SEASONAL CYCLE OF REPRODUCTIVE PHYSIOLOGY IN THE BLACK-BILLED MAGPIE

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The Black-billed Magpie (*Pica pica*) is a prominent species in many parts of western North America. However, with the exception of a report by Davis (1955) in which a determinate laying pattern was noted, information on reproductive physiology in magpies is not available. Recent studies of behavioral and ecologic factors influencing reproduction in birds (see reviews by Lehrman 1961; Marshall 1961; van Tienhoven 1961; Farner and Follett 1966) have shown the value of field investigations in studying reproductive processes and have suggested a need for further work which correlates environmental, behavioral, and physiological events. The present study was performed concurrently with an ecological-behavioral study (Erpino 1968b) in an attempt to determine gonadal correlates of reproductive behavior in a non-migratory magpie population near Laramie, Wyoming.

Ovarian histology is accorded special attention in this paper because characteristics of seasonal ovarian changes and factors influencing these changes in birds are poorly understood (Marshall 1961; Farner 1964; Farner and Follett 1966). While histology of yolk formation has been studied in periodic breeders (Bissonnette and Zujko 1936), details of seasonal changes in ovarian microstructure are available only for the Starling, *Sturnus vulgaris* (Bullough 1942), and the Rook, *Corvus frugilegus frugilegus* (Marshall and Coombs 1957). The limited older literature on ovarian cycles in birds is reviewed by Matthews and Marshall (1956: 207–217) and Brambell (1956: 497; 505–506).

Most field investigations of avian reproductive physiology have involved examination of gonads obtained on a chronological basis without regard to the precise behavioral status of the bird collected (Johnston 1956; Marshall and Coombs 1957; Selander and Hauser 1965; and others). In the present study, intense predation and consequent renesting as well as individual variation in time of nesting resulted in an extended and asynchronous breeding period. Therefore, concerted effort, facilitated by marking, observing, and subsequent collecting of individuals, was made to procure birds of known reproductive status.

METHODS

Magpies were collected in an area 15 miles southwest of Laramie, Wyoming, from November 1964 to February 1967. A total of 47 males (29 adults, 13 first-year, 5 nestlings) and 43 females (41 adults and first-year, 2 nestlings) was examined. Behavioral subdivisions of the annual cycle from which birds were collected include (1) winter (November to mid-January), (2) early breeding season or "pursuit period" (late January to late February, depending upon the year), (3) nest-building (early March to late April when known to be building), (4) egg-laying, (5) incubation, (6) nestling, and (7) postbreeding season. Methods of marking and aging birds followed those of Hester (1963) and Erpino (1968a), respectively.

Gonads, a portion of the oviducal magnum in females, adrenal glands, thyroids, and incubation patches were excised within 20 min after collection and placed in Bouin's fluid. Weight (nearest mg) of both testes was recorded prior to embedding. Diameter (nearest 0.1 mm) of the largest ovarian follicle was recorded in nonlaying females, and diameters of the larger, graded follicles (van Tienhoven 1961: 1114) were noted in laying birds. All tissues but incubation patches were embedded in paraffin by usual procedures.

Selected 6 μ sections of testes and oviducts were stained with hematoxylin and eosin. Interrupted 8 μ serial sections of ovaries were stained with a modification of Mallory's triple connective tissue staining technic which involved prestaining with hematoxylin for nuclear detail (Humason 1962:146–148). Sections were made in the plane of the long ovarian axis; from 15 to 35 sections were retained from each ovary.

Histologic analysis of testicular material comprised recording abundance of spermatozoa by means similar to those of Johnson (1966) and noting general condition of the tubules and seminiferous epithelium regarding activity and regressional characteristics.

Qualitative features of the ovarian stroma, healthy (non-atretic) follicles and their thecae, atretic follicles, and postovulatory follicles were noted. Mitotic frequency in the granulosa of healthy follicles was subjectively divided into three classes; common mitoses, uncommon mitoses, or an apparent absence of mitotic activity. Atretic follicles were distinguished from

TABLE 1. Histologic characteristics of postovulatory and atretic follicles in Black-billed Magpies.

Characteristic	Postovulatory follicles	Atretic follicles	Source
Shape	Irregular or angular	Round	Davis 1942a
Lumen shape	Irregular	Round	Payne 1966
Granulosa cells	Remain in close association with basement membrane in early stages, necrosis occurs when cells slough	Separate and move away from basement membrane in early stages, cellular hypertrophy common	Marshall and Coombs 1957; Payne 1966; present study
Features of contained tissues	Widespread necrosis, lack of vascularity, phagocytic invasion, yolk absent	Relatively few phagocytes, persistent thecal vascularity, yolk often present	Marshall and Coombs 1957; Payne 1966; present study
Rupture of thecal layers	Present, at least in early stages	Absent in all stages of non-bursting (yolky) atresia	Davis 1942a, b; Payne 1966
Thecal features	Thecae folded in early stages, merge imperceptibly with ovarian stroma in older examples	Thecal layers retain round appearance and persist, segregated from stromal elements	present study

older postovulatory follicles according to criteria listed in table 1. On a minimum of three representative and non-overlapping ovarian sections from each bird, all healthy and atretic follicle types were counted, and relative estimates of size were made by noting the number of intersects occupied by the follicular diameter on a Whipple ocular disc. Primary oöcytes were not included in these counts.

Oviducal features recorded included characteristics of albumen-secreting tubular glands and measurements (nearest 0.1 mm) of total oviducal diameter.

Weight (nearest 0.1 g) was used to assess the degree of edema in incubation patches.

RESULTS

GENERAL CYCLE

Breeding ecology and behavior, much of which has previously been reported in detail (Erpino 1968a), are briefly summarized here.

Winter. From November through early or mid-January, magpies associated in larger flocks (5–15 or more birds) and exhibited vigorous interspecific aggression (mobbing) against resident birds of prey. Intraspecific aggression was more common than at other times of the year and usually occurred at feeding sites.

Early breeding season. Pursuit flights were the earliest annually appearing sexual behavior. This activity was first noted in mid-January in 1966 and 1967 and may have been involved in pair formation. Pursuit flights terminated shortly after widespread and intensive nest-building became evident, but pair bonds persisted for at least the duration of the breeding season. Adults and first-year birds participated in chases although first-year males rarely participated in breeding.

Nest-building. Nest construction was most intense from mid-March through late April. Stages in nest-building and time involved in construction included a twig superstructure (2–3 weeks), a mud bowl (about 9 days), and grass lining (about 14 days). The building period was characterized by a decrease in flocking activity but a simultaneous increase in intrapair sociality. Both birds participated in nest construction but males contributed more than females. Sexual displays became prominent during the lining of the nest. No evidence of territoriality was noted.

Egg-laying. Vigorous intrapair social interaction occurred in the laying period. Major activities included intense courtship feeding of females by males with associated postural and vocal displays, and, most probably, copulation. Eggs were laid daily, and clutches in 63 carefully observed nests (29 in 1965 and 34 in 1966) averaged 6.26 eggs (the modal clutch was seven). Adult females laid eggs somewhat earlier in the season than did first-year females. Old or unoccupied nests were always used for renesting, which was common in both nesting seasons. Modification of old nests while renesting involved rebuilding of the grass lining. Renesting intervals, the time between destruction of the first clutch and initial oviposition in the second (Sowls 1955: 133–134), averaged 13 days; variation was apparently not related to the stage of incubation at the time of nest destruction. While renesting was common in the population, field observations indicated that individual pairs

TABLE 2. Pooled averages of testicular weights of adult and breeding male Black-billed Magpies collected 1964–1967.

Stage in breeding cycle	Average weight of both testes (mg)	Number of birds
Winter	12	4
Pursuits	37	3
Nest-building		
Early	166	3
Mud-bowl	700	3
Lining	1250	2
Laying[a]	1220	3
Incubating[b]	521	4
Nestling	31	2
Postbreeding molt	4	3

[a] All were collected while engaged in original nesting attempts.
[b] All collected had not yet passed the half-way point in the 18-day incubation period.

renested only once. That is, when the first renesting attempt of a particular pair was destroyed, further renesting was not observed. Moreover, renesting probably occurred primarily when nests were destroyed during incubation. Brood destruction was common but renesting following predation of young was noted only once.

Incubation. Courtship feeding persisted during incubation, but accompanying displays were less intense than during egg-laying. Courtship feeding during the 18-day incubation period probably aided energy maintenance of the female, who alone incubated. Incubating birds showed increased ferocity in nest defense in comparison with that observed in earlier nesting stages.

Nestling period. Parental behavior including participation of both sexes in feeding of nestlings and in vigorous nest defense, was prominent during this period. Male–female social interaction was passive. Brooding attentiveness by females gradually decreased during the 27-day nestling period. More males than females fledged.

Postbreeding period. Renewal of flocking activities and the annual molt occurred during the period from mid-July through early September. Some birds began molting while still feeding nestlings. Segregation of recently fledged and molting birds may have occurred during the peak of molting.

MALE CYCLE

Investigation of the male reproductive system revealed no important differences between magpies and other seasonal breeders. Thus only a brief summary of aspects of the male cycle is given here.

Changes in testicular weights are summarized in table 2, which shows that increase in weight began in the pursuit period, reached a peak just before or during laying, and declined rapidly during incubation and nestling periods. In three first-year birds obtained in the pursuit period, the testes averaged 8 mg. Testes from three first-year birds collected early in the nest-building period averaged only 35 mg while those of three first-year birds obtained during the mud-bowl period averaged only 162 mg. This suggests that rate of gonadal development in first-year males lagged behind that in adults.

Spermatozoa appeared in small numbers in adult testes in the mud-bowl stage of nest-building and increased to a peak during the egg-laying period. Testes of nonbreeding first-year birds collected at this time also contained spermatozoa. A decline in numbers of spermatozoa occurred during incubation and none were seen in testes from birds feeding nestlings. Thus spermatozoa were present in the testes for at least six weeks. Simultaneously with the decline in numbers of spermatozoa during incubation, testicular tubules became occluded with lipids (suggested by vacuolation of the intratubular area) and detritus and were invaded by phagocytes. Males collected near the end of the postbreeding molt exhibited testes in which the seminiferous epithelium appeared healthy, lipoidal remnants were rare, and detritus and phagocytes were absent.

One of three adult males whose mates were laying showed complete defeathering of the ventral apterium. This area, similar to incipient incubation patches in females, was neither highly vascular nor edematous and weighed only 0.1 g. Males collected during incubation did not have structures resembling incubation patches, but in one nonmolting adult male taken while feeding nestlings, the ventral apterium was nearly completely defeathered. Those few feathers remaining were easily removed.

FEMALE CYCLE

Follicular cycle. Diameters of single largest ovarian follicles averaged 0.5–1.0 mm in winter ($n = 4$) and in the early breeding season ($n = 2$), about 1.5 mm early in the nest-building period ($n = 5$), 2.2 mm during the mud-bowl period ($n = 3$), and 2.4 mm during the grass-lining period ($n = 1$). Largest ovarian follicles in the egg-laying period were 13.5–14.0 mm in diameter. This represents, then, ovulable follicular size in magpies. A reduction in numbers of large follicles was noted in birds nearing completion of the clutch.
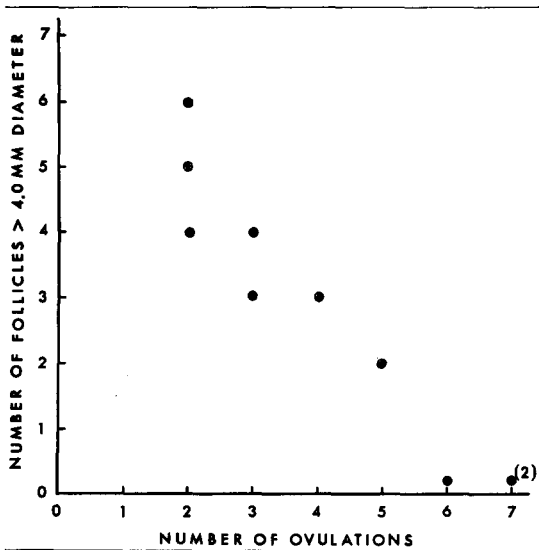
FIGURE 1. Number of ovarian follicles greater than 4.0 mm in diameter correlated with the number of ovulations in 10 laying magpies.

Bursting atresia of larger follicles, as noted upon cessation of laying in Argentine Cowbirds (*Molothrus bonariensis*) by Davis (1942b), or flaccid atresia of mature follicles, as seen in laying Atlantic Brant (*Branta bernicla*) by Barry (1962), were not seen in magpies. Follicles attaining a diameter of about 4.0 mm in magpies continued growing to ovulable size and were shed (fig. 1).

Postovulatory follicles regressed rapidly after ovulation. Up to two days after ovulation, the granulosa layer thickened but cells remained integrated and juxtaposed to the basement membrane. Erythrocytes appeared in small numbers in lumina of recently ovulated follicles. Thecal layers shortened and thickened and some proliferation of presumed thecal gland cells persisted shortly after ovulation. Beginning about three days after ovulation, granulosa cells and phagocytes began sloughing into the follicular lumen. Shortly after granulosa dissociation occurred, sloughed cells became vacuolated (lipoidal) and showed pycnotic nuclei. Subsequent degeneration was rapid, and as early as day five of incubation, older postovulatory follicles could no longer be distinguished with certainty from larger, degenerating yolky atretic follicles (described below). Sections through unruptured ends of older ovulated follicles disclosed structures similar to developmental stages of lipoglandular atretic follicles, but attendant phagocytosis of luminal granulosa cells, necrosis of cells in thecal layers, and an absence of thecal vascularity indicated that postovulatory follicles were short-lived. Regression of postovulatory follicles in magpies is thus similar to that reported in other species (Davis 1942a; Payne 1966).

Measurements of the largest ovarian follicles in incubation and nestling periods reveal characteristics of follicular regression. In six incubating birds, the largest ovarian follicle averaged 2.78 (SD = 0.44 mm), while in seven females with nestlings the largest follicle averaged 0.97 (SD = 0.34 mm) in diameter. Largest follicles in two molting birds averaged 1.1 mm.

TABLE 3. Characteristics of healthy and atretic follicles (AF) in ovarian sections from Black-billed Magpies collected 1964–1967.

Stage in Cycle	Number of birds	Healthy follicles $\bar{x}$ size[a] ± SD (n)	Glandular AF $\bar{x}$ size[a] ± SD[b] (n)	Lipo-glandular AF $\bar{x}$ size[a] ± SD[b] (n)	Yolky AF $\bar{x}$ size[a] ± SD[b] (n)
Winter	4	3.5 ± 0.75 (146)	8.0 (1)	2.3 (4)	
Pursuits	2	4.1 ± 0.68 (167)	4.8 ± 0.28 (3)	2.8 ± 0.28 (3)	
Nest-building					
Early	5	4.8 ± 0.76 (418)	6.2 ± 0.90 (20)	2.7 ± 0.20 (18)	
Mud-bowl	3	5.8 ± 0.81 (154)	7.2 ± 0.73 (11)	3.5 ± 0.89 (8)	
Grass-lining	1	8.0 (20)	4.5 (2)	4.5 (4)	
Egg-laying					
Four or fewer ovulations	4	15.1 ± 3.80 (131)	7.5 ± 0.97 (23)	4.2 ± 1.02 (19)	8.5 ± 5.00 (4)
More than four ovulations	5	7.7 ± 1.43 (215)	7.8 ± 1.15 (19)	3.4 ± 0.70 (24)	16.4 ± 5.30 (15)
Incubation					
9 days or less	4	7.4 ± 0.30 (116)	7.7 ± 0.79 (9)	4.0 ± 0.82 (26)	20.6 ± 10.91 (10)
10 days or more	2	7.1 ± 0.10 (54)	10.0 ± 1.90 (6)	3.9 ± 1.18 (16)	15.9 ± 4.35 (7)
Nestling					
1–14 days	2	4.9 ± 0.32 (64)	6.8 ± 1.25 (5)	3.7 ± 0.17 (21)	5.0 (5)
15 or more days	5	4.7 ± 0.78 (131)	7.0 ± 1.10 (12)	3.0 ± 0.45 (57)	8.3 ± 3.90 (2)
Postbreeding molt	2	5.6 ± 0.99 (70)	9.7 ± 0.28 (3)	3.0 ± 0.45 (33)	7.0 (1)

[a] Each unit of measurement represents 84 μ.
[b] SD computed from mean values among birds from a particular collection period and is not, therefore, indicated when value is from a single bird.
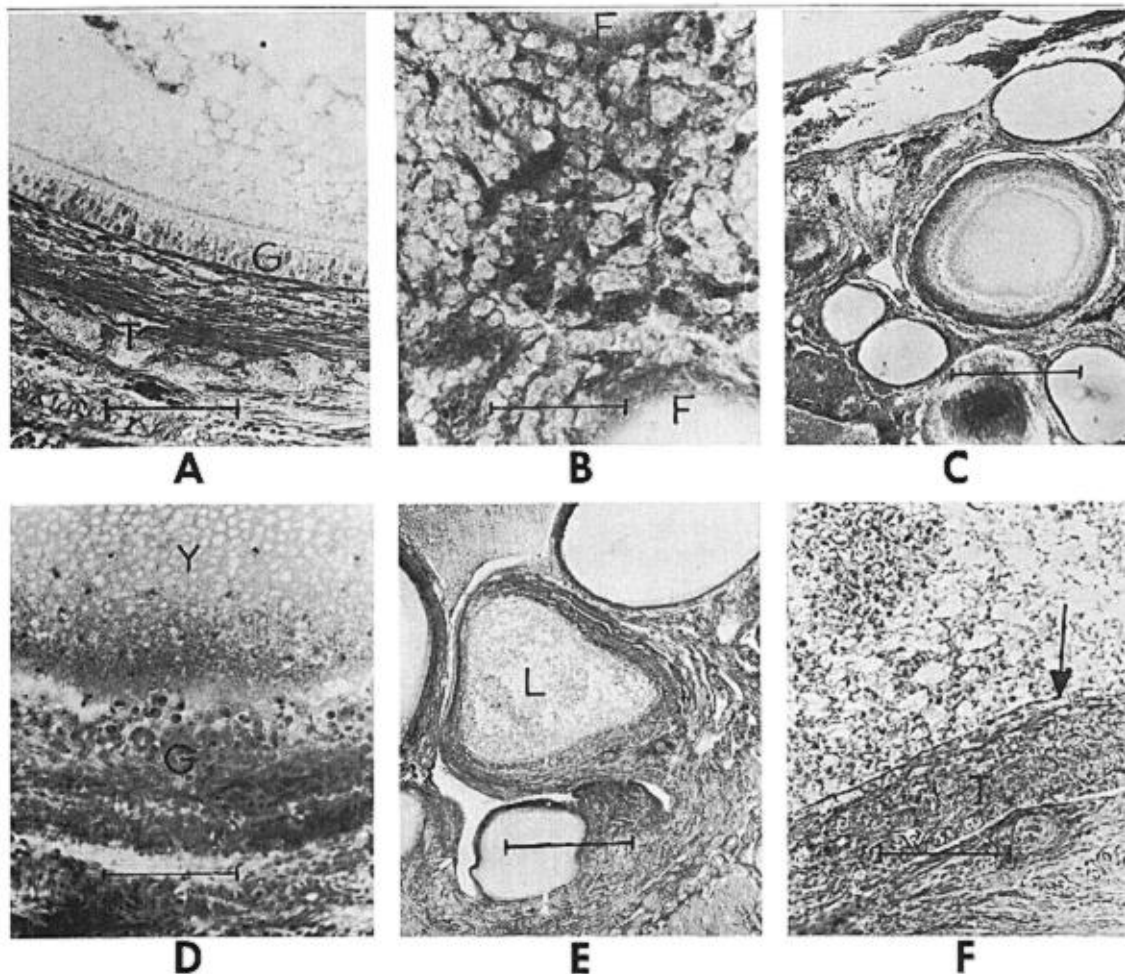
FIGURE 2. Photomicrographs of ovaries of Black-billed Magpies. A. Healthy follicular wall (high power). T = thecal glands cells; G = follicular epithelium (granulosa). Line = 100 μ. B. Well-developed stromal glands as seen in ovaries during the mud-bowl stage of nest-building (high power). F = ovarian follicles. Line = 20 μ C. Follicle in early stage of glandular atresia (low power). Line = 0.50 mm. D. Portion of wall of follicle in 2C (high power). Granulosa cells (G), along with dark-nucleated phagocytes have begun to move into the yolk (Y). Line = 100 μ. E. Follicle in more advanced stage of glandular atresia than that in figure 2C (low power). Follicular lumen (L) is now occupied nearly entirely by former granulosa cells and phagocytes. Line = 0.50 mm. F. Portion of wall of follicle in 2E (high power). Vacuolation of granulosa cells is pronounced and phagocytes remain abundant. Arrow indicates position of wall of fibroblasts which has arisen between the follicular lumen and outer thecal layers (T). Line in theca = 100 μ.

Results of histologic measurements of healthy follicles are presented in table 3, and show that growth of all healthy follicles paralleled that of the largest ovarian follicles as outlined above. In particular, decreases in size were noted late in the laying period as compared with early in this period, and in the nestling period as opposed to the incubation period. Follicles larger than 5 mm in diameter were removed before embedding and measurements from these are not included in table 3.

In all collection periods large healthy follicles had stratified granulosa cells. Mitotic activity was noted in the granulosa in all seasons but was most common during the early and mud-bowl nest-building periods, the egg-laying period, and during incubation. Granulosa mitotic activity in the nestling period was restricted to one or two of the largest follicles in each ovary. Thecal gland cells (fig. 2A) were present in larger follicles in all collection periods but trends in development were not evident since in all seasons some birds had small, widely separated gland cells while others had larger, more abundant gland cells. Only one possible peak in gland-cell development occurred; this coincided with massive development of stromal glands in two of three females believed to be constructing the mud bowl.
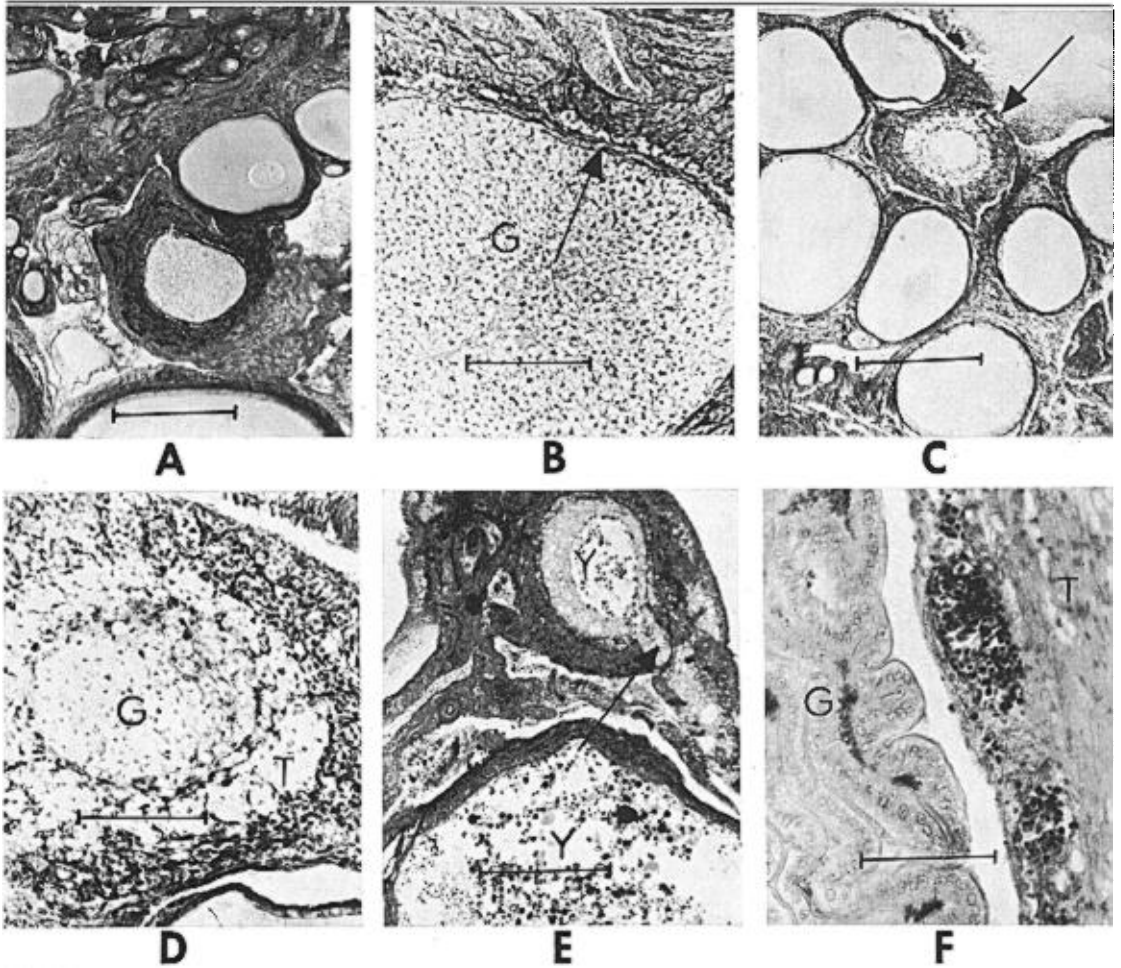
Atretic follicles. Three types of atretic

FIGURE 3. Photomicrographs of ovaries of Black-billed Magpies. A. Terminal stage of atresia (low power). The rarity with which this stage was encountered suggests a rapid transition from previous stages of glandular atresia to lipo-glandular atresia. Line = 0.50 mm. B. Portion of follicle in figure 3A (high power). Steatogenesis of granulosa cells (G), which now occupy the entire follicular lumen, is advanced; fibroblast wall (arrow) persists between lumen and thecal layers. Innermost thecal gland cells are also lipoidal. Line = 100 μ. C. Lipo-glandular atresia (arrow) (low power). Line = 0.50 mm. D. Lipo-glandular atresia (high power). Ex-granulosa cells (G) are separated from thecal gland cells (T) by fibroblast partition; central cells of granulosa origin are highly lipoidal and show shrunken, eccentric nuclei. Innermost thecal gland cells are also heavily lipoidal. Line = 100 μ. E. Adjacent yolky atretic follicles, Y (low power). Yolk platelets are abundant in lumen of larger follicle; lipoidal granulosa cells are moving outward through rupture in thecal layers of smaller follicle (arrow). Line = 0.50 μ. F. Portion of follicular wall in large yolky atretic follicles (high power). Thecal layers (T) are thickened; follicular epithelium (G) remains attached to warped and folded basement membrane. Line = 20 μ.

ovarian follicles (AF) were distinguished in magpies: (1) glandular AF, (2) lipo-glandular AF, and (3) yolky AF. These are described below.

Glandular atretic follicles in various degrees of development are shown in figures 2C, D, E, F, and 3A, B. This class of atresia represented initial stages of follicular degeneration in all birds. When first forming, glandular AF showed a slight dissociation of epithelial cells and often the formation of a narrow band of dense connective tissue immediately peripheral to the granulosa. Further development involved movement of sloughed granulosa cells,

usually with large numbers of phagocytes, into the ovum. The subgranulosa ring of connective tissue, apparently composed of fibroblasts, maintained separation of granulosa cells and thecal layers. In early stages of dissociation, granulosa cells hypertrophied; mitotic activity was rare in the granulosa once cells had begun to slough. In later stages of glandular atresia, granulosa cells often became vacuolated and probably lipoidal with shrunken, eccentric nuclei (fig. 3B). Separation of the central cells of granulosa origin from outer thecal layers by connective tissue persisted.

Lipo-glandular atretic follicles consisted of

a central cluster of lipoidal cells of granulosa origin that was ringed by a wall of fibroblasts (fig. 3C, D). Connective tissue septa, radiating outward from the fibroblast ring in a spoke-like manner, enclosed clusters of gland cells of thecal origin. Often, the innermost of these gland cells was also lipoidal. Thecal layers of lipo-glandular AF were highly vascular. In contrast to glandular AF, lipo-glandular AF were rather uniform in size in all ovaries (table 3) and were nearly always embedded within the ovarian stroma and not elevated on stalks. When originally observed, it was unclear whether lipo-glandular AF were of follicular (atretic) origin or were formed directly from stromal gland cells. Their round appearance and similarity to advanced stages of both glandular and yolky AF indicated follicular origin. This suggests that lipo-glandular AF originated from both glandular and yolky AF.

Yolky atretic follicles fall into two categories. Intermediate-sized (2–4 mm) follicles during the laying and postlaying periods often showed rupture of thecal layers and yolk extrusion. Concomitant collapse of follicular walls and constriction of spiral arteries resulted in structures resembling ovulated follicles but containing nests of yolk (fig. 3C). In other cases, yolk extrusion was not apparent and yolky AF resembled massive glandular AF. Granulosa cells either remained integrated and attached to the warped basement membrane (fig. 3F) where they continued to proliferate, or dissociated and streamed into the follicular lumen as was seen in the smaller glandular AF. Usually, however, yolky AF had spheroids of yolk within the follicular lumen and showed less conspicuous phagocytosis of yolk than observed in glandular types. Some ruptured yolky AF in several ovaries exhibited extensive areas peripheral to the thecae which were occupied by light-staining cells which may have originated from the follicular epithelium (fig. 3E). Regressing yolky AF and blind ends of early stages both resembled lipo-glandular AF and thus may have added to the ovarian complement of this atretic type.

Seasonal trends in abundance of AF are shown in table 3. Proportions of glandular AF increased slowly during the breeding season, reached a peak during egg-laying, and declined in frequency thereafter. Frequencies of lipo-glandular AF increased significantly ($x^2 P < .05$) in seasonal transitions from winter and pursuit periods to nest-building, from nest-building to egg-laying, from egg-laying to incubation, and incubation to
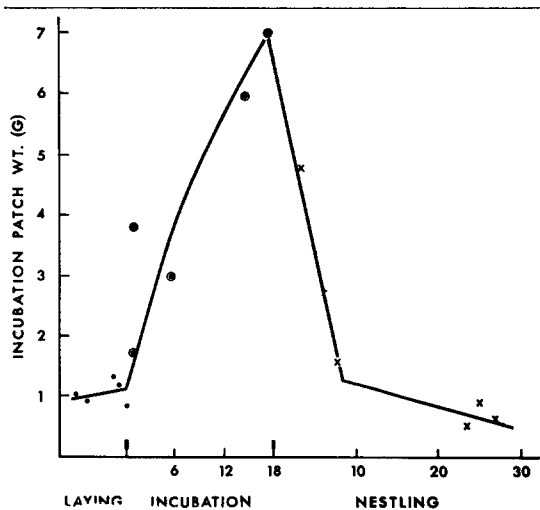

FIGURE 4. Incubation patch weights from female magpies collected in various nesting stages. Solid dots = laying birds; encircled dots = incubating birds; x = birds with nestlings. Numbers on abscissa indicate days of incubation (6, 12, 18) and nestling (10, 20, 30) periods.

nestling periods. Lipo-glandular AF from birds with nestlings and from molting birds differed from those in earlier periods in showing reduced cellular vacuolation and a decrease in nuclear compression. Presence of yolky AF was largely confined to egg-laying, incubation, and nestling periods.

Stromal glands. Ovarian stromata in most collection periods had few clusters and strands of gland cells which were widely separated by stromal connective and vascular tissue. As indicated above, however, two of three females collected during the mud-bowl stage of nest-building had ovarian stromata occupied almost entirely by large, light-staining gland cells (fig 2B).

Oviducal cycle. Magnum diameters averaged 0.5 mm in winter, 0.7 mm in early building stages, 0.8 mm in the mud-bowl period, 1.3 mm in the grass-lining period, 4.0 mm during egg-laying, 2.3 mm during incubation, 1.8 mm in the nestling period, and 0.8 mm in the postbreeding period. Infoldings of the lining epithelium, which may have represented tubular gland rudiments, were detected during the mud-bowl period. Tubular glands engorged the mucous membrane in oviducts from laying birds but shrank rapidly during incubation. Vestiges of tubular glands were absent in oviducts from birds with nestlings.

Incubation patch. Incipient development of the incubation patch in the female occurred while lining the nest. Defeathering became
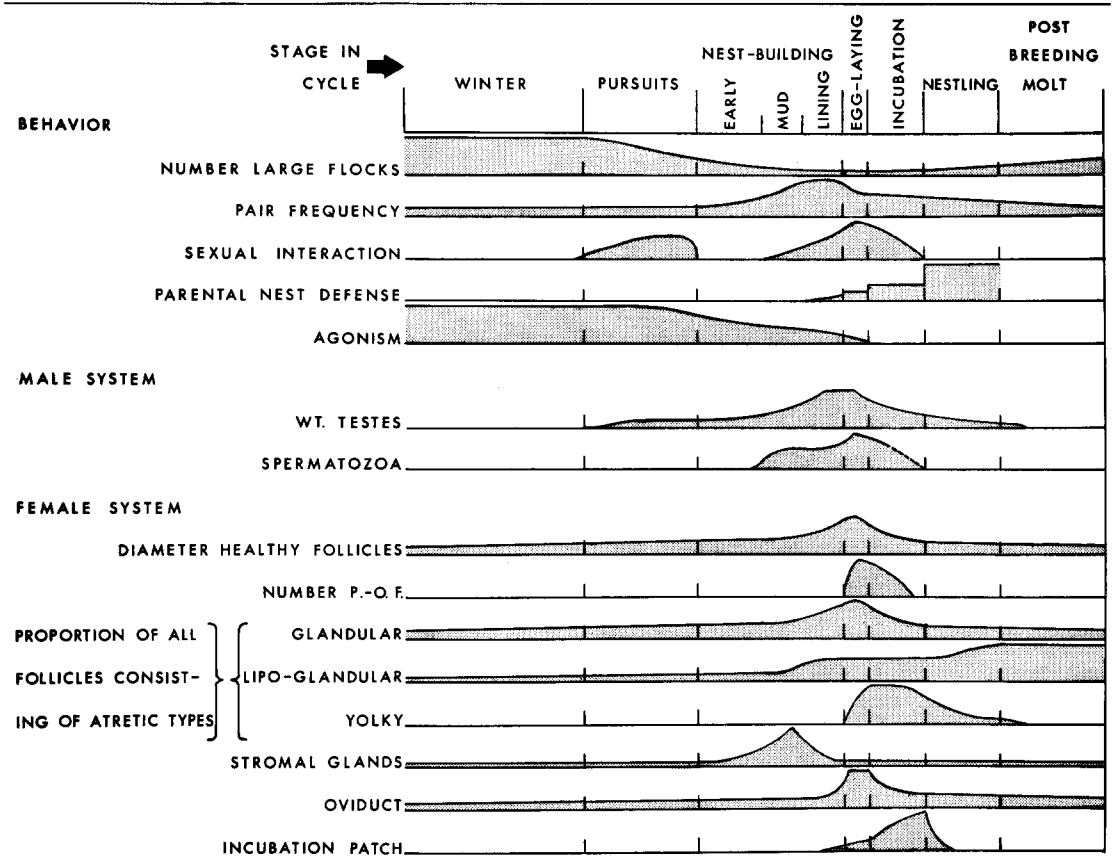
FIGURE 5. Integration of trends in some major behavioral and physiologic features (ordinate) with that portion of the annual cycle investigated (abscissa). The distance between perpendicular lines on abscissae indicates relative length of time involved in each stage of the cycle by a pair of magpies. Curves for each behavioral and physiologic category were derived by designating time of maximum activity or development as 100 per cent and then plotting seasonal variations thereof. Agonistic behavior, for example, was never common but was most frequently observed in winter. In females, measurements of healthy follicles were histologic; P.-O. F. indicates post-ovulatory follicles.

complete, and increased vascularity (as suggested by increased tissue redness) occurred during egg-laying. Edema started early in the incubation period, apparently proceeded throughout incubation, and subsided rapidly after hatching (fig. 4).

DISCUSSION

MALE

A slower rate of gonadal development in first-year males compared to adults has been verified in other passerines (Marshall and Coombs 1957; Selander and Hauser 1965; King et al. 1966). Differences in rate of development have been attributed to differential photoperiodic responses in the two age classes (King et al. 1966). The lack of breeding by first-year male magpies may have been a corollary of (1) a monogamous mating system and an excess of males in the population, and (2) a behavioral disadvantage in accomplishing

pair formation imposed upon first-year males by delayed testicular development. Maximum testicular size in adults preceded and outlasted maximum ovarian development (fig. 5). The rapid reduction in testicular size in magpies after the onset of incubation corresponds with that inferred in certain other species collected on a chronological basis (e.g., Johnston 1956).

Tubule steatogenesis and tissue degeneration during incubation and nestling periods suggests that the testis may be limited in frequency of activity. That is, testicular regression appeared to be direct and complete and displayed no maintenance of components from which a rapidly-renewed spermatogenic apparatus could be drawn. Marshall (1961) noted that, in male birds, the regeneration phase (during which the testis returns from an inactive to an active condition) began shortly after the final ovulation in single-

brooded species. One might speculate that testicular regression in magpies and other forms, once initiated, is irreversible. This poses several problems. For example, since renesting (with a renewal of vigorous sexual behavior) occurred commonly, it might be asked whether spermatozoa fertilizing the second clutch were stored in the male's deferent ducts or in the female tract. Similar problems might confront polygynous forms in which males secure additional mates during incubation and nestling periods (Zimmerman 1966; Willson 1966) and species which nest more than once in a particular season (Hanson and Kossack 1963).

The possibility exists that behavioral interactions between mates, which normally are probably the immediate "timers" of ovulation and insemination in annual breeders (Marshall 1961; Lehrman 1965), can effect dissipation of tubule lipids and renewal of testicular function. These questions could be answered through field studies involving experimental nest destruction and subsequent collection of renesting males. Regeneration of spermatogenic components appeared to have occurred by the end of the postbreeding molt. Few remnants of tubule lipids remained and the seminiferous epithelium appeared active.

Occurrence of incubation patches among male corvids is inconsistent. In the Clark Nutcracker (*Nucifraga columbiana*) Mewaldt (1952) found that breeding males commonly had well-developed incubation patches. Johnston (1959) reported that in Common Crows (*Corvus brachyrhynchos*), males did not have incubation patches. The lack of incubation behavior along with partial development of incubation patches in some individuals has also been noted in domesticated canaries (*Serinus canarius*) by Hinde (1962). This suggests that the male role in incubation in magpies and some other species may be changing from either a greater or lesser participation. That some male magpies had incipient incubation patches also poses questions of endocrine factors involved. Selander and Kuich (1963), for example, found that the skin of the ventral apterium in female Brown-headed Cowbirds (*Molothrus ater*), which do not develop incubation patches, did not respond to exogenous hormones normally involved in incubation patch formation.

FEMALE

Seasonal variations in ovarian follicular diameters in magpies correspond to those recorded in many other annual breeders (Bissonnette and Zujko 1936; Peterson 1955; Johnston 1956;

Phillips and van Tienhoven 1962; Barry 1962; King et al. 1966). Maximum follicular growth occurred late in the long nest-building period when little actual building but intense sexual behavior took place (fig. 5). Follicular regression began immediately after cessation of laying. This short span of peak ovarian activity (less than two weeks) in comparison with the longer period of maximum testicular activity agrees with observations in California Gulls, *Larus californicus* (Johnston 1956); Atlantic Brant (Barry 1962); White-crowned Sparrows, *Zonotrichia leucophrys* (King et al. 1966); and others.

Thecal gland cells, the presumed source of estrogen (Marshall and Coombs 1957), showed various degrees of development in most behavioral stages. One discrete peak of thecal gland abundance occurred simultaneously with a peak in stromal gland development during the mud-bowl stage of nest-building (fig. 5). Maximum oviducal development and sexual behavior, both of which are estrogen-induced (van Tienhoven 1961:1117), occurred two to three weeks after peak development of thecal glands, however.

Two other aspects of follicular growth which can be considered are determinate laying, and post-laying follicular regression and implications thereof in renesting.

Birds can be placed in two general categories regarding egg-laying patterns. Determinate layers ovulate a relatively fixed number of eggs regardless of the fate of previously laid eggs. Indeterminate layers continue ovulating until a certain number of eggs accumulate in the nest. Destruction or removal during laying of some eggs from the clutch of a determinate layer results in a deficient clutch. But removal during laying of eggs from the clutch of an indeterminate layer effects maturation and ovulation of additional ova so that the resultant set of eggs corresponds to the normal clutch for the species (Lehrman 1961:1282–1283). It has been shown that Black-billed Magpies are determinate layers (Davis 1955). Little information is available on physiologic aspects of laying patterns but indeterminate laying would logically require maturation of more follicles than normally appear in the clutch, since additional ova must be available to supplement deficient clutches. Dilger (1960:651) observed that more follicles matured than ovulated in determinate laying African parrots (*Agapornis* spp.) but that atresia of remaining follicles occurred when three to eight eggs were laid. Barry (1962) believed that while

a limited number of follicles matured in Atlantic Brant, environmental factors which delayed oviposition induced a flaccid type of atresia (in mature follicles) in which yolk was resorbed. Thus in two of three nesting seasons, unfavorable weather prevented egg-laying and resulted in atretic loss of over 60 per cent of mature follicles in brant. Therefore, determinate laying in brant and African parrots appears to entail different processes. Observations in the present study indicated that only ova destined to ovulate attained diameters greater than 4.0 mm (fig. 1) and that clutch size was fixed very early in (or perhaps before) the laying period. Determinate laying in magpies involved a mechanism whereby follicular growth to a size approaching only 30 per cent of the diameter of ovulable follicles (13.5–14.0 mm) was restricted to ova appearing in the clutch. This may be similar to the mechanism in *Branta* but appears more efficient than that of *Agapornis*, since energy was not consumed in development of excess large follicles. Although pituitary control of laying patterns remains unclear (see Dunham and Clapp 1962), data from magpies suggest that gonadotropic hormones might act preferentially on those follicles that ovulate.

Ecologic and behavioral studies of renesting in birds include those of McCabe (1963) on Alder Flycatchers (*Empidonax traillii*), Fankhauser (1964) on Red-winged Blackbirds (*Agelaius phoeniceus*), and Gates (1966) on Ring-necked Pheasants (*Phasianus colchicus*). In addition many studies have shown the importance of renesting in population maintenance (e.g., Stotts and Davis 1960). Little is known of the physiologic processes involved in renesting. Phillips and van Tienhoven (1962) reported gonadal correlates of renesting behavior in Pintails (*Anas acuta*), and Barry (1962) presented data on gonadal regression in brant, from which he inferred that the species was physiologically incapable of renesting. Some information from the present study gives additional insight regarding physiologic aspects of renesting. In magpies, field observations of marked birds suggested that renesting attempts occurred only once and that renesting was probably not attempted after brood destruction. This is interpreted as a result of selection against prolongation of nesting in an area with a potentially very short nesting season. Barry (1962) suggested that short nesting seasons in the Arctic acted against renesting in Atlantic Brant. Physiologic correlates of renesting behavior and ecology in female magpies were as follows: unlike testicular regression and degeneration, which took place during incubation, the ovary showed maintenance of some healthy follicles throughout the incubation period. Thus, follicular development slightly above that noted during other nonlaying phases of the cycle persisted during incubation. Diameters of largest follicles in incubation were greater ($P < .05$) than during the nestling period. Renest clutches might arise from those follicles actively maintained during incubation. Histologic features of follicular regression, including a reduction in size of healthy follicles, a decrease in mitotic activity in the granulosa, and a general increase in atretic follicles (table 3), observed in the transition from incubation to nestling periods support macroscopic observations suggesting a marked decrease in activity of healthy follicles. Aspects of gonadal regression in both sexes, therefore, suggest that physiologic constraints acted against persistent renesting. Renesting intervals corresponded with the length of the grass-lining period in original nests. Sowls (1955:133–134) noted that renesting intervals in certain waterfowl increased as nest destruction occurred later in the incubation period. This was believed to reflect the extent to which the ovary had regressed; later in incubation, more time would be required for ovarian development. The constancy of renesting intervals in magpies and the similarity of this period to that during ovarian maturation in original nests suggests that processes inducing follicular maturation in both original and renests are similar. Since little building activity occurred during this period in either original or renests, perhaps behavioral interactions between mates (cf. Lehrman 1961; Brockway 1965; Brown 1967) and between birds and their completed nests (Hinde 1965; Lehrman 1965) were of major importance in eliciting ovarian maturation in both cases. In this context, it appears significant that complete regression of the testis may have occurred prior to that of the ovary. As pair bonds persisted for the duration of the breeding season, and assuming that ovarian regression was similar in original and renests, renesting frequency may ultimately have been limited by the male. For example, repeated destruction of nests during incubation could result in prolonging ovarian activity but testicular regression might render males incapable of behavioral responses needed for inducing ovarian development.

Descriptions of mature atretic follicles presented here agree in general with those ob-

served in the Rook (Marshall and Coombs 1957), although bursting or yolky atresia of intermediate-sized follicles was not reported in the Rook. Early stages of atresia in the Rook, moreover, involved a lipoidal metamorphosis within the ovum, and dissociation of granulosa cells was not reported. Marshall and Coombs (1957) believed that cells finally occupying the core of AF were derived from fibroblasts which invaded the follicle, hypertrophied, and became lipoidal. Fibroblasts in AF in magpies, however, apparently persisted and formed a partition between the central lipid core and outer thecal layers. Central lipoidal cells appeared to stem from the granulosa rather than from fibroblasts or from thecal gland cells as reported by early workers (Brambell 1956:505). Atresia in magpies, particularly in early stages, resembled that seen in Starlings (Bullough 1942:205–208), Fulmars, *Fulmaris glacialis*, (Wynne-Edwards 1939), and mammals (Ingram 1962). Marshall and Coombs (1957) found that lipoidal AF were short-lived and that connective tissue infiltration of AF resulted in obliteration of central lipoidal cells and dispersal of peripheral gland cells into the ovarian stroma. Initial stages of destruction of AF were noted in molting magpies but intact lipoglandular AF remained the most conspicuous gland-like ovarian unit through the molt (fig. 5). Since winter ovaries had few AF, study of autumn ovaries should provide information on regression of mature AF. Published data on frequency of various AF are not available. Davis (1944), however, found that in ovaries of a large number of species bursting atresia occurred most often during active and regressing stages of the ovarian cycle.

Progesterone is produced in the avian ovary but the site of synthesis remains unclear (van Tienhoven 1961:1120). Repeated lipoidal AF were proposed as sites of progesterone production in Rooks (Marshall and Coombs 1957). The seasonal incidence of lipo-glandular AF in magpies paralleled events influenced by progesterone in some species. These include oviducal development (Brant and Nalbandov 1956), incubation behavior (Lehrman 1965), parental nest defense (Vowles and Harwood 1966), and the molt (Kobayashi 1958). Occurrence of lipo-glandular AF in magpies differed from that in Rooks since nestling Rooks, but not magpies, had lipoidal AF, and frequency of lipoidal AF declined after the breeding season in Rooks. Certainly the possible endocrine role of atretic ovarian follicles in birds warrants further attention.

Ovarian stromal glands were observed in Starlings by Bullough (1942:199–204) and in Rooks by Marshall and Coombs (1957). Dual origin of stromal glands from (1) stromal fibroblasts and (2) cells formerly in atretic follicles which have been dispersed by connective tissue was proposed by Marshall and Coombs (1957). Gland cells of the former type are thus homologous to male Leydig cells. In Rooks, Marshall and Coombs observed a tremendous increase in stromal glands prior to laying. Origin of these glands from AF, which also increased prior to laying, was proposed. A similar increase in stromal glands occurred in magpies two to three weeks before oviposition and intense sexual behavior. Lipo-glandular AF, destruction of which could provide stromal glands of follicular origin, remained uncommon during this period (fig. 5). Massive stromal glands in magpies, therefore, probably formed directly from connective tissue precursors. Gland cells of follicular origin were believed to be estrogenic while those of connective tissue origin were suggested to be androgenic by Marshall and Coombs (1957). Histochemical evidence of androgen secretion by stromal glands in the domestic fowl was reported by Woods and Domm (1966). Stromal glands in magpies reached a peak in development during a time of intense nest-building activity but apparently declined to a resting state before development of the oviduct and vigorous sexual behavior.

Seasonal variations in oviducal size in magpies were similar to those in most species for which comparable data are available (Johnston 1956; Phillips and van Tienhoven 1962), but Marshall and Coombs (1957) believed that incubating Rooks maintained the oviduct in laying condition. Data in figure 5 indicate trends in oviducal diameter, but not in the histology of secretory units. It seems significant that remnants of tubular glands persisted in oviducal mucosae during incubation but were absent in birds with broods. Oviducal features, therefore, paralleled features in healthy ovarian follicles during incubation and nestling periods.

Stages in incubation patch development and regression seen in female magpies agree with those observed in most other birds (Bailey 1952). Weights of incubation patches from a large number of Bank Swallows (*Riparia riparia*), however, appeared to decline late in the incubation period (Peterson 1955: fig. 18). Raitt and Ohmart (1966) also found that some ovulating Gambel Quail (*Lophortyx gambelii*) showed no signs of incubation patch

development. Apparently species differences exist in the temporal aspects of incubation patch development.

SUMMARY

The reproductive physiology of Black-billed Magpies from winter through the postbreeding molt was studied near Laramie, Wyoming. Emphasis was on gonadal correlates of reproductive behavior and the female cycle.

Testicular development in first-year birds lagged behind that of adults and may contribute to non-breeding by first-year males. Testicular regression began during incubation and appeared irreversible; thus renesting may have been limited through the male. Although males did not incubate, some showed signs of incubation patch development.

Maximum ovarian follicular development occurred after cessation of vigorous nest-building activity. Determinate laying in magpies involved a mechanism in which only follicles appearing in the clutch attained diameters of 30 per cent ovulable size. Renesting was attempted only once and did not occur after destruction of young. Ovarian correlates of these observations included a tendency for partial follicular maintenance through incubation and existence of oviducal glands through incubation. Postovulatory follicles were short-lived.

Atretic follicles of three types were distinguished. Two of these, glandular and yolky, appeared to give rise to the third, lipo-glandular. Frequencies of lipo-glandular atretic follicles corresponded with increasing intensity of events influenced by progesterone in some species.

Ovarian stromal glands, believed to stem from connective tissue precursors, showed a single well-defined peak in development during a portion of the nest-building period characterized by intense building activity.

Developmental and regressional characteristics of the oviduct and incubation patch in females generally agreed with that of other birds.

ACKNOWLEDGMENTS

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