THE STRUCTURE AND POSSIBLE FUNCTION OF THE AVIAN SEMINAL SAC

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Although the presence of the cloacal protuberance in male passerine birds has been recognized for a long time (Wolfson 1952, 1954a), it is only recently that its structure and function have been investigated (Wolfson 1952, 1954a, b; Bailey 1953; Salt 1954). The protuberance is formed by a much coiled extension of the posterior end of the vas deferens lying beneath the skin, dorso-lateral to the cloaca (Wolfson 1952; Salt 1954). This organ has been called variously a seminal glomus (Witschi 1945; Salt, 1954), a seminal vesicle (Bullough 1942; Bailey 1953; Wolfson 1952, 1954a), and a seminal sac (Marshall 1961; Mann 1964). For the reasons given by Marshall (1961), the term seminal sac seems most appropriate and has thus been adopted by the author.

The seasonal development of the seminal sac is closely synchronized with testicular recrudescence and regression, and, as a result, its size fluctuates greatly throughout the year (Wolfson 1952; Salt 1954). In cross-section the seminal sac is tubular, each tubule being surrounded by a layer of smooth muscle and the entire structure being encapsulated in loose connective tissue (Bailey 1953; Salt 1954; Wolfson 1954a). The epithelium, lining the tubules, has been described as being cuboidal (Salt 1954; Wolfson 1954a), columnar (Bailey 1953; Wolfson 1954a), and ciliated columnar (Bullough 1942).

While checking on the sperm content in the seminal sacs of European Goldfinches, *Carduelis carduelis*, several observations were made which led to a detailed study of the anatomy and function of the seminal sac. These studies are the subject of this paper.

METHODS AND MATERIALS

Studies were made of the seminal sacs from 103 European Goldfinches and 58 American Goldfinches, *Spinus tristis*, collected throughout the year from wild populations, and 25 canaries, *Serinus canarius*, maintained under laboratory conditions. At death, the left seminal sacs were dissected out and fixed in either Bouin's or Allen's fluid for 24 hr. Following fixation, the seminal sacs were transferred to 70% ethanol, embedded in wax, and sectioned at 8 μ. Sections, representative of the entire length of the organ with serial sections from five birds, were mounted and stained with Weigert's haematoxylin and eosin. In addition, representative sections from the American Goldfinch and canary material were stained by the Periodic Acid-Schiff method (identification of polysaccharides), with Best's carmine (specific identification of glycogen) and with Alcian blue (identification of mucopolysaccharides). Amylase treated control slides were stained with the former two stains. The right seminal sacs were dissected out and placed in 10% formalin, sectioned on the freezing microtome at 10 μ and representative sections mounted, stained with Sudan IV (identification of lipids), and counterstained with Weigert's haematoxylin.

RESULTS

HISTOLOGY

The results are based on the examination of the seminal sacs from all three species. No significant difference in the histological structure of the organ was detected between the species examined.

During the nonbreeding season, the histology of the seminal sacs was as described by Bailey (1953) and Salt (1954) (fig. 1). Accompanying testicular recrudescence, a gradual structural transition occurred within the seminal sacs which culminated in the expansion of the tubules, the production of a uniformly smooth cuboidal epithelium, and the reduction in thickness of the smooth muscle capsule (fig. 2). As the season progressed, the tubular epithelium gradually changed from a cuboidal to a columnar to a pseudo-stratified columnar type (fig. 3). Ciliated columnar epithelium was not identified in any of the sections. However, the transition did not occur uniformly throughout the organ, and 85% of all seminal sacs examined during the breeding season had tubules with the epithelium in varying stages of development (fig. 3). During the breeding season, the epithelium appeared to be secretory, a condition commented on by Bailey (1953), Salt (1954), and Wolfson (1954a). Following its initial pre-breeding expansion, the diameter of the lumen became variable throughout the organ, so that tubules of varying diameter (range 80–350 μ) were commonly observed in any one cross section of the seminal sac during the breeding season.

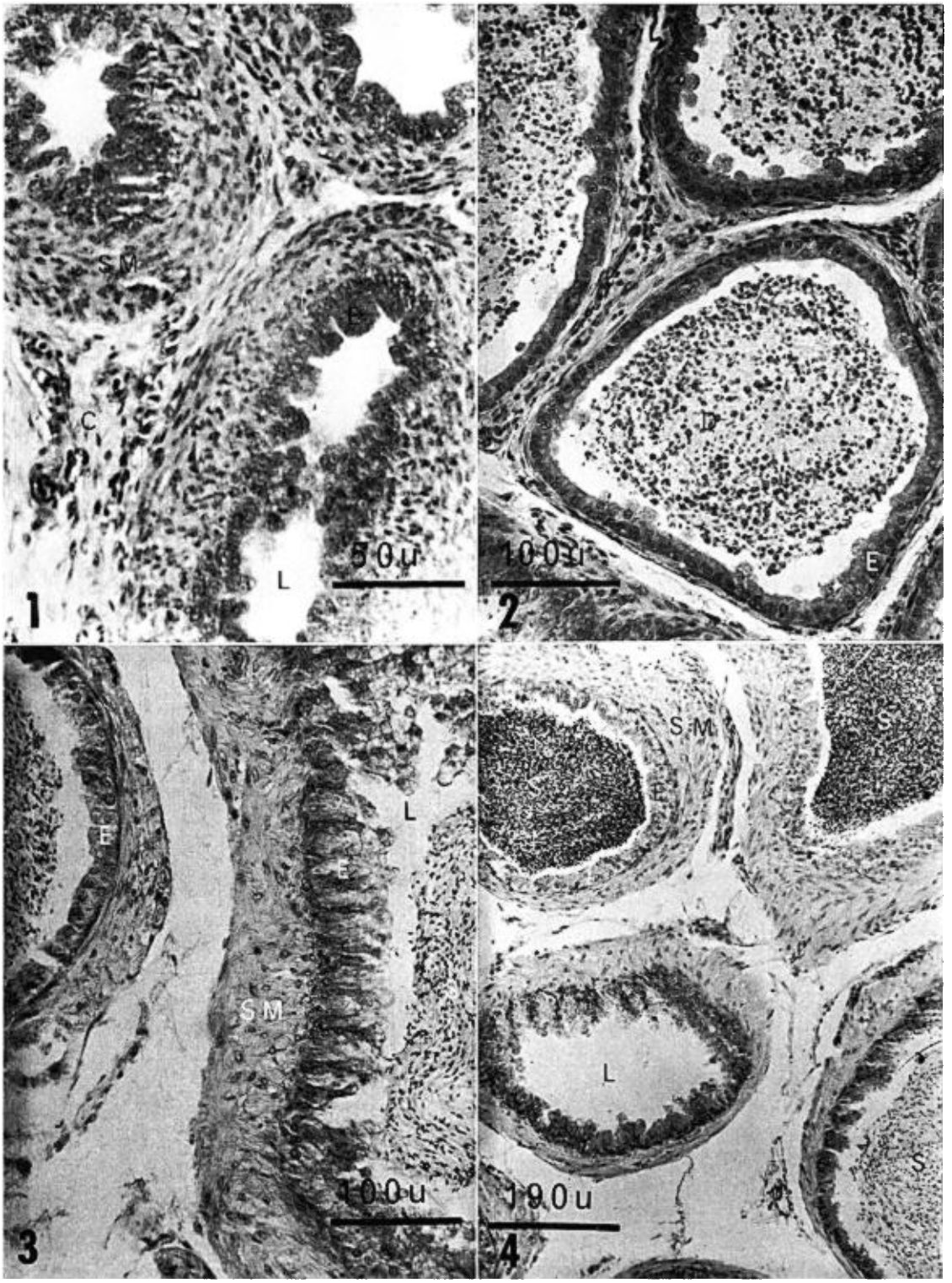

FIGURE 1. (Upper left) Seminal sac in pre-breeding condition. Haematoxylin and eosin (H and E). (American Goldfinch).

FIGURE 2. (Upper right) Seminal sac at start of breeding season, with uniform cuboidal epithelium, reduced smooth muscle sheath, and a few spermatozoa mixed with cellular debris. H. and E. (European goldfinch).

FIGURE 3. (Lower left) Sections of two adjacent tubules showing epithelium in differing stages of development. H. and E. (European Goldfinch).

The first spermatozoa occurred in the seminal sacs shortly after they were identified in the testes. At the beginning of the breeding season, the sperm concentrations were low and the sperm were mixed with cellular debris (fig. 2), but gradually the concentrations increased until many of the tubules were packed with sperm, a condition which persisted throughout the breeding season. However, the sperm concentration within the tubules of the seminal sac of any bird was variable (fig. 4). Frequently, the spermatozoa were oriented with their heads touching the epithelium, but in other cases the sperm heads penetrated the epithelium (fig. 5), causing its disruption and eventual erosion to the basement membrane (fig. 6).

HISTOCHEMISTRY

The identity of the secretions, noted by Bailey (1953), Salt (1954), and Wolfson (1954a,b) and observed by the author, had not previously been established. Thus sections were stained by the PAS method, with Best's carmine, Alcian blue, and Sudan IV. The PAS method consistently gave positive results, sections from one seminal sac gave a positive test with Best's carmine, and negative results were obtained with the remaining stains.

Study of the PAS-treated material revealed large cells with PAS-positive droplets within the cytoplasm, interspersed throughout the epithelial lining of the seminal sacs (fig. 7). In addition, extracellular droplets of PAS-positive material occurred along the border of the epithelium and free within the lumina of the tubules. Spermatozoa were oriented with their heads touching droplets of PAS-positive material, both during the invasion of the epithelium and while free in the lumen (fig. 8). A similar distribution of secretory material was observed in the positive test with Best's carmine.

DISCUSSION

Although Wolfson (1952, 1954a,b), Bailey (1953), and Salt (1954) did much to clarify the function of the seminal sac, they did not define its precise role. Clearly, the seminal sac functions in sperm storage, but how long viable sperm may be stored here has not been determined. The penetration by spermatozoa of the epithelial lining, observed in this study, may be a long-term storage mechanism. Storage of sperm by penetration of the epithelial lining of the female genital tract has been reported for as long as 28 days in the fowl, *Gallus gallus* (Fujii and Tamura 1963), and for several months in temperate zone bats (Chiroptera; Asdell 1965). However, sperm are known to pass through the genital ducts of the male fowl in 1–4 days (Munro 1938), and since many passerine species copulate before and during the egg-laying period, it seems unlikely that a long period of sperm storage is required within the seminal sac. Thus, some alternative explanation for epithelial invasion by the spermatozoa seems essential.

Perhaps epithelial invasion is related to the release of the secretory material from the epithelial cells. Salt (1954) and Wolfson (1954a,b) suggested that the epithelial secretions were released by apocrine activity. However, the widespread concentrations of PAS-positive material within the epithelial cells, the distribution of the extracellular PAS-positive droplets, and the quantities of cellular debris within the lumina of the tubules, in conjunction with the epithelial invasion of the sperm heads, lead to the suggestion that the PAS-positive material is released by holocrine activity. The invading sperm heads may stimulate the breakdown of the epithelial cells and the subsequent release of the secretion by releasing lytic enzymes similar to those produced by the acrosome during fertilization (Mann 1964; Austin 1965). Despite the drastic effects of epithelial invasion by the sperm heads, the damage is apparently restricted to localized areas in any one seminal sac. Thus the damage is never widespread and could be easily repaired without impairing the function of the seminal sac.

The precise identity of the epithelial secretion remains unknown. The negative results with Sudan IV and Alcian blue rule out the presence of lipids and mucopolysaccharides. The positive reaction with PAS indicates the presence of a polysaccharide with a glycol grouping, the most likely compound being glycogen. It is known that fowl semen contains quantities of glucose (41 mg/100 ml) with only traces of fructose (4 mg/100 ml) (van Tienhoven 1961:1110). Since glycogen is formed by a polymerized chain of glucose molecules, glycogen could readily be con-

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FIGURE 4. (Lower right) Seminal sac of a breeding bird showing variable sperm concentrations in tubules of the same organ. H. and E. (European Goldfinch). C = connective tissue, D = debris, E = epithelium, L = lumen, S = spermatozoa, SC = secretory cell, SD = secretory droplets, and SM = smooth muscle.

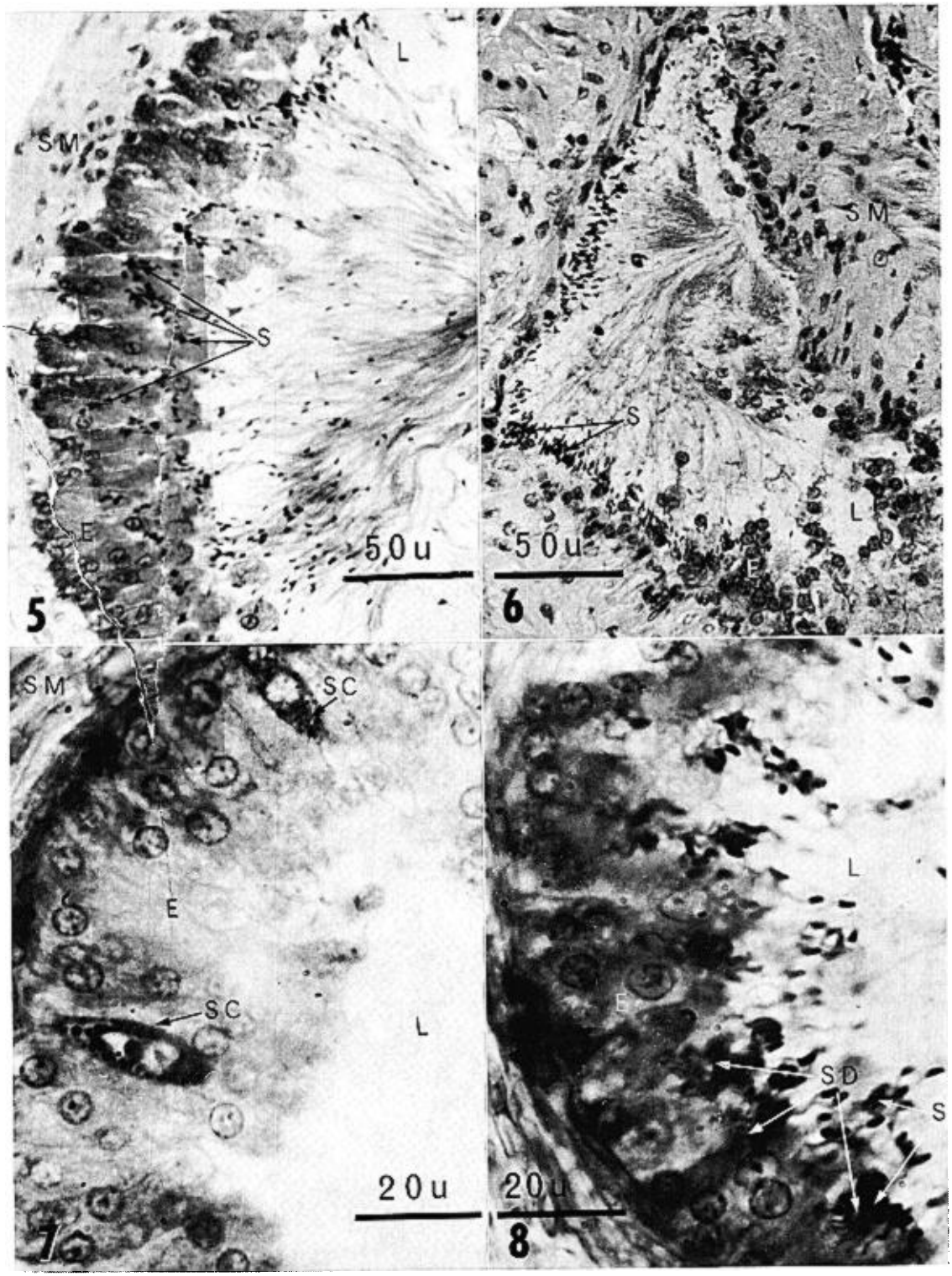

FIGURE 5. (Upper left) High power view of a single tubule showing penetration of the epithelium by spermatozoa. H. and E. (European Goldfinch).

FIGURE 6. (Upper right) High power view of a single tubule in which severe epithelial disruption has followed sperm penetration. H. and E. (European Goldfinch).

FIGURE 7. (Lower left) Portion of epithelium in which secretory cells are clearly visible. PAS. (Canary).

FIGURE 8. (Lower right) Portion of epithelium showing relationship between PAS-positive secretions and spermatozoa. PAS. (Canary). C = connective tissue, D = debris, E = epithelium, L = lumen, S = spermatozoa, SC = secretory cell, SD = secretory droplets, and SM = smooth muscle.

verted to free glucose by enzymatic action. Thus, if glycogen is produced by the epithelial cells of the seminal sac, it could be the source of the glucose characteristic of avian semen. Thus, there is support for the theory that the PAS-positive material identified in the seminal sac is glycogen.

Since glycogen seemed to be a likely component of the epithelial secretions, it was hoped that Best's carmine, a selective stain for glycogen (Lynch et al. 1963; Chayen et al. 1969), would provide a definitive answer, but the results were inconclusive. By this method glycogen was identified within the secretions of one seminal sac. However, Best's carmine is an empirical stain which is capricious, unpredictable, and frequently subject to failure (Lynch et al. 1963; McManus and Mowry 1965). Thus the failure of Best's carmine to stain glycogen in the bulk of the seminal sacs, though tentatively suggesting the absence of glycogen, may have been due to the unpredictable nature of the stain.

The role of the PAS-positive secretion also remains unknown at this stage, but it seems probable that it may play a vital role in the activation of the spermatozoa. Mann (1964) indicated that spermatozoa from the mammalian epididymis remain immobile until they contact some glycolyzable substrate. In the fowl it is known that sperm taken from distal parts of the male genital tract are capable of completing fertilization, whereas those from the testis are not (Munro 1938). Since spermatozoa are seldom found in the passerine epididymis (Bailey 1953) but are abundant in the seminal sac, the PAS-positive secretions about which the sperm are clustered could provide the glycolyzable substrate necessary for the activation of sperm. Wolfson (1954b) demonstrated that a significantly lower temperature exists in the seminal sac than in the body of passerine birds and suggested that, in addition to storage, the seminal sac could function as a temperature-sensitive maturation area. Thus the activation process, as suggested by this paper, fits well with Wolfson's (1954a, b) theories that the seminal sac serves as a maturation area for sperm in addition to its storage function.

SUMMARY

The seminal sacs of 103 European Goldfinches, 58 American Goldfinches, and 25 canaries were prepared for histological and histochemical examination. Histological studies showed that structural changes occur in synchrony with testicular development and regression. During the breeding season, sperm cells were observed to penetrate the epithelium of the seminal sacs, causing its disruption and erosion. Histochemical tests suggest that glycogen is produced by the seminal sac epithelium during the breeding season. This, in turn, suggests that the seminal sac may serve as a maturation area in addition to its role in sperm storage.

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