagnosis.

Diagnosis	Average plasma levels (pg/mL)	
	Estrogen	Progesterone
Ovarian adenocarcinoma	252 ± 22[a] (31)[b]	495 ± 60 (36)
Oviductal adenocarcinoma	191 ± 43 (11)	647 ± 112 (14)
Granulosa cell tumor	664 ± 187 (12)	397 ± 58 (13)
Other tumors	384 ± 103 (4)	617 ± 146 (4)
Leiomyoma	217 ± 24 (7)	876 ± 324 (9)
Visceral gout	260 ± 44 (9)	477 ± 110 (13)
Salpingitis	240 ± 88 (9)	911 ± 269 (10)
No lesions	300 ± 49 (38)	523 ± 55 (44)

[a]Mean ± SE.
[b]Numbers in parentheses indicate number of cases.

Ovarian Adenocarcinoma: Gross and Microscopic Morphology

Ovarian adenocarcinomas in their early stages of growth are seen as nodular, very firm, white growths

Table 5. Amounts of total estrogen, progesterone, and testosterone in the plasma of hens with or without ovarian tumors compared with nontumorous hens.

Number of hens	Description	Number of samples	Hormone plasma levels in pg/mL[a]		
			Estrogen	Progesterone	Testosterone
49	Nontumorous (in production)	59 112 74	213 ± 191	889 ± 1072	67 ± 43
86	Nontumorous (out of production)	247 277 49	272 ± 230	498 ± 600	138 ± 260
28	Adenocarcinoma	114 170 29	278 ± 182	446 ± 419	99 ± 210
7	Granulosa cell tumor	14 29 18	1211 ± 857	406 ± 319	78 ± 148
5	Sertoli cell tumor	9 15 8	286 ± 149	333 ± 390	65 ± 40

[a]Values are mean ± SE.

Table 6. Sequential plasma estrogen and progesterone levels in a hen with ovarian adenocarcinoma.

Date of sampling	Estrogen, pg/mL	Progesterone, pg/mL
4–22	382	428
4–27	285	698
5–4	389	333
6–14	302	572
6–24	440	432
8–16	271	253
9–2	393	118
10–7	190	266
10–20	281	168
11–1	130	126
11–9	185	83

Table 7. Sequential plasma estrogen and progesterone levels in a hen with a granulosa cell tumor.

Date of sampling	Estrogen, pg/mL	Progesterone, pg/mL
4–1	438	765
8–16	ND	257
9–2	1042	ND
10–7	2046	521
10–20	1493	218

ND = not done.

that resemble atretic follicles (Plate 1). Ovarian adenocarcinomas are less symmetric than atretic follicles and may be partially buried within the ovarian stroma or growing on the surface of follicles (Stage 1). With progressive growth, these tumor nodules coalesce so that the ovary loses maturing follicles and resembles a cauliflower with irregular masses bulging from the central tumor, which usually replaces all the normal ovarian structures (Stage 2). Such ovaries seed the abdominal cavity with tumor cells, and large numbers of individual foci (Plate 2) start to grow on serosal surfaces of the oviduct, mesentery, and intestines, and on the pancreas (Stage 3). Presumably this seeding process is facilitated by the fact that the ovary lies dorsad, immediately under the vena cava, so that as tumor cells break off, they have multiple chances for implantation. Growth of such implanted cells appears to be rapid, with pronounced reaction of the muscularis of the oviduct and intestine. As the mesentery contracts and the bowel wall thickens, ascites develops, so that often 500 mL of fluid may be withdrawn from the abdominal cavity (Stage 4). Occasionally, cystic structures project from the surface of the ovary. These look like follicles filled with clear, amber-colored fluid. Although they are not tumor structures, they are invariably associated with the development of ovarian adenocarcinomas. Although adenocarcinomas are highly malignant, they are not hematogenously borne to distant sites, and even such sites of implantation as the liver or spleen rarely are affected.

The earliest histologically detectable structures are small groups of cells forming small round acini. These are found either in the ovarian stroma or growing near or within the theca externa (Plates 3 and 4), and in some cases, early acini look somewhat like thecal gland cells (Plate 5) (*33,34*). Individual acini often enlarge and become slitlike (Plate 6), or a cribriform pattern predominates (Plate 7). In some tumors, individual acini are separated by wide bands of dense fibrous connective tissue (Plate 8). A characteristic of all these forms is the single layer of low columnar or cuboidal cells surrounding the lumen. Ultrastructurally the layer of cells forms a tight adherent ring of cells joined along their apical borders by prominent desmosomes. Their most notable feature is the presence of short microvilli projecting into the acinar lumen, which contains variable amounts of moderately electron-dense material (Plates 9 and 10). There are, however, other forms that the tumor takes, including nests of darkly staining cells either within the ovarian stroma (Plate 11) or as loose clusters attached to the cortical surface (Plate 12). Ul-

trastructurally such cells appear to have undergone degeneration with formation of cytoplasmic vacuoles and densely osmophilic, irregularly shaped inclusions (Plate 13). Glandular forms of tumor cells are also seen (Plate 14). Another infrequently seen structure is nests of cells that appear to have undergone some degree of squamous metaplasia, with intervening areas containing structures similar to acini with transitional forms in adjacent areas (Plate 15).

Granulosa Cell Tumor: Gross and Microscopic Morphology

The gross appearance of granulosa cell tumors is distinctly different from ovarian adenocarcinoma. The external surface is smooth, covered by a glistening capsule, and the general shape is ovoid with some degree of lobulation. Instead of the gray color of the adenocarcinoma, the granulosa cell tumor tends to be yellow. Old and new hemorrhages are often a feature because they are very friable. Tumors are often attached to the hilus of the ovary by a thin stalk. Although they may grow to enormous size, occupying a large portion of the abdominal cavity (Plate 16), they very seldom implant within the abdominal cavity.

Histologically there appears to be distinct progressive steps leading to the formation of large granulosa cell tumors. The first stage is the accumulation, usually within localized areas in the cortex, of small round follicular structures (Plates 17 and 18) composed of granulosa cells surrounded by a thin capsule. Identification of granulosa cells was made possible by the presence of so-called transosomes (Plate 19), an ultrastructural feature unique to avian granulosa cells (*35*,*36*). These follicles do not contain oocytes, and as they continue to grow, they tend to form cordlike structures (Plate 20). With progressive growth there is a general loss of follicular structures so that large tumors are composed of granulosa cells forming sheets of cells interspersed by a delicate, vascular stroma, with individual tumor cells loosely joined (Plate 21). Transosomes continue to be seen, sometimes in large numbers in the tumor cells (Plate 22). In some tumors this progression of growth from isolated follicles to sheets of granulosa cells is not so orderly. Mixed among the follicles in early stages may be thecal gland (Plate 23) and tubular structures (Plate 24). It is from such tubular structures that well-defined Sertoli tumors (Plate 25) develop. In some areas foci of granulosa cell proliferation and tubular structures may be seen in the same ovary.

Differentiation of Ovarian from Oviductal Adenocarcinoma

Because of the aggressive nature of oviductal adenocarcinoma, which implants on the ovary as well as on the mesentery and pancreas, it is often difficult to be sure whether the primary tumor is of ovarian or oviductal origin. Implants of these carcinomas have a similar gross appearance; the characteristic eosinophilic cytoplasmic granules, presumably ovalbumin, are often not seen in oviductal adenocarcinomas, and acinar structures are characteristic of both types of adenocarcinomas. Also, in some cases, it appeared as though both ovarian and oviductal tumors developed in the same hen. An attempt was made to reduce misdiagnosis by carefully examining the oviductal mucosa. If typical sessile tumor masses were present in the luminal mucosa, the case was diagnosed as an oviductal adenocarcinoma, but tumors growing only on the serosal surface were presumed to have been ovarian in origin.

Differentiation of Ovarian Adenocarcinoma from Leiomyoma

Leiomyomas grow as firm, circumscribed, nodular masses composed of smooth muscle tissue with few mitotic figures and are benign. They were most often seen in the supporting ligament of the oviduct and less frequently as small nodules projected from the wall of the oviduct or intestines. Their size, shape, consistency, and location were quite different from ovarian tumors.

Discussion

Tumor Incidence

This study for the first time allows some definition of the incidence and types of neoplastic disease in chickens that were allowed to live out a considerable portion of their potential life-span. Previous reports have dealt mainly with field cases from flocks for which there was relatively little background information and which were composed of hens 3 years old at the most. Nevertheless, observations from field cases have been corroborated by this study. There is indeed a unique propensity for hens to develop cancer of the reproductive system in the almost total absence of tumors at other sites. Freedom from tumors induced by oncogenic viruses helped make this observation quite clear.

There are several observations regarding the data presented on tumor diagnosis. It is clear that the number of cases per year did not appreciably decrease but continued to climb at a steady rate, especially among hens of Flock 29. Thus, there appears to be no age protection in development of genital tumors. What is perhaps not very clear is that the number of benign granulosa tumors becomes an increasingly large proportion of the total number of cases with progressive age. Increased age, therefore, appears to increase risk, but it was considered a possibility that ovarian tumors simply grew at slow rates, and many are initiated earlier than our results indicated. For this reason a series of biopsies was done. The indications obtained from biopsies done on hens prior to their development of ovarian tumors was that some tumors grew very quickly, while others appear to have been fairly indolent. It does appear, however, that if ovarian tumors often started to grow in the pullet year, then many more ought to have

been seen in younger hens in this study and in the field among hens inspected in slaughterhouses when they are killed at 2 years of age.

Difference in the Number of Genital Tumors among Flocks

An unexpected finding was the disparity of the number of tumors among flocks. Flock 29 had about five times the tumor incidence of Flock 31. Possible explanations for this, other than the relative ages of each flock are: possible genetic differences, as Flock 31 was not a direct descendant of Flock 29; difference in fertility patterns; and more frequent occurrence of nonneoplastic disease, especially salpingitis, in Flock 31. One possible cause that can be ruled out is difference in feed and environmental conditions. All hens were raised in the same house and fed and maintained under similar conditions. Flock 29 was much more similar to previous flocks with regard to the incidence of genital tumors, and Flock 31 was an exception.

Fertility and Development of Ovarian Tumors

Despite a considerable effort to relate fertility to genital cancer, two entirely contradictory results were obtained. On the one hand, Flock 29 was the most fertile and had the greatest number of cases of cancer. Flock 31 was the least fertile and had the fewest number of cases of cancer. Yet within Flock 29 the tendency for a hen to remain in production appeared to be protective (data not shown). Few cases of cancer occurred when the ovary remained functional. An observation supporting the idea that in otherwise healthy hens infertility predisposes to cancer was that, with few exceptions, ovarian tumorigenesis was preceded by loss of mature follicles. This was the case for both adenocarcinomas and granulosa cell and Sertoli cell tumors of the ovary. In establishing this relationship, early cases were essential because advanced tumor growth replaced most of the normal ovarian tissue, and birds with the clinical aspects of advanced tumor growth and abdominal seeding would be expected to go out of production because of malnutrition. However, even in cases with minimal tumor growth, follicles were usually atretic, with the exception of 10 cases where hens had mature or intact follicles.

Sex Steroids and Development of Ovarian Tumors

Levels of steroid sex hormones were followed throughout most of the study period to find out if alterations from normal levels preceded development of tumors. There are several problems in assessing the data; the greatest problem is the variation from sample to sample for the same bird. This is reflected in the overall tabulation of the average levels for progesterone and estrogen that gave a large standard error of the mean. There appeared to be no discernible trends with time for birds developing adenocarcinoma from their first to last plasma assays. The one exception to this rule was that the high estrogen levels for hens with granulosa cell tumors reflected their steroidogenic capacity. Small tumors or earlier samples from birds with large tumors did not show above-normal levels of estrogen. Elevated levels were seen when the tumor had grown to an appreciable size.

The difference in progesterone levels when comparing nontumorous hens to all others is apparent. This is because of maintenance of their ovulatory cycle. There was no indication that testosterone values varied among groups. It is not known what the source of estrogen is in old, infertile hens. The thecal glands of the ovary have been implicated (*33*,*34*); however, follicular loss accompanying senescent sterility would reduce the number of glands. Thus, an extraovarian source of estrogen seems likely. The relatively even plasma levels of estrogen were consistent for all groups, and the role of the hormone in birds with ovarian atrophy or cancer is difficult to understand. It is known that tamoxifen, an antiestrogen drug, causes follicular involution and sterility, as well as oviductal atrophy. Yet sterility and oviductal atrophy occur among old hens, although plasma estrogen levels are similar to those of young hens. This must mean that estrogen cytoplasmic or nuclear receptors or possibly receptors for progesterone are dysfunctionally processed in ovarian and oviductal tissue. Indications are that progesterone receptors are those most affected by age, and this may be a factor in development of cancer in either organ. Cytosolic and nuclear estrogen receptors and cytosolic progesterone receptors were measured in seven ovarian adenocarcinomas (*32*). Data from these studies revealed no pattern for the number of receptors in any type of tumor. The wide variation was in contrast to the relatively uniform numbers measured in oviducts of nontumorous hens.

Histogenesis of Ovarian Tumors of the Hen

In women, about all of the malignant ovarian tumors are thought to be derived from the germinal epithelium (*37*). Those of the chicken are from an unknown derivation, but there are several possible sources. One source may be the germinal epithelium, although no clear-cut ingrowths of these cells into the ovarian stroma have been observed. Also, the structure of the ripening follicle and its swift postovulatory atrophy make it difficult to see how infolding could occur. However, there is a moderate degree of ultrastructural similarity to germinal epithelial cells, and the malignant nature of the ovarian adenocarcinoma cells is consistent with such an origin.

Another possible cell of origin is suggested by the frequent development of adenocarcinoma cells in the thecal area with what could be interpreted as transitions of thecal glands to tumorous acini. Thecal cells have a role similar to interstitial cells in the mammalian ovary

and are considered to be sources of estrogen. However, lack of any detectable steroidogenic or ultrastructural similarity makes it doubtful that ovarian adenocarcinomas arise from the cells of the thecal gland.

Another possible structure which could be implicated as the origin of adenocarcinomas is the mesonephros. There is a close similarity in the histologic appearance of cells lining the tubules of the mesonephros and the neoplastic acini. The initial location of the adenocarcinomas is, however, in the ovarian cortex and not near the mesonephros. Thus, the question of the cellular origin of avian adenocarcinomas remains open to further investigation.

With regard to sex cord tumors, there appears to be a lot of similarity between chickens and mammals. Ovarian atrophy seems to be a predisposing factor. Cortical accumulations of sterile follicles, and less frequently, tubules and interstitial cells, go on to form either granulosa cell or Sertoli cell tumors. In chickens the granulosa cell tumors are identified by the presence of transosomes, although steroidogenesis is toward production of progesterone, according to *in vitro* studies (*3,38*). Transition between the follicular pattern to that of the well-developed tumor is clear-cut. The Sertoli cell tumors, for which no steroidogenic activity was found, have a typical tubular morphology, perhaps because of their small size. A possibly interesting line of study would be to exploit the vestigial right ovary (*39*) as a site in which sex cord tumors could be experimentally induced. Surgical and radioactive ablations of the functional left ovary have resulted in sex cord tumors, but the amount of experimental work done so far is extremely limited.

Conclusions

The following conclusions are drawn from this study: 1) Hens do have a high rate of ovarian tumors; however, the rate observed in this study may not be representative of chickens of other strains and breeds. Possibly, there were more ovarian as opposed to oviductal tumors than usually occur in hens. 2) Ovarian tumors are unusual in hens less than 2 years of age. 3) Adenocarcinomas were most commonly observed, and sex cord tumors were present in a few older hens. 4) Adenocarcinomas often have a highly variable morphology. This is, however, not representative of different tumor types but of some degree of histologic variability of the same cell type. Granulosa cell tumors are steroidogenic, generally benign, and have a distinctive morphology. Their histogenesis is clearly from follicles appearing in atrophic ovaries. These studies do not implicate hormonal imbalances as a factor in the development of adenocarcinomas.

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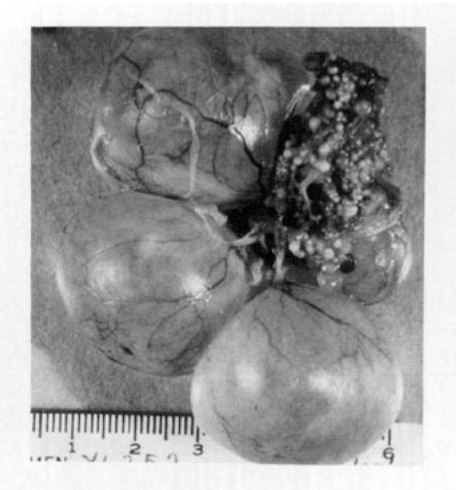

PLATE 1. Hen 2926 from Flock 29, killed at 5 years of age because of visceral gout, had atretic ovarian follicles typical of a hen out of production. The large cysts were nonneoplastic. The oviduct was filled with caseous material and had no tumors associated with it. This was one of the earliest cases of ovarian adenocarcinoma observed in this study and represents Stage 1. Shown just under life size. H & E.

PLATE 2. Hen 3362 from Flock 30 died at 4 years of age as a result of hemorrhage from ovarian biopsy site. The massive, glistening, firm tumor implants throughout the abdominal cavity are typical of ovarian adenocarcinoma, Stage 3. Note the cauliflowerlike primary tumor (←). Shown one-half of life size. H & E.

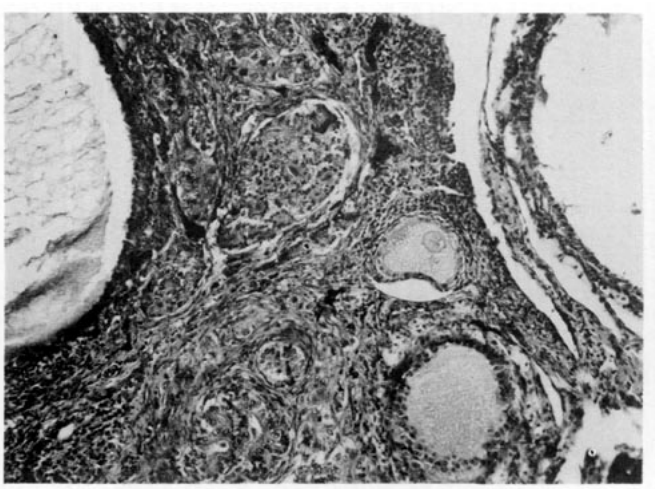

PLATE 3. Adenocarcinoma (hen 2926) composed of isolated small nests (→) lying between atretic follicles. This area was the only one in which a tumor was detected, and it represents one of the earliest adenocarcinomas. H & E, ×88.

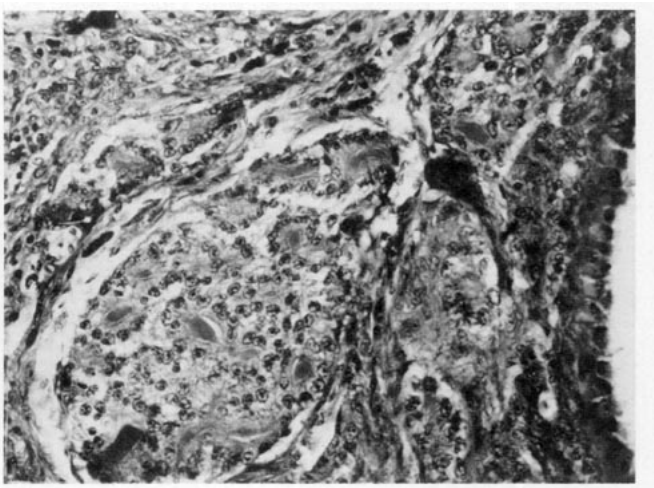

PLATE 4. Adenocarcinoma (hen 2926). The tumor nests are collections of small acini containing eosinophilic material. They are lined by a single layer of cuboidal to low columnar cells. The granulosa cells of a neighboring follicle at the right are clearly different in structure. H & E, ×352.

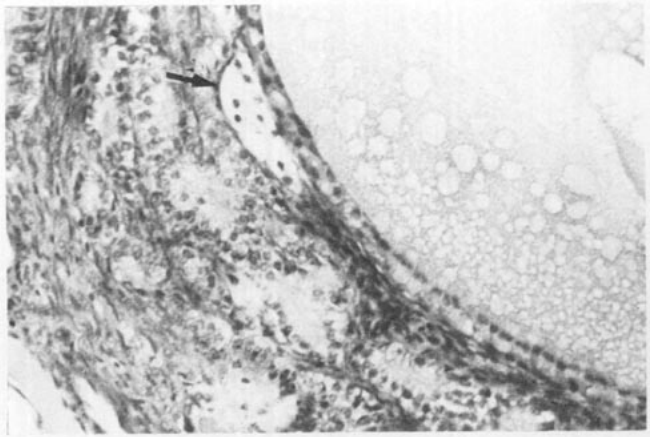

PLATE 5. Hen 3302 from Flock 30 killed at 4 years of age with an early ovarian adenocarcinoma, Stage 1. The oviduct was atrophic and without tumors. A thecal gland (→) is shown next to the granulosa cell layer of a small follicle; irregularly shaped acinar structures composing the tumor are scattered through the theca externa. H & E, ×882.

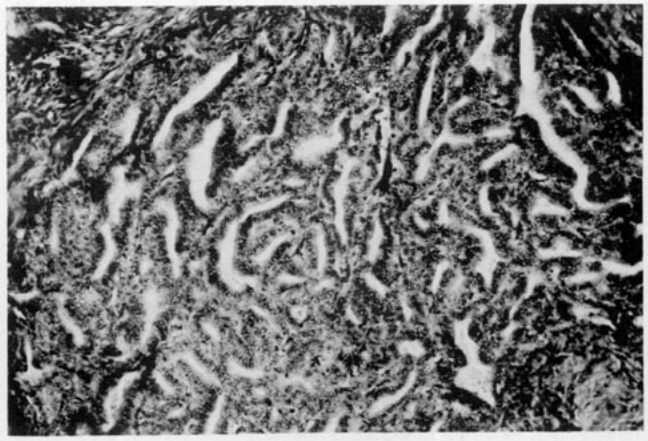

PLATE 6. Hen 2384 from Flock 29 killed at 6 years of age with an ovarian adenocarcinoma, Stage 4. The tumor is composed of single layers of cuboidal cells lining slitlike spaces. H & E, ×147.

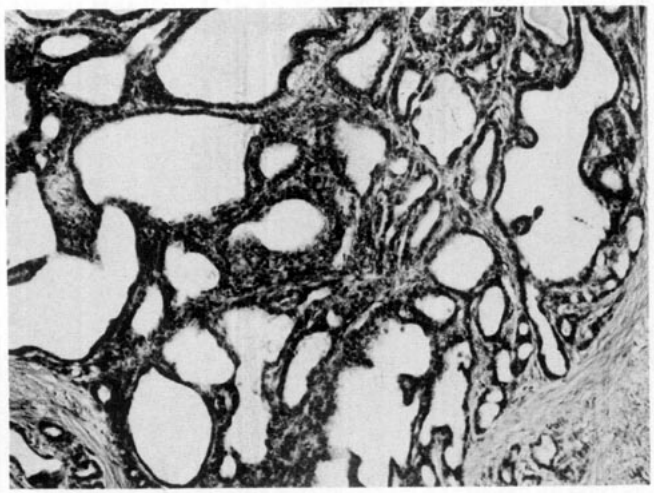

PLATE 7. Hen 2944 from Flock 29 killed at 5 years of age with an adenocarcinoma, Stage 3. This portion of the tumor was composed of a single layer of cuboidal cells lining widely dilated, empty spaces in a cribriform pattern. H & E, ×140.

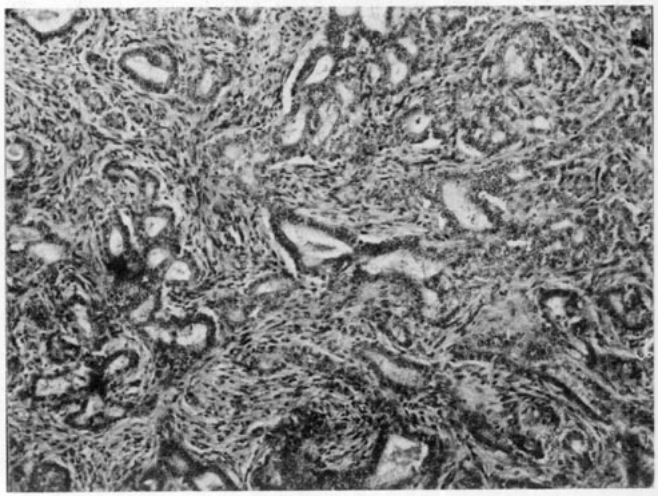

PLATE 8. Hen 2384. Acini separated by a connective tissue stroma composed of fusiform cells with dark staining nuclei. H & E, ×144.

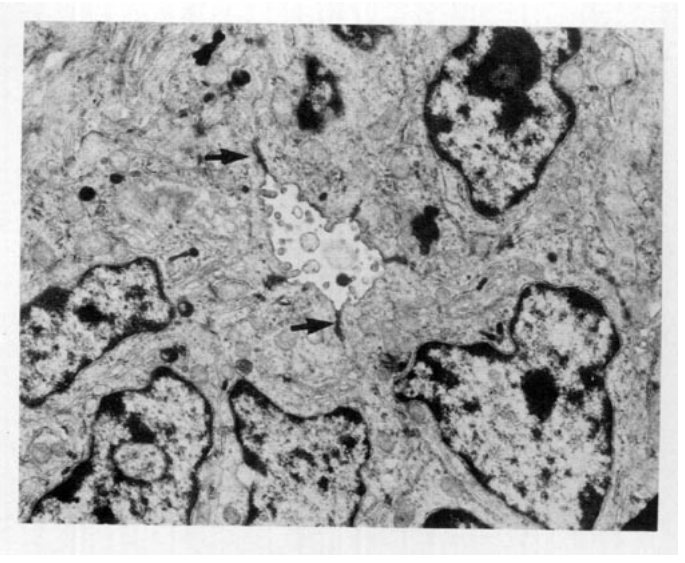

PLATE 9. Hen 2923 from Flock 29 killed at 7 years of age with an adenocarcinoma, Stage 3. In this electron photomicrograph of a typical acinar structure, the desmosomes are clearly defined (→); note the short microvilli. There are relatively few polyribosomes and a sparse amount of smooth and rough endoplasmic reticulum. H & E, ×9400.

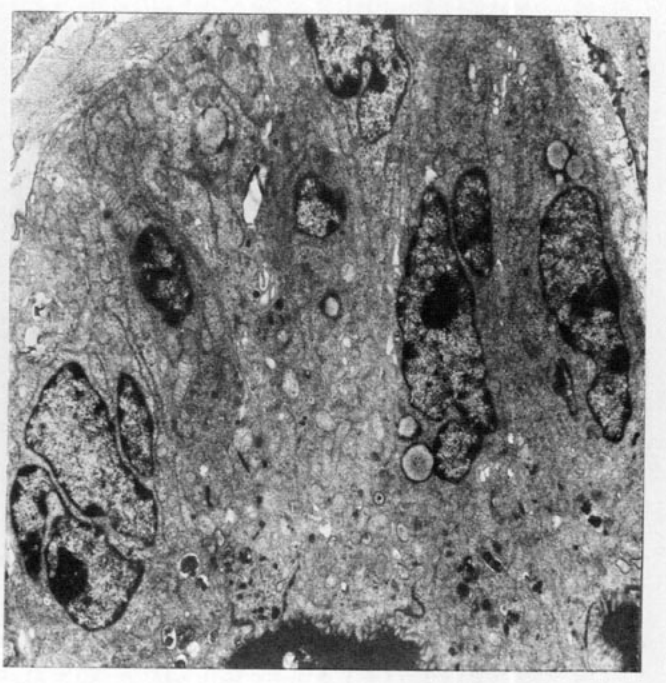

PLATE 10. Hen 3026 from Flock 29 killed at 6 years of age with an adenocarcinoma, Stage 3. An acinar structure is present similar to that shown in Plate 4 is lined with columnar cells, and the lumen is filled with electron-dense material. Dense bands of collagen surround the tumor cells. H & E, ×4320.

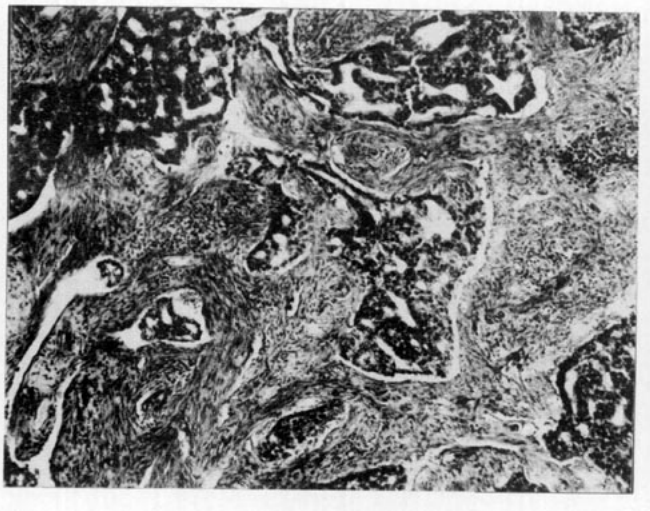

PLATE 11. Hen 2944. Small, darkly staining tumor cells arranged in irregular patterns filling spaces between bands of dense connective tissue. H & E, ×138.

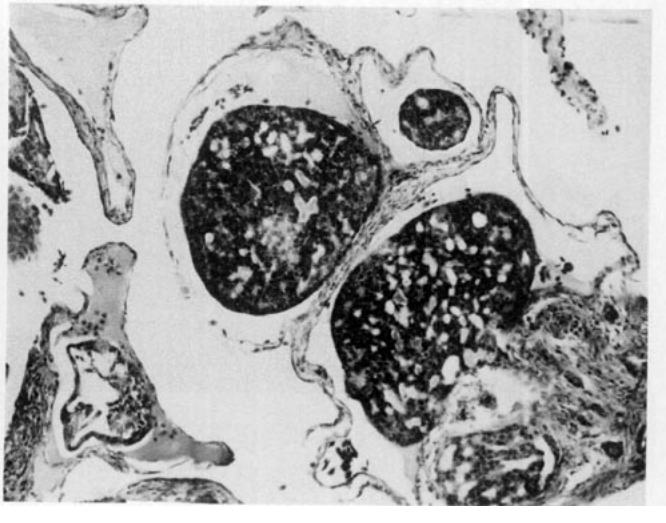

PLATE 12. Hen 2944. Spherical masses of small tumor cells, similar to those in Plate 11, projecting from the surface of the ovary and enclosed within a fluid-filled, cystic structure. H & E, ×140.

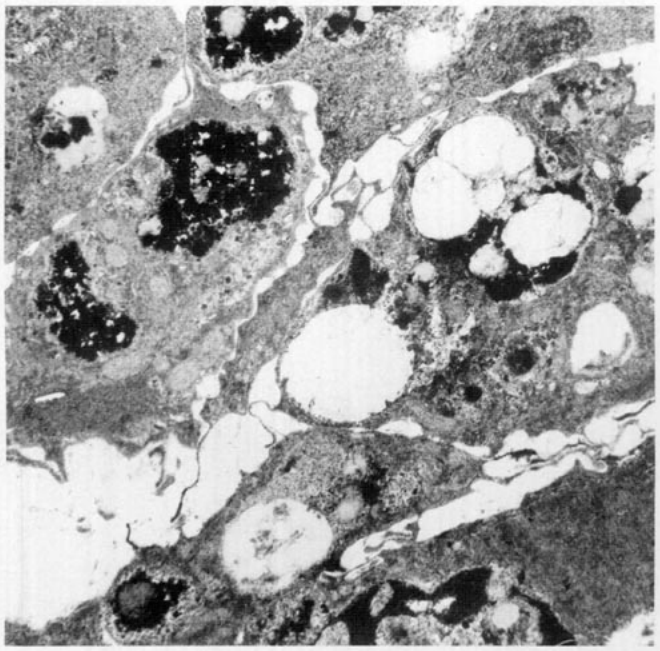

PLATE 13. Hen 3371 from Flock 30 killed at 4 years of age with adenocarcinoma, Stage 3. An electron micrograph of cells similar to the small, darkly staining cells is shown in Plates 11 and 12. Severe degenerative changes have taken place, with formation of vacuoles and irregular, very electron-dense masses within the cytoplasm. H & E, ×16,730.

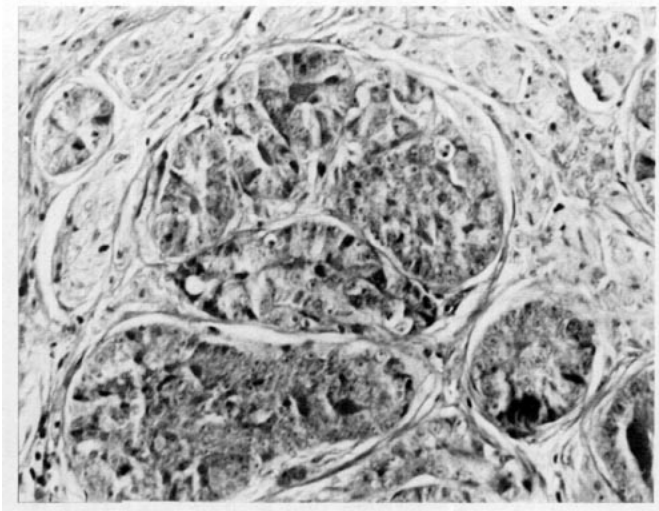

PLATE 14. Hen 2955 from Flock 29 killed at 6 years of age with adenocarcinoma, Stage 1. Tumor cells are arranged in a glandular pattern separated by bands of dense stroma. H & E, ×360.

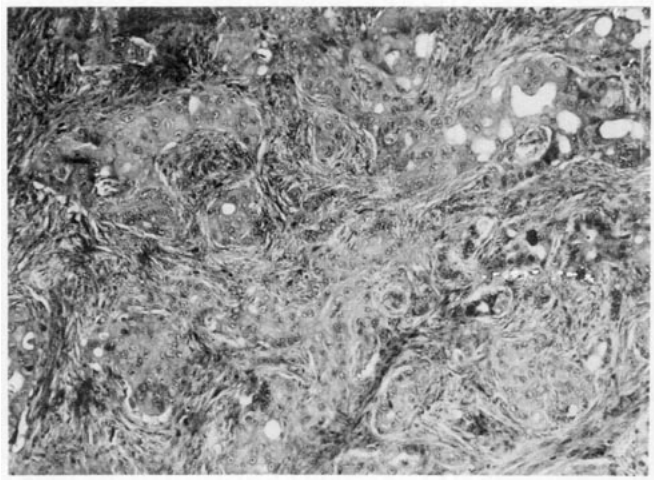

PLATE 15. Pronounced atypia of acinar cells with transition to a squamous type. It should be noted that this adenocarcinoma contained a variety of phenotypes (Plates 7, 11, 12, 15), which are believed to represent different forms of the same tumor. H & E, ×140.

PLATE 16. Hen 3049 from Flock 29 killed at 5 years of age with a large granulosa cell tumor. The oviduct (not shown in this photograph) was the size of the oviduct of a laying hen. Although there were microscopic implants on the intestinal serosa, no macroscopic tumors can be seen. Note the large hematomas on the surface of the tumor. Shown 0.8 of life size. H & E.

PLATE 17. Hen 3068 from Flock 29 killed at 7 years of age with two small ovarian tumor masses which proved to be granulosa cell tumors. There were no maturing follicles in the rest of the ovary but rather large numbers of individual follicles, not containing oocytes (→), scattered under the germinal epithelium and through the ovarian stroma. H & E, ×93.

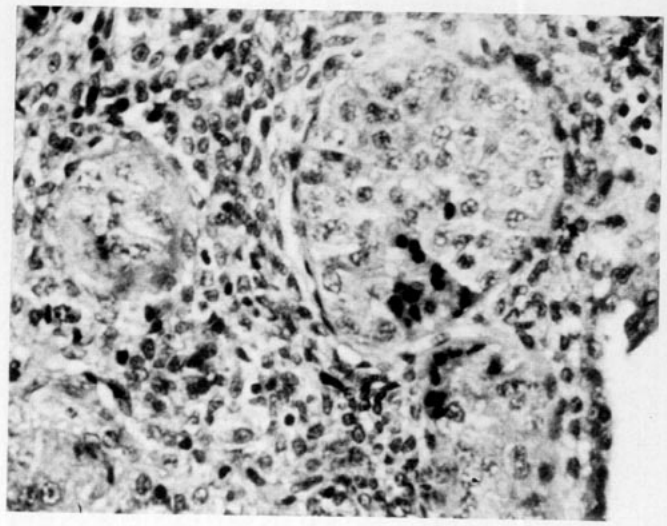

PLATE 18. Hen 3068. Typical appearance of follicular structures, surrounded by a cellular stroma, next to the germinal epithelium. H & E, ×368.

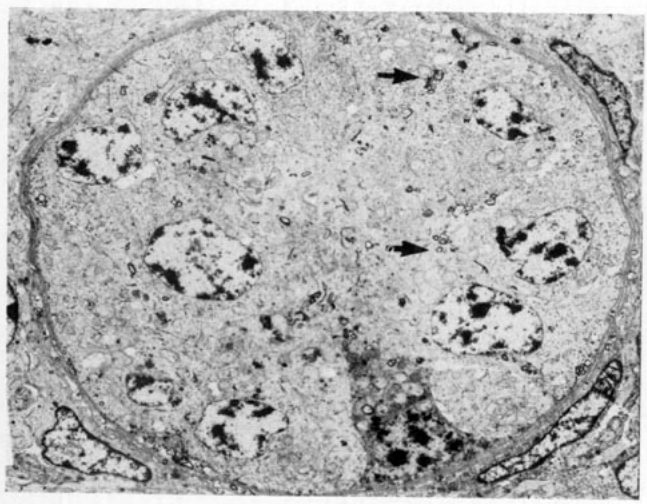

PLATE 19. Hen 1896 from Flock 29 killed at 10 years of age with a small, cystic granulosa cell tumor. The round follicular structure contains granulosa cells identified by the presence of transosomes (→). H & E, ×8100.

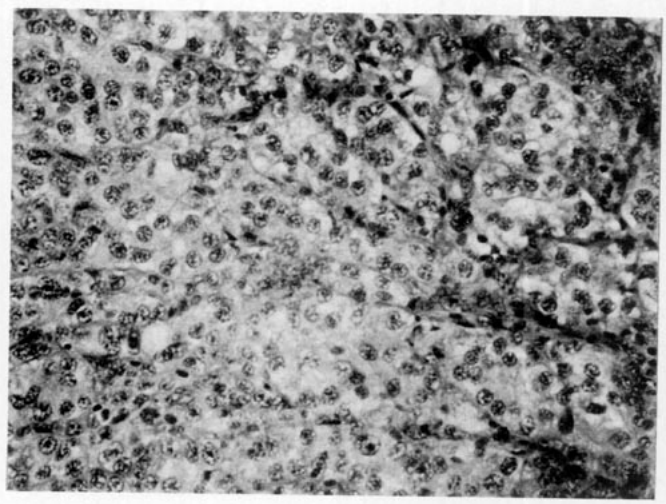

PLATE 20. Hen 3124 from Flock 30 killed at 4 years of age with a large granulosa cell tumor which had implanted onto the serosal surfaces of the intestines. The primary tumor was typical, with sheets of uniform granulosa cells interlaced by a delicate stroma. Some other areas of the tumor were necrotic and hemorrhagic. H & E, ×352.

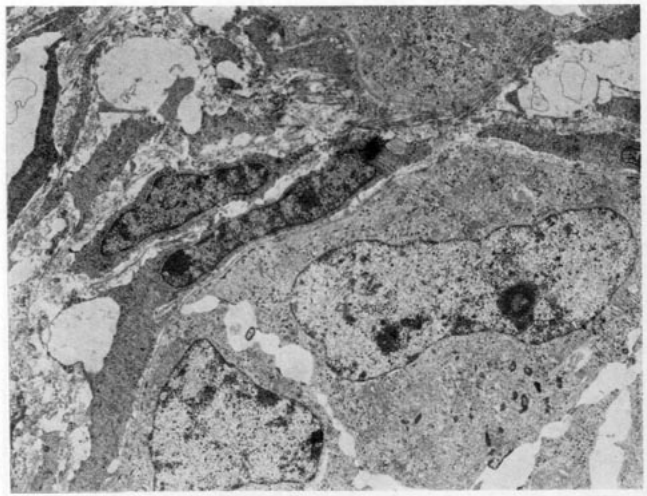

PLATE 21. Hen 1906 from Flock 29 killed at 10 years of age with a small granulosa cell tumor. The granulosa cells, containing transosomes, numerous polyribosomes, and ovoid mitochondria, are separated by a capillary. H & E, ×8280.

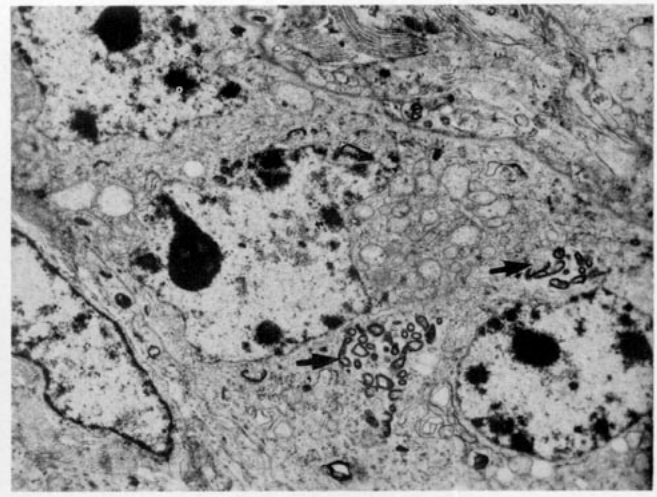

PLATE 22. Hen 1896 showing internal transosomes (→) in granulosa tumor cells. Bundles of collagen are shown in upper right. H & E, ×7500.

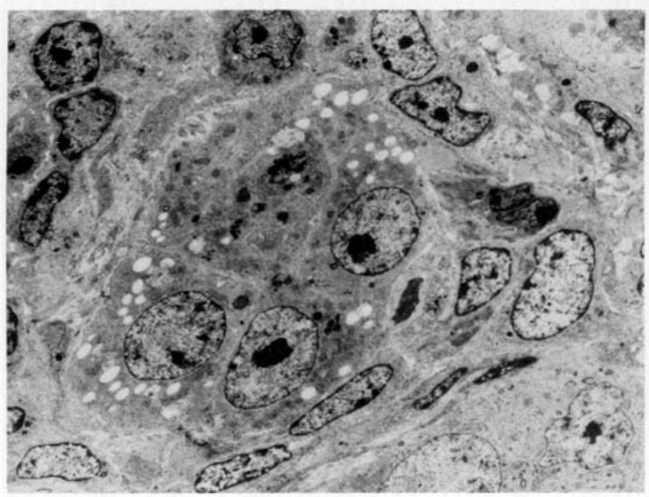

PLATE 23. Hen 298 from Flock 29 killed at 7 years of age with multiple small granulosa cell tumors. Scattered among the follicular structures, typical of early tumors, were numerous thecal glands (center). The clear cytoplasmic vacuoles are typical of thecal gland cells; transosomes are absent. H & E, ×1060.

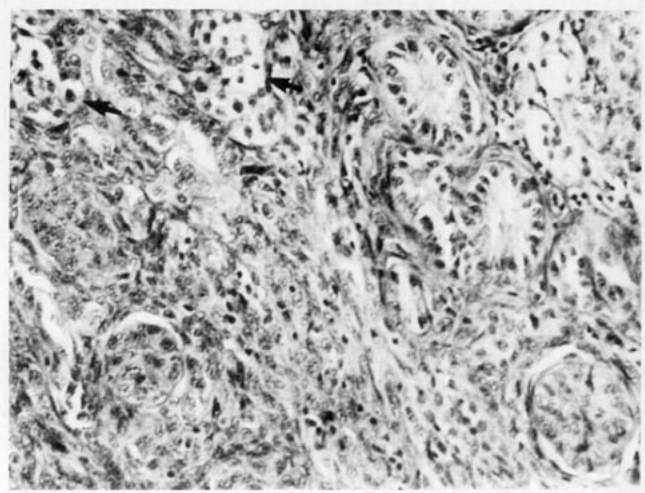

PLATE 24. Hen 3065 from Flock 29 killed at 7 years of age with oviductal adenocarcinoma and a very early ovarian tumor which contained three types of structures: follicular (lower right corner); tubular (upper right); and thecal cells (←). Bands of stromal connective tissue appears on the left. H & E, ×140.

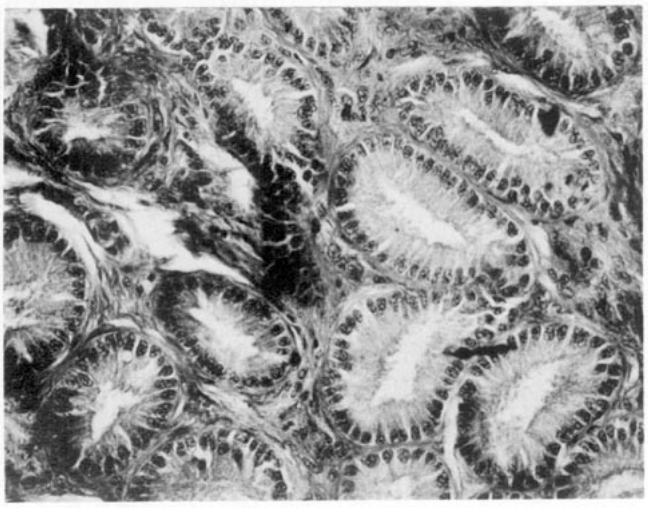

PLATE 25. Hen 3130 from Flock 29 killed at 5 years of age. The ovary appeared normal, but microscopically, small Sertoli cells tumors containing well-defined tubular structures were found. No follicular structures were seen. H & E, ×144.