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Seasonal Variation in the Tubular and Interstitial Areas of the Testes in Sternotherus odoratus L.

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SEASONAL VARIATION IN THE TUBULAR AND INTERSTITIAL AREAS OF THE TESTES IN STERNOTHERUS ODORATUS L.

(TITLE)

BY

Robert Hans Spaet

THESIS

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I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
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SEASONAL VARIATION IN THE TUBULAR AND INTERSTITIAL AREAS OF THE TESTES IN STERNOTHERUS ODORATUS L.

Presented by

ROBERT HANS SPAET

a candidate for the degree of Master of Science and hereby certify that in their opinion it is acceptable.

ABSTRACT

Seasonal changes within the tubular and interstitial areas of the testes in Sternotherus odoratus are investigated, histologically and histochemically. Forty specimens were collected in Illinois, Tennessee and Louisiana between May, 1969 and August, 1972.

Many of the histological and macroscopic observations made by Risley (1938) on the spermatogenic cycle of S. odoratus in Michigan are confirmed. Contrary to his findings, fluctuations in interstitial cell nuclear diameters are observed during spermatogenesis. Greatest diameters coincide with what Risley has considered " . . . period of most active breeding." Sertoli cell nuclear diameters undergo similar fluctuations being greatest during spring breeding and fall steatogenesis.

The testes undergo a sequence of well-defined cyclical events. These include a tubular and interstitial lipid cycle correlated with spermatogenesis and a PA/S-glycogenic cycle which can be traced within the Sertoli elements. Roughly, an inverse correlation exists between the two lipid cycles. Lipid concentrations decrease as spermatogenesis advances and accumulate during the interim between cycles. The Sertoli cell is the intratubular locus of lipid accumulation while the interstitial cell is the intertubular locus. Sertoli lipids accumulate more rapidly than do interstitial lipids and are present for a longer time. Whereas interstitial lipids become depleted as secondary spermatocytes are forming, Sertoli lipids remain through the spermatid stage. In the PA/S-gylcogenic cycle, glycogen and carbohydrate materials accumulate

within the Sertoli cytoplasm in granular form during the fall and spring. These substrates become depleted during the early stages of spermiogenesis.

Latitudinal geographic variation in the timing of spermatogenic, glycogenic and lipid cycles is evident. Spermatogenesis in Illinois is two to three weeks ahead of that found in Michigan, two weeks behind the cycle in Tennessee and five weeks behind in specimens from Louisiana. The PA/S-glycogenic cycle in Illinois is two weeks behind that found in Tennessee and four weeks behind that in Louisiana specimens. Intertubular lipid cycles in Tennessee and Louisiana are similar to those found in Illinois for corresponding spermatogenic stages. The Sertoli lipid cycle follows the same pattern of timing but differs in that lipids are retained for a longer time in turtles from more southerly locations.

Spring spermatogenesis accelerates with increasing temperature and photoperiod. Following the summer solstice and despite decreasing photoperiod, the cycle continues unabated. With the onset of cooler temperatures in the fall, the cycle regresses and stops. These facts suggest that temperature is the main environmental factor regulating spermatogenesis once it has started.

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INTRODUCTION

Spermatogenic cycles have been described for a variety of reptiles but for few Chelonian species: Pseudemys scripta (Burger, 1937; Moll and Legler, 1971); Sternotherus odoratus (Risley, 1938); Terrapene carolina (Altland, 1951); Terrapene ornata (Legler, 1960); Clemmys caspica (Lofts and Boswell, 1961); and Chrysemys picta (Hutchinson and Kosh, 1964; Gibbons, 1968; Ernst, 1971). Most studies have attempted to define spermatogenic changes while largely ignoring concomitant histochemical variations involving interstitial and Sertoli cells (Lofts, 1969). Only three of these studies dealing with Chelonian species have correlated cyclic changes in the above cell types: T. carolina (Altland, 1951); C. caspica (Lofts and Boswell, 1961) and P. scripta (Moll and Legler, 1971). Sertoli and interstitial cells undergo cyclic variation involving seasonal accumulation and depletion of cytoplasmic lipids. In addition, a well-defined glycogenic cycle occurs in Sertoli cells. Any information regarding lipid and glycogenic cycles should be correlated with spermatogenesis to formulate a more accurate picture of gonadal activity.

Risley (1938) investigated seasonal changes in the testes of S. odoratus in Michigan. Interstitial cells were reported to lack demonstrable cyclic changes. Lofts (1968) believed that without thorough histological examination and appropriate histochemical evidence, such a conclusion was premature. The exclusive use of the traditional paraffin embedding techniques results in the dissolution of cytoplasmic and other lipids (Lofts, 1969). Thus, an important aspect of the cycle goes unnoticed.

The present study investigates three aspects of the male reproductive cycle in the stinkpot turtle, Sternotherus odoratus, family Kinosternidae: (1) comparison of latitudinal variation in spermatogenic timing for various populations; (2) description and correlation of cyclic events involving the tubular and interstitial areas with the spermatogenic cycle with special reference to the Sertoli and interstitial cells; (3) comparison of the cycle with that described for other reptiles and vertebrates in general.

Geographic Range and Habitat

S. odoratus ranges from the Parry Sound area of southern Ontario eastward through southern Maine and southward to Dade county, Florida. Westward, its range covers all of Michigan south of a line extending east-west through Saginaw bay and reaches southeastern and northcentral Missouri, western Iowa, extreme southeastern Kansas and the eastern parts of Oklahoma and Texas (Carr, 1966). Raun and Gehlbach (1972) show its range extending further westward into central Texas. Moll and Williams (1963) have described the only known specimen of S. odoratus from Mexico (collected by S. E. Meek in 1903 from Sauz, Chihuahua). Stinkpots inhabit almost any type of fresh water habitat, particularly shallow, clear-water lakes, ponds and rivers (Conant, 1958; Pritchard, 1967). The turtle is primarily aquatic and shuns temporary bodies of water (Carr, 1966).

PROCEDURE

Stinkpots were collected from May, 1969 through August, 1972; 40 turtles were captured representing all months from March through November. Geographic location of capture and the corresponding number caught were: 14 from Illinois; 16 from Tennessee; and 10 from Louisiana. Gaps appear in monthly collections for all three geographic regions. As a consequence, no yearly spread of specimens for any one of these areas could be obtained. During April, May, June, October and November, collections from Illinois were made in Pope county (one); Coles county (nine); and Cass county (four). Sixteen turtles from Tennessee were taken in Lake county during May, June, July, August, October and November. Stinkpots from Louisiana were taken during March, June, July and September from St. Charles Parrish (one) and Concordia Parrish (nine).

Turtles were captured primarily in baited hoop-net traps. Carapace length was measured for all specimens excluding four from July: 2 from Tennessee and 2 from Louisiana. Testes and epididymides were removed, weighed, measured, fixed and preserved in Baker's neutral formalin within five days of capture. Cross-sections from the mid-region of the testes were sliced at a thickness of two millimeters, washed in tap water, dehydrated in two changes of ethyl cellosolve (four hours each), cleared in methyl benzoate (one hour) and rinsed in two changes of benzene (one hour each). Infiltration was accomplished by immersion into a 50:50 solution of Paraplast[1] (one

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[1]Tissue embedding media manufactured by Sherwood Medical Industries Inc., St. Louis, Mo.

hour at 58° C) and two subsequent changes of molten Paraplast (two hours each at 58° C). Tissues were blocked in Paraplast at 0° C for fifteen minutes and at room temperature (23-25° C) for one day, then sectioned at about three microns on the rotary microtome. Other material was washed, infiltrated with Lipshaw M-1 Embedding Matrix[2] (10-12 hours) and frozen-sectioned at eighteen microns with a cryostat.

Part of the Paraplast-embedded tissue was stained by a regressive technique using Harris' alum haematoxylin (Mallory, 1944) and eosin Y. Another segment of tissue was subjected to the PA/S test for carbohydrates (with 1,2-glycol linkages) as developed by McManus (1948). Additional PA/S sections of the same tissue underwent diastase digestion for the removal and subsequent demonstration of glycogen as suggested by Pearse (1960).

Frozen-sectioned tissue was stained with Sudan IV for simple lipids (triglycerides) as recommended by Chiffele and Putt (1951). In addition, October 21 material from Coles county, Illinois was subjected to Sudan Black B (Sumner and Sumner, 1970) for both simple and complex lipids (triglycerides, phospholipids and glycolipids) and the fumes of a two percent aqueous solution of osmium tetroxide (Guyer, 1941) for phospholipids.

Sections stained with Harris' alum haematoxylin and eosin Y underwent: 1) deparaffinization in two 15 minute changes of xylol; 2) hydration beginning in 100% methanol and proceeding in a "down" sequence of ethanol solutions (95, 70 and 50%) 3-5 minutes each and ending in a bath of distilled water for 5 minutes; 3) overstaining in Harris' haematoxylin for 5-10 minutes; 4) removing the excess stain in tap water for 3-5 minutes; 5) dehydration in 50 and 70% ethanols; 6) differentiation in 1% acid alcohol

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[2]Frozen sectioning embedding media made by Lipshaw Manufacturing Co., Detroit, Mich.

for 2-3 minutes; 7) bluing in 70% alcoholic Scott's solution and subsequent dehydration in 95% ethanol; 8) counterstaining for 15-30 seconds in 1% eosin Y in 95% ethanol containing two drops of glacial acetic acid per 100ml; 9) dehydration in 100% methanol; 10) clearing in two 5 minute changes of xylol; 11) mounting in Permount[3]. This procedure is a modification of a schedule prescribed by Humason (1967). Modifications include: 1) a shortened period of overstaining; 2) alcoholic Scott's solution; 3) a longer period for differentiation; 4) a substitution of 100% methanol for absolute ethanol.

Procedure for the PA/S method for carbohydrates (McManus, 1948) was as follows: 1) dehydration of sections to water; 2) immersion in a .5% aqueous solution of periodic acid for 5 minutes; 3) rinsing in distilled water for 5 minutes; 4) exposing to Schiff's reagent (Lillie, 1965) for 15 minutes; 5) rinsing in sulfurous acid for 5 minutes; 6) washing in tap water for 5 minutes; 7) dehydration to 70% ethanol; 8) counterstaining with .5% light green in 70% ethanol for 15 seconds; 9) further dehydration in 95% ethanol and 100 methanol; 10) clearing in two changes of xylol and mounting in Permount. Coupled with the above procedure were the control slides for glycogen. These tissue sections were exposed to the PA/S method described but underwent a modification in that before being submitted to the 0.5% aqueous periodic acid, incubation took place in 1% malt diastase for one hour at 37° C.

Sudan IV staining for simple lipids (Chiffele and Putt, 1951) required the following steps: 1) dipping of the sections in two changes of ethylene glycol (3-5 minutes in each) and subsequent agitation; 2) staining in Sudan IV for 15 minutes with occasional agitation; 3) differ-

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[3]Synthetic mounting media manufactured by Fisher Scientific Co., Fair Lawn, New Jersey.

entiation in a solution of 85:15 ethylene glycol and distilled water with subsequent agitation; 4) rinsing in two changes of distilled water for 5 minutes; 5) counterstaining in Harris' alum haematoxylin (Mallory, 1944) for two seconds; 6) tap water rinse and mounting in glycerine jelly.

Sudan Black B staining followed a schedule similar to that outlined by Gurr (1958) and required: 1) dehydration of the sections to 70% ethanol; 2) immersion in Sudan Black B for 10 minutes; 3) immersion in 50% ethanol for five seconds; 4) mounting in glycerine jelly.

The procedure for the demonstration of phospholipids was run in accordance with that outlined by Guyer (1941). Tissue sections were exposed to a 2% aqueous solution of osmic acid for five hours. The preparation stood in a closed petri dish to prevent excessive volatilization and in a hood to avoid exposure to toxic fumes.

Macroscopic measurements were made in millimeters using vernier calipers. Greatest carapace length for each turtle was measured to the nearest millimeter. Greatest length and width of each testis were measured to the nearest tenth of one millimeter. Volumetric measurements were made using the formula for the area of a prolate spheroid. Testes and epididymides were weighed to one-tenth of a gram on a triple beam balance.

Microscopic measurements were made using a calibrated ocular micrometer with maematoxylin and eosin Y-stained sections. Measurements involving the interstitial cell included only an account of nuclear diameter as the cell boundries were poorly delineated. Fifty interstitial cell nuclei were measured across the widest point in all sections excluding the following specimens in which only 25 could be located: 1) June 15, St. Charles Parrish, Louisiana; 2) March 24 and June 15, Concordia Parrish, Louisiana; 3) June 17 and May 6, Lake county, Tennessee. Systematic right-left, left-right scanning assured that interstitial cell nuclei were not

measured repeatedly. No positive measurement could be made of Sertoli cell height. Maximum diameters of ten nuclei per tubule cross-section in each of five randomly chosen tubules were measured.

OBSERVATIONS

Macroscopic observations of testes in S. odoratus reveal a seasonal trend of enlargement and regression (Fig. 1). Mid-March testes from Illinois specimens are smaller than those noted during the previous November (testes are not available for specimens from Louisiana - November and Tennessee - March). Turtles from all three geographic regions attain minimum testicular size during May until mid-June. Two mid-June specimens from Louisiana show testes enlargement. Testes from Tennessee and Lousiana specimens reach maximum size during August and September (no Illinois testes are available).

The pattern of testicular size fluctuation for animals in this study is basically similar to that found in S. odoratus from Michigan but with slight variations with regard to timing. Risley (1938) noted that stinkpot turtles from this state: 1) emerge from hibernation in late March or early April, at which time the testes are smaller than those from the previous November; 2) exhibit testes which attain a minimum size during the first two weeks of May, probably coincident with a period of most active breeding; 3) display a maximum testicular size during July and August when sperm production and storage is greates; 4) demonstrate testes measurements which decline during October and November when sperm are being transferred to the epididymides for winter storage. Size fluctuations in testes from turtles investigated in this study also suggest a correlation with the period of most active breeding in spring, maximum spermatogenic activity during the summer and transfer of sperm from the testes to the epididymides during the fall.

Other studies involving Chelonians have revealed seasonal changes in testes size similar to those outlined above: T. carolina (Altland, 1951); T. ornata (Legler, 1960); C. caspica (Lofts and Boswell, 1964); C. picta (Gibbons, 1968; Ernst, 1969) and P. scripta (Moll and Legler, 1971). In S. odoratus from Michigan, testes vary from a minimum of 9.1 mm x 7.0 mm in May to a maximum of 19.6 mm x 16.0 mm in July-August (Risley, 1938). As the testes are nearly ellipsoid in shape, these figures correspond to a volumetric increase of about 11 times. The specimens from Louisiana and Tennessee investigated in this paper give values approximating this. Testes from Louisiana enlarge from a minimum of 5.2 mm x 4.4 mm in June to a maximum of 16.9 mm x 14.2 mm by the end of August corresponding to a volume increase of 11.5 times. Tennessee gonads demonstrate a minimum size of 8.6 mm x 5.9 mm in June and a maximum of 19.7 mm x 17.3 mm in August equivalent to an increase of volume of about 11 times.

Few reptilian studies have used interstitial cell nuclear diameter as an index of cyclic metabolic activity: Phrynosoma solare (Blount, 1929); Eumeces fasciatus (Reynolds, 1943); Terrapene carolina (Altland, 1951); Uromastix (Kehl and Combescot, 1955); Vipera berus (Marshall and Woolf, 1957); Uta stansburiana (Hahn, 1964); and Leiolopisma fuscum (Wilhoft and Reiter, 1965). Only the study on T. carolina has correlated cyclic changes in Sertoli cell nuclear diameter with spermatogenesis. An increase in cell size has been interpreted by most authors as indicative of increasing cellular activity in reptilian testes (Fox, 1952). Concomitant size fluctuations in nuclear diameter probably imply the same. It is known that an artificial increase in nuclear size leads to a corresponding increase in cytoplasmic dimensions and that the nucleus is the primary agent in the constructive processes of the cytoplasm (Wilson, 1947).

Nuclear measurements taken from interstitial and Sertoli cells show a seasonal cycle of increasing and decreasing diameters correlating with the spermatogenic cycle (Fig. 2 and 3). In one September specimen from Louisiana (the only turtle collected during this month), interstitial cell nuclear diameters have increased. Other animals from Louisiana and missing representatives from Illinois and Tennessee may demonstrate similar increases. Nuclear diameters of specimens from all three geographic regions gradually increase during October and November attaining maximum size in the spring (April-June). These diameters generally decline during late June and July reaching a minimum in August. Sertoli cell nuclear diameters parallel those of the interstitial cell except that a greater increase occurs during the fall (October, November). Assuming that nuclear measurements reflect metabolic activity, both cell types are more active during the spring prior to and during early stages of spermatogenesis, when most active breeding may be occurring. The almost three-fold increase in Sertoli cell activity in the fall may be correlated with a time of increasing steatogenic activity. The three March specimens collected (one from Louisiana and two from Illinois) show Sertoli nuclear diameters conspicuously smaller than those from October-November. This may indicate a reduction in metabolic activity resulting from hibernation.

Spermatogenesis

The following is a description of spermatogenesis in stinkpot turtles from Illinois. The events described are depicted in Fig. 4D.

Mid-March to mid-April: The spermatogenic condition of mid-March Illinois specimens is similar to that found in the seminiferous tubules of turtles captured during the previous November. Approximately one-quarter of the tubule cross-sections are filled with spermatozoa and

residual spermatid nuclei from the preceding cycle. Spermatogonia are scattered at intervals around the periphery of each tubule cross-section. Sertoli nuclei (6.5 microns in diameter) are located nearer the basement membrane than the spermatogonial cells and outnumber them by almost 5:1. Sertoli cytoplasm extends one-quarter of the way towards the center of the lumina. Intertubularly, interstitial nuclear diameters are between 5.5 and 6.0 microns.

Mid-April through mid-May: The Sertoli cytoplasm of mid-April specimens partially occludes luminal areas which contain loosely aggregated sperm and moderate amounts of cellular detritus. Recrudescence (beginning of a new spermatogenic cycle) is evident by an increase of spermatogonia. Sertoli nuclei (7.1 microns in diameter) now outnumber them by only 3:1. Interstitial nuclei increase to about 6.3 microns in diameter. Sections from mid-May specimens show no sperm in the lumina but some cellular debris. Sertoli cytoplasm fills a portion of the tubule system but many lumina resemble those found in mid-April in this respect. Spermatogonia now outnumber Sertoli elements and surround them.

End of May through mid-June: By the end of May, spermatogonial cells predominate and hold positions around and above Sertoli nuclei which have expanded to about 7.2 microns. The Sertoli syncytium no longer occludes the luminal spaces and extends only half of the way into the tubule. No sperm and little cellular detritus remain in most instances. A number of primary spermatocytes and many spermatogonia are present at this time. Interstitial nuclear diameters have enlarged to between 6.4 and 6.8 microns. A considerable number of primary and secondary spermatocytes are seen by mid-June. Sertoli nuclei are pushed to a more basal position and show reduced diameters (6.7 microns). Sertoli cytoplasm is

poorly defined at this time. Interstitial cell nuclear diameters are reduced to approximately 6.1 microns.

An interesting variation is observed in mid-June specimens from Louisiana (with one exception). Intratubular areas exhibit curious, finger-like projections of the Sertoli cytoplasm into the tubule lumina. This condition takes place during a period of heightened spermatogenic activity. Due to a lack of summer specimens, it is not known if this phenomenon also occurs in Illinois turtles, yet this may be a transitory stage in the cycle and occur 4-5 weeks later than in mid-June Louisiana animals. June 17-19 and July 17-18 turtles from Tennessee do not show this condition. The latter may appear sometime between these dates or not at all.

July, August, September: Illinois specimens are missing for these months but the spermatogenic events occurring during these times can be reconstructed using gonadal material from Tennessee and Louisiana as well as the information given by Risley (1938) for S. odoratus in Michigan. Observations indicate that the cycle in Illinois is about 2-3 weeks ahead of that found in Michigan, 2 weeks behind the cycle in Tennessee and approximately 5 weeks behind in specimens from Louisiana. From this, it can be postulated that between the beginning and middle of July spermatids appear and spermiogenesis begins. Both spermatogenesis and spermiogenesis reach a zenith sometime between the end of August and the beginning of September. Following this peak, spermatogonial divisions stop, spermatogenic stages decrease and the cycle undergoes a gradual degeneration. Nuclear changes in both the Sertoli and interstitial cells have already been given in the previous section.

End of October, beginning of November: Illinois representatives are again present. At this time, numerous sites of bunched, feathery-

appearing spermatozoa are noted along the tubule peripheries. Sertoli nuclei maintain a position inferior to a few secondary spermatocytes, spermatids and the rapidly maturing sperm many of which have already been freed into the lumina. Sertoli cell nuclear diameters have expanded to between 6.8 and 7.2 microns. Interstitial nuclear dimensions have slowly increased with diameters ranging between 5.4 and 5.7 microns.

Seasonal Variation in PA/S+ and Glycogenic Substances

Glycogenic carbohydrates are regarded as stored fuel, deposited when glucose is present in sufficient amounts and available as fuel when glucose is less abundant (Carlson et al., 1962). Thus, a depletion of stored glycogen in cellular entities suggests a utilization of this substrate in the course of heightened metabolic activity (Fig. 4C).

Few studies have been made regarding the occurrence and distribution of PA/S-positive and glycogenic material in reptilian testes. Cavazos and Melampy (1954) and Cavazos and Feagans (1960) have investigated the incidence of testicular PA/S-reactive carbohydrates and glycogen in the horned lizard, Phrynosoma cornutum, as affected by seasonal variation. Only one such study has involved a Chelonian: Kinosternon flavescens flavescens (Christiansen and Dunham, 1972).

Varying amounts of PA/S-reactive material are observed inter- and intratubularly throughout the spermatogenic cycle in the stinkpot. Most of these carbohydrates are non-glycogenic in nature. PA/S-positive substances which occur within the tubule take a granular form and stain a magenta color. Basement membranes are strongly reactive most of the time, and during times of maximum tubule response possess minute, deeply-staining particles susceptable to diastase digestion. Within the interstitium, reactive carbohydrates do not assume an agranular appearance but

but instead stain homogeneously. Exceptions to the latter arise in the case of an occasional reactive granule observed in the wall of a blood vessel. Due to a lack of discrete, packeted carbohydrate in the interstitium (i.e. granules), it is difficult to define cyclic changes therein. As mentioned, PA/S-reactive carbohydrate (including glycogen) is present in granular form intratubularly and seasonal variation becomes evident.

Mid-March to mid-April: Mid-March specimens from Illinois demonstrate a relatively intense PA/S staining reaction. Moderate amounts of glycogen are observed within the Sertoli cytoplasm and spermatogonia as evidenced by a decrease in the number of PA/S-positive granules following diastase digestion. The majority of granules are diastase-resistant, however, indicating that they are non-glycogenic. The staining intensity of the interstitial area remains the same after diastase digestion suggesting that this area contains little glycogen.

Mid-April through mid-May: By mid-April, tubule cross-sections show a slight increase in glycogen content. Granules of this substrate are distributed in a seemingly random fashion within the Sertoli cytoplasm and do not appear to be localized in any particular area or relationship therein. Residual spermatogenic debris does not appear to stain specifically. The interstitium remains glycogen poor. Sections from mid-May show a sizeable increase in the number of glycogenic particles within the Sertoli cytoplasm and intratubular areas. Many prominent, brightly-staining non-glycogenic granules are also noted at this time. Within the interstitium, a few granules of glycogen are dispersed in the media of arterioles and venules.

End of May through mid-June: Specimens from the end of May remain in approximately the same condition as for mid-May but exhibit a buildup

of glycogen in the basement membrane. By mid-June, PA/S-reactive carbohydrates are more abundant in the Sertoli cytoplasm than at any previous time. No changes were evident in the amounts of glycogen within the interstitium.

The finger-like projections of Sertoli cytoplasm, as seen in mid-June specimens from Louisiana, contain many PA/S-positive granules as well as a good number of glycogenic particles. Many of these structures are seen in the distal end of the projections. This occurs at a time when many of the secondary spermatocytes are maturing into spermatids.

July, August, September: Observations taken from Tennessee and Louisiana specimens enable one to reconstruct the PA/S-glycogen cycle in Illinois testes during these missing months. The cycle in Illinois is about 2 weeks behind that found in Tennessee and 4 weeks behind specimens from Louisiana. Large amounts of glycogen and PA/S-positive material probably remain in the Sertoli cytoplasm until sometime between mid-July and the beginning of August. No glycogen is located in the interstitium by mid-July. Glycogen and carbohydrate stores are depleted during August corresponding with an acceleration of spermiogenesis. A "low point" is reached in September and continues through most of October.

End of October, beginning of November: Only a few scattered PA/S-reactive granules are seen lining the tubule peripheries along with maturing spermatozoa. Non-glycogenic granules quite variable in size are observed in the lumina together with freed sperm. The only detectable intertubular glycogen seems to be localized in the heads of these sperm. No changes in staining intensity are noticed in the interstitial areas following diastase digestion.

Seasonal Variation in Lipids

The testes of S. odoratus display a well-marked seasonal variation

in the appearance and disappearance of lipids from interstitial and Sertoli cells (Fig. 4A and B). Seasonal changes involving testes lipids have been reported in such reptiles as the box turtle, Terrapene carolina (Altland, 1951), European viper, Vipera berus (Marshall and Woolf, 1957), horned lizard, Phrynosoma cornutum (Cavazos and Feagans, 1960), Caspian terrapin, Clemmys caspica (Lofts and Boswell, 1961), cobra, Naja naja (Lofts et al., 1966) and Neotropical slider, Pseudemys scripta (Moll and Legler, 1971). Generally, changes in testes lipid content appear consistent for these reptiles in relation to seasonal spermatogenic changes. During a period immediately prior to or during mating, testicular lipids are gradually depleted whereas during the interim between spermatogenic cycles, they are reaccumulated. The testes lipid pattern in S. odoratus follows this pattern closely.

Mid-March through mid-April: Illinois turtles from mid-March are accumulating intertubular sudanophilic droplets in the Sertoli cytoplasm. An occasional globule may be seen free within the luminal areas. Only moderate amounts of lipid are concentrated in the interstitial cell cytoplasm. Accumulation of lipoidal material continues within the Sertoli and interstitial cells of mid-April representatives. Lipid droplets are present in greater amounts in the interstitium though not to the extent found intratubularly.

Mid-May through mid-June: Sertoli cells from mid-May tubule cross-sections contain intensive amounts of lipid material. Interstitial cell cytoplasm continues to build up sudanophilic lipids. By the end of May, only moderate concentrations of intratubular lipid remain. In contrast, the interstitial cells are packed with lipid. By mid-June, the intertubular areas contain relatively little fatty material. Only a few

droplets are present in the general interstitial areas and these are abutting the tubules in scattered locations. The Sertoli elements continue to be moderately lipoidal and hold a number of droplets close to the basement membrane.

The oddly-shaped Sertoli cytoplasm in mid-June specimens from Louisiana contains heavy concentrations of lipid. The fact that lipids remain so plentiful at a time of advanced spermatogenesis indicates that southern populations retain tubule lipids much longer than do northern individuals.

July, August, September: Events pertaining to the Illinois testes lipid cycle during these months may be reconstructed as for the spermatogenic and PA/S studies. Following the onset of spermiogenesis in early July, tubule lipids vanish. Both inter- and intratubular lipids are at a minimum during the time of most active spermatogenesis in August and September. A gradual accumulation of lipid occurs in both interstitial and Sertoli elements sometime during October. Lipid accumulations within the tubule seem to take place at a faster rate than in the interstitium. By March, considerable intratubular lipoid is present while the interstitial areas contain only moderate amounts.

Early November: A moderate buildup of sudanophilic material is noted among scattered interstitial cells. A noticeable amount of lipoidal material is concentrated in the Sertoli cytoplasm by this time indicative of a fall tubule steatogenesis.

A variation to the general pattern occurs in the testes of an October 21 specimen from Coles County, Illinois making it particularly noteworthy. Sections of the testes are highly vascularized and clusters of atypical, polyhedron-shaped blood cells pack the interstitium. Selective staining indicates that these clusters and the interstitium in general

contain large quantities of lipids particularly phospholipids. The absence of this phenomenon in other specimens from the same general time period suggests that this condition may be pathological or a short-lived transitory stage, perhaps involved with lipid mobilization and reclaimation.

Animals from Tennessee show a testicular lipid cycle similar to that described for Illinois turtles but probably retain intratubular lipids for a longer period of time much as in turtles from Louisiana. Aside from these observations, lipid fluctuations in Illinois, Tennessee and Louisiana spermatogenic cycles correspond to similar spermatogenic stages during the early spring, late summer and fall.

Summary of Spermatogenic, PA/S+ Glycogenic and Lipid Cycles

Geographic variation in timing for turtles from Tennessee and Louisiana has already been given. It should be made clear that spermatogenic, PA/S and lipid cycles in these animals are basically similar to those found in animals from Illinois of corresponding spermatogenic stages. The prolonged retention of tubular lipids in specimens from Tennessee and Louisiana may be the only exception.

Timing of lipid and glycogenic cycles in the testes appear to be strongly correlated with the spermatogenic cycle. Combining observations from all specimens studied results in a composite description of the male reproductive cycle in S. odoratus in Illinois.

In March when the germinal epithelium is quiescent and the tubules are congested with residual cellular material from the previous cycle, lipids accumulate within the Sertoli cytoplasm and interstitium. PA/S- reactive granules increase within the Sertoli cytoplasm only.

In April the germinal epithelium recrudesces as spermatogonia begin to proliferate. Sertoli cells continue to accumulate lipid, glycogen

and PA/S-reactive carbohydrate. The interstitial cells also continue to collect lipids but contain little glycogen.

By May no sperm and little cellular debris remain in the tubules. Spermatogonia proliferate throughout May and primary spermatocytes appear by the end of the month. A sizeable increase in glycogen and PA/S-reactive carbohydrate occurs in the Sertoli cells. Sertoli cytoplasm reaches maximum lipid content during early May after which a depletion takes place towards the end of the month leaving only moderate amounts. Interstitial cells continue to gather lipids and reach a peak by the end of the month. Small amounts of glycogen are present in the interstitium.

Numerous primary and secondary spermatocytes appear by late June. Virtually all lipids disappear from the interstitium by this time, and no glycogen can be detected. Levels of lipid and carbohydrate within the Sertoli cytoplasm remain constant.

Following the onset of spermiogenesis in mid-July, lipids, glycogen and other PA/S-positive granules are gradually depleted from the Sertoli cytoplasm. Large accumulations of carbohydrate and lipid may concentrate in finger-like projections of the Sertoli cytoplasm as in specimens from Louisiana at this time. These projections and their contents would probably disappear upon spermiogenic acceleration.

Spermiogenesis continues to accelerate in August, reaching a peak towards the end of the month. Tubules contain bunched sperm around the peripheries during the latter part of August and September. Gonial divisions stop in September and spermatogenic stages are reduced. Little carbohydrate and no lipids are present during August and September.

During October maturation of spermatids and sperm continues and many are now free in the tubule lumina. Most of the glycogen is concentrated

in the heads of these sperm and a few non-glycogenic granules are seen in the Sertoli cytoplasm. The interstitium contains no glycogen. Lipids are accumulating in both the Sertoli and interstitial cytoplasm at this time.

By late November many sperm have entered the epididymides. Tubule cross-sections contain only moderate amounts of sperm (peripheral and free), inactive gonial cells and scattered spermatids. The Sertoli cytoplasm continues to accumulate lipids, glycogen and PA/S-reactive material. Interstitial cells also continue to buildup cytoplasmic lipids while no glycogen can be detected. Hibernation and testicular quiescence occur in December.

Fig. 1. Monthly fluctuations in the size of 40 testes from Illinois, Tennessee and Louisiana. Average testis length is expressed as a percentage of carapace length for all but four July specimens: 2 from Tennessee and 2 from Louisiana. Carapace length was not available for these animals. Volumetric measurements of the gonads were made instead followed by an estimate of their graphic placement. Dots represent specimens from Illinois; Circles - Tennessee; Triangles - Louisiana.

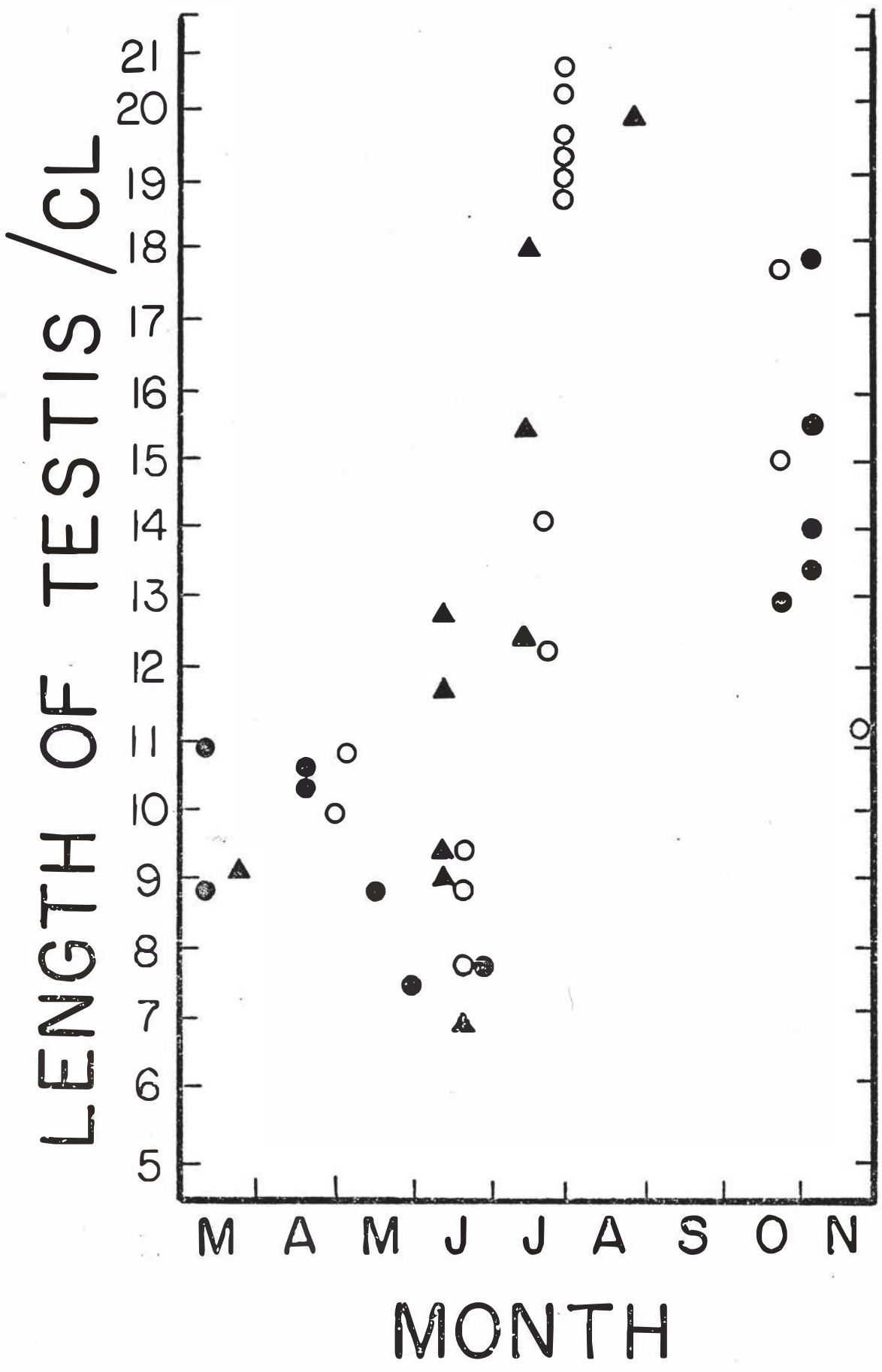

LENGTH OF TESTIS /CL
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
M
A
M
J
J
A
S
O
N
MONTH

Fig 2. Sertoli cell nuclear diameters (in microns), by month, in 40 turtles from Illinois, Tennessee and Louisiana. Dots represent specimens from Illinois; Circles - Tennessee; Triangles - Louisiana.

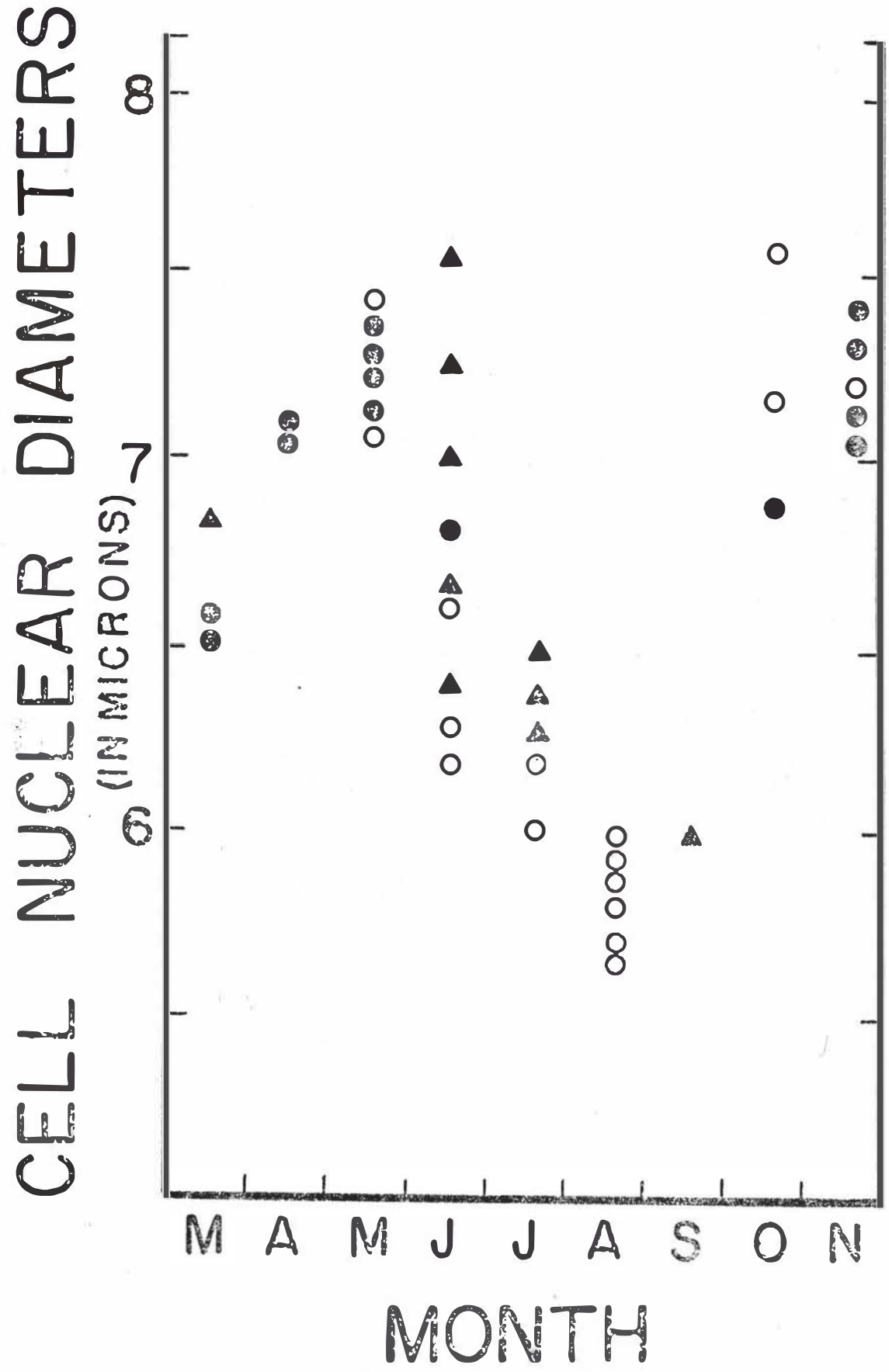

CELL NUCLEAR DIAMETERS
(IN MICRONS)
8
7
M A M J J A S O N
MONTH

Fig. 3. Interstitial cell nuclear diameters (in microns), by month, in 40 turtles from Illinois, Tennessee and Louisiana. Dots represent specimens from Illinois; Circles - Tennessee; Triangles - Louisiana.

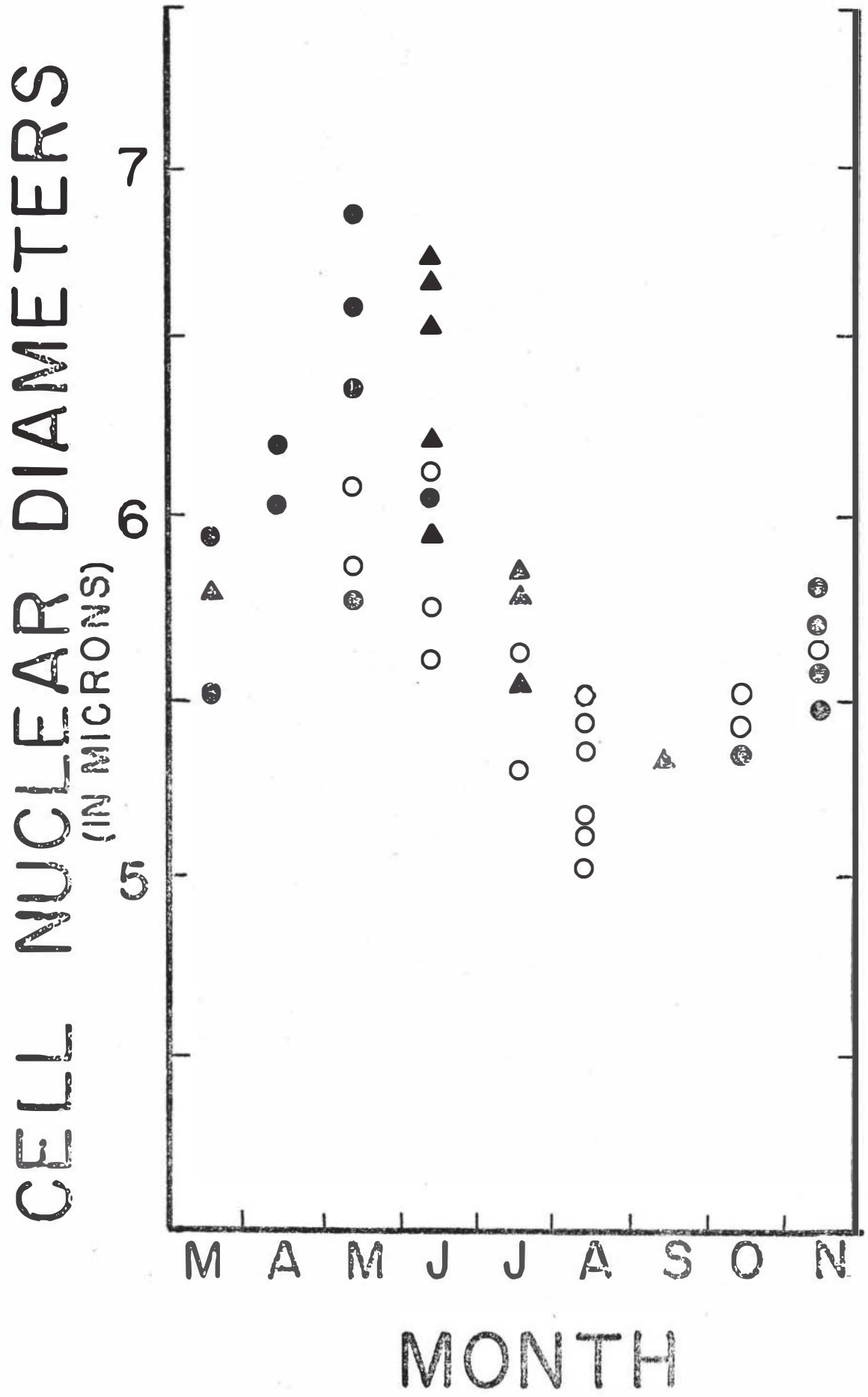
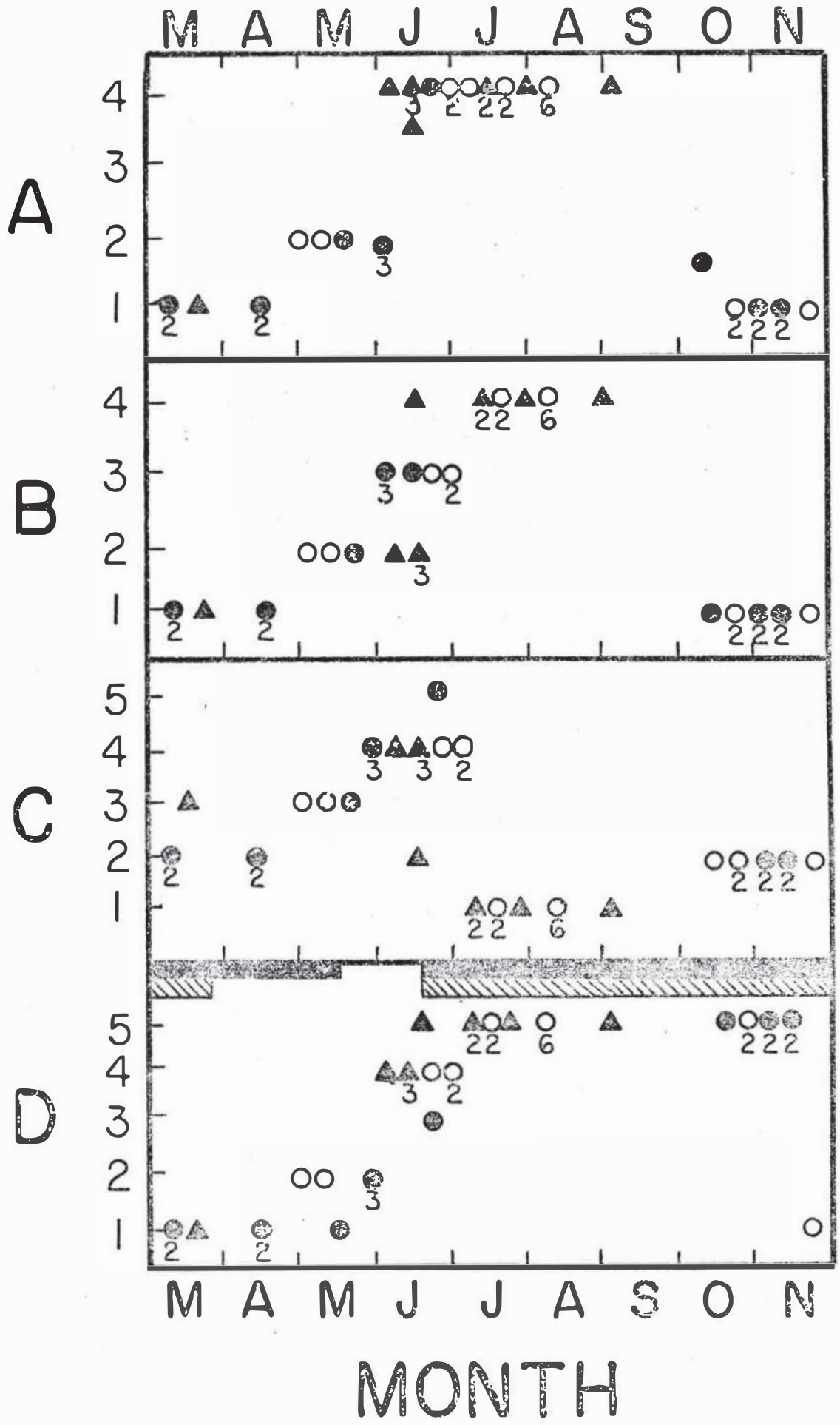

CELL NUCLEAR DIAMETERS
(IN MICRONS)
7
6
5
M
A
M
J
J
A
S
O
N
MONTH

Fig. 4. Seasonal fluctuations in inter- and intratubular lipid, glycognic and spermatogenic cycles. Turtles from Illinois, Tennessee and Louisiana are represented. Dots represent specimens from Illinois; Circles - Tennessee; Triangles - Louisiana. Graph A represents the intertubular lipid cycle. Graph B depicts the intratubular lipid fluctuations. Four arbitrarily chosen categories are used to trace each cycle: 1) An accumulation of lipid droplets; 2) Heavily charged with dense sudanophilic droplets; 3) Depletion of lipid material to moderate amounts; 4) Very little if any lipid. Graph C represents the glycogenic cycle in the Sertoli cell. Five categories are used to follow this cycle and are based upon the relative amount of glycogen present: 1) None; 2) Little; 3) Moderate; 4) Moderate to high; 5) High. Graph D represents spermatogenesis. Five categories are used based upon the predominance of one of the five spermatogenic stages: 1) Spermatogonia; 2) Primary spermatocytes; 3) Secondary spermatocytes; 4) Spermatids; 5) Spermatozoa. Solid bar represents freed sperm within the lumina. Striped bar denotes sperm which are attached to luminal peripheries. During the months of June and July, solid and striped bars pertain to stage 5 representatives only.


M A M J J A S O N
A
B
C
D
MONTH

DISCUSSION

The spermatogenic cycle in S. odoratus suggests that temperature, not photoperiod, is the primary environmental factor in the regulation of testicular recrudescence once it has started. The cycle responds in a manner similar to that observed by Lofts and Boswell (1961) in Clemmys caspica. An acceleration of spring spermatogenesis with increasing temperature and photoperiod occurred in this turtle. Following the summer solstice (June 21) and despite decreasing photoperiod, the spermatogenic cycle continued unabated. The latter seemed more closely coordinated with temperature, coming to a standstill with the onset of cooler temperatures in the fall. In contrast, Burger (1937) reported that in the turtle Pseudemys elegans, an artificial increase in day length would interrupt the spermatogenic cycle and start a new one. Although artificial conditions were imposed, his study suggests that increasing photoperiod is the chief regulator of spermatogenic timing.

In the absence of experimental data and because specimens are collected from a variety of localities and different years, it is difficult to assess the interaction of water temperature and day length with respect to control of latitudinal variation of spermatogenesis. Certain a priori assumptions may be made, however, using spermatogenic conditions of specimens from Tennessee and Louisiana. Both temperature and photoperiod tend to increase at an earlier date for stinkpot populations at lower latitudes. Perhaps recrudescence tends to begin earlier as a result of these increases. Thus, differences arise in the spermatogenic timing

of turtles varying in latitudinal distribution. Also, animals living further south experience an earlier increase in day length and temperature during the spring, leaving a shorter period for hibernation and a longer time for reproductive activity. Longer periods of reproductivity have been described for southern populations of the painted turtle, Chrysemys picta (Moll, personal communication).

The seasonal cycle of testicular glycogen and PA/S-reactive carbohydrate in S. odoratus differs considerably from that noted in the horned lizard, Phrynosoma cornutum (Cavazos and Feagans, 1960) for corresponding spermatogenic stages. Unlike the stinkpot, lizards obtained at a stage of accelerated spermiogenesis demonstrated an intense PA/S reaction intratubularly. Sertoli cells and attached clusters of immature spermatozoa contained much glycogen. Also, a number of glycogenic granules were seen within the cytoplasm of the germinal epithelium. Interstitial areas contained generous amounts of PA/S-positive material in granular form. During the height of the breeding season in P. cornutum, little glycogen and carbyhydrate material were noted, whereas a moderate to high amount can be seen in S. odoratus.

In contrast, the carbohydrate cycle in the yellow mud turtle, Kinosternon flavescens flavescens (Christiansen and Dunham, 1972) parallels that seen in S. odoratus for the most part. "Projections" of the Sertoli cytoplasm, perhaps similar to those noted in mid-June Louisiana stinkpots, were observed. These also held many strongly PA/S-positive granules which the authors interpreted as an accumulation of glycogen. As spermiogenesis began in early July, no glycogen remained in the Sertoli cytoplasm. Unlike K. f. flavescens, S. odoratus retains reactive carbohydrate and glycogen throughout early spermiogenesis and does not lose these substances until

an increase of the latter in late July and early August. Both Chelonians accumulate this buildup in late fall and during the early stages of the following spermatogenic cycle.

Seasonal changes were also evident in the interstitial cell cytoplasm of the yellow mud turtle. During May, when these cells were greatest in size, they contained more glycogenic substances than at any other time. In the stinkpot, no well-defined cycle can be established in the interstitium. Nevertheless, some non-granular glycogen can be observed during June.

The most obvious areas of carbohydrate and glycogen presence in S. odoratus are localized intratubularly, particularly within the Sertoli cytoplasm. Spermatogonia in March Illinois specimens are the only germinal elements to demonstrate an accumulation of cytoplasmic glycogen along with the Sertoli cells. Although slight variations in staining reactivity are seen within the interstitium, PA/S reactions are not as evident nor do they reach the magnitude found in the Sertoli elements. This seems reasonable if the latter are to perform a sustentacular function in terms of the germinal epithelium and a nutritional role in the development of spermatids into sperm (Miller, 1959). The Sertoli cells are separated from the intertubular blood supply by the basement membrane. Presumably these cells store substrates such as glycogen in order to provide a source of reserve carbohydrate for the developing germinal elements. According to Free (1970), Sertoli cells and spermatogonia are the main sites of pentose cycle activity intratubularly.

Sertoli cells in vertebrates contain glycogen so that as well as providing a means of transporting blood nutrients to the germinal epithelium, they can also buffer the local fluctuations in nutrient requirements during cyclical changes in the germ cell population (Free, 1970). The

variation in Sertoli cell glycogen with the cycling of spermatogenesis is generally thought to represent a utilization of this substrate by the developing spermatids. This reason may be suggested to account for the seasonal fluctuations of glycogen and PA/S-carbohydrate in the Sertoli cells of S. odoratus.

In his experiments on the glycogen content in the testes of domestic and laboratory animals, Nicander (1957) observed that much of this carbohydrate is often concentrated in the heads of the maturing sperm intimately associated with the Sertoli cells. In a November stinkpot from Illinois, the heads of sperm free within the lumen hold minute amounts of glycogen. This may suggest that the substrate is not used immediately for metabolic energy during maturation, but perhaps stored until the sperm reach the epididymides.

Gierke (1937) reported a low glycogen content in intratubular areas showing advanced spermiogenesis as compared to earlier stages in prepubertal human testicles. He also showed that this carbohydrate is stored during the early stages of spermatogenesis. The glycogenic cycle in S. odoratus seems to follow this pattern closely.

The interstitial cells in the testes of the stinkpot require a substrate such as glycogen just as the Sertoli cells do, but evidently have no need to store it in a compact form as do the intratubular elements. This occurrence seems unusual when compared with the seasonal accumulation of granulations in the interstitium of P. cornutum and K. f. flavescens. It would appear that interstitial areas contain a generous blood supply and that most of their cells are closely juxtaposed with blood so that little stored glycogen would be needed. Interstitial cells are the major sites of pentose cycle activity intertubularly, enhancing the importance of this substrate in these locations (Free, 1970).

Burgos and Vitale-Calpe (1967a; 1967b) have noted that all germ cell types are surrounded by deep recesses of the Sertoli cells in the toad, Bufo arenarum. Free (1970) noted an exception in terms of the first gonial stages of spermatogenesis which were not connected in any way with the Sertoli cytoplasm. Working on rat testes Vilar et al. (1962) stated that the Sertoli cells are the obligatory anatomical pathways for metabolic interchanges between the germinal cells and the bloodstream. This latter fact could explain the glycogen stores within the newly awakened spermatogonia in March specimens of S. odoratus. These cells need a stored substrate for metabolic energy since they have no connections with the Sertoli cells. Vilar et al. (1962) and Burgos and Vitale-Calpe (1967b) have observed that in order to implement such metabolic interchanges, the Sertoli cytoplasm posseses many highly developed microtubules, cisternae, vesicles and a number of inclusions. In addition, Sertoli cells in the mammal have elaborate enzyme systems with which to cope with these metabolic interchanges (Tice and Barrnett, 1963).

Coincident with the accumulation of glycogen within fall Sertoli cytoplasm is a similar pattern of nuclear expansion in S. odoratus. Working on the mouse testes, Firlit and Davis (1965) have observed that glycogen can be synthesized from the residual body which becomes detached from a maturing spermatid. These residual bodies become phagocytized by the Sertoli cells preceding their conversion into glycogen. In light of these observations, it may be possible to make a correlation between the accumulations of this substrate during the fall and the enormous increases in Sertoli nuclear diameter also occurring at this time. No correlation is apparent between the expanded Sertoli nuclei in spring and the glycogen cycle. As nuclear diameters decline in size during June, cytoplasmic glycogen reamins high.

The cyclic events of intratubular lipids in S. odoratus are basically similar to those obtained in the cobra, Naja naja (Lofts, et al.,

1966) and the Neotropical slider, Pseudemys scripta (Moll and Legler, 1971) for corresponding spermatogenic stages. In the cobra, intratubular lipids begin to gather during the summer months following the final stages of spermiogenesis in spring. Dense accumulations of lipid gather during this four month interim between spermatogenic cycles. Spermatogenesis resumes in September with the gradual depletion of these lipids by November when spermiogenesis begins. P. scripta from Panama follows a similar pattern of intratubular lipid accumulation and depletion. During the four month interim between January and May, Sertoli cells are heavily lipoidal. As spermatogenesis resumes in late May, a depletion occurs such that only small amounts remain from August to November.

Intratubular lipid cycles in the European viper, Vipera berus (Marshall and Woolf, 1957), horned lizard, Phrynosoma cornutum (Cavazos and Feagans, 1960) and Caspian terrapin, Clemmys caspica (Lofts and Boswell, 1961) differ from the above pattern. The viper demonstrates premature lipid concentrations within tubules still containing intermediary and late stages of spermiogenesis from the previous cycle. This tubule lipid accumulation does not take place until after the cycle has stopped in S. odoratus, N. naja and P. scripta. Also, the period during which tubular lipids are present is much longer in V. berus. Following hibernation in P. cornutum, a marked accumulation of lipid is observed in the Sertoli cell cytoplasm. Unlike any of the reptiles noted thus far, this gathering of intratubular lipid takes place during the early stages of spermiogenesis. Standard accumulations of these lipids continues prior to and during breeding as in the other reptiles. During the early stages of spermatogenesis and hibernation, few intratubular lipids are noted. The cycle in C. caspica differs in that post-nuptial steatogenesis does

not begin until November. However, Sertoli cells in the former reach a maximum lipid content and already become depleted by the appearance of the first primary spermatocytes the following April. Intratubular lipids are present, therefore, only four months during the year owing to their slow accumulations in fall and rapid depletion during early recrudescence.

The duration of a lipoidal tubule condition varies from species to species (Lofts, 1969). The mechanism whereby a succeeding spermatogenic rhythm is actively proceeding while the tubules remain clotted with lipids is not restricted to reptiles but also occurs in teleosts such as the pike, Esox lucius (Lofts and Marshall, 1957). It has been suggested by these latter authors that this phenomenon may be an adaptation related to the poikilothermic condition and the thermal fluctuations of the environment to which these organisms are subjected.

The intertubular lipid cycle in S. odoratus is somewhat similar to that found in the box turtle, Terrapene carolina (Altland, 1951), P. cornutum (Cavazos and Feagans, 1960), C. caspica (Lofts and Boswell, 1961) and P. scripta (Moll and Legler, 1971). All of these reptiles undergo an accumulation of interstitial lipids prior to breeding at which time a depletion occurs. The cycle in T. carolina is almost identical with that found in S. odoratus. Only moderate amounts of lipid are accumulated during the interim between spermatogenic cycles. Shortly before breeding, interstitial cells become densely lipoidal. Lipids are depleted following this time. Histochemical work on P. cornutum reveals an abundance of cytoplasmic lipid in the interstitial cells during and immediately prior to the breeding season from May to July. During tubule atrophy and following breeding, interstitial lipids decline. Interstitial cells begin gathering lipids following hibernation and at an early stage of spermiogenesis

This condition does not occur in the testes of other reptiles. A similar cycle is observed intratubularly. In C. caspica, an accumulation of lipid material within the interstitium begins in October coincident with advanced spermiogenesis. These sudanophilic lipids reach maximum proportions from December through April. During April, when active breeding is occurring and spermatogenesis has already started, the interstitial areas become denuded of lipids. Interstitial cells remain lipid-free until the following October when accumulations resume. A longer period of interstitial lipids is noted in S. odoratus, perhaps owing to a slower rate of spermatogenesis. P. scripta shows a yearly increase and decrease of lipids in the interstitial cells, simultaneous with intratubular lipid fluctuations. In S. odoratus, interstitial and tubular lipids levels do not correspond during the spring and early summer.

The cycling of intertubular lipids differs from the above in two species of snakes: V. berus (Marshall and Woolf, 1959) and N. naja (Lofts et al., 1966). In the viper, interstitial cells become denuded of lipids for only a short time during the breeding season in April. Some cells already begin a rapid accumulation of lipoid material by mid-May, coincident with recrudescence. The interstitium is fully recharged by the end of July and remains in this condition until the following April. An almost identical cycle is observed in the cobra.

The significance of and mechanisms involved in the intratubular lipogenesis of male amphibia, birds and reptiles is poorly understood. It has been suggested that the Sertoli cell may be involved in steroid biosynthesis and the production of androgens (Lofts, 1969). In two species of lizards, P. cornutum (Cavazos and Feagans, 1960) and Hemidactylus flaviviridis (Sanyal and Prasad, 1965), estimation of testes cholesterol content

(a sex hormone precursor) supports histochemical lipid evidence that Sertoli cells are involved in hormone production. Cholesterol levels are highest during intratubular lipid buildup. With the onset of spermatogenesis, both cholesterol and Sertoli lipids decline, suggesting a mobilization of steroids for androgen production. In S. odoratus, moderate amounts of Sertoli lipids are lost during the breeding season and as spermatogenesis proceeds, suggesting a similar period of hormone production.

In the rat, Lacy and Lofts (1965) have shown that injections of estrogen decrease the number and type of spermatogenic elements within the seminiferous tubules until only spermatogonia remain. Accompanying this decline was an increase in lipid accumulation in Sertoli cell cytoplasm. This intratubular lipid was sterol-negative at first but became predominately sterol in time. With the administration of FSH, there was a resurgence of spermatogenesis and a depletion of Sertoli lipids. According to these workers, these trends suggest that spermatogenesis is impaired by a lack of FSH due to an inhibitory effect of the estrogen on the pituitary. Impaired spermatogenesis in turn causes an increased lipid content in the Sertoli elements. This increase appears to be a result of phagocytosis of the degenerating germ cells by the Sertoli cells. The sub-microscopic residual bodies of the maturing spermatids along with the degenerating germinal cells are phagocytized. These residual bodies appear to be laden with lipids which accumulate when not utilized in active spermatogenesis.

The development of steroids from the tubular lipids of the rat following the phagocytizing activities of the Sertoli cell may be of endocrine significance. Analysis of similar intratubular lipids in birds have shown them to contain progestogens (Lofts and Marshall, 1959). In S. odoratus, Sertoli cell phagocytosis is not actually observed during

fall steatogenesis. According to Miller (1959), Sertoli cells in reptilian testes not only play a nutritive role in the development of the germinal cells but also act as phagocytic elements after shedding of the sperm. He further suggested that the salvaging of lipids by the Sertoli cytoplasm was important to the "endocrine economy" of an organism.

Lofts (1969) believed that in view of histochemical and ultrastructural data, the main locus of intratubular steroidogenesis is the Sertoli cell. According to this worker, these cells contain the characteristic agranular endoplasmic reticulum and tubular mitochondrial cristae associated with steroid-production. The pentose cycle present in this cell is closely associated with tissue lipogenesis as a generator of extra-mitochondrial NADH (Free, 1970).

In addition to having a possible endocrine role, Sertoli cell lipids may serve as an energy reserve to be used during hibernation and early spring spermatogenesis. As previously noted, the result of Sertoli cell phagocytosis includes glycogenesis in addition to lipogenesis. Both substrates may be used in order to buffer fluctuating intratubular needs in S. odoratus.

Sertoli nuclear diameters are greatest during May and June coincident with what Risely (1938) has called " . . . period of most active breeding" and in the fall following the end of spermatogenesis. During the time of intense breeding, Sertoli cells may be active converting intratubular lipids into steroids for the production of the male sex hormone. During and following the late stages of spermiogenesis in fall, Sertoli elements may be phagocytizing residual bodies and degenerating germ cells in their genesis of lipids.

It is widely accepted that a profuse accumulation of cytoplasmic lipid in the interstitial cell indicates reduced androgen secretory activity.

Conversely, a discharge of interstitial lipids reflects an increase in androgenic activity (Lofts, 1968). Thus in the stinkpot, maximum androgen production is probably correlated with the phase of marked lipid depletion during late May and June. A conversion of precursor material into the sex hormone may also occur at this time. The succeeding period during which interstitial lipids accumulate may be attributed to a decline in androgen synthesis and a consequent buildup of precursor material. Interstitial cell nuclear measurements parallel these histochemical observations. Nuclear diameters are greatest during the " . . . period of most active breeding" in May and June.

SUMMARY

1. The present investigation has served to confirm many of the histological and macroscopic observations made by Riseley (1938) on the spermatogenic cycle of Sternotherus odoratus in Michigan. Contrary to his findings, definite fluctuations in interstitial cell nuclear diameters are noted during the spermatogenic cycle. Greatest diameters coincide with what this worker has considered the " . . . period of most active breeding." In addition, Sertoli nuclear diameters undergo similar fluctuations, being greatest during spring breeding as well as during fall steatogenesis.

2. A PA/S-glycogen cycle can be traced intratubularly in the Sertoli cell. Glycogen and carbohydrate materials accumulate in granular form during the fall and spring. They become depleted during the early stages of spermiogenesis.

3. An intratubular and interstitial lipid cycle can be correlated with the spermatogenic cycle. Roughly, an inverse correlation is observed between these two cycles. Lipid concentrations decrease as spermatogenesis advances and accumulate during the interim. The Sertoli cell is the locus of lipid accumulation intratubularly. Intertubularly, the interstitial cell is the site of lipid activity. Sertoli lipids build upmore rapidly than do interstitial lipids and are present for a greater length of time. Whereas interstitial lipids become depleted about the time that secondary spermatocytes are forming, Sertoli lipids are maintained through the spermatid stage.

4. Latitudinal variation in the timing of the spermatogenic, glycogenic and lipid cycles in S. odoratus is evident. Spermatogenesis in Illinois is 2-3 weeks ahead of that found in Michigan, 2 weeks behind the cycle in Tennessee and about 5 weeks behind specimens from Louisiana. The PA/S-glycogen cycle in Illinois is about 2 weeks behind that found in Tennessee and 4 weeks behind Louisiana specimens. The intertubular lipid cycles in Tennessee and Louisiana are similar to that found in Illinois for corresponding spermatogenic stages. The cycle of intratubular lipids follows the same pattern of timing but differs in that these lipids are retained for a longer time in turtles from more southerly locations.

5. Spring spermatogenesis accelerates with increasing temperature and photoperiod. Following the summer solstice and despite decreasing photoperiod, the cycle continues uninterrupted. With the onset of cooler temperatures in the fall, the cycle regresses and stops. These facts suggest temperature as the main environmental factor regulating spermatogenesis once it has started.

6. The intratubular PA/S-glycogen cycle in K. f. flavescens is similar to that found in S. odoratus. In contrast, P. cornutum differs from these reptiles in maintaining a generous supply of glycogen in the Sertoli cells during accelerated spermiogenesis. Also, both K. f. flavescens and P. cornutum hold glycogen in granular form within the interstitium whereas no such phenomenon is noted in S. odoratus.

7. Cyclic events of Sertoli cell lipids in S. odoratus are basically similar to those observed in N. naja and P. scripta. Sertoli lipids accumulate during the interim between spermatogenic cycles and gradually become depleted as spermatogenesis advances. The cycle in V. berus, P. cornutum and C. caspica deviates from this pattern in a number of ways.

8. The interstitial lipid cycle in T. carolina, P. cornutum, C. caspica and P. scripta from Panama is somewhat similar to that found in S. odoratus. An accumulation of interstitial lipids occurs prior to breeding, at which time a rapid depletion takes place. The cycle in two species of snake, V. berus and N. naja, differs in that the interstitial areas become denuded of lipids for only a short time during the breeding season after which these lipids are rapidly reaccumulated.

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