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STUDIES OF REPRODUCTION IN THE BLUE SPINY LIZARD, SCHELORUS CYANOGNYS COPE

by

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ABSTRACT

The blue spiny lizard, *Sceloporus cyanogenys*, an ovoviviparous lizard from Texas and Mexico was studied in order to contribute further information to the physiology of reproduction in reptiles.

Seasonal changes in gonadal microanatomy and the sex accessories could be demonstrated in this lizard as a criteria for the reproductive activity of testicular and ovarian tissue. The animal has a single breeding season which occurs during the winter months.

Spermatogenesis commences in August, followed immediately by a period of spermiogenesis; producing mature sperm which are shed into the epididymides and vas deferens prior to the time ovulation occurs in the female.

Interstitial cells were seen to develop and regress with the waxing and waning of the sex accessories and secondary sexual characters. These sex accessories were demonstrated to be under the influence of the male sex hormone, testosterone.

Ovogenesis and yolk deposition begins in Autumn; producing large eggs which are ovulated during mid-winter. A corpus luteum is formed from the old Graffian follicle. Stages in the development of this structure are presented. Seasonal changes in the oviduct appear to be controlled by ovarian estrogens.

Gestation lasts approximately four months, Apposition be-
between fetuses and oviducal epithelium is loose. A demonstration of the ovoviviparous nature of the lizard indicates that there is no exchange between oviduct and fetuses, other than water and possibly gases.

Mating behavior and parturition is described. The average number of young born to an adult female is 17.68.

Mammalian gonadotrophins were incapable of inducing another reproductive cycle in females with quiescent ovaries; but oxytocin was able to facilitate parturition.

A hypothesis for the ovoviviparous reproductive mechanism, and the winter breeding season, unique among reptiles, but found in S. cyanogenys, is proposed.
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INTRODUCTION

The problems concerned with reproduction in vertebrates have interested biologists for many years. The anatomical and physiological requirements for successful breeding, fertilization, gestation, and birth; the evolution of reproductive patterns; the mechanisms of these patterns and the phylogenetic significance of these systems are only a few of the questions concerned with the perpetuation of the species through reproduction.

Vertebrate animals, whether warm or cold blooded, have adapted to a great variety of aquatic and terrestrial conditions. Fish, in view of their habitat, are limited or restricted to an aqueous environment; so too their developing young. All but a few amphibians must return to water to lay their eggs. However, the conditions imposed by a land environment have posed certain problems to be solved by the terrestrial vertebrate with regards to reproduction. The appearance of the cleidoic egg, which offers a developing embryo the protection and the necessary aquatic environment for growth, was certainly an adaptation for successful survival of some reptiles and all of the birds. The Marsupial and Eutherian mammals solved the problem by retaining the embryo for a time within the abdominal, reproductive tract of the female, where the developing fetus may be nourished by substances transversing the placenta.
Intermediate between these two conditions of oviparity (egg-laying) and viviparity (giving birth to live young) is another condition, somewhat less well defined, in which the developing embryo is retained in the reproductive tract of the female, receiving protection from the external environment, permitting an exchange of gases and water, but without obtaining nutritional materials from the mother. This condition, referred to as ovoviviparity, is found in some fish and a few reptiles.

While considerable knowledge has been obtained concerning the physiology of reproduction in mammals, little is known with respect to the reproductive status of the lower vertebrates.

In a recent review on the endocrine basis for reproduction in reptiles, Miller (1959) mentions that of some 6000 species of turtles, alligators, lizards and snakes, adequate data concerning reproductive physiology is available for less than one per cent of the known number of species. Our present understanding of reptilian reproduction has come from two main sources; one from life history studies of particular reptilian species, and the other from a few experimental observations, employing the endocrinological method of surgical ablation of a gland, and the subsequent replacement therapy with a suspected hormone. While both ecological and physiological studies have contributed to our understanding of reproduction in these forms, much more is yet to be learned.
STATEMENT OF THE PROBLEM

In order to contribute further information to reptilian reproduction, an investigation of the ovarian and testicular cycles of *Sceloporus cyanogenys*, an ooviviparous lizard, was undertaken in extensive detail. The purpose of the study was three-fold: First, the determination of the seasonal reproductive cycle of *S. cyanogenys*, with a description of the morphological changes in the testis, male accessory organs, secondary sexual characteristics, the ovary and oviduct; Secondly, the description of mating behavior, gestation, and parturition of newborn lizards; Thirdly, a study of the effects of surgical ablation of the gonads and hormone replacement therapy during different times of the reproductive cycle.

It was hoped that such a study of the seasonal changes in the sexual structures of this lizard would contribute a better understanding to the reproductive physiology of a lower vertebrate. Interest in the seasonal cycle is further justified by the fact that this is the first investigation of reproduction in a subtropical lizard; all previous investigations being conducted with North and South Temperate-living fauna.

Due to the ooviviparous nature of the species, and the presence of well-developed corpora lutea, such
an investigation might offer an insight into the phylogenetic and evolutionary significance of this reproductive mechanism, and could possibly broaden our understanding of the endocrine control for the retention of the fetuses during oviducal incubation.
SURVEY OF THE LITERATURE

It is beyond the scope of this paper to site all the references made to reptilian reproduction. Relatively recent and detailed reviews of reptilian reproductive histology and endocrinology have been provided by Herlant (1933), Bretschneider and Duyvene Dewit (1947), Kehl and Combescot (1955), and by Miller (1959).

For the sake of clarity, I have divided the work into two major sections, one dealing with male reproduction and the other concerning the female.

The Male

A study of the literature concerning the seasonal changes occurring in the reptilian testis shows a variety of cyclic variations. The Lacertilia are by far the best known, while little concerning the Ophidia (Serpentes, snakes), Chelonia (turtles) and Crocodilia has been described.

With few exceptions, the species so far studied may be considered seasonal breeders; the relative extent, time of occurrence, and duration of testicular activity varies from species to species.

Of the lizards so far studied, a group of European forms including Lacerta agilis, Reiss (1923); L. viridis, L. vivipara, and L. muralis, Herlant (1933); the Amer-
ican *Seeloporus gracious*, Woodbury and Woodbury, (1945); *S. occidentalis*, Wilhoft and Quay, (1961); and *S. orcuti*, Mayhew, (1963) exhibit a testicular cycle in which the histological details may differ, but the primary picture is one in which spermatogenesis occurs in early summer, producing the primary spermatocyte as the predominant cell of late summer, with the formation of spermatids during the autumn and winter. In spring, spermiogenesis or the metamorphosing of spermatids to spermatozoa begins. This process is accelerated during April and May and the mature sperm are shed into the epididymis in late spring and early summer.

The old world slowworm, *Anguis fragilis*, Dalgo (1921), exhibits a somewhat more sharply delimited cycle, wherein the general picture is similar to the above, but the testis shows an inactive period during the winter. No division of primary spermatocytes occurs during the hibernal rest and maturation division and spermiogenesis are purely a spring phenomena. This pattern is also seen in *Xantusia vigilis*, Miller (1951); and in the Gecko, *Platydactylus muralis*, Herlant (1933).

Dutta (1946) has observed that *Hemidactylus*, a Gecko, attains a maximum testicular size in November and December, losing weight in February and March; but that interstitial cells are maximally developed in late spring. In *Eumeces laticutatus*, Kidata (1951), spermatozoa are absent from the seminiferous tubules bet-
ween April and the beginning of June. Primary and secondary spermatocytes with subsequent maturation stages appear in July and August, followed by spermiogenesis in November and December.

Following a suggestion of Courrier in 1928, Kehl started investigations of Algerian Sauria. The seasonal cycles of Uromastix, Varanus and Scincus similar, in that they are sexually active during the spring. On superficial examination of Acanthodactylus, the fringetoeed lacertid, sexual activity appears to be continuous, or at least displays two periods of activity, one quite long in the spring and the other shorter in the autumn, Kehl and Combescot (1955).

Hamlett (1952) and Fox (1958) pointed out that the testicular cycle is unusual in the more southern living American anole; in that the testes and sex accessories are maintained in a fully mature condition throughout the summer (from April to August). Of even greater interest, is the recent report of Wilhoft (1963), in which the tropical Australian skink, Leiologisma rhomboidalis, is sexually active throughout the year. This situation makes more apparent the fact that our knowledge of reptilian reproductive cycles is based almost exclusively upon North and South Temperate fauna, and that very little is known of tropical and subtropical forms.

Observations on Ophidians have been limited; but from studies of Tropidonotus matrix, the oviparous water
snake distributed throughout central Europe, western and central Asia and Algeria; and the common European viper, *Vipera berus*, Herlant (1933), the testicular cycle is similar to that occurring in Lacertilia; with the primary spermatocyte being the overwintering cell. Volsoe (1944), Petter-Rousseaux (1953), Marshall and Woolf (1957), in studies on other European snakes, have shown that the sperm are matured and a new cycle is reinstituted in the fall. In the North American garter snakes of the genus Thamnophis, the testes are maximally developed during the summer and are regressed in the winter. Spermatogenesis and spermiogenesis occur in the spring, Cieslak (1945); Fox (1952).

In turtles (Chelonia), possibly the most primitive of existing reptiles, spermatogenesis occurs primarily in the late spring and summer; spermiogenesis takes place in the fall. The mature sperm usually pass the winter hibernation period in the sex accessories, Burger (1937); Risley (1938); Hansen (1938); Altland (1951) and Combescot (1954).

Little is known of the testicular cycle of alligators.

In the male reptile, the sexual accessory organs are the epididymides, the vas deferens, and modified elements of the metanephros - the so called "renal sex segment". In all reptiles so far examined, the epididy-
mal epithelium becomes hypertrophic and secretory at the
time mature sperm are formed in the testis. As long as
sperm are retained in the lumen, the epididymal epithe-
lium becomes and remains secretory. This appears to be
true irrespective of the condition of the interstitial
cells; except that in most species studied, there is
some correspondence between activity of leydig cells,
and that of the sex accessories. In most lizards, where
the sperm are matured in the spring and shed into the
epididymis shortly before copulation, the epididymides
need be enlarged for a short time. In turtles and some
snakes, the epididymis retains the sperm throughout the
winter hibernation, and the secretory lining persists
during the entire period, Dalcq (1921); Reiss (1923);
Padoa (1933); Herlant (1933); Regamey (1935); Takewaki
and Fukuda (1935); Reynolds (1947) and Fox (1952).

According to Fox (1952), the ductus deferens is
non-secretory in snakes. Unfortunately, little is known
concerning the lower portion of the genital tract in
male lizards.

Excellent discussions on the renal sex segment
have been published by Fox (1952); Forbes (1941) and
Takewaki and Fukuda (1935). The renal sex segment does
not appear in female lizards and snakes, or male and
female turtles and alligators. During the time of
maximum testicular development, the epithelial lining
of the terminal portion of the nephron undergoes marked hypertrophy and the cytoplasm of the columnar cells is packed with eosinophilic-staining fluid of a secretory nature.

The development of the secondary sexual characters in reptiles is not nearly as pronounced as in fishes and birds. Among the reptiles, the lizards are the best endowed; whereas snakes and turtles exhibit little in the way of sexual diamorphism. In many lizards, such features as body size, post-anal swelling due to the hemipenis, femoral pore secretion, dorsal crests, gular folds and patches, post anal scales and color differences distinguish males from females, Pope (1956). Some turtles show differences in concavity of plastron, the color of the iris, and length of tail and hind claws, Evans (1951, 1952).

Experimental findings for the hormonal control of the sex accessories in male reptiles have been elucidative. Unfortunately, the number of investigations of this kind have been all too few. Employing the classical techniques that led to our present understanding of mammalian reproductive endocrinology, workers have studied the effect of castration on male reptiles, and the subsequent replacement therapy by means of testicular grafts and the administration of the male sex hormone.

Matthey (1929) reported that castrated lizards of
Lacerta agilis, sacrificed one year after the operation, appeared identical to the female with respect to femoral pores and general body coloration. Padoa (1933) confirmed Matthey's findings on the femoral gland, and in addition, observed that castration caused suppression of secretory activity of the epididymis.

Herlant (1933) studied the effects of castration on the renal sex segment of Lacerta and Anguis. If the operation was performed during the resting phase, the development of the segment, which normally occurs in the spring, is hindered. On the other hand, if castration was done in the season of sexual activity, then regression followed after a latent period of fifteen days.

Takewaki and Fukuda (1935) castrated winter specimens of Tachydromus tachydromoides. After the surgery the activity of the epithelial cells of the reproductive ducts and sex segment gradually diminished. After forty days glandular activity had ceased and secretory granules had disappeared. Viability of spermatozoa in the epididymis was reduced with castration.

The early experiments with testicular grafts met with little success. Testes implanted into Lacerta agilis degenerated, Matthey (1929). Takewaki and Fukuda (1935) transplanted testes into male and female castrates of Tachydromus. They showed that the renal sex segment and the epididymis became secretory at the time of seasonal sexual activity.
Certain other references are informative. In the experiments of Forbes, (1940, 1941), pellets of testosterone, implanted into the lizard, *Sceloporus spinosus floridanus* (olivaceus), brought about development of the sex accessories. Noble and Greenberg (1940) administered male sex hormones to *Anolis carolinensis*; and Gorbman (1939) did the same for *Sceloporus occidentalis*. The results were similar to the work of Forbes. Evans (1951, 1952) has investigated the action of androgens upon the tail and claw growth in the slider, *Pseudemys scripta*; and studied the effects of gonadotrophic and androgenic hormones upon the dorsal crest of the anole, (1948). Altland (1943) noticed that the hemipenis hypertrophied in lizards treated with androgens during the period of sexual rest.

**The Female**

Leydig (1853), Gegenbauer (1861), Braun (1877), and Hoffman (1890), described the general features of the reptilian female urogenital tract. Braun (1877), in particular, described the structure of the reptilian ovary. Loyez (1906), studied the process of oogenesis and the formation of the follicle in lacertan types. Accounts of the corpus luteum in reptiles began with Mingazinni (1893). A full description of variations in the reproductive tract is available for *Lacerta agilis*, (European form), Regamey (1935). The process of oogenesis and the formation of the corpus luteum has been
investigated in considerable detail in the New Zealand viviparous lizard (Gecko), *Hoplodactylus maculatus*, Boyd (1940). Miller (1948), working with the viviparous lizard, *Xantusia vilis*, elucidated the histological changes in the ovary and corpus luteum. The presence of corpora lutea have been described in oviparous lizards by Weekes (1934), Boyd (1941) and by Dutta (1944); and in snakes by Bragdon (1952) and Betz (1963).

Several types of ovarian cycles are found in reptiles. It has not yet been possible to correlate the type of cycle with taxonomic, geographic, or climatic conditions. The following types of ovarian cycles are found in lizards: (1) a large amount of yolk is deposited shortly before ovulation, but is subsequent to a long, slow initial growth of the ova - type found in *Phrynosoma*, Blount (1929); *Hemidactylus*, Dutta (1944); and *Xantusia*, Miller (1948); (2) yolk deposition occurs gradually during most of the year preceding ovulation - type found in *Lacerta agilis* and other European lizards, Regamey (1935); (3) yolk deposition occurs shortly after ovulation and mature ova remain in the ovary throughout the winter - type found in *Sceloporus gracilis*, Woodbury and Woodbury (1945); in *S. occidentalis*, Wilhoft and Quay (1961); and in *S. orcuti*, Mayhew (1963); (4) Two or more sets of ova are produced and ovulated annually, each set formed directly following ovulation of the pre-
ceeding set - type found in *Amphibolurus*, Weekes (1934); *Sceloporus olivaceus*, Blair (1960); *S. undulatus*, Crenshaw (1955); and *Uta stanburiana*, Tinkle (1961); a modification of this theme is seen in *Anolis carolinensis*, where the female deposits single eggs in regular succession from April to August, Hamlett (1952); (5) Non-seasonal breeders which may reproduce at any time of the year - type found for the New Hebrides lizard, *Lycosoma*, Baker (1929); a slight modification of this type is seen in the tropical Australian skink, *Leiolopisma rhomboidalis*, Wilhoft (1963), where the female has a seasonal cycle and the male has a continuous spermiogenesis.

Both male and female of some species of lizards and snakes follow a biennial schedule of reproduction. This has been described in *Crotalus viridis*, Rahn (1942); *Vipera berus*, Volsoe (1944); in *Heloderma suspectum*, Bogert and deCampo (1956); and in the mountain living individuals of *Xantusia vigilis*, Miller (1959).

Of even greater interest is the reproductive behavior of the viviparous *Lacerta* (Zootoca), distributed over the middle and northern parts of Eurasia and the only lizard living within the artic circle. At this high latitude where there are only three months of activity, so that eggs would never hatch, there is a retention by the female to incubate her own eggs (possibly by behavioral thermoregulation). In the mountains
of Central and Eastern Europe, where the summer season is longer, this same species (*L. vivipara*) becomes an egg layer, Schmidt and Inger (1957).

The ovary of reptiles is composed of ovarian epithelium, stroma, the germinal bed, corpora atretica, corpora lutea, and graffian follicles, Bolk (1933).

Excellent studies, reviews and discussions of the reptilian corpus luteum and follicular atresia have been published by Betz (1963); Panigel (1956); Matthews (1955); Amoroso (1955); Bragdon, et al (1954); Bragdon (1951, 1952, 1953); Harrison (1948); Miller (1948); Cunningham and Smart (1934); Boyd (1940, 1942); Rahn (1938); Weekes (1934); Asdell (1928); Hett (1924); and Luciem (1903).

Apparently all reptiles, whether oviparous or viviparous, develop a post-ovulatory corpus luteum, Weekes (1934). This structure is produced by the hyperplasia of both granulosal epithelium and the surrounding thecal elements. The degree of involvement of each of these is variable and seems to bear no relation to the taxonomic affinities or to the degree of viviparity. The most common type of corpus is composed of a central mass of enlarged, lipid containing, granulosa derived, luteal cells; surrounded by a connective tissue envelope, which may or may not send penetrating strands into the luteal tissue, Miller (1959).

According to Miller (1959) and Weekes (1934, 1935), there is a definite correlation between the longevity of
the corpus luteum and the egg-laying or retaining habit of the species. Oviparous reptiles have a corpus luteum regressing shortly after egg laying. This is particularly true for species producing a new clutch of eggs within the same breeding season, Mayhew (1963). According to Miller and Weekes the average gestation time for lizards which give birth to live young is about three months. In these species, the corpus luteum remains developed for approximately two months and begins to regress during the latter third of gestation.

The specific function of the corpus luteum in reptiles, however, has remained an enigma. The earlier studies of Clausen (1940) and Fraenkel et al (1940) indicated that the corpus was essential for maintaining pregnancy in viviparous snakes. In their experiments, deluteinization during early pregnancy was followed by abortion or resorption of embryos. The more recent work of Bragdon (1951) on the viviparous garter snake, and of Panigel (1956) on the ovoviviparous lizard, Zootoca, would indicate that the corpus luteum is not essential for the maintenance of gestation.

Ovariectomy results in regression of the oviducts, while administration of estrogens stimulate the growth and development of these structures, Noble and Greenberg (1941). It is interesting that testosterone given in proper dosages will also stimulate the oviduct, Kehl and Combescot (1955); just as estrogens may sometimes stimu-
late the epididymides. The effects of progesterone, alone, or in combination with estrogens, has not been studied adequately in reptiles. One report by Panigel (1956) shows that although progesterone will stimulate the oviduct, it is less effective than either estrogen or testosterone. Panigel is of the opinion that estrogens may act most effectively on the glandular portion of the oviduct and progesterone on the muscular wall. Studies on the effect of female sex hormones in reptiles have been reported by a number of workers: Cunningham and Smart (1934); Turner (1935); Regamey (1935); Mellish and Meyer (1937); Fraenkel and Martins (1938); Forbes (1938); Gorbman (1939); Takewaki and Hatta (1941); and Panigel (1956).

The female sex hormones have been isolated from several species of reptiles. Progestins have been demonstrated in extracts of ovaries containing corpora lutea, Porto (1942); Valle and Valle (1943); and in the plasma of pregnant viviparous snakes, Bragdon et al, (1954). Furthermore, in certain viviparous reptiles, if only one oviduct is occupied by a developing embryo, the other duct will remain in an enlarged state throughout the entire period of gestation, indicating the presence of ovarian or placental hormones, Miller (1959).

The effects of anterior pituitary extracts on the genital system of the horned lizard, Phrynosoma cornutum,
has been studied, Mellish (1936). Turner (1935) studied the effect of Antuitrin (anterior pituitary extract) on the male lizard, *Eumeces laticeps*. In *Phrynosoma*, treatment with extracts from the pituitary caused an increase in weight of the testes, epididymis and vas deferens; while no effect was seen on the ovary.

Mayhew (1961) studied the photoperiodic response of the female fringe-toed lizard; while Burger (1937) did a similar study on the male turtle, *Pseudemys elegans*. Bartholomew (1950) found that light was more important than heat in producing a reproductive reaction in *Xantusia vigilis*.

Placentation in reptiles has been reviewed by Weekes (1935). Boyd (1942) studied the oviduct, fetal membranes, and placentation in *Hoplodactylus*; and more recently, Bellairs and co-workers (1955) have studied placentation in the adder, *Vipera berus*.

Noble and Bradley (1933) offer an excellent treatise on the mating behavior of lizards. Field observations by Woodbury and Woodbury (1945) mention the behavior of breeding lizards of *Sceloporus gracilus*. The urogenital anatomy, growth, reproductive cycle in several species of *Sceloporus* have been described to variable extents by Altland (1941); Fitch (1940); Forbes (1941); and Mulaik (1946).

Parturition in two other ovoviviparous lizards of
the genus *Sceloporus* have been reported. Ramsey and Donald (1949) and Axtell (1950) reported on birth in the lizard, *S. poinsetti*. Zweifel (1949) mentions the young of *S. jarrovi*. Apparently the only accounts of parturition in *S. cyanogenys* is that of Smith (1939); Hunsaker (1959); and Kennedy (1960).
MATERIALS AND METHODS

Adult male and female lizards were obtained from an animal dealer (Zoological Supply Co., Laredo, Texas); and were presumably collected in that vicinity. Upon collection at regular intervals throughout the year, they were immediately shipped to us. In the determination of the seasonal changes of the gonads and reproductive ducts, animals were sacrificed shortly after being brought into the laboratory. Testes, vas deferens, ovaries and oviducts were first weighed to the nearest one-tenth of a milligram, and then fixed in a variety of preservatives depending upon the different staining procedures to be used. Neutral formalin, Bouins, and Zenker's formol were the main fixatives employed. Kidneys and occasionally femoral pores were examined periodically to determine the condition of these sex accessories. All tissues, unless otherwise stated, were dehydrated in a series of alcohols, transferred and cleared in chloroform, and embedded in paraffin. Sections were cut at seven microns and stained by a variety of methods. The most common of these procedures were hematoxylin and eosin, and a modified (unpublished) Kornhauser tetrachrome stain. In the study of the corpus luteum, two histochemical methods were employed. The standard Periodic acid Shiff reaction was used to determine the presence of glycogen, and a modified McManus method for lipid was employed using Sudan Black B and
S. cyanogenys lends itself well to experimental study. Animals were placed in wooden cages (four feet X two feet X two feet), with one side of the cage covered by a fine screen. Alight and heat source was provided by a 60 watt electric bulb. Cages were kept in a large animal room. Twelve hours of light and twelve hours of darkness were maintained within the cages during experimentation and controlled by a time switch. During the daylight hours, temperatures in the cages ranged from 32 to 35 degrees centigrade. Cage temperatures during the night hours were in the range of 20 to 24 degrees centigrade. During the daylight hours, lizards were frequently observed to be basking under the artificial illumination. At night the animals were seen to bury themselves in the dirt and sand provided for them in the bottom of the cage. Water was provided for them in preparation dishes; and food, consisting of insects caught in the spring and summer, and meal worms cultured during the winter were placed periodically in their cages.

All surgical procedures were performed antiseptically under hypothermic anesthesia. The latter was accomplished by packing the lizard in crushed ice for about ten minutes or until his body temperature dropped to about 5 degrees centigrade. Lizards may be kept in this state for extended periods of time. Removal of the gonads was accomplished through a parasagittal incision, one
centimeter from the mid-line of the abdomen. The incision was closed with nylon thread and a thin film of colloidin was spread on the epidermal surface over the closed hiatus. Animals responded well to the surgical techniques, and within 15 minutes after rewarming, could be seen scurrying around their cages.

To test the effects of the male sex hormone, testosterone propionate (USP Sigma Chemical Co.) was given to juvenile and adult male lizards with seasonally quiescent reproductive ducts and testes. Dosages of 1.0 milligram of the hormone, dissolved in sesame oil, was injected intraperitoneally every other day for 30 days. Control animals received an equivalent amount of sesame oil (0.10 cc/injection). All animals were killed the day following the last injection; and the testes and reproductive ducts were excised, weighed to the nearest tenth of a milligram, and then fixed for histological observation according to the methods already specified.

In experiments designed to test the effects of the female sex hormones on the oviduct; eight adult females were ovariec tomized at a time when yolk was being deposited in the ova. These were divided into two groups; one receiving 5 micrograms of estrogen (Delestrec, E.R. Squibb and Sons) every other day for 60 days; and the other receiving an equivalent amount of sesame oil (0.10cc/injection). Four adult female lizards served as controls. The day following the last injection, the animals were
sacrificed and the oviducts of the experimentals and the ovaries and oviducts of the control animals were excised, weighed to the nearest tenth of a milligram and fixed for sectioning.

In an attempt to initiate another cycle immediately following the natural cycle, an experiment was set up to test the effect of the pituitary control on the ovary during the anestrus phase of the female. Four adult females received 100 I.U. of human chorionic gonadotrophin (CH-10, Sigma Chemical Co.) every day for a period of one month. Four females received 0.1 mgms. FSH every day for two weeks, followed by 0.1 mgms. of LH for an additional two weeks (NIH-FSH-S1, NIH-LH-S3, Ovine, Endocrinology Study Section). Four lizards receiving an equivalent volume of isotonic saline served as controls. Injections were given intraperitoneally; and the day following the last injection, the animals of all three groups were killed by decapitation. The ovaries and oviducts were excised, weighed and prepared as mentioned previously.

In order to determine the hormonal facilitation of parturition, a single dose of one USP oxytocin (Syntocinon, Sandoz Pharmaceuticals) in 0.10 cc. of saline was injected intraperitoneally into 5 pregnant females. Isotonic saline was given to 5 control animals. Both groups consisted of pregnant females at different gestational stages. Examination of the oviducts of all animals was done 18 hours after the single administration.
To determine the ovoviviparous nature of the lizard, two methods were employed. The first, consisted in taking the weight of recently ovulated ova and comparing these to the weights of newborn lizards. Wet, dry, and ashed weights were read to the nearest tenth of a milligram on a Mettler balance. Wet weights were taken immediately. Dry weights were obtained by exposing the specimen in a crucible to a temperature of 100 degrees centigrade for 48 hours. Ashed weights were obtained after the specimens were placed in an electric furnace for 24 hours at 500 degrees. The second method, consisted in measuring the transport of Carbon-14, labeled amino acids from the female to the developing fetuses. This was accomplished by injecting 0.5 microcuries of $^{14}$C$^2$H$^2$O$^2$ glycine (labeled in the first carbon position) and $^{14}$C$^2$H$^2$O$^2$ methionine (methyl carbon labeled) into adult females. Some of these lizards had not as yet ovulated, but contained large, yolk-filled ova within their ovaries. Others which had ovulated contained developing fetuses (of different embryological stages) within their oviducts. Twelve hours after the administration of the isotope, the animals were killed by bleeding; and liver, oviducts, ova or fetuses were removed, blotted, and weighed. Samples were then homogenized in 5 mls. of isotonic saline. The samples were centrifuged and the supernatant was planchotted, dried down, and counted in a windowless flow counter. Each sample was analyzed in triplicate. Results were expressed as counts/minute/gram of tissue.
GENERAL ASPECTS OF THE LIFE HISTORY OF THE LIZARD

The blue spiny lizard, *Sceloporus cyanogenys*, is one of three species of this genus which gives birth to their young as the natural means of reproduction. The other two are *S. jarrovii* and *S. poinsetti*. These lizards belong to the Torquatus species group, the largest group of this genus. *S. cyanogenys* was first described by Cope in 1885, who listed it as a subspecies of *Sceloporus torquatus*. Taylor in 1931 was the first to discover and record this species in the United States. In 1936, Smith, in a revaluation of the Torquatus group, elevated *S. cyanogenys* to the level of a species.

According to Smith (1939), *S. cyanogenys* inhabits southern Texas along the Rio Grande from Devil's River to Starr County, southward in Mexico to central Tamaulipas and central Nuevo Leon, Mexico. Taylor says that in the small hills near Rio Grande City, Texas, the specimens were extremely numerous; as many as ten or fifteen might be seen at one time running over the face of the outcropping rock which caps the hill. The largest males were most wary and would disappear in the deep holes in or under the rocks (rather than in cracks or crevices); the larger females and the younger specimens were less wary and instead of disappearing to safety would frequently hide from sight behind a jutting rock and then expose their heads to view a moment later.
The blue spiny lizard is the largest of all Sceloporine species; and unlike most iguanids, the males are larger than the females, and may attain a length of 141 mm. The females measure 130 mm., snout to vent. Table 1. lists the measurements for adult males and females, as well as that for newborn male and female lizards. The average snout-vent length of 65 adult male lizards was 121.49 mm., with an average weight of 68.37 grams. The average weight of 80 adult females was 55.42 grams, with an average snout-vent length of 109.17 mm. Tail length of both male and female lizards is approximately 119 mm. Regeneration of the tail is a common phenomena in these lizards. In captured specimens the average regenerated tail length is about 35 to 40 mm.

Full-grown males are brilliantly colored. They are a greenish blue above, often showing a general metallic iridescence (unless about to shed or during cold spells). The head is sometimes brown to black. Along the posterior part of the neck passes a jet-black collar, some three scale lengths long, somewhat wider on the shoulders and narrower on the throat. The black collar has a yellow to white border behind, sometimes broken in the middle of the back and ending on the shoulder of each side. The dorsum is unmarked with the exception that a few light flecks, frequently arranged in pairs down the middle of the back, are almost invariably present. There are no distinct markings on the limbs, and the dark bands on
Figure 1.

A. Dorsal-lateral view of an adult male lizard. *Sceloporus cyanogenys* can be easily distinguished from other members of this genus by the dark black neck collar, dorsal flecks, and the ill-defined tail banding.

B. Ventral view of the same lizard in figure 1. A; FP, femoral pores; PAS, enlarged post anal scales; BP, blue belly patches. The animal in question measured 130mm., snout-vent; tail length was approximately 160 mm.
the tail are usually never clear cut and distinct. Below, the males have the entire throat and chin pale blue; this color ending posteriorly at the black neck collar. The belly patches range from a pale to very deep blue, and some males, especially during the breeding season, may possess blue areas bordered with a narrow band of darker blue to almost black extending laterally to the groin. The chest, a broad, median band on the abdomen, and the ventral surfaces of the tail are an off-white to cream color.

Females are always darker than the males above, and the belly is cream color and devoid of the blue pigmentation characteristically found in mature males. The dorsum is an olive drab to brown and not unlike the coloration found in males about to shed. The abdomen of some females, especially during the time of gestation, is a very pale green to light blue along the lateral surfaces of the belly.

The young are much like the female; the ventral surface of both male and female newborns is cream color with the exception that the throat and neck area is occasionally mottled by a faint, dark-bordered band. Dorsally, the dark neck collar is bordered by a yellow band posteriorly. A streak of yellow extends posteriorly from the orbit, and the lateral, labrial scales are also yellow. Sex is determined by the presence of enlarged post-anal scales in the male. Table 1 gives measurements for newborns.
TABLE 1.

MEASUREMENTS OF ADULT AND NEWBORN MALE AND FEMALE LIZARDS

<table>
<thead>
<tr>
<th>Group</th>
<th>Range</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>80 Adult Females:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snout-vent</td>
<td>129-75mm</td>
<td>109.17mm</td>
<td>10.5</td>
<td>±1.17</td>
</tr>
<tr>
<td>Weight</td>
<td>93-17gms</td>
<td>55.42gms</td>
<td>15.2</td>
<td>±1.62</td>
</tr>
<tr>
<td>Ave. Tail</td>
<td>172-3mm</td>
<td>118.89mm</td>
<td>31.8</td>
<td>±3.57</td>
</tr>
<tr>
<td>Unbroken Tail</td>
<td>172-70mm</td>
<td>130.09mm</td>
<td>26.1</td>
<td>±4.02</td>
</tr>
<tr>
<td>Regen. Tail</td>
<td>93-3mm</td>
<td>40.29mm</td>
<td>22.0</td>
<td>±3.77</td>
</tr>
<tr>
<td><strong>65 Adult Males:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snout-vent</td>
<td>146-89mm</td>
<td>121.49mm</td>
<td>13.3</td>
<td>±1.65</td>
</tr>
<tr>
<td>Weight</td>
<td>122-20gms</td>
<td>68.37gms</td>
<td>22.2</td>
<td>±2.25</td>
</tr>
<tr>
<td>Ave. Tail</td>
<td>192-22mm</td>
<td>119.48mm</td>
<td>45.0</td>
<td>±5.71</td>
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<tr>
<td>Unbroken Tail</td>
<td>192-95mm</td>
<td>141.03mm</td>
<td>26.1</td>
<td>±4.77</td>
</tr>
<tr>
<td>Regen. Tail</td>
<td>77-7mm</td>
<td>35.33mm</td>
<td>22.2</td>
<td>±4.27</td>
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<tr>
<td><strong>50 Newborn Females:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snout-vent</td>
<td>31-22mm</td>
<td>28.19mm</td>
<td>2.85</td>
<td>±0.41</td>
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<tr>
<td>Weight</td>
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<td>0.07</td>
<td>±0.01</td>
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<tr>
<td>Tail</td>
<td>40-28mm</td>
<td>35.80mm</td>
<td>2.79</td>
<td>±0.40</td>
</tr>
<tr>
<td><strong>50 Newborn Males:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snout-vent</td>
<td>32-22mm</td>
<td>28.66mm</td>
<td>2.16</td>
<td>±0.31</td>
</tr>
<tr>
<td>Weight</td>
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<td>0.8556gms</td>
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<td>±0.01</td>
</tr>
<tr>
<td>Tail</td>
<td>41-29mm</td>
<td>35.75mm</td>
<td>2.79</td>
<td>±0.40</td>
</tr>
</tbody>
</table>

Sex Ratio of Newborns: 1:1
RESULTS

The Testis

Gross Morphology of the Testis:

The testis of *Sceloporus cyanogenys*, as is true for other lacertan forms (van den Broek in Bolk's Handbuch, 1933), is an ovoid body suspended in the abdominal cavity by the mesorchium, the right being only slightly more cranial than the left. During the summer months the testis is a brick-orange color and extremely flaccid. In late autumn and winter it assumes a light cream color. Due to its turgid nature at the height of spermatogenesis, the outlines of the convoluted seminiferous tubules may be seen through the thin translucent tunica albuginea. The anterior aspect of the testis is marked by an area of dark pigmentation in the shape of a butterfly, the wings extending posteriorly to the lateral and medial surfaces about 4 mm. The average testis during the period of minimal activity measures 6 mm. in length, 4 mm. in width, and 3 mm. in breadth, and has an average weight of 28.6 milligrams. The average testis at the height of spermatogenesis measures 14 X 10 X 10 mm., and is 505.7 milligrams in weight.

For seasonal changes in the gross size and volume of the reptilian testis, many authors have utilized many different methods of representation; Figure 2 gives a graphic description of seasonal changes of the testis.
Figure 2.

A graphic representation of the testicular cycle of *Sceloporus cyanogenys*. The testis and reproductive ducts are expressed in per cent of the total body weight of the lizard. The dark unbroken line represents changes in weight of the testis; the small dotted line represents that of the reproductive ducts (epididymis and vas deferens). The arrow on the left indicates the commencement of spermatogenesis; the one on the right marks the beginning of spermiogenesis. The hatched area represents maximum development of the sex accessories and secondary sex characteristics, along with a concomitant breeding activity. Each point on the graph represents the average weight of eight organs taken from four adult males.
seasonal testicular cycle of *S. cyanogenys*

% body wt., testis, rep. ducts

- **TESTIS**
- **REP. DUCTS**
and reproductive ducts expressed as per cent total body weight.

Histology of the Testis:

The microscopic anatomy of the testis is similar to that described for other lizards (not unlike other members of the genus *Sceloporus*). The tunica albuginea is thin and composed of several layers of collagenous connective tissue with a few scattered fibroblasts. The tunica vasculosa is relatively meager, with only scattered blood vessels periodically situated around the gland when viewed in cross section. Similar thin connective tissue septa penetrate the substance of the testis between the convoluted seminiferous tubules, of which there are some 200 to 300 in a mid-cross section during the breeding season. The seminiferous tubules are composed of a basement membrane, Sertoli cells, and seminal elements. The tubules vary from 91.3 micra in diameter in the most regressed state to 425.5 micra at the height of spermatogenesis. The lumen of these tubules is entirely closed during the onset of spermatogenesis in summer and become approximately 100 micra wide in the mature testis. Spermatogenic activity occurs in all parts of the tubules; there being no regional differentiation in activity as occurs in fish and amphibians.

The arrangement of the interstitial cells is in agreement with a majority of species studied. In *S. cyan-*
ogenys, they are in the form of small nests, situated in the midst of fine, connective tissue septa and small capillaries between the seminiferous tubules. A definite increase in interstitial cell activity is coincident with the actual time of breeding.

Seasonal Changes in the Testis:

During late June, a reconstitution of the germinal epithelium is inaugurated. The testis at this time has an average weight of 28.6 milligrams, and is representative of minimal reproductive activity. The interstitium is composed of dense connective tissue septa and many arterioles are present. At this time there remains a decreased number of interstitial cells (due to cell death) from the previous breeding season. By July the seminiferous tubules of the testis is composed of 3-4 cell rows, representing mature spermatogonia and primary spermatocytes. A generalized hyperemia is apparent with an engorgement of a capillary network permeating the connective tissue septa; and is probably the reason for the brick-orange color of the testis in situ. The average weight of the testis in August is approximately 200 milligrams, and the seminiferous tubules have increased their germinal bed to about 7-9 rows of cells, the most progressive ones being secondary spermatocytes. The increased diameter of these tubules almost obliterates the interstitium, making it difficult to distinguish the presence of interstitial cells, of which there
are few, if any.

During September there is a further increase in mitotic activity of the germinal bed, along with some reduction division of the secondary spermatocytes to spermatids. A new crop of interstitial cells is evident and mitotic figures can be seen at this time. The month of October represents the maximum size of the testis. The diameter of the seminiferous tubules is 425.5 micra. Nine to ten rows of cells line the tubule, and folliate layers of metamorphosing spermatids fill the central zone. A few, free spermatozoa are found in the lumina, and these mature sperm begin to pass from the tubules into the epididymides by mid-October. The number of interstitial cells per nest have increased. The interstitial cell averages about 18 microns in diameter; and an oval nucleus with a definite nucleolus is present in the center of the granular cytoplasm.

By November there is a decrease in weight of the testis due to the release of mature spermatozoa into the reproductive ducts. The interstitial cells have undergone a further hyperplasia as well as an increase in size. The cytoplasm of these hypertrophied cells have passed from a granular to a seemingly lipoidal consistency, probably indicative of their secretory nature in liberating the male sex hormone, testosterone. During December the germinal epithelium consists of
six to eight rows of cells; the folliate appearance is reduced as mature sperm are shed to the lumen. The cytoplasm of the interstitial cells is extremely vesicular, and these elements are in close apposition to the capillary bed.

By January the folliate appearance of the seminiferous tubules of the testis has disappeared, and the germinal epithelium consists principally of spermatogonia, many spermatid cells, a few remaining spermatozoa, and certain other cellular debris of unknown origin. Shortly after mating, the seminiferous tubules collapse, decreasing from 400 microns in diameter (during their peak of reproductive activity) to approximately 125 micra.

In the month of February, the lining of the seminiferous tubules is 1-2 cell rows thick; the tubule collapsed, and no sperm is present in the lumen. The interstitium shows only a fair degree of vascularity; the cytoplasm of the interstitial cells is highly vesicular and vacuolated. Irregular shaped nuclei are evident. By March, the cytoplasm of these cells is represented by fine reticular strands; and cell death is apparent as seen by the pyknotic nuclei and the reduced cytoplasmic content. The seminiferous tubules consist usually of only one cell layer, namely that of spermatid syncytium and a few spermatogonia. At no time do the spermatogonia
ever completely disappear from the tubule.

It is difficult to distinguish the testes taken from lizards during the months of April, May and June, from one another; with two possible exceptions. During this period of reproductive quiescence there is a slight decrease in diameter of the seminiferous tubules, and a decrease in the number of interstitial cells.
Sexual Accessory Organs

Gross Morphology of the Reproductive Ducts:

The coiled epididymis and the comparatively straight vas deferens of *S. cyanogenys* lie on the dorsal aspect of the pleuroperitoneal cavity. Anteriorly, the epididymis is craniad and lateral to the testis and extends posteriorly to the cloaca as the vas deferens. During the breeding season the epididymis is tightly coiled in the region of the gonad. Before entering the cloaca, the vas deferens passes over the anterior-ventral portion of the metanephric kidney. During minimal sexual activity, the average wet weight of the epididymis and ductus deferens taken collectively is approximately 10 milligrams. At the height of the breeding season, the average wet weight of these ducts is 114.4 milligrams; representing an eleven-fold increase. Figure 2 depicts the seasonal changes in the epididymis and vas deferens, expressed as a per cent of the total body weight.

Histology of the Reproductive Ducts:

The histology of the reproductive ducts of *S. cyanogenys* is similar to that described for other species of lizards. Histological sections taken through the epididymis reveals a series of convoluted tubules lined by a single row of low cuboidal to tall columnar epithelium, depending upon the time of the year the ducts were taken. The epithelium rests on a thin basement membrane.
the ducts are invested with loose connective tissue; and small blood vessels are present in this stroma.

Correlated with an increased testicular activity can be seen a concomitant increased activity of the male reproductive ducts (figures 3 and 4). During minimal sexual activity the diameter of the epididymis measures 63.2 micra. The cuboidal epithelium has an average cell height of 12.0 microns. The nucleus is centrally located. As the breeding season approaches, there is a steady increase in the diameter of the ducts and cellular height of the luminal epithelium; reaching a maximum in the month of December. At this time the diameter of the epididymis is 360.6 micra and the hypertrophic cell measures 67.3 micra in height. The nucleus of the tall columnar cell is situated toward the basal portion of the cell. During the breeding season the epithelium is secretory and eosinophilic staining granules are dispersed throughout the cytoplasm in a gradient; the apical portion of the cell has the greatest concentration of this material. The epididymis and vas deferens are packed with sperm and secretory fluid. As the breeding season comes to a close, only trace amounts of this secretory material and a few clusters of spermatic debris are present in the lumina of the ducts. The diameter and cell height of these ducts decrease; and by late April, no signs of secretory activity are evident. The histo-
The bar graph represent a correlation of the seasonal activity of the sex accessories, expressed as cell height in micra, with interstitial cell activity. The vertically directed bars depict changes occurring in the epididymis; while the horizontally directed bars represent variations of activity of the renal sex segment. The dark circles depict the activity of the interstitial cells, expressed as a give number per nest of cells. At least four adult males are represented for each months determination.
logical changes of the vas deferens parallels that of the epididymides.

**Histology of the Renal Sex Segment:**

During late May and the following summer months, the renal sex segment of the male is in a state of repose. At this time the terminal nephric tubule measures approximately 110 microns in diameter, with a low columnar cell height of 15-25 micra. The cells are non-secretory at this time. A steady increase in cell height of the tubules, along with a gradual increase in secretory activity is seen through the months of September, October, and November. By December the hypertrophic state reaches a maximum, with an average cellular height of 70 micra. Eosinophilic-albuminoid material (not unlike that found associated with the epididymides) is seen at the apical portions of the tall columnar cells. Large amounts of these granules is also seen within the lumina of these ducts. While there is a significant difference between the diameters of the renal sex tubules of breeding and non-breeding lizards, the variations in diameters of these tubules is not as pronounced as those observed in the reproductive ducts. As the breeding season terminates, there is a gradual diminution in size of the tubules and a decrease in secretory activity. By March and April, the secretory granules have diminished, along with the tubular diameter and cellular height (figures 3 and 4).
Figure 4.

A line graph representing the correlation of the seasonal testicular cycle with the diameter of the seminiferous tubules, epididymides, and renal sex segment. Each point on the graph represents the average monthly determination of at least four adult male lizards. The heavy dark line represents the diameter of the seminiferous tubule, the small dashed line depicts the activity of the epididymis, and the dotted line demonstrates the seasonal fluctuations in the diameter of the renal sex segment.
Secondary Sexual Characteristics

In the present study, such secondary sex characters as the hemipenes, femoral pore secretion, and the blue coloration of the gular and abdominal patches were observed to develop and regress concomitantly with the waxing and waning of testicular activity. The hemipenes is enlarged during the months of November, December, January and February; i.e., at the time of breeding.

Femoral pore secretion is nil during the late spring, the entire summer, and early autumn. During the month of November, femoral pore secretion is initiated, commensurate with the onset of breeding. Paralleled with this, at this time, is an intensification of the blue gular and belly patches. During the month of December, a steady increase in femoral pore secretion and a darkened coloration of gular and belly patches is observed. By January femoral pore secretion reaches its peak, and yellow colored cones protrude several millimeters from their bases along the femoral margins of the hind extremities. Thereafter, there is a gradual decrease in secretory activity, with a corresponding diminution in the intensification of the gular and abdominal coloration.
Effects of Testosterone

The results of testosterone administration to adult male non-breeding lizards are expressed in table 2. It was found that testosterone administration induced precocious growth and development of the epididymis, vas deferens; significantly decreased the volume of the testes in the adult non-breeding male; and brought about the early commencement of blue pigmentation in juvenile males. The weight of the reproductive ducts (expressed as per cent of the total body weight of the animal) in the treated lizard was four times that of the untreated control. The decrease in weight of the testes in the experimentally treated animal was ten times less than that of the control lizard. Testosterone had a remarkable effect on the sex accessories. It approximately doubled the diameter of the renal sex tubule and almost tripled the diameter of the epididymis. The results clearly demonstrate, that at the dose levels employed, we were successful in mimicking the reproductive status of an actively breeding lizard with respect to the diameter, cell height, and secretory nature of the epididymis as well as the renal sex segment. Testosterone failed to elicit femoral pore secretion.
**TABLE 2.**

**THE EFFECTS OF TESTOSTERONE ADMINISTRATION**

**IN THE NON-BREEDING MALE**

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO. OF ANIMALS</th>
<th>% TOTAL BODY WEIGHT</th>
<th>DEVELOPMENT OF SEX ACCESSORIES</th>
<th>RENAL SEX SEGMENT</th>
<th>EPIDIDYMIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TESTIS</td>
<td>REP. DUCTS</td>
<td>DIAMETER</td>
<td>CELL HEIGHT</td>
</tr>
<tr>
<td>CONTROL</td>
<td>4</td>
<td>0.372 ± 0.040</td>
<td>0.018 ± 0.001</td>
<td>123.7 μ</td>
<td>26.4 μ</td>
</tr>
<tr>
<td>EXPERIMENTAL</td>
<td>5</td>
<td>0.037 ± 0.002</td>
<td>0.071 ± 0.002</td>
<td>200.6 μ</td>
<td>72.7 μ</td>
</tr>
</tbody>
</table>
The Ovary

Gross Morphology of the Ovary:

The ovary of *Sceloporus cyanogenys* was studied primarily with the intention of determining the level of activity of this organ at the various seasons of the year. Indications of ovarian activity are the rate of growth of the contained ova, the relative activity of the germinal bed, and the presence or absence of a corpus luteum. Consequently, observations on the ovary included a seasonal study of the changes in the mass of the whole ovary, the number and size of the developing ova, and a histological investigation to determine the level of activity of the germinal bed.

The ovaries of *S. cyanogenys* are oval to oblong bodies lying posteriorly and dorsally in the pleuro-peritoneum and suspended by the mesovarium. The completely transparent ovarian epithelium permits easy recognition of the individual ova. The ovary of a large adult non-breeding lizard contains 10 to 15 white or gray, opaque ova arranged in somewhat linear order along the perimeter of the gland. The ova measure approximately 0.5 to 1.0 mm. in diameter. During the year from January to July, the ovary does not contain any very large ova awaiting ovulation, such as those of the Gecko, described by Boyd (1940). In *S. cyanogenys*, the period of extensive yolk deposition does not occur until the month of September. At this time and the subsequent months
thereafter (up until the time of ovulation), a moderate deposition of yolk is laid down.

Inspection of the abdominal cavity of a female just prior to ovulation, reveals large yellow-colored and yolk-laden follicles measuring 8 to 10 mm. in diameter. Each ovary of a large adult female usually produces eight or ten completely developed ova; however, the number of ova produced by a single lizard depends much upon the size, weight, and nutritional condition of the animal. It is therefore not surprising to find some females which will produce a larger number of ova than others. This would certainly seem to be the case with females of different ages, Blair (1960).

Immediately following ovulation, the collapsed follicles begin to develop into corpora lutea. The post-ovulatory corpora are cream colored, and appear as uniconcave discs on the surface of the ovary. As gestation progresses, the corpora lutea become spherical, and yellow pigment is deposited in them. At the time of parturition, the corpus luteum has undergone rapid degeneration, and within 4 to 6 weeks it is almost completely resorbed and no longer visible to the naked eye.

Histology:

The ovarian epithelium consists of a single layer of low cuboidal to flattened cells, as is mentioned by
van den Broek in Bolk's Handbuch. This is the germinal epithelium which encapsulates the ovary. As in all reptiles so far reported, the stroma ovarii is of a limited quantity and is composed of collagenous fibers and scattered fibroblasts. Small blood vessels are situated in this meshwork. The stroma forms the thin wall of the ovary, the thecal coat of the ova, and contributes to the structure of the germinal bed. The germinal bed, in turn, is composed of primary oogonia and oocytes in varying stages of development.

The histology of the ovary will differ from time to time, depending upon the functional reproductive status of this gland. Just prior to ovulation, the most prominent structure is the Graffian follicle containing its large, yolk-laden ovum. The follicle of S. cyanogenys exhibits a granulosa unlike the general type reported for other lacertilians and ophidians. The granulosa consists of several rows of small follicular or nurse cells and large pyriform cells. These may be divided into three classes of cells based on their size and shape. Peripheral to the zona pellucida, there are several rows of small follicular cells. An outer layer of one or two rows of medium, spherical cells separates the smaller cells from the large pear-shaped cells. A thin thecal envelope encases the follicle and separates it from other developing follicles.
In our hands, the vitelline membranes of the oocyte could not be resolved into a striated and homogeneous layer, as have been described in Xantusia and Hoplodactylus.

Contrary to that which has been reported for several species of lizards, no cytoplasmic prolongations were ever seen to communicate from the large cells to the interior of the egg. In addition, corpora atretica, unlike that found for Xantusia, may be found only rarely; and this is confined to the time immediately after ovulation. In Sceloporus, as with Xantusia, the atretic follicle is easily distinguished from the newly formed corpus luteum by the presence of yolk. Epitheloid-like macrophages are scattered throughout the atretic follicle, but are found in greatest numbers toward the periphery of the corpus. It is assumed that these cells are responsible for the resorption of the unused yolk.

A post-ovulatory corpus luteum is formed from the old Graffian follicle. At this time I would like to propose three phases or stages in the development and life of the corpus luteum of S. cyanogenys. The three phases are differentiated on the basis of the following parameters: 1.) the gross morphological appearance with respect to size, shape, and coloration; 2.) the presence or absence of lipid and pigment as verified by histochemical techniques; 3.) and the term or period of gestation. While the stages are quite distinct-
ive from one another, one must bear in mind that the histological differences seen in each phase represents a continuous spectrum of growth, maintainence, and necrosis in the life cycle of this structure. Any precise limits where one stage terminates and the other commences are difficult to make in view of the fact pregnant females were sacrificed at intervals of one month, and intermediate stages of the cycle could not be observed. Suffice it to say, that our description of the three stages is an arbitrary one; representing several sequences of events that occur in the life span of the corpus luteum.

The first phase of the corpus is confined to the first four to six weeks of gestation. Immediately following ovulation the follicular cells of the collapsed follicle undergo a very rapid and proliferative hyperplasia, producing a compact mass of large cells that within several weeks completely fills the old follicular cavity. The corpora are cream colored and appear as uniconcave discs on the surface of the ovary. An unhealed cicatrix is apparent and is invariably found on the ventral aspect of the corpus. The thecal envelope is thick and completely surrounds the lutein cell mass, except at the point of rupture. The well vascularized theca is divisible into a thin theca interna and an outer, thick externa. The externa cons-
ists of many collagenous fibers and a few scattered fibroblasts, while the theca interna contains few fibers and many fibroblasts. At this time the lutein cell mass and the outermost layer of the theca interna are highly lipid positive. The innermost layer of the theca interna is devoid of lipid but is demonstrably PAS positive. Within several weeks (three to four), a few fibroblasts from the theca invade the adjacent lutein cell mass and tend to produce thin septa. The fibroblasts do not surround the individual cells; and at no time during this stage of development are blood vessels carried into this developing mass. The lutein cells near the periphery of the cell mass form a zone of usually larger and apparently more secretory elements than the central zone of more compact, smaller and more deeply staining cells. The lutein cell toward the periphery of the mass is large and vesicular. It has ill defined cytoplasmic membranes, and the cytoplasm takes only slight acidophilic stain. The nucleus of these cells has a characteristic granular nucleoplasm at this time, and is strongly basophilic with hematoxylin and alizarin blue dyes. In histological sections, it is not unlikely to see portions of the theca in the center of the corpus. This is the result oblique sectioning and the deep infolding of the theca after collapse of the follicle.

By the fourth week after ovulation, the corpus
luteum is fully formed and is a spherical body on the surface of the ovary. The old follicular cavity is completely obliterated by the growth of the luteal cells. Unlike that found for Xantusia, a plug of lutein cells, representing the point of follicular rupture, does not persist for the entire life of the corpus; but rather is eventually covered by the growing thecae.

The second phase in the life history of the corpus luteum is confined to the middle one-half of gestation. It is represented by its characteristic yellow color, due to the deposition of pigment. The lutein cell mass is much more compacted within its thecal envelope. The lutein cells are more vacuolated; their cytoplasmic membranes are less distinct; and the distinction between cells of the inner and outer zones is more apparent. The lutein cells have lost their affinity for Sudan Black, indicating a depletion of lipid within their scanty cytoplasm. The second phase is terminated approximately a month before parturition takes place.

The third stage represents the necrotic phase in the life span of the corpus luteum. Degenerative changes appear in the central zone and extend peripherally. At this time (close to the time of parturition and shortly thereafter), there is an extensive invasion of fibroblasts from the theca into the remaining lutein tissue, often times carrying a capillary bed. By the fourth to sixth week after parturition, almost
all of the corpora have been resorbed and the last vesti¬
ges are present as light orange colored flecks which
persist on the surface of the ovary for some time.

Seasonal Changes of the Ovary:

The seasonal ovarian cycle of the female lizard
is depicted in figure 5. The ovaries and oviducts are
expressed as percentage of the total body weight of the
animal. During the summer months the ovary and oviducts
are quiescent. The minimum average weight of the summer
ovary is approximately 20.0 milligrams. During the month
of August, minute follicles may be seen on the surface
of the ovary, appearing as white opaque spheres ave-
raging 1.0mm. in diameter. By September, the first de-
posits of yolk are laid down and each Graffian follicle
has increased to about 2.0mm. in diameter. The average
weight of the ovary is 267.3 milligrams. This weight
is doubled by the month of October, and the individ-
ual ova now measure 4 mm. in diameter. Throughout the
month of November, there is a steady increase in size
and weight of the ovary; due to the increased yolk
deposition. Each ovum measures between 5 and 7 mm. in
diameter. The ovaries with their yolk-laden ova com-
pletely fills the abdomen of the female. During the
month preceding ovulation, there is a much more rapid
deposition of yolk; and the absolute, average weight
of the ovary just prior to ovulation is between 2.5 to
3.7 grams, depending upon the size and nutritional status
Figure 5.

Graphic representation of the seasonal reproductive cycle of the female lizard, *Sceloporus cyanogenys*. The weight of the ovary and oviduct is expressed as a per cent of the total body weight of the female. The upturned arrow indicates the commencement of yolk deposition; the downturned arrow depicts the time of ovulation. The hatched area represents the gestational period. Each point on the graph represents a minimum of four females sacrificed at monthly intervals. From November to January, samples were taken at two week intervals so that the time of ovulation could be precisely determined.
seasonal ovarian cycle
of S. cyanogenys
of the female. The abdominal cavity is swollen and distended.

The majority of females mate and ovulate during the later part of December and the early weeks of January. Evidence of this is based upon the following facts: 1.) actual observation of mating behavior, 2.) the presence of sperm in the oviduct of the female, 3.) the presence of freshly ovulated ova and newly formed corpora lutea, and 4.) the variable length of gestation.

Immediately after ovulation the ruptured follicles form corpora lutea. By mid February, all of the adult females that have successfully mated contain embryos of varying embryonic stages within their oviducts. The average weight of the ovary at this time is approximately 100 milligrams. The cream colored corpora can easily be seen as an uniconcave disc with an unhealed cicatrix.

During the month of March, the corpora have reduced in size and appear as characteristic yellow spheres on the surface of the ovary. They measure about 1.0mm. in diameter. The average weight of the ovary is 57.7 milligrams. Corpora lutea persist throughout the months of April and May; and the absolute weight of the ovary is about 20,0 milligrams. While necrosis begins in May, it is not grossly apparent until parturition and thereafter. The period of gestation is approx-
imately four months for *S. cyanogenys*; being somewhat shorter at the warmer temperatures of an animal room. By late June all of the females that were pregnant had given birth. The month of July is represented by an ovary at rest - the reproductive cycle having been completed. The initiation of the next cycle commences in late August.
The Oviduct

Gross Morphology of the Oviduct:

The oviduct was studied to assess the endocrine control of this female sex accessory by the ovary. The oviduct of *S. cyanogenys* is a slightly coiled tube, superficially pleated, flattened and folded, lying against the dorsal aspect of the pleuro-peritoneum. It is lateral to the ovaries, and extends from the posterior portion of the pleural cavity to the cloaca. The anterior aspect of the oviduct is thin walled, but as it approaches the level of the ovary it becomes larger in diameter and the walls are considerably more muscular.

Figure 5, depicts the seasonal changes of this organ with that of ovarian activity. The gross morphology will vary with the season and the reproductive status of the ovary.

During the time of ovarian quiescence, the oviduct is a white to gray colored and slightly coiled tube. As the breeding season approaches and yolk is deposited in the ova of the ovary, the oviduct becomes a faint pink color (representing a hyperemia) and is slightly swollen. During the period of gestation, it is distended and transparent, allowing the individual fetuses to be seen when the abdominal cavity is surgically opened. Figure 6 is a photograph of an
adult pregnant female after laparotomy demonstrating this condition. During the incubatory period of gestation, the developing fetuses within the oviduct appear as links in a sausage; measuring 12 mm. at its largest diameter, and approximately 7 mm. in diameter at the constricted portion of the tube between the developing lizards. The striking increase in weight of the oviduct during gestation represents the increase in weight of the developing embryos and not that of the oviduct itself.

Histology of the Oviduct:

Two considerations must be taken into account in a histological description of the reptilian oviduct; first, the portion of the oviduct under consideration; and secondly, the time of the year the organ was taken. That is, the histology of the oviduct fluctuates with the season, as well as, the different areas along its length. One further point to consider is that of terminology. The term oviduct will be used instead of uterus, since "uterus" gives the connotation that this organ is a place of nourishment. For reasons that will become obvious later, we will prefer the term "oviduct" as the chamber of incubation for the developing embryo and fetus.

The oviduct of S. oyanogenys may be differentiated into two portions: the thin, anterior-most end of the
Figure 6.

Photograph of a large, adult pregnant female upon laparotomy; demonstrating the gravid nature of the oviduct. This lizard was sacrificed at mid-term. Note that the individual fetuses with their yolk sacs may be seen through the thin transparent oviducal wall. Notice further, the absence of enlarged post anal scales in the female (arrow).
tract or infundibulum; and the body, or oviduct proper. In cross section the infundibulum is weakly pleated and consists of a mucous membrane, thin, circularly arranged muscular layer, and an external serosal membrane. Blood vessels are present in the stroma between the basement membrane (upon which rests the columnar epithelium) and the circularly disposed musculature. Prior to ovulation, the mucosal epithelium, consisting of ciliated and non-ciliated columnar cells, hypertrophies. The ciliated cells are in greater number than those which lack cilia. Eosinophilic material may be seen within the lumen of the infundibulum. During reproductive quiescence, the once columnar epithelium has decreased in height and these cells are low columnar to cuboidal in shape.

The body or oviduct proper, when viewed in cross section, is considerably larger than the anterior fourth of the duct. The outer most layer of the tubule is lined by a serosal membrane. Just inside this membrane is a thick coat, well pleated, consisting predominately of collagenous fibers and a few longitudinally arranged, muscle bundles. Between the mucosa and the longitudinally directed external layer is a circular layer of muscle, which is thicker than that portion of the infundibulum.

During the period of reproductive inactivity, the mucous membrane is drawn up into ruffled folds, con-
sisting of longitudinally directed, muscle fibers and small blood vessels. The mucous membrane consists of low columnar to cuboidal epithelium which rests upon the basement membrane. During the period of yolk deposition the oviduct undergoes several striking histological changes. Glands appear in the stroma. These are the simple alveolar type. The epithelium of these glands consist of tall columnar cells with nuclei situated towards the basement membrane. Eosinophilic material is present throughout the cytoplasm. This is in heaviest concentrations at the apical ends of the cells. Just prior to ovulation, the mucosa is thrown up into folds, and the luminal portion is engorged with sperm - especially numerous in the crypts between villi. The crypts are presumably formed from the glandular epithelium. During gestation the oviduct is well vascularized and extremely distended. The luminal epithelium is stretched, due to the presence of the fetuses; and the once columnar epithelium lining the duct is flattened. Just following parturition, the oviduct is again well pleated; this time due to the collapse of this tubule. The lumen is filled with cellular and non-cellular debris.
Ovariectomy and Replacement Therapy

The results of the experiments designed to test the effects of the female sex hormones on the oviducts are expressed in table 3. As can be seen from the table, ovariectomy prevented an increase in weight of the oviduct; while exogenous estrogen administration reversed the effect on the oviducts produced by removal of the ovaries. It is of interest that exogenous estrogen, in concentrations of 5 micrograms every other day, had a greater stimulatory capacity on the oviduct than that produced by the control ovary.

Attempts to demonstrate the endocrine control of maintaining the gestational state by the corpus luteum were unsuccessful. In both sham-operated and ovariectomized pregnant females, all females aborted within three days.
Table 3.

Effects of Ovariectomy and Estrogen Replacement Therapy on the Oviduct of the Female Lizard, *S. cyanogenys*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Weight of Ovaries (grams)</th>
<th>Weight of Oviducts (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>3.279 ±0.347 **</td>
<td>-</td>
</tr>
<tr>
<td>Ovar-x *</td>
<td>8</td>
<td>1.412 ±0.284 ***</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>-</td>
<td>0.380 ±0.072</td>
</tr>
<tr>
<td>Ovar-x</td>
<td>4</td>
<td>-</td>
<td>0.188 ±0.018</td>
</tr>
<tr>
<td>Ovar-x plus Estrogen</td>
<td>4</td>
<td>-</td>
<td>0.420 ±0.010</td>
</tr>
</tbody>
</table>

Ovar-x * - means ovariectomized

** - ovarian weight at the time of autopsy

*** - ovarian weight at the time of ovariectomy
Reproductive Behavior and Fertilization

The courtship and mating behavior for *S. cayanogenys* is similar to that described for other species of this genus. Only one observation of the mating ritual was seen in the laboratory throughout its entirety; however, isolated sequences of this behavior have been observed on several occasions. The male bobs rhythmically up and down on its fore legs. These movements are synchronized with a pronounced swelling and subsiding of the throat and belly patches. Occasionally this action is interrupted with scurries toward the female. Females also have a definite posture which is peculiar to their sex. It consists of raising high on their four legs and hunching the back until it forms a dome. Occasionally she would hop up and down on all fours in a stiff-legged fashion. The male would seize the female by the integument of the neck before attempting copulation. The copulatory act is short, lasting about 10 to 15 seconds. The insertion of the hemipenis into the cloaca of the female could not be seen, but the everted hemipenis was visible after coition was completed. Once the male has hold of the female's neck and back, the female is submissive throughout the copulatory act, except for the slight raising of the tail.

Homosexual attempts at mating were observed on four different occasions between two males which were receiving testosterone. The smaller of the two would
assume the role of the male. This phenomena has been reported in several species of lizards, especially when kept in good health in large laboratory cages, Noble and Bradley (1933). Since the cloaca of the male permits the insertion of the hemipenis of another male; there is no structural hindrance to this performance. There are isolating mechanisms, however, which would prevent males from copulating with other males as frequently as with females. Hunsaker (1962) has reported that different species can recognize other species by the frequency and duration of the characteristic head bobbing. In addition, males when confronted with other males during the breeding season tend to fight. The result is that males tend to occupy discrete territories, which are difficult to recognize in the laboratory; but which have been described in the field.

Copulation occurs before ovulation; and possibly triggers the ovulatory process, as it does in some mammals. Evidence for this is based on the finding of sperm in the lumen of the oviducts while ova are still within the ovary. Fertilization probably takes place in the anterior horn of the infundibulum; since sperm is found in considerable numbers in this region of the duct. As mentioned previously, ovulation occurs in the majority of cases, somewhere during the last two weeks of December and the first two weeks of January.
Gestation

The period of gestation in S. cyanogenys is approximately four months. Under artificial conditions, as in an animal room, the length is somewhat shorter.

Of twenty-five females which had ovulated, the average number of eggs per female was 18.32. The average number of eggs fertilized per female, as verified by the number of young lizards born was 17.68; or 96.5 percent of the total number of eggs ovulated. The percentage of eggs which failed to develop and probably represent those which had not been fertilized was 3.5%.

Implacement of the fertilized eggs within the body of the oviduct is accomplished by a constriction of the extremely thin, transparent oviducal wall between each embryo, giving the gravid tubule a segmental appearance (figure 6). The germinal disc, as well as the developing fetus of a later stage, lies dorsally in the oviduct. The apposition between the ectodermal chorion and serosa of the fetuses and the oviducal epithelium is loose. There is no implantation and no structural attachment between the soft egg membranes and the oviduct. As gestation proceeds, the yolk sac has disappeared completely. Laparotomy of several newborn lizards revealed an absence of any unused yolk.
Parturition and Litter Size

Parturition may occur anywhere from late April to June. The earliest date of birth in our laboratory was February 24th., in which a 48 gram female (after parturition) gave birth to 13 young. Two unfertilized eggs were also found in the cage. Only three accounts of parturition have been reported for this lizard. Smith (1939) stated that eleven young were born June 2, 1937 by a female specimen collected at Arroyo Los Olmos, three miles southeast of Rio Grande City, Starr County, Texas. Hunsaker (1959) reported the births from seven gravid females collected in Webb County, Texas. These seven females gave birth from May 5th. to June 11th. Kennedy (1960) reported that a female measuring 108 mm. snout to vent, had given birth on the morning of the 28th. of February to 17 young. In addition, two small eggs which had failed to develop were found on the floor of the animal cage.

Parturition has been observed on five occasions in our laboratory. Birth invariably occurred during mid-morning on all occasions. One female was observed after she had given birth to one lizard. The length of time for the entire birth process of this female, which eventually gave birth to 14 young and deposited two unfertilized eggs, was approximately an hour and forty-five minutes. The average length of time between the
birth of any two lizards was about four to six minutes. Abdominal contraction, unlike that reported by Kennedy, (1960), was not noticeable. During birth the female is sluggish in her movements, unless molested. Just prior to giving birth to one of her young, the female raises the base of her tail and the young lizard emerges. Usually the young emerged from the cloaca enclosed in the extraembryonic membranes. About thirty seconds after delivery, the young would give a quick wiggle forward and the delicate, transparent membranes would rupture, leaving the lizard about three-fourths free of his capsule. After running a short distance, the adhering membranes on the tail would be left behind.

Occasionally a lizard would be born free of his extraembryonic membranes. On other less frequent occasions, the membranes would dry before the lizard could break through his capsule, thus sealing him in, unless the membranes were artificially broken.
Demonstration of Ovoviviparity

Two techniques were employed in this study to determine and demonstrated the ovoviviparous nature of S. cyanogenys. The first consisted of comparing the weights of freshly ovulated ova with weights of newborn lizards. The results are given in table 4. As can be seen from the data presented, the wet weight of the newborn was twice that of a freshly ovulated ovum. When expressed as dry weight, however, the newly ovulated ovum is not statistically different from that of the newborn. This implies that the gain in weight is due to water and not to nutritional materials supplied from the mother.

The second method employed to demonstrate that the mother is not contributing nutritive material to the developing fetuses within the oviduct was to measure the transport and uptake of carbon\textsuperscript{14}-labeled amino acids. The results of these experiments are found in table 5. With the use of glycine, there was no difference in the amount of radioactivity in blood, liver and oviduct, between pregnant females and females which contained large ova in their ovaries but had not as yet ovulated. Notice, however, the marked differences between the amount of radioactivity found in the pre-ovulatory ovum with that found in the fetus. It is also interesting to observe the marked concentration difference between oviduct and developing fetus.
Table 4.

Weights of Ova and Newborn Lizards

<table>
<thead>
<tr>
<th>Stage of Development</th>
<th>No. of Specimen</th>
<th>Wet Weight (gms)</th>
<th>Dry Weight (gms)</th>
<th>Ash Weight (mgms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovum</td>
<td>20</td>
<td>0.4183 ± 0.022</td>
<td>0.2163 ± 0.043</td>
<td>16.2 ± 5.4</td>
</tr>
<tr>
<td>Newborn</td>
<td>20</td>
<td>0.8658 ± 0.024</td>
<td>0.1603 ± 0.030</td>
<td>18.6 ± 2.2</td>
</tr>
</tbody>
</table>

Table 5.

Radioactivity Experiments

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of Animals</th>
<th>Pre-ovulatory Cts./Min./Gm.</th>
<th>No. of Animals</th>
<th>Post-ovulatory Cts./Min./Gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Glycine:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
<td>1092.4</td>
<td>2</td>
<td>1045.4</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>1237.7</td>
<td>3</td>
<td>1138.8</td>
</tr>
<tr>
<td>Oviduct</td>
<td>2</td>
<td>1477.7</td>
<td>3</td>
<td>1451.0</td>
</tr>
<tr>
<td>Ova-Fetus</td>
<td>3</td>
<td>90.0</td>
<td>3</td>
<td>3.7</td>
</tr>
<tr>
<td>B. Methionine:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>2</td>
<td>2801.0</td>
<td>2</td>
<td>2752.2</td>
</tr>
<tr>
<td>Liver</td>
<td>2</td>
<td>3829.3</td>
<td>2</td>
<td>4184.3</td>
</tr>
<tr>
<td>Oviduct</td>
<td>2</td>
<td>1364.0</td>
<td>2</td>
<td>3971.9</td>
</tr>
<tr>
<td>Ova-Fetus</td>
<td>4</td>
<td>1391.4</td>
<td>4</td>
<td>23.2</td>
</tr>
</tbody>
</table>
In our methionine experiments, there were essentially no differences in blood and liver of pregnant females with that of females with pre-ovulatory ova. There was however, an unexplained difference in the amount of radioactivity within the oviducts of gravid females as compared to non-pregnant females; the oviducts of pregnant females being approximately three times that of the non-gravid lizards. A most significant difference is seen in the amount of radioactivity found in pre-ovulatory ova and the developing fetuses. Again notice the concentration differences between oviduct and fetuses of pregnant females.

The results would indeed indicate that there is little transport of nutritional materials from mother to developing young.
Relationship of Other Hormones to Reproduction

Gonadotrophins:

In an attempt to observe the pituitary control over the ovary and its influence on gestation, several experiments were undertaken. The results are expressed in table 6. The data indicates that under our experimental design, neither the purified gonadotrophins nor the human chorionic gonadotrophins were able to elicit another reproductive cycle in the non-breeding male. No experiments were performed to test this in adult non-breeding male lizards.

Oxytocin:

The results of a single dose of 1 USP oxytocin on the facilitation of parturition are expressed in table 7. In every instance, all five females receiving oxytocin either gave birth or aborted within 18 hours after hormone administration. No effect was seen in those lizards which received isotonic saline. The data is highly suggestive that oxytocin has an important effect on maintaining the gestational state; and that its action in lizards is to cause contraction of the oviducal musculature, as it is known to do in mammals.
Table 6.

Lack of Effect of Gonadotrophins on the Ovary and Oviduct

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>Ovarian Weight</th>
<th>Oviduct Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Total Body Wt.</td>
<td>% Total Body Wt.</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>0.042 ± 0.003</td>
<td>0.334 ± 0.021</td>
</tr>
<tr>
<td>HCG</td>
<td>4</td>
<td>0.046 ± 0.005</td>
<td>0.349 ± 0.030</td>
</tr>
<tr>
<td>FSH+LH</td>
<td>4</td>
<td>0.044 ± 0.005</td>
<td>0.339 ± 0.029</td>
</tr>
</tbody>
</table>

Table 7.

Effects of Oxytocin upon Parturition

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Animals</th>
<th>No. giving birth or aborting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (saline)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Experimental (oxytocin)</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
DISCUSSION

A cyclic histological change in the testicular and ovarian tissue can be demonstrated in the blue spiny lizard, Sceloporus cyanogenys. Along with this seasonal development of the gonads is a concomitant development of the sex accessories and the secondary sexual characteristics of both male and female lizards. As expected, the reproductive cycle of the male is coincident with that of the female.

The histological changes occurring in the testis of S. cyanogenys is in general similar to that described for other lizards; especially that reported for other species of this genus, Woodbury and Woodbury (1945); and Wilhoft and Quay (1961). The organization of the testis is like that of the higher vertebrates. True seminiferous tubules are present. The period of spermatogenesis is evident by the proliferation of germ cells within these tubules. This phenomena is seen on a macroscopic level in the gradual increase in size and weight of the testis. Spermogenesis, or the transformation of spermatids to mature spermatozoa, is continuous with that of the spermatogenic process.

The role played by the Leydig or interstitial cells in the elaboration of the male sex hormone in reptiles has been the subject of much debate. Because the sex accessories like the epididymis, renal sex segment, and such secondary sexual characters as femoral pore secre-
tion and abdominal coloration become conspicuous during the breeding season (and the interstitial cells give indication of activity at this time); the presumption is strong that the two phenomena are related in *S. cyanogenys*.

Increase in the epididymides is due to a hyper trophy of the epithelium as well as the accumulation of mature spermatozoa from the seminiferous tubules. The secretory activity of the epididymis and renal sex segment is under the control of the male sex hormone, testosterone. These results of exogenous testosterone administration to males with seasonally quiescent testes confirms the work of Gorbman (1939), Forbes (1941), Altland (1943), Evans (1948, 1951). Although it was possible to duplicate the secretory activity of the epididymis and renal sex segment with the administration of the male sex hormone; it was not possible to elicit femoral pore secretion with the dose levels employed. Possibly a longer time was needed to witness this effect.

The presence of pigmentation in male lizards as a possible secondary sexual character has long been thought to be due to the male sex hormone. Apparently this is the first verification of this hypothesis to date. Other workers have demonstrated the effects of testosterone on such sexual characters as the dorsal crests, femoral pores, cloacal glands, and the hemi-
penes, Gorbman (1939); Kehl (1938); Forbes (1938, 1940, 1941); Noble (1941, 1943); Altland (1943); Hartley (1945); Reynolds (1947); and Evans (1948, 1951). No attempt was made to test the effects of testosterone on the female; but it would be interesting to see whether one could achieve the degree of pigmentation as we found in juvenile males.

As previously mentioned in the introduction, there is considerable variation among groups of reptiles, and differences between members of the same group. Alligators and member of the order Chelonia (turtles), which are all oviparous forms, lay their eggs in spring to insure summer hatching. In the suborder Serpentes (snakes), mating occurs in the autumn, and sperm is stored in secretory pouches over the hibernation period; ovulation occurs in the following summer. The seasonal changes in the ovary among members of the suborder Lacertilia (lizards) are, for the most part, quite similar. Most species ovulate in spring and, or, early summer. In general, it can be said that breeding in reptiles is a spring phenomena.

The seasonal changes in the ovary of S. cyanogenys is similar in many details to that described for reptilian species; but in other respects, it is unique. In the present study we have shown the possibility of a winter breeding season. Speculation on the reasons for this will be mentioned.
The most prominent structure of ovarian tissue throughout the breeding season is the individual ova, appearing as large, yellow, yolk-filled bodies (8 to 10 mm in diameter) awaiting ovulation. Unlike the ovary of the Gecko, described by Boyd (1940), the ovary of S. cyanogenys does not contain any very large ova awaiting ovulation during the greater part of the year, i.e., from January to July. For Hoplodactylus maculatus, Boyd says that deutoplasmic deposition occurs immediately after ovulation. In S. cyanogenys, the period of extensive yolk deposition does not occur until September, and is continuous through the next several months until shortly before ovulation. This agrees with the general aspect of ovarian development for certain European lizards, Loyez (1906), and for Xantusia, Miller (1948).

As mentioned earlier, the female exhibits a granulosa unlike that found in most reptiles. No cytoplasmic prolongations, as described recently by Betz (1963), for Natrix, were ever seen to communicate from the large pyriform cells to the interior of the ooplasm. The vitelline membrane could not be resolved into a striated and a homogeneous layer, as mentioned for Xantusia, Hoplodactylus, and Natrix. However, the inability to distinguish this zonation of the zona pellucida is more than likely due to our microscopic resolution, and I would not like to make an issue whether there is one or
two layers. Also, unlike many reptiles, follicular atresia is found only rarely in *S. cyanogenys*, and is confined to the time immediately following ovulation. This agrees with the report of Tinkle (1961) for *Uta*, where atresia occurs in less than one per cent of the observed number of females.

The corpus luteum, which is formed from the old Graafian follicle, has remained an enigma to investigators of reptilian physiology. In general the corpus luteum resembles those of mammals, except, that the theca does not undergo luteinization to form paralutein cells, Betz (1963). However, according to Mingazini (1893), Weekes (1934), Altland (1951), and Bragdon (1952), the theca does form these elements. In *S. cyanogenys*, we were not able to demonstrate any elements which resembled theca lutein cells of mammals; but did observe that the theca interna was markedly lipid positive, at least in the early stage of development of this structure.

A curious feature of the reptilian corpus is the absence of blood vessels in the central parenchyma. It is of interest that no sinusoidal elements were present in this region of luteal cells when progesterone is presumably being liberated (as indicated by the positive sudan black histochemical test); and when such a test is performed at a later stage in the life
of the corpus, blood nests are present. At this point, however, we must emphasize that we are only assuming that progesterone is giving the positive reaction with the sudan black, and that our assumption is based solely on the similarity of physiological function found in mammals.

The suggestion that a hormone from the corpus luteum prevents further follicular growth and ovulation was made by Cunningham and Smart (1934) and Panigel (1951). Boyd (1940), calling attention to the corpora lutea of oviparous reptiles, offers the hypothesis that in this vertebrate class, the corpus luteum may have evolved before the placenta and only subsequently acquired an endocrine function related to viviparity. Bragdon (1951), working with several species of viviparous snakes has also come to this conclusion.

Bio-assays of alcoholic extracts of corpora lutea of Bothrops and Crotalus revealed progestins, Porto (1941); as did bioassays of plasma from Natrix and Thamnophis, Bragdon, Lazo-Wasem, Zarrow and Hisaw, (1954).

I was unsuccessful in experiments to deluteinize the ovary in females during the early and mid-gestational period. In both ovariecomized and sham-operated animals, all of them aborted three days after surgery. No explanation can be given for this abortion at this time, except that it is doubtful that we were inter-
ering with the adrenal gland, since replacement with cortisone was ineffective in preventing this abortion.

Besides the presence of progesterone in the ovary, other female sex hormones have been found in the ovaries of reptiles. Daily injections of follicular fluid from the ovaries of *Crotalus terrificus* provoked vaginal cornification in castrate female mice, Fraenkel and Martins (1938). Alcoholic extracts of the ovaries of crotalid snakes on bio-assay contained the equivalent of 200 estrone units per kilogram of fresh ovaries, Valle and Valle (1943).

During the course of maturation of the ova in *S. cyanogenys*, estrogens are presumably being liberated by the ovary. Justification of this conclusion is seen in our experiments of ovariectomy and replacement therapy in female lizards. Surgical ablation of the gonads prevented an increase in weight of the oviduct. Estrogen in small concentrations (5 micrograms), reversed this effect and even stimulated the oviduct to some extent above the sham-operated controls. This is in agreement with that found for *Lacerta*, Regamey (1935), and for *Matrix*, Takewaki and Hatta (1941).

Except for a few ill-defined reports, information on the histological changes in the reptilian oviduct is lacking. In *S. cyanogenys*, the oviducal glands appear prior to mating and ovulation. Eosinophilic material appear as secretion droplets in the lumen of
these glands. This is a confirmation to the report of Takewaki and Hatta (1941), who observed secretion granules in the oviducal glands of *Matrix tigrina*. Prior to ovulation, but following mating, deep crypts are seen extending downward from the lumen of the oviduct. I am of the opinion that these crypts are formed from the oviducal glands. Since these crypts are packed with sperm pre-coitus, I would like to suggest that the purpose of these glands (now crypts), is to serve as storage depots or chambers for sperm before ovulation takes place and fertilization occurs in the infundibulum of the oviduct. It is interesting to notice that the secretory fluid liberated by these glands is of the same consistency and stains with the same dyes as the fluid found in the epididymis of the male when sperm is present there.

The behavioral courtship and mating process is quite similar to that described for others of the genus *Sceloporus*, Noble and Bradley (1933). The period of gestation in *S. cyanogenys* is approximately four months, and is probably longer than that reported for most saurian species which give birth to their young, Miller (1948, 1959).

Because the apposition between oviduct and fetus is loose, and because our radioactivity experiments and weight measurements indicate that the mother is contri-
buting no nutritional material to the developing fetuses; it is reasonable to posit that *S. cyanogenys* is indeed ovoviviparous. Some workers, Miller (1948); Barwick (1959) have noted that the weight of the fully developed newborn lizard is approximately twice that of the freshly ovulated ovum (as we have found for *S. cyanogenys*). They therefore come to the conclusion that the animal is viviparous, i.e., receiving their nutritional materials from the mother's circulation. They have failed to realize that the increase in weight is due to absorption of water. It is well known that oviparous lizard and turtle eggs developing outside the mother's body gain considerable weight by absorption of water through the egg shells, Cunningham and Huene (1938); Portman (1934).

Reasons for the lack of effect of mammalism gonadotrophins in eliciting another reproductive cycle in *S. cyanogenys* is more than likely due to the immunological differences in pituitary hormones between widely separated classes. It is interesting, however, that oxytocin (a small polypeptide), was able to stimulate the oviduct and facilitate parturition, as it is known to do in some mammals.

Reasons for the winter breeding and ovoviviparous nature of *S. cyanogenys* can now be made. A very important observation in relation to ovoviviparity and viviparity in reptiles is that those species living at the
highest latitudes both North and South, and at the highest altitudes are almost always members which give birth to their young, Darlington (1957); Weekes (1935). With such cooler temperatures, there is little time for incubation of reptilian eggs, lizards which can thermoregulate (raise their body temperature by basking) would then be able to supply the warm temperatures that appears essential for incubation of embryos.

On the other hand, those animals which are faced with high ambient temperatures for extended periods of time have to solve yet another problem. Eggs laid under these severe environmental conditions suffer the possible effects of dehydration. It would therefore seem advantageous to retain the developing embryos, and to offer a protective aqueous environment that is also required for the development of the fetuses.

In addition, many young lizards are insectivorus, relying on an abundant food supply which is confined to the spring and summer months. Since most live-bearing lizards give birth to their young in late spring and early summer; and because most oviparous species lay their eggs in spring to insure summer hatching; it is not surprizing that S. cyanogenys follows the general pattern of giving birth to their young in early summer by extending their gestational period, and at the same time take advantage of the moderate winter temperatures for
its breeding season.

As mentioned earlier, this is only a hypothesis and more information must be obtained on tropical and sub-tropical fauna, before a general rule can be made concerning the evolution of this reproductive mechanism.
SUMMARY

The blue spiny lizard, *Sceloporus cyanogenys*, an ovoviviparous lizard from Texas and Mexico was studied in order to contribute further information to the physiology of reproduction in reptiles.

Seasonal changes in gonadal microanatomy and the sex accessories could be demonstrated in this lizard as a criteria for the reproductive activity of testicular and ovarian tissue. The animal has a single breeding season which occurs during the winter months.

Spermatogenesis commences in August, followed immediately by a period of spermiogenesis; producing mature sperm which are shed into the epididymides and vas deferens prior to the time ovulation occurs in the female.

Interstitial cells were seen to develop and regress with the waxing and waning of the sex accessories and secondary sexual characters. These sex accessories were demonstrated to be under the influence of the male sex hormone, testosterone.

Ovogenesis and yolk deposition begins in Autumn; producing large eggs which are ovulated during mid-winter. A corpus luteum is formed from the old Graffian follicle. Stages in the development of this structure are presented. Seasonal changes in the oviduct appear to be controlled by ovarian estrogens.
Gestation lasts approximately four months. Apposition between fetuses and oviducal epithelium is loose. A demonstration of the ovoviviparous nature of the lizard indicates that there is no exchange between oviduct and fetuses, other than water and possibly gases.

Mating behavior and parturition is described. The average number of young born to an adult female is 17.68.

Mammalian gonadotrophins were incapable of inducing another reproductive cycle in females with quiescent ovaries; but oxytocin was able to facilitate parturition.

A hypothesis for the ovoviviparous reproductive mechanism, and the winter breeding season, unique among reptiles, but found in S. oyanogenys, is proposed.


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Figure 1. Cross section of testis, removed from an adult lizard during spermatogenesis. The seminiferous tubules contain primary and secondary spermatocytes. (ST) represent the lumen of the seminiferous tubule.
Magn. 100X Hematoxylin and Eosin

Figure 2. Cross section of testis from an adult male lizard at height of spermatogenesis, demonstrating the folliate appearance of the germinal lining. Spermatids (S) represent the most progressive cell type at this time.
Magn. 400X Kornhauser Tetrachrome

Figure 3. Interstitium of testis. Gonad was removed from a male after the breeding season. Notice the degeneration of the germinal lining. (S) represents a Sertoli cell; arrow points to a nest of interstitial cell elements.
Magn. 400X Hematoxylin and Eosin

Figure 4. Interstitium of testis of a breeding male. A capillary (CAP) is associated with a nest of interstitial cells. Notice the lipoidal and vesicular cytoplasm.
Magn. 400X Hematoxylin and Eosin
Figure 5. Cross section of the epididymis of a non-breeding adult male lizard. The low columnar epithelium is non-secretory.
Magn. 100X Hematoxylin and Eosin

Figure 6. Cross section of the epididymis of an actively breeding adult male lizard. The epididymal epithelium is of the tall columnar type, and the cells are secretory. The lumen of these ducts are engorged with sperm.
Magn. 100X Kornhauser Tetrachrome

Figure 7. Longitudinal section of the femoral pore apparatus. (P) indicates the pore canal; (Gd) the glandular element is found at the base of the papilla.
Magn. 100X Hematoxylin and Eosin

Figure 8. Cross section of epididymis removed from an adult non-breeding male lizard after exogenous testosterone administration. The epithelium is of the columnar type. The lumen is filled with secretory fluid.
Magn. 100X Hematoxylin and Eosin
Figure 9. Photomicrograph of renal sex segment of a non-breeding adult male lizard. Epithelium of the tubule is low columnar to cuboidal in height and is non-secretory.
Magn. 100X Kornhauser Tetrachrome

Figure 10. Photomicrograph of renal sex segment of a non-breeding adult male lizard after exogenous testosterone administration. Epithelium is columnar and secretory.
Magn. 100X Kornhauser Tetrachrome

Figure 11. Longitudinal section of the terminal metanephric segment. The dark areas at the apical or luminal end of the renal sex tubule represent eosinophilic material.
Magn. 35X Kornhauser Tetrachrome

Figure 12. Photomicrograph of Renal Sex Tubule (R) from an adult male taken during the breeding season. Kidney elements (K) are also seen. Notice accumulation of secretory granules at apical ends of the renal tubule.
Magn. 400X Hematoxylin and Eosin
Figure 13. Photomicrograph of ovary showing little stroma and large yolk-filled ova (O); three of which can be seen in this field.
Magn. 100X  Hematoxylin and Eosin

Figure 14. Cross section of ovary indicating a portion of the Graffian follicle. (GE) with arrow depicts the cuboidal germinal epithelium. Nurse or granulosa cells (NC), of varying sizes surround the ovum and lies between the thecal wall and zona pellucida. Yolk-filled cytoplasm (Y) and Nucleus (N) can be seen at left of micrograph.
Magn. 900X  Hematoxylin and Eosin

Figure 15. Photomicrograph of ovary from a pregnant female. Eight corpora lutea (c) and three miniture follicles (f) are seen in this field.
Magn. 100X  Hematoxylin and Eosin

Figure 16. Section of newly formed corpus luteum. Dark area represents the central lutein cell mass. Pointer indicates PAS positive reaction of theca interna.
Magn. 100X  McManus: Sudan black B
Figure 17. Cross section of the body of the oviduct taken from a female during the period of ovarian quiescence. The luminal epithelium is composed of low columnar cells, some of which are ciliated.
Magn. 100X Hematoxylin and Eosin

Figure 18. Higher magnification of the above, figure 17., demonstrating the ciliated cells that line the oviducal lumen (L).
Magn. 400X Hematoxylin and Eosin

Figure 19. Cross section of the body if the oviduct taken from a mature female which was depositing yolk in the ova. Glands (Gd) are found in the stroma.
Magn. 400X Kornhauser Tetrachrome

Figure 20. Cross section of the body of the oviduct from an adult female after mating but prior to ovulation. Dark areas in the lumen and crypts (C) represent sperm.
Magn. 100X Hematoxylin and Eosin