Chapter 4

Discussion
4.1 Reproductive Biology:

The gonadal parts of Male *Calotes versicolor* are somewhat simple. Like that of the other reptiles, the lizards are egg laying animal which show that ovipersness of the animal. The fertilization is internal which occurs through cloaca there is no such organ like penis of Mammals is seen. There is variations of somatic variations. The variation in the size & shape of gonad of the lizard. There are changes of weight of testes is observed which is highest in the month of May and the it decreases as non-breeding seasons starts, from the month of September the values decreases and it becomes lowest in the month of January. In this respect *C. versicolor* compares favorably with *Scoloporus oculatus* (Mayhew, 1963) Steinberger, E. and Steinberger, A, 1975. In *lizard and Urosaurus ornatus* (Asplund and Lowe, 1964) with minor exceptions. In contrast to the observation made in *Calotes versicolor*, complete cessation of spermatogenesis does not occur in the above two species as the mitotic divisions of the spermatogonia leading to slight increase in the testicular weight have been noticed after the completion of the preceding cycle. However, the observations made in *Calotes versicolor* are in agreement with those made in the Cobra, *Naja naja* (Lofts et al., 1966 Zaher, et al. 1999) where the breeding period is immediately succeeded by a rapid testicular regression and complete suppressions of spermatogenesis for a period of four months. Once the spermatogenic process sets in it is very rapid and leads to the formation of mature sperms within a very short time. The breeding period of land animals varies greatly. In the temperate zone the majority of the
animals breed during summer. In the tropics food supply, rain and other factors have a bearing on the time of reproduction. The variation in the breeding activity of animals is more in tropics than in temperate regions where more or less a uniform breeding rhythm is observed. And this affect on the population of the animals. The population of the animals in the surrounding area varies according to the seasons also. The percentage of male and females differs at this time. 

The data of collected animals is always recorded. The male and female identification is somewhat difficult in non-breeding seasons but when there is breeding seasons there is the morphological differentiation is seen. In the breeding season the throat of the male becomes red and black, as it help in the identification of male. In month of April the percentage of male and female out of 20 collected animals is 57.4 % male and 42.6% female found. In the month of May it about 59.8% and 40.2% respectively At that time the activity of maturation goes to pick, in month of May testes shows the highest maturity and release of sperms. But in month of September and October these values changes as 41.1% male 58.9% female, 37.0% male and 63% female respectively when there is a period of the egg deposition on the ground

The size and sex dependent maturity is seen in the most of the animals. The seasonal variation affect directly on the various physiological activities and metabolism of the animals. The behavioral changes occurs in the animal as per maturity of the animal. In reptiles many of the species show changes in their reproductive system of the animals.


In gonads of reptiles there are changes occurs. Some of them are
seasonal breeder or some are regular breeder. But those who are in the temperate zone the majority of the animals breed during summer. In the Tropics food supply, rain and other factors have a bearing on the time of reproduction. The season at summer time is somewhat favorable for breeding, the food is somewhat rich at this time. Even also in insects the month of June to September is favorable for breeding otherwise there is effect of temperature or light. Those who are nocturnal in habit the time of photoperiod affect on the animals.

Generally, photoperiod affects on reproduction of birds. The duration of light plays an important role in stimulating the migration of birds but in reptiles photoperiod will not affect on the animals, the temperature affect on them. As the observation of lizard Calotes versicolor on sexes are shows activity depending upon the seasonal conditions, Generally food, temperature, light, water plays an important factor for reproducing activity of animal. The seasonal variation is seen in the activity of Calotes versicolor. The breeding time of the lizard is also dependent on various factor which is generally from the month of May to the month of July. The male Calotes versicolor show the full activity during the breeding season. The weight of testes increases during this period but in non-breeding seasons.

The data of the gonad-somatic activity, which is shown in table, conform the seasonal changes in the gonadal activity. In the month of May which is full breeding time of the animals. From the month of September the average values being 26.1±1.25 mg. This decline in the weight continues through October and November and reaches its minimum in the month of December (average
value 8.4 ± 0.78 mg) At there is non breeding season (in the month of January) GSI is 0.430. This show that GSI and weight of testes changes there structure, size and weight of testes, but in the breeding season it increases higher rate of gonadal growth continues till the month of May, when the maximum average value of testicular weight is noticed. The Gonado-somatic index of male Calotes versicolor varies per month and season. The GSI of this month is highest in month of May i.e.0.672. 

The period of the annual reproductive cycle during which the testes display their greatest gametogenic activity varies from species to species. There are mainly two distinct patterns of spermatogenic activities.

**Pre-nuptial spermatogenesis**

This type of spermatogenesis, characteristic of homoeothermic animals (aves and mammals) is characterized by the production of the first spermatozoa immediately prior to the onset of the breeding period and the process continues throughout the duration of the nuptial cycle. At the termination of the breeding period the testes regress rapidly and remain spermatogenically inactive until the succeeding year when the cycle is repeated.

**Post-nuptial spermatogenesis.**

The majority of the poikiloothermic species (viz. Fishers, amphibians and the reptiles) show a characteristic pattern of spermatogenesis, wherein advanced germinal stages are found in the seminiferous tubules of the testes, soon after the breeding period. This spermatogenic activity continues till the formation of spermatozoa, well before the onset of the next breeding season. Once the spermatozoa are produced the spermatogenic activity is retarded and the testes are maintained in this stage for quite some time. The
spermatozoa thus produced are retained either in the testes or the accessory ducts for a considerable time and are utilized in the next breeding season. The testicular cycles in reptiles show variations among the different species. In general, there are four different patterns of testicular cycles found in reptiles.

(a) In tortises and turtles the greatest spermatogenic activity, with advanced germinal stages occurs immediately after the earlier breeding season. It is a typical example of post-nuptial spermatogenesis. In this respect these reptilian species resemble the amphibians. Thus, the occurrence of post-nuptial spermatogenesis has been described in the turtle, Clemys caspica (Lofts and Boswell, 1961) in which breeding takes place in early spring (late March to the end of May) and recrudescence of spermatogenic activity takes place in April and continues until September. The testes then become sexually quiescent until after the succeeding copulatory period. However, the testicular cycle in this species differs from the frogs in that the spermatozoa do not remain in the seminiferous tubules but pass into the epididymis and are stored there. A similar storage of spermatozoa in the accessory ducts occurs in the turtles, Pseudomys elegans (Burger, 1937); Stenotherus odoratus and Terrapene carolina (Hensen, 1938; Mayhew, W.W. 1960.)

(b) The most common type of breeding cycle found in the species of temperate zone lizards is the one, where a new wave of spermatogenesis becomes established soon after the completion of the earlier breeding cycle as in turtles. Thus the period of true inactivity of the testes characterized by the presence of resting spermatogonia.
is very short and a marked proliferation of the spermatogonia takes place immediately after the breeding season. The process of spermatogenesis continues leading to the formation of spermatids before the onset of winter and active spermatogenesis occurs only in the following spring. During the intervening period of winter hibernation the process of spermatogenesis is retarded. Such a testicular cycle has been described in *Lacerta muralis* (Heriant, 1933) *Lacerta agilis* and *Sceloporus occidentalis* (Wilhoft 1961).

Similar testicular cycles with slight variations have been described in non-hibernating species of lizards viz. Uta stanburiana (*Asplund and Lowe, 1964; Hahn, 1964*); Anolis carolinesis (Fox, 1958) Prasad MR, Sanyal MK 1969 and Hemidactylus flaviviridius. In these forms, the spermatogenesis that follows the earlier reproductive session leads to the production of mature sperms, without any winter arrest.

(c) A third type of testicular cycle has been described in the lizards *Urosaurus ornatus* (*Asplund and Lowe, 1964*) and *Sceloporus orcutti* (Mayhew, 1963) where in a distinct arrest in the spermatogenesis follows the preceding reproductive cycle. In *Urosaurus ornatus*, through January, February and March the size of the testes is similar to that attained in autumn (November, December) which is slightly larger than that reached in September. From October to March spermatogonial increase in number to some extent through mitotic divisions, thus causing a slight increase in testicular weight. Further, developmental stages in the processes of spermatogenesis do not occur till April. Thereafter the Process is stimulated leading to spermatogenesis in May. This pattern of spermatogenesis which
occurs in the cobras, *Naia naia* (Lofts et al., 1966) the garter snake, *Thamnophis radix* De Queiroz 2001 in the lizard (Cleislak, 1945; Fox, 1952) and the checkered water snake, *Natrix piscator* is similar to the pre-nuptial spermatogenesis described above. In this respect the reptiles show resemblance to aves.

(d) The fourth type of testicular cycle has been reported in some of the tropical species of lizards namely *Cosymbotus platyrurus*, *Hemidactylus frenatus*, *Perpus mutilatus* (Church, 1962) and *Leiolepis rhomboidalis* (Wilhoff, 1963 a) *Emoia* (Baker, 1947) *Agama agama* (Daniel, 1960) in the snake *Norell, Mark A.*. This cycle is characterized by the continuous breeding activity in the males.

The present data on the testicular cycle of *Calotes versicolor* clearly show that it is of the pre-nuptial type. In this species, the breeding takes place in summer (from May to August), followed by a rapid regression of the gonads in September, which continues until the minimum size of the gonads is reached in the month of December. New spermatogenic wave does not follow immediately after the copulatory period is over but there is a distinct arrest in the process of spermatogenesis till January. From January to March, the testes undergo spermatogonial mitosis and thereby increase slightly in weight. However, the spermatogenesis is accelerated only in April, leading to the formation of advanced germ cells. The spermatogenesis takes place at the end of April and the beginning May.

In this respect *Calotes versicolor* compares favorably with *Sceloporus occutii* (Mayhew, 1980) and *Urosaurus ornatus* (Asplund and Lowe, 1964) with minor exceptions. In contrast to the observation made in *Calotes versicolor*, complete
cessation of spermatogenesis does not occur in the above two species as the mitotic divisions of the spermatogonia leading to slight increase in the testicular weight have been noticed after the completion of the preceding cycle. However, the observations made in *Calotes versicolor* are in agreement with those made in the Cobra, *Naja naja* (Lofts et al., 1966) where the breeding period is immediately succeeded by a rapid testicular regression and complete suppressions of spermatogenesis for a period of four months. Once the spermatogenic process sets in it is very rapid and leads to the formation of mature sperms within a very short time.

Many workers who have studied seasonal reproductive cycles of reptiles, have tried to correlate the change in these cycles with those in the environment, Temperature, Light and rainfall are among the principal environmental factors which have been considered to be influencing the regulation of breeding activity.

Marshall (1960) and Danial (1960) working on two species of Agama have suggested a relationship between the rainfall and the reproductive activities in these tropical lizards. It was also suggested by Wilholt (1960) that the fluctuations observed in the reproductive efforts by the females of *Leiolepis rhomboidalis* may be attributed to the annual fluctuations in the rainfall. However, he has not explained the non-cyclic behavior of males inhabiting the same locality. The reproductive activities of two closely related species of lizards, *Leiolepis rhomboidalis* and *L. fusca*, further they taking into consideration the environmental temperature, relative humidity, rainfall and the day lengths of the two areas from which the two species were collected. They observed that there
was little difference in the ambient air temperature between the two areas and the difference in the day length amounted to less than 15 minutes for the six month period of study (October to March). The most significant difference in the climatic condition was encountered in the annual rainfall, 83.6 inches in the habitat of *L. fuscum* and 139.1 inches in the habitat of *L. rhomboiiddis*. The reproductive cycle may be affected indirectly by the rainfall through its influence on the supply of insects or other proteinous food or perhaps directly in some unknown way. But, these authors have tried to correlate the differences in the reproductive activity of these two species to their microhabitats. *L. rhomboiiddis*, which lives in or about the rain forests is subjected to less variations in the environment than *L. fuscum* which inhabits an open forest.

The estimation of the relative importance of the day length and the temperature in the control of gonadal cycle has been difficult. Photoperiod has been reported to influence the testicular recrudescence in several species of reptiles (*Burger, 1937; Clausen and Poris, 1937; Miller, 1948; Bartholomew, 1950, 1953; Fox and Desaures, 1958; Fischer, 1967*). But recent experimental studies of *Licht* (1966, 1967a, 1967b) and *Licht et al.* (1969) have raised doubts regarding the importance of photoperiod in the control of testicular cycles. They have concluded that the temperature is more effective in initiating the testicular recrudescence that he photoperiod. However, they have not completely ruled out the effect of day length.

When the annual meteorological data of Amaravati (table 3C) are examined the following facts stand out :- the maximum difference between mean daily
temperatures is about 6°C. The difference in the maximum and minimum relative humidity is about 11.9 while a difference of about two hours and twenty-five minutes is observed between maximum and minimum day lengths. Thus it is seen that the climatic fluctuations at the subtropical Bombay habitat at sea level are of lesser magnitude when compared to the more drastic climatological changes of the temperate region. The breeding period of land animals varies greatly. In the temperate zone the majority of the animals breed during summer. In the tropics food supply, rain and other factors have a bearing on the time of reproduction. The variation in the breeding activity of animals is more in tropics than in temperate regions where more or less a uniform breeding rhythm is observed.

With regard to the breeding habits of *Calotes versicolor* the rainfall possibly does not have a direct effect on the reproduction of at least the males of the species, as there is hardly any difference in the amount of rainfall in May (when the testes show maximum activity) and in December (when the testes are at the minimum level of activity). As seen from table 20, heavy rainfall occurs in June and July and maximum spermatogonic activity is reached in May, well in advance before the rains start.

The maximum gonadal development is observed in the month of May, when a maximum relative humidity is recorded. Therefore, it is likely that the relative humidity may have a bearing on the gonadal growth indirectly, as the prevailing temperature conditions also determine the humidity of the atmosphere.

Highest values for maximum temperature and mean daily temperature are registered in the month of May. A warm temperature
promotes higher metabolism in poikilotherms and so the period is suitable for reproduction. The changes in the temperature although of lesser magnitude, parallel with the changes in the gonads. The maximum gonadal development coincides with the maximum mean daily temperature (May) and the process of spermatogenesis is complete when the maximum temperature is recorded. However, once the testes reach peak activity a steady state is maintained, even though there is a slight fall in the environmental temperature from June till August. Therefore, it is quite likely that the mean daily temperature of 39.4°C in the month of May is favorable for the stimulation of gonads. As there is always a parallelism between the increasing temperature and the increasing day length it is very difficult to say which of these two factors has a more direct effect on the testicular cycle of Calotes varicolor unless it is proved by experimental means. In the following chapters experiments on these lines are performed, and the results obtained are discussed.

The interstitial or Leydig cells of reptiles have been reported to occur as isolated groups of small number of cells or more obvious compact masses which in the majority of the species studied, show a definite increase in the activity coincident with maximum breeding activity and the appearance of secondary sexual characters.

(Fox, 1958; Lofts and Boswell 1961, Hahn, 1964; Lofts, 1968 and Prasad, et al. 1967; Keli De et al. 1995; Forbes, T.R. 1938). In a few cases, such as the lizards, Xentus visis and L. rhomboidalis the musk turtle, Stenotherum odoratus the grass snake, Natrix natrix (Herlant, 1933) and the chequered water snake, Natrix piscator the interstitial cells are
reported to lack demonstrable seasonal changes. However, the observations on the diameters and stainability of the nuclei of the interstitial cells of *Calotes versicolor* indicate that a parallelism exists between the activities of these cells and the epithelial cells of epididymis and also the process of spermatogenesis. The interstitial cells show lowered activity as evidenced by the decreased size and the increased stainability of the nuclei, during the period when spermatogenetic activity is at its minimum. This period also coincides with the least activity of the ductus epididymis.

As the spermatogenic process advances, there is a gradual increase in the nuclear diameter of the interstitial cells and is significantly greater in May, when the process of spermatogenesis reaches peak activity. At this time, most of the nuclei are large, faintly stained and are more or less spherical. Thus the present observation in *Calotes versicolor* agrees with the findings made earlier in *Sceloporus occidentalis* (Wilhoft 1961); *Laelopisma fuscum* Anolis carolinensis (Fox, 1958), *Cemmys Caspica* (Lofts and Boswell, 1961) *Vipera berus* (Marshall, 1967); *Uta stanburiensis* (Hahn, 1964) *Naja naja* (Lofts et al., 1966) and *Hemidactylus flaviviridis*. In *L. rhomboidalis* both gonads and the accessory sex organs are non-cyclic, while in *N. piscator* the gonads show the seasonal changes in the accessory organs. The non-cyclic nature of the interstitial cells of *L. rhomboidalis* and that of *N. piscator* may be correlated with the non-cyclic nature of the accessory sex organs, indicating that the activity of the interstitial cells has a more direct relation with the necessary sex organs than the spermatogenic elements. The present observations and those of
on the effect of exogenous steroids in *Calotes versicolor* give support to the above observations.

The supporting evidence also comes from the observations on the interstitial cells of those animals in which spermatozoa are evacuated from the testicular tubules and are stored in the accessory ducts.

In these animals it is seen that the development of accessory ductus is maximum (at the time when the spermatozoa are stored in them) when the testes are fully regressed and show minimum spermatogenic activity. Thus the development of the accessory ducts is out of phase with the spermatogenic activity but the interstitial cell cycle operates simultaneously with the development of the accessory ducts. Such a condition occurs in the turtle, *Clemmys caspica* (Lofts and Boswell, 1961).

### 4.2 EFFECT OF ENVIRONMENTAL PARAMETERS ON TESTES:

Attempts to determine the roles of various environmental factors in timing the reproductive cycles of seasonally breeding animals have led to conflicting results. In most of the birds and certain animals, it has been shown that it is the day length (either a long day or a short day) that plays a major role (Farmner, 1959, 1964; Wolfson, 1959; Farner and Follett 1966; Lofts and Morton, 1968; Bissonnette, 1931; and Andrews T J 1996. Clark and Kennedy 1967 a Aragon P. et. al. 2000 Asplund. K.K. and Lowe C.R. 1964) in this respect. On the other hand, some of the lower vertebrates such as the fishes and amphibians (Cordt Cordt, 1955) react not to changing light but to changing.
Early studies on the environmental control of reproduction in reptiles led to the conclusion that reptiles show a close similarity to birds. Thus, Burger (1937) reported that artificially increased day length stimulated a new spermatogenic cycle in the turtle, *Pseudonyme scripta* elegans. Clausen and Poris (1937) noticed that male Annul receiving additional six hours of illumination showed unseasonal gonadal hypertrophy and an increased rate of spermatogenesis. Fox and Dessauer (1958), in their extensive studies on *Anolis carolinensis* observed a direct relation between the day length and the testicular response. Similar reports of Photo-stimulation of the gonads have been described in other species of reptiles (Calgane, 1951; Fischer, 1967). Earlier workers at variance from those report the present findings on the effect of increased day length on the testes of *Caiotes versicolor*.

The experimental lizards although receiving daily seven hours of additional light than the controls (which were subjected to natural day length) did not show any statistically significant increase in the testicular weight (table 4). Moreover, no histological changes were observed in the testes of the treated animals. The testes of both experimental and control animals simulated a typical resting state.

The discrepancy observed in the results of earlier workers and those obtained in the present investigation may probably be due to the difference in the temperature conditions. All the earlier results reported in the reptilian species are based on the photoperiodic experiments conducted at a relatively higher temperature than normal room temperature and therefore, it is not very clear from their observations whether the greater development
observed in the gonads of the experimental animals is solely due to the increased day length or the higher temperature. In the present experiments in Calotes, the effect of temperature on the testes is eliminated as both experimental and control animals were maintained at room temperature and the illumination to the experimental animals was provided by day light fluorescent lamp which emits negligible amount of heat.

In this connection it is interesting to note the results obtained by Bartholomew (1950) working on Xanthusia vigilis. He exposed the animals to three sets of experimental conditions.

Normal day length and normal room temperature. Normal day length and increased temperature, and increased day length and normal room temperature.

The conditions given under experiment ‘C’ of Bartholomew (1950) are almost similar to those in the present experiments. However, he observed maximum gonadal response under these conditions, which is not in agreement with the findings made in the present experiments.

It has been shown in many birds (Burger, 1947; Miller, 1948, 1951) that during a certain period of the year animals failed to respond to the increased day length while at other times of the year long days provided an effective stimulus. That period of the year when increased day length fails to evoke a gonadal response is termed as refractory period. It is, therefore, attractive to postulate that the non-stimulation of the gonads observed in Calotes under increased day length may perhaps be related to the state of photo-refractoriness of the animals during the period of experimentation.

Further more, the present observation on G. versicolor were made over only
a short period of the year ranging from December to February. The argument in favour of the occurrence of the state of refractoriness during this period appears plausible. This concept, however, finds little support when the effects of increased temperature on the gonads of this species are taken in consideration.

Thus it is more likely that temperature alone has a stimulatory effect on the testicular development of *C. versicolor* and that the non-responsiveness of the gonads to increased day length is the result of absence of photostimulation rather than occurrence of a photorefractoriness. Moreover; photorefractoriness has been reported to be absent in other species of reptiles, which respond very well to the increased photoperiod (Fox and Desure, 1958; Bartholomew, 1950, 1953).

The recent findings of Licht (1967 a,b) in *Anolis carolinensis* and that of Licht et al. (1969) in *Lacerta sicula* and *Lacerta muralis* support the observations in *Calotes*. These authors have reported that at lower temperatures photoperiod is ineffective in stimulating the gonads of these animals. The logical conclusion is that the photoperiod alone cannot induce testicular recrudescence in *C. versicolor*.

All the three sets of experiments covering a period from January 20th to April 8th (Table 5, and 6) carried out to study the effect of increased temperature on the recrudescence of the testes in the lizard under investigation gave identical results. The testes of experimental animals from all the three groups showed a significant increase in weight over those of their respective control (table 5, 6 and 7).
The histological examination of the testes of the experimental animals revealed advanced stages of spermatogenesis, especially in the testes of animals' spermatogenesis, especially in the testes of animals spermatogenesis including the mature spermatozoa were noticed. In group c all the stages of spermatogenesis with the exception of spermatozoa were represented and no signs of spermatogenesis were evident.

In this connection it may be pointed out that the experimental animals under group c were subjected to increased temperature for a period of 20 days, while those in groups a and be were similarly treated from 30 days and 32 days respectively.

It would, therefore, be quite logical to conclude that the difference in the testicular response observed in the experimental animals under group 'c' is due to the lesser duration of the experimental period than in the other two groups of animals.

The other conclusion that one can draw is that under the experimental conditions a period of about 30 days is necessary for the completion of the process of spermatogenesis.

Thus it is seen from the above observation that increased temperature can initiated testicular recrudescence about two months earlier than the normal seasonal timing.

Earlier workers have reported similar observations on the role of increased temperature on gonadal recrudescence. According to Licht (1967a and b), Anolis carolinensis, which was shown to be responsive to increased day length by Fox and DeSSAure (1958), was not obligatorily dependent on the long day lengths for testicular recrudescence as, this photoperiodic effect is completely abolished by lowering the temperature to 20°C. on the contrary, these lizards
when maintained at constant temperature of 28°C. with 6 hours of light and 18 hours of darkness or even complete darkness, in the month of November, showed a rapid testicular recrudescence. Similar effects have also been noticed in the females of Uta stanturiana. The present observations on Calotes are fairly in agreement with these findings. The observations of Licht et al. (1969) on two species of Lacerta give further support to the contention that temperature is primarily responsible in stimulating gonadal recrudescence. In these experiments following combinations of light and temperature have been employed. When the lizards were kept in the complete darkness and subjected to different temperatures it was seen that at 12°C, there was complete arrest of spermatogenesis even after 80 days of treatment, whereas at 20°C. The process of spermatogenesis paralleled that with the controls maintained at the same temperature but under natural conditions of light. It means that the arrest of spermatogenesis noted in the animals kept in darkness at 12°C. is not the effect of absence of light but is directly related to the lower temperature.

In another experiment, the lizards were kept at 33°C. and the light period ranged from 8 to 16 hours. Under these conditions, it was shown that the difference in the light period was of no particular consequence so long as the temperature was maintained at 33°C. The testes of the lizards at this temperature showed sperms in their seminiferous tubules indicating completion of spermatogenic process. These experiments clearly emphasized the role of temperature in influencing gonadal recrudescence. Unfortunately,
Licht et al. (1969) have not subjected their lizards to complete darkness at the temperature of 33°C and, therefore, it is not very clear from their observations whether complete spermatogenesis can take place in the absence of light. In our observations on *Calotes versicolor* the lizards were maintained at higher temperature in complete darkness and thus effect of light was completely avoided. Our results indicate that higher temperature is the primary factor controlling the initiation of a new spermatogenic cycle and that it is effective even in the absence of light. It is interesting to note that thermal refractoriness during a certain period of the year was reported in *Anolis carolinensis* (Licht, 1967 a and b), *Lacerta simula carpestris* (Galgano, 1951), *Uta stanburiana* (Tinkle and Irwin, 1965) and *Lacerta sicula sicula* (Licht et al., 1969). However, it is difficult to say whether such thermal refractoriness occurs in the animal under study as the present observations on the thermal control of testicular recrudescence were restricted to a period of three months, ranging from January to April and it is quite likely that by chance a right period was perhaps absent in the animal. Further studies in this direction may help to clarify this point.

4.3 EFFECTS OF HORMONES

The histological observations on the effect of increased temperature on the thyroids were timed during a period when the thyroid activity in the animals under natural conditions is low or moderate. From the present observations it is seen that elevation of environmental temperature does not have any appreciable effect on the thyroidal activity as judged by the histological criteria. This observation, is in agreement to a certain extent, with those of S. Girons and Dugue (1962a) in Vipera and of Evans and
Hegre (1940) in the turtle, *Emys europea*. These authors also did not observe any appreciable effect of the increased temperature on the thyroid histology. However, our observations are at variance with those in the lizards, *Sceloporus occidentals* (Eakin et al., 1959) and *Anolis carolinensis* (Lynn et al., 1965) in which a marked hypertrophy of the thyroid gland was observed when the animals were exposed to a higher temperature.

These workers have interpreted the increase in the thyroid activity as a consequence of high level of metabolic activity (as judged by the higher rate of food consumption and the faster rate of heart beat.)

In the present study the animals were also subjected to increased temperature which might have induced increased metabolic rate. However, there was no appreciable change in the thyroid histology of the experimental animals indicating that the rise in temperature is not the causative factor influencing thyroid function in *C. versicolor*. Differences in the response of thyroid gland to higher temperature in various species suggest the possibility of species specificity.

Another point to be noted is that earlier workers correlated the thyroid activity with that of the reproductive activity. They showed that in males, the increased thyroid activity. They showed that in males, the increased thyroid activity was observed during the period when increased spermatogenic activity was noted. However, in the present investigation it is observed that although there is no significant increase in the thyroid activity as judged by the histological criteria, there is a definite stimulation of testicular function resulting in the completion of spermatogenic process. Therefore, it appears that there is no
direct relationship between the thyroid activity and the process of spermatogenesis can be completed in the presence of a favorable external stimulus (temperature in the present case) irrespective of low thyroidal activity. The present observations on the thyroid of the lizard, *Calotes versicolor* clearly demonstrate that the administration of testosterone propionate (TP) has a stimulatory effect on the gland.

This finding is in agreement with that of Kumaresan and Turner (1967), who observed a rise in the rate of thyroxin secretion in male rats after injections of 2 mg. of testosterone propionate daily for 10 day. However, in the present study on *C. versicolor* it is not very clear from the data obtained, whether this action of TP is a direct one or is mediated through the pituitary gland. Van Rees et al. (1965) observed that castration decreases both pituitary and serum TSH levels in rats, indicating thereby that the absence of circulating androgens affects the pituitary resulting in the low production of TSH. On the other hand, the administration of TP to castrated male rats increases the TSH level in both, pituitary and serum and the ultimate effect is an increase in the production of thyroxin. In the present work, the effects of testosterone propionato on the thyroid activity were studied in an intact animal and not in a hypophysectomized animal and therefore, it is difficult to decide whether the action of the injected androgens on the thyroid gland is direct indirect (viz. Through stimulation of TSH secretion in the pituitary). Non theless, there is sufficient evidence to suggest that the androgens cause stimulation of the thyroid.

Hoar et al. (1967) have reported that in Methallibure treated fishes, initial stages of gametogenesis which depend upon mitosis are not affected
but the consequent stages which depend upon reduction division are blocked. In the present investigation on male Calotes treated with Methallibure, the seminiferous tubules show only spermatozoa, although no mitotic divisions are noticed. The subsequent stages through which the spermatogonia normally pass are completely blocked. This is in agreement with the findings made in fishes under similar treatment. As identical effect is seen in Calotes, thirteen days after the removal of hypophysis. It appears, therefore, that the arrest of spermatogenesis in Calotes versicolor following methallibur treatment may be due to interference in the hypophysial gonadotrophin production. In male mammals it is established that LH controls the development and function of the intestinal cells and is responsible for the production of androgens (Gorbman and Bern, 1962). Although, in the non-mammalian vertebrates below the evolutionary scale of aves, almost nothing is known about the naturally occurring androgens, their pathway of synthesis starting from cholesterol has been established in the higher forms, and this may well be true for lizards.

In the present investigation however, there is a significant increase in both cholesterol and ascorbic acid contents in the testes of the methallibure treated animal.

These observations strongly suggest that cholesterol is not utilized in the synthesis of androgens. Further support for this interpretation is given by the observation made on epithelial cell height of the ductus epididymis of experimental animals. In these animals the epididymal epithelium shows significant atrophy, an effect closely similar to that resulting from castration in a mammal. Obviously methallibure treatment leads to failure

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of the biosynthesis of androgens.

The observations made after the administration of ICI compound 33.828 on the cessation of the estrus cycle and the regressive changes in the ovary of rat fall in weight, atrophic changes in the testis and secondary sex organs in male rats, blocking of ovarian compensatory hypertrophy after hemicastration in rats are indicative of the possible gonadotrophic inhibiting action of the substance administered. A more direct evidence in support of the selective blocking of the pituitary gonadotrophin production is given by the findings on post-menopausal woman (Brown et al., 1963).

In the rats and mice (Brown, 1963) and fishes. In the present investigation on Calotes there is an arrest of spermatogenesis after the administration of ICI 33.828. The substance may act in two ways, either at the pituitary level by inadequate production and release of gonadotrophins, or directly on the testes making them refractive to the action of the pituitary gonadotrohins.

The regression in the testes, the selective suppression of only those stages in the spermatogenesis depending on reduction division, non-utilization of cholesterol in the biosynthesis of androgens and consequent atrophy of the ductus epididymal epithelium are pointing to the interference in either the production or release of pituitary gonadotrophins.

From the histological studies made on the thyroid, it clear that ICI 33.828 treatment in lizards leads to inhibition of thyroidal activity. As Methylthiouracil contains thiourylone groups it is likely that it may act as an antithyroid compound, blocking the production of thyroid hormone, with a consequent stimulation of TSH release due to reduced feedback. But the
reduced height in the thyroidal secretory epithelium in the treated animals clearly suggests a lowered output of TSH from the pituitary. It appears, therefore, that the compound acts at the pituitary level, by blocking the secretion of TSH, have also noticed that the compound when administered in mice leads to a decrease in the secretion of thyrotrophin.

4. 4 EFFECT OF ENDOCRINE GLANDS AND TESTES:

From the present observations it is clear that the testes of C. versicolor readily respond to the treatment of FSH of mammalian origin. The testes of treated animals showed marked increase in size and weight than the controls. Histological observations revealed a rapid proliferation of the spermatogonia and the primary spermatocytes. This effect was manifested within 8 days of treatment and it was further observed that the degree of response was directly proportional to the duration of treatment. However, it was noticed that FSH could stimulate only the rate of spermatogonial mitosis and the process of spermatogenesis could not proceed beyond the formation of primary spermatocytes inspite of a prolonged treatment of 30 days.

The failure of sperm production in the testes of treated lizards is difficult to explain when it is recalled that in hypophysectomized rats it is shown that a dose as low as 0.05 ug. Of FSH given daily brings about complete restoration of spermatogenesis within 15 days of treatment (Greep and Ferold, 1937; Greep et al., 1942). On the other hand, in the present investigations on the lizard, Calotes versicolor the treatment of 100 ug. Of FSH on alternate days, for 30 days, has permitted proliferation of spermatogonial layers in the testes but
meiosis and subsequent stages are completely blocked.

In male mammals it is well established that there are two gonadotrophins (FSH and LH) distinct in their physiological effects. LH controls the development and function of the interstitial cells and is responsible for the production of androgens. The second hormone, FSH stimulates spermatogenesis. It appears that in the lizard under study the process of spermatogenesis in the tests is not completed under the influence of FSH alone and the participation of a second hormone is necessary.

A failure in the completion of spermatogenesis was also observed in the immature rodents treated with only FSH. In the tests of the treated animals he observed only the proliferation of spermatogonia and primary spermatocytes. A similar result is obtained in the present study on *Calotes versicolor* after FSH treatment. FSH may be responsible for the proliferation of spermatogonia and primary spermatocytes and he further speculated that androgens may be participating in the final stages of spermatogenesis.

The suggestion that for completion of spermatogenesis in *Calotes versicolor* in addition to FSH an second hormone probably ICSH is necessary gains support from the following. In a recent paper, Van-Berswardth et al. (1968) have demonstrated that the effect of FSH in maintaining the spermatogenesis in hypophysectomized rats, is probably due to the synergistic actions of endogenous androgens, which are resulted due to the action of ICSH contamination in FSH preparation. They observed that when FSH treatment was combined with cyproterone acetate (a known androgen antagonist which prevents
the synergistic action of androgens) the spermatogenesis in the testes of the hypophysectomized rats is completely abolished and accessory sexual glands are atrophied.

The testicular stimulation and non-stimulation observed following the injections of crude extracts made from the pituitaries of sexually active and inactive lizards respectively clearly indicate that the extracts prepared from the pituitaries of active animals contain higher titers of gonadotrophins. Despite these high titers of gonadotrophins (FSH and ICSH) the process of spermatogenesis advances only up to the formation of primary spermatocytes. The effect is therefore, similar to that obtained following treatment with bovine FSH. The failure in the completion of spermatogenic process, despite the presence of both FSH and ICSH in the injected extracts of active pituitaries may be attributed to the short duration of this treatment (10 days). But taking into consideration the testicular weight and the number of cells per seminiferous tubule it appears that the effect of purified mammalian FSH is more pronounced. This greater effect observed following FSH treatment may be attributed to the purified nature of the hormone used. In the interpretation of these results one should remember that the lesser effect seen in the pituitary extract injected group, is perhaps due to the lower does used (1 pituitary equivalent/day) and that the injection of large amounts of FSH is not equivalent to normal physiologic situation.

The action of androgens of the germinal elements of the testes is found to be different under different physiological conditions. Thus, in intact male mammals the administration of small doses of androgens leads to testicular inhibition and atrophy of the germinal epithelium.
This effect has been clearly established to be the result of feedback mechanism causing inhibition of pituitary gonadotrophin secretion with a consequent involution and atrophy in the germinal elements of the testes.

Ludwig (1950) showed that in rats, the effects of androgens on the testes are dose dependant. A dose as small as 0.1 mg. given daily inhibits the spermatogenic process whereas a larger dose of 2.0 to 3.0 mg./day prevents atrophy of the germinal epithelium and the normal condition of the testes is maintained. The bioassay data on the pituitaries in the above injected groups showed lowered concentrations of pituitary gonadotrophins and the extent of inhibition of the gonadotrophs was directly proportional to the dose of the injected androgens. The higher dose is effective in preventing the atrophy of the germinal epithelium. This effect is attributed to the direct action of androgens on the germinal epithelium. This direct effect is manifested only at a stage when gonadotrophic output of the pituitary is very low or entirely absent. With the lower dose of androgens the degree of inhibition of the pituitary gonadotrophs is comparatively lower and the resultant effect is an inhibition of spermatogenesis in the testes.

Ludwig's observations are supported by the results of experiments performed on hypophysectomized mammals (Nelson, 1941; Albert, 1961; Hervey, 1966 and Bocabella, 1963 Brown. P.S. 1963). When androgen is injected in the hypophysectomized rat the resultant effect is similar to that observed after injection of a high dose of androgen in the intact rat.

The present experiments were timed during the period when the gonadotrophin secretion in the pituitaries of the lizards under study
was negligible and in this respect it is probable that their physiological state simulates that of hypophysectomized mammals. In spite of this the injections of both high and low doses of androgens failed to stimulate the testes. In this respect our results differ from those made by Lofts (1962, 1968) in sexually quiescent birds, where a distinct stimulation of gonad was observed. They also differ from those obtained with hypophysectomized mammals (Bocabella, 1963; Albert, 1961). However, the present findings in the lizard under study are in agreement with those in other species of reptiles (Gorban, 1939;). It is interesting to note that the gonadal response to exogenous androgens appears to vary in immature and sexually quiescent reptiles (Forbes, 1941; Ramswami and Jacob, 1963, 1965). In immature lizards (Forbes, 1941) and crocodiles (Ramswami and Jacob, 1965) androgen appears to stimulate the gonads. Where as the same treatment in adults with regressed gonads is found to be ineffective (Ramswami and Jacob, 1963). The present observations are solely on adult sexually quiescent lizards and are in accordance with those reported in similarly treated Uromastix hardwicki (Ramswami and Jacob, 1963); Hemidactylus levis and Sceloporus occidentalis (Gorban, 1939). The present observations in the adult sexually inactive lizard, C. versicolor and those made in other species of reptiles under similar condition clearly indicate that the exogenously injected androgen is without any effect on the germinal epithelium of the testes. In this respect the reptiles completely differ from birds and mammals.

The cord like arrangement of the cells of the pars anterior of C.
versicolor agrees with the generalized reptilian anterior pituitary pattern and is found in almost all the forms studied so far (Atland, 1939; Cieslak, 1945; Poris and Charrier, 1937; Miller, 1948; Gabe and Rancural, 1958; Poris, 1941 and Grignon, 1963) with the solitary exception described in Mabuva carinata (Annamalai, 1962 Cieslak, E. S. 1945) where as follicular arrangement of cells occurs in the pars anterior. Another distinguishing feature of the reptilian pituitary is the distinct and uniform localization of the various cell types in the appears anterior (Saint Girons, 1963). In C. versicolor it is observed that the basophils are more concentrated towards the cephalic end, whereas the caudal half of the pars anterior is predominantly acidophilus. This observation is in agreement, but differs from that in anolis (Poris and Charrier, 1937), where the cephalic region is predominantly acidophilic and the caudal region is more basophilic. The observations by Atland (1932) in Sceloporus and by Annamalia (1962) in Mauve are also at variance with those made in the present investigation. In these two species all the three types of chromophil cells are distributed throughout the entire pars anterior.

Since a long time, workers interested in the pituitary cytology of reptiles were depending only on the classical trichrome method of staining and thus, they could identify only three types of chromophils (two types of acidophils and a single type of basophil). In recent years, a number of staining methods have been introduced in the study of different cell types of the anterior pituitary (Halmi, 1950, 1952; Purves and Griesbach, 1951, 1954, 1957; Adams and Sweetenham, 1958). However, among these, MacManus’s (1946)
periodic acid Schiff (PAS) procedure occupies a key position. The PAS reaction divides the adenohypophysial chromophil cell into two primary groups: (1) basophils with (PAS) positive granules and (2) acidophils with PAS negative granules. The combination of PAS with other dyes such as acidic blue and methylene blue allows further differentiation of basophils into various types. Thus, Adams (1958) using PAS alcolian blue combination demonstrated two types of basophils. The first type stained blue with alcolian blue and the other which resisted performic oxidation, stained red with PAS. These cells were designated by them as S and F cells respectively. In the present investigation on the adenohypophysis of *C. versicolor*, PAS reaction has been used to distinguish between the basophils and acidophils. All the PAS positive cells are termed as basophils and those that are stained with orange G or Azocarmine are called as acidophilus. By combining the PAS reaction with other dyes such as alcolian blue (ADAMS and Sweetenham 1958) or orange G methylene blue, further differentiation of the basophils into three types has been achieved (table No. 14). Although, type 1 acidophilus stained orange and type 2 acidophilus yellowish in colour their precise differentiation into these two types was not possible by any of the above methods. The results obtained with Heidenhain's Asan method were relatively better as type 1 and type 2 acidophils stained orange and carminophilic respectively. On the basis of the tinctorial affinities, shape and size of the constituent cells, six different cell types are identified in the anterior lobe of the pituitary of *Calotes* (table 14) Saint Girons (1963) in his excellent article on the comparative account of the reptilian pituitary stated that by combining the various modern
staining techniques one could recognize six cell types in the anterior lobe of reptilian pituitary. A comparison of the different cell types in the pars anterior of *Calotes* with those described by S. Girons (1963) in several reptilian species brings out interesting similarities. The type 3 basophils of *Calotes* are comparable with LH gonadotrophs described by S. Girons (1963) in having strongly PAS positive granules, elongated shape and their concentration around blood capillaries. They take blue to blue-black stain with haematoxylin and are stained violet with Azan. The type 2 basophils of *Calotes* and the FSH gonadotrophs described by the same author, have common properties. Both are PAS positive and stain light blue with Azan. The type 1 basophils in the present study show close similarity to thyrotrophs described by S. Girons (1963), as both are strongly PAS positive to alcian blue and are stained dark blue with Azan.

The type 1 acidophils in the present study agree with the cells of *S. gironi* in the following respects. Both occur in the cephalic region of the pars anterior in large numbers and are scarce at the caudal extremity and in the medial region of the pars anterior. More over, orangeophilic granularity is a common feature in both. The type 2 acidophils in *Calotes versicolor* and the X-cells observed by S. Giron appear to be more or less similar as they are both distributed in the median and medioleteral regions, and both are carminophilic. And localization within pars anterior is not very precise as is seen from the following differences. The LH gonadotrophs described by S. Girons are alcian blue positive whereas type 3 basophils of *Calotes versicolor* are alcian blue negative. The type 2 basophils of *Calotes* are also alcian
blue negative whereas the FSH gonadotrophs of *S. Girons* are described to be having a slight affinity to alcin blue. In contrast to the localization of FSH gonadotrophs of *S. Girons*, exclusively in the cephalic region, the corresponding type 2 basophilis of Calotes in addition to their presence in the cephalic region are also found in the caudal region of the pars anterior.

These minor differences observed in the stainability, distribution and relative proportions of the different cell types in the pars anterior of *C. versicolor* from those described by *S. Girons* (1963) may be species specific. Such differences have also been recorded in the members of the different families of reptiles (*S. Girons*, 1963). Thus, in *chameleon*, LH gonadotrophs are very rare and faintly chromophil while those in Trogonophis do not stain with haematoxylin. The staining reaction of the X-cells in *chameleon* differs from that of the corresponding cells of the majority of reptiles studied (*S. Girons*, 1963), as in the former the cells show strong PAS positive reaction, while in the latter they are PAS negative. With regard to localization, the α-cells described by *S. Girons* (1963) are generally found in the caudal region and the X-cells in the mediolateral region. On the contrary, in the anterior pituitary of the snake, *Natrix natrix* the cells are found in the mediolateral region and the X-cells are scattered among the LH gonadotrophs in the rostral region (*S. Girons*, 1963).

It is difficult to ascribe specific functions to different cell types in the anterior pituitary in the absence of experimental data. In order to decide for example, that the basophilis of the pars anterior are associated with TSH secretion, it is necessary to study the changes in the various basophile of the pars anterior are associated with...
TSH secretion, it is necessary to study the changes in the various basophile types of the pars anterior after thyroidectomy. Nevertheless, on the basis of the present observations on the cyclic changes in the different cell types of the anterior lobe of the pituitary of *Calotes versicolor* it can be argued that a possible relation exists between certain physiological events (maturation of testes) and simultaneous cytological changes in the pars anterior. The increase in the granularity and the number of purple basophils (type 2 basophils) of the pars anterior of *C. versicolor*, during the period ranging from March to July (table 15) coincides with the greater testicular development. From table 15, it is clear that this increase in the number of basophils is first noticed in the month of March, but, its effect on the development of the testes is negligible, perhaps due to insufficiency of the hormones produced. However, a rapid testicular development ensues in the month of April and reaches a maximum in the month of May. These changes in the testicular development and in the number of purple basophils occur simultaneously during the pars anterior and the peak in testicular development is attained in May and June. The number of purple basophils remains high till the end of July but surprisingly a decline in average testicular weight is observed in the same month (311.5 mg). The histological observations made on the testes in the month of July indicate that the process of spermatogenesis is in full swing with the presence of sperms within the lumina of the tubules and this picture is maintained even in the month of August. The surprising fall noticed in the average weight of the testes in the month of July is perhaps related to a greater rate of mobilization of fat present in the organs for its utilization in the

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synthesis of androgens in the interstitial cells.

The earlier workers (Attand 1939; Miller, 1948 and Hertmann, 1944), using the known staining technique of their time, observed three cell types in the anterior lobe of the pituitary — basophils, acidophils type 1 and acidophils type 2. Further, they noticed an increase in the number of basophils when the animals were in a breeding state. It was observed that in the female lizard, *Zootoca vivipara*, the basophils were particularly active during the period of follicular maturation. In the turtle *Emys leprosa*, Combescot (1955) observed an annual variation in the number of the basophils and acidophils in the anterior lobe of the pituitary. He observed that the basophils were more in number during the period of active spermatogenesis while the acidophils increased in tugs with the activity of the interstitial cells.

On the basis of these observations he suggested that the basophils were responsible for the secretion of FSH whereas the acidophils secreted LH or ICSh. However, these results should be accepted with some reservation as their findings were based on old staining techniques. Heriant and Grignon (1961a and 1961b) in the land turtle, *Testudo mauritanica*, S. Girons (1962) in the snake *Cerastes cerastes* and S. Girons and Duguey (1962b) in *Vipera aspis*, were successful in distinguishing two types of gonadotrophs comparable to beta and gamma cells of mammals, in the anterior lobe of the pituitary of these animals. These authors have attributed the role of FSH secretion to beta cells and that of LH to gamma cells. The assignment of functions to these two cell types was easy in the turtle, *Testudo mauritanica* as the spermatogenic activity and the interstitial cell activity in this animal are
evident during different seasons. The changes in the beta cell activity and spermatogenetic process occur simultaneously. Similarly, the gamma cell activity coincides with the activity of the interstitial cells. The beta cells described by Herlant and Grignon (1961) in the lead turtle, Testudo and by S. Girons and Duguey (1960) in the snake, Vipera, as also the FSH cells of S. Girons (1963) and the purple basophils of C. Versicolor have identical tinctorial affinities and distribution in the anterior lobe of the pituitary. Therefore, it is logical to surmise that the beta and FSH cells described in other reptilian species and the purple basophils of Calotes are homologous and may have identical function of FSH production.

In the earlier part of the discussion it was shown that the type 1 and type 3 basophils of Calotes versicolor have morphological and tinctorial similarities with the thyrotrophs and LH gonadotrophs (described by S. Girons) respectively and therefore they can be considered as homologous to each other. The cyclic changes are also observed in the type 1 and 3 basophils (red basophils) of the anterior lobe of the pituitary of C. versicolor. However, since these two types of basophils have not been counted separately, it is difficult to assess their significance with regard to thyroid and interstitial cell functions separately. Nevertheless, the alterations in the number of these red basophils and in the activities of thyroid and the interstitial cells of the testes suggest that one of the two red basophils and in the activities of thyroid and the interstitial cells of the testes suggest that one of the two red basophile types is responsible for the production of TSH the other, for the production of ICSH or LH. It may, therefore be surmised that the maturation of the testes which occurs
in the month of April is brought about by the release of gonadotrophins elaborated by the purple basophils and type 3 basophils of the pars anterior. The rise in the proportions of these two cell types during the period ranging from March till July offers support to this contention.

The present observations on the neurosecretory system of Calotes fairly agree with those made in birds by George and Naik (1965) and John and George (1967) in that the amount of NSS in SON and PVN is less during the period ranging from October to November. The amount of NSS in these cells in birds was found to be increasing during the period ranging from December to April (George and Naik 1965; John and George 1967). In the present observations the accumulation of NSS in the cells of PVN and SON did not start till the end of January. Thereafter, there was an increase in the amount of NSS in these cells. The increase in the amount of NSS in the cells of PVN and SON in birds from October till November was accompanied by heavy accumulation of CHP-positive material in the median eminence (Furner, 1962). Similar accumulation of NSS in the median eminence during the non-breeding period was observed in sheep (Clark, 1961) and Vole (Clark and Kennedy, 1967b). However, in the present work on C. versicolor, the accumulation of NSS in the median eminence was seen only in the month of March and there was no sudden depletion of this material as was observed in birds. Moreover, the decline in the amount of NSS in the median eminence of Calotes versicolor was slow, indicating a gradual release of NSS from this region.

The interpretation of the changes in the neurosecretory system of Calotes versicolor during the
period ranging from February to August is comparatively easy. The accumulation of the NSS in the cells of SON and PVN can be attributed to the failure of its release from the neurosecretory cell bodies during this period. In the month of April, there is a decrease in the amount of NSS in the neurosecretory cells accompanied by an increase in the stainable material in the axons and also its accumulation in the median eminence. Thus it appears that the NSS discharged from the neurosecretory nuclei is transported along the collateral fibers extending from the hypothalamo- hypophysial tract into the median eminence. The relative increase in the size of the nuclei of the neurosecretory cells belonging to both SON and PVN during the period ranging from April to August may be attributed to the higher rates of synthesis and release of the NSS from these cell bodies.

The present observation in the lizard, *Calotes versicolor* that there is an accumulation of NSS in the median eminence during the period immediately preceding breeding, is in conformity with the studies made in birds *John* and *George*, 1967) and mammals (Clark and Kennedy, 1967a). However, the amount of NSS stored in the median eminence of *C. versicolor* is relatively lesser. In birds (George and Naik 1965) there was a sudden depletion of NSS from the median eminence and neurohypophysis a few days prior to migration. These authors considered this depletion in NSS as a trigger for migration. observed a similar depletion in NSS of the median eminence of the migrating birds, *Motacilla alba* and *M. flava*, followed by changes in the other endocrine glands. Therefore, these authors considered the possibility that the changes in the hypothalamic NSS might be affecting the various tropic
functions of the pituitary. Cleark and Kennedy (1967) similarly made an attempt to correlate the changes in the NSS of the medium eminence in the seasonally breading mammal, vole with those associated with gonadal development.

In Calotes versicolor also there is a depletion in the amount of NSS from the median eminence during a period when the gonads are undergoing development. However, this process is slow and continuous till the end of the breeding period.

The neurosecretory nuclei, during the period ranging from September to January appeared inactive as judged by the loss of cytoplasmic granularity, poor staining ability of the cells and reduction in blood supply. The axon fibres of the hypothalamo-hypophysial tract reaching the neurohypophysis and the median eminence were completely devoid of NSS. The reason behind this state of affair is not far to seek as during this period, the animal under study, goes into sexual quiescence and the reproductive activity ceases completely.

The gradual accumulation of NSS within the neurosecretory cells from February till March, a period of low testicular and thyroid activity, its concentration within the median eminence in the last week of March and depletion in the third week of April, with a simultaneous rise in thyroid and spermatogenic activity afford circumstantial evidence in support of the participation of hypothalamus in the control of these two endocrine glands.

The deletion of NSS in the neurohypophysis and specially within the median eminence in the third week of April with a consequent increase in the functional activity of the testes strongly suggested that this NSS on reaching the pars anterior via portal
circulation stimulates the gonadoïtophys.

From the seasonal cyclic changes occurring in the hypothalamic SON, PVN and the median eminence, and the simultaneous histological changes observed in the adenohypophysis and the testes, it can be surmised that the hypothalamus – hypophysis – testis endocrine axis operates in the lizard under study.

The activation of this endocrine axis to set off the reproductive response is in turn dependent on certain environmental stimuli. In chapter II of this thesis, it was shown that the unseasonal development of gonads could be achieved by elevating the environmental temperature. It is, therefore, concluded that a rise in temperature is the primary stimulus which exerts its influence on testicular activity through higher nervous center which eventually communicate with the hypothalamus.

In the adult lizards, Lacteria, agilis, L. muralis, L. vivipara (Eggart, 1935), Xantusia vigilis (Miller, 1955) belong to the temperate zone, the increased thyroidal activity has been directly correlated with the reproductive activity. The males of S. occidentalis exhibit an increase in the thyroid epithelial height during May and June the period. When spermatozoa are abundant in the seminiferous tubules. Similarly, the thyroid gland of male Xantusia vigilis (Miller, 1950) shows two peaks in its activity which may correspond with the increased spermatogenesis and spermatogenesis respectively.

Our observations, on the thyroid of Calotes versicolor agree to a certain extent with those of the above mentioned workers. There appears to be a correlation between the thyroidal and testicular activities.
The minimum thyroid activity as judged by the histological appearance is recorded in December (epithelial cell height 4.65 ± 0.13 μ and percent activity 5.9%). This period corresponds with the minimum gonadal activity. From January till March, there is a gradual increase in the thyroidal epithelial cell height which becomes quite striking in April accompanied by a sudden spurt of spermatogenic activity. The functional activity of the thyroid gland attains its peak in June (epithelial height 11.42 ± 1.05 μ and percent activity 14.22%) which coincides with the period of maximal spermatogenic activity. This parallelism between the activities of thyroid and gonads is a persuasive evidence though perhaps still not convincing to support the presumption that a direct interrelationship exists between the activity of the thyroid gland and the process of spermatogenesis.

However, this contention becomes questionable when our observations on the thyroid histology of the animal under investigation during the period ranging from July to December are taken into consideration. It is observed that the testicular activity after reaching the peak level, is maintained in that steady state till August but there is a decrease in the thyroidal activity from July onwards. Thus the process of active spermatogenesis continues inspite of the fact that there is a decrease in the thyroidal activity. This can be explained, if we presume that the testicular activity is dependent on the thyroid activity only initially and once the testes are primed by the increased level of thyroid hormone, there is no further necessity of their continued stimulation. However, this hypothesis, cannot explain the sudden decline in the spermatogenic activity during the month of September.

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During this and the following months gonads are completely inactive, but no histological signs of the thyroid gland being non-functional are evident. Therefore, it appears that the increased thyroid activity during the period ranging from April to June might have some other explanation.

Some alternative explanations have been suggested by other workers. The increased thyroid activity in *L. rhomboidalis* during spring, may be due to increased locomotory activity involved in territory maintenance and the act of copulation, because, in this animal, the process of spermatogenesis is continuous whereas actual mating takes place in spring and the males maintain their breeding territories during this period only. The observation made on the thyroid activity in hibernating species of reptiles gives support to the above concept. The thyroids of hibernating species *Lacerta agilis* *L. vivipara* *L. muralis* (Eggart, 1935) *Chrysemys Evans* and *Hegre*, 1940) and *scoloperus occidentals* are completely inactive during the period of hibernation when their locomotor and metabolic activities are at minimal level. In contrast the non-hibernating forms *Anolis carolinensis* (Evans and Hegre, 1936) and *Xantusia vigilis* possess thyroids which are active all the year round.

According to Miller (1955) the parallel changes in the activities of the gonads and thyroids may be a coincidence resulted from the influence of some external factor such as temperature which is influencing both but independently. In chapter II it was shown that temperature plays a major role in controlling the testicular cycle. Therefore, it is likely that the same may be affecting the thyroid function. Therefore, the supposition that the thyroid activity may perhaps be due to the effect of increased
environmental temperature deserves experimental evaluation. On the other hand, there are examples where the thyroidal function has been affected by the gonadal steroids. For example, Kumaresan and Turner (1967) found that injections of testosterone propionate into the normal and castrated rats increase the thyroxin secretion rate. Evans and Hegre (1940) observed that the estrogens had a stimulatory effect on the thyroid of female lizards. The greater production of steroid hormones that accompanies increased testicular activity may influence the thyroidal function. This aspect merits further investigation.

It was clearly shown that the secretion of hormones of adrenal gland causes effect on reproductive system of male Calotes versicolor and even also plays important roll in maturation of testes if there is removal of adrenal gland it directly effect on proliferation and retarding growth with higher doses. In present study on male C. versicolor role of adrenal in reproduction was very much observed. In different months of observe period the gonado-somatic index, followed the breeding and non-breeding phases of reproductive cycle. Similar pattern was observed in Adreno-somatic index. The gonado-somatic index increased towards breeding period (May to Sept.) and decrease was observed during post breeding period (Sept. to Jan.)

Adreno-somatic index also followed similar pattern that is index increase from pre-breeding period to breeding and their after decreased in index was observed. During pre breeding and breeding period the appearance of adrenal was very prominent and lustery. Similar observations are made in differ animals belonging to birds and mammals.
Histological studies of gonad that is testes and adrenal were found to be correlated during pre-breeding conditions. The adrenal cells were found to be enlarged which may be correlated with spermatogenesis in the testes. Unilateral adrenalectomy reflected in partial arrest in spermatogenetic stages, which was very much obvious from the sections of testes at 24, 48, 72 & 96 hrs. Unilateral adrenalectomized Calotes versicolor.

Bilateral adrenalectomy at very much effect on spermatogenesis, the different stages of spermatogenesis, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are very much influence. These stages were found to be recovered with adrenal extract injection. The prominent effect was noticed with increase in injection doses.

The animal receiving extra adrenal extract injection shows proliferation, increase in size of adrenal cells, which in turn acted on gonads that is testes. Similar reports were noticed in mammals and other animals. The correlating differences between adrenal and testes might be the interference of adrenal in reproduction of Calotes versicolor. Further physiological, biochemical and enzymological studies may help in detecting clear role in reproduction.

The adrenal gland and gonads are closely related to the same embryological origin found degenerative changes in the seminiferous tubules in the testes of mature rats after adrenalectomy. It was noted by Altermann (1956) that gonadotrophin concentration in the blood was decreased after adrenalectomy in adult rat and that this might cause histological changes in the testes. Many workers also
observed that exogenous ACTH can maintain spermatogenesis in mature hypophyseal-ectomized rats presumably by the stimulation of the adrenals.

To observed the effect of adrenalectomy and the administration of androgen in the testicular cells of the immature rats.

Histological observations on the testes of the intact animals treated with DEAT (Dehydroepiandrosterone) revealed the presence of primary and secondary spermatocytes in the seminiferous tubules with elongation and hypertrophy of the interstitial cells.

Bilateral adrenalectomy produced oedema in the interstitial cells and a marked degeneration of seminiferous tubules where as supplementation with DEAT after bilateral adrenalectomy corrected these defects.

Testicular degeneration in adrenalectomized adult rats indicated the role of the adrenal gland in the process of spermatogenesis (Freed, S. C et. al. 1931;).

The variation in the size of an endocrine gland, the period of activity, dormancy and reproductive cycle are interrelated phenomenon. The studies on the adrenal gland in relation to reproductive cycle among the mammals and the available literature concerning the activity of the rodents are limited. Although the adrenal glands of many small mammals have been studied Jain, 1970, 1971; Gastone 1986 a, b). Comparatively not much attention of the adrenal gland in relation to the reproductive cycle.

Seasonal changes seen in the adrenal gland also correlated with the changes in the ovary. The cortical cells of the Zona-fasciculata and Zona-reticularis in the adrenal glands of sexually active gerbils show decrease in cell as well as nuclear size during quiescent period. The zona-
glomerulosa and medulla do not show any changes in their structure during different reproductive phases.

The left adrenal gland weight more than the right gland. The percentage of difference in weight between right and left adrenal glands in a gerbil is 11-13%. The changes in the adrenal weight is correlated with that of the ovarian weight fluctuation seen during the different months. Maximum weight of adrenal gland is seen during breeding seasons and loss during non-breeding season. The seasonal variation seen in the adrenal cortex of the gerbils may be explained on the rhythmic life process that are compatible with the environmental factors which are prevalent with their range and ecological niche. The account for the cortical enlargement which is seen during the breeding season, Christaian (1955, 1956) & Louch (1966) demonstrated that there is a increase in thee adrenal size with an increase. In the population until the population reach a point when there is a breakdown in social organization. In case of field mouse, it is observed that the adrenal weight increases with reduction in floor area up to a certain population density. The secretory activity of the adrenal cortex is determined by adenohypophysis. The zona glomerulosa is the site of synthesis and secretion of aldosterone, the fasciculate synthesizes and secretes glucocorticoids (Gastone et. al. 1978, Gastone 1980) and the zone reticularis is concerned with secretion of the androgenic steroids (Long, 1957).

Adrenalectomy or adrenal hormonal insufficiency causes degeneration of testes (Chowdhaury A.R. and Chatterjee A.K. 1976). The elevating effects of adrenalectomy after attainment of puberty (50 days) are contrast to its effect at prepubertal
ages (upto 40 days), the trend of testicular growth stimulating, effect of corticosterone of the prepubertal rats. From the present results it appears that both the hormones have growth promoting effects on the testes and overall body.

The age dependent balance of these hormones probably governs the normal testes-body weight relationship.

Bilateral adrenalectomy, although shows reduction of the testicular enzymes activity at the age of Puberty (40-50days), after attainment of adulthood the testes appears to become resistant to adrenalectomy. However the testicular tissue becomes responsive to either corticosterone or DHA (Dehydroepiandrostone) in the stimulating the enzyme activity corticosterone being the better of the two hormones. Nevertheless, these hormones demonstrate antagonistic effects by showing a sharp reduction in activity (Neera Sexana and Paul, P.K. 1988).

Adrenalectomy during prepubertal ages (days 21 to 40) caused a significant reduction in the testes weight in relation to the body weight. Administration of DHA and corticosterone independently or in combination, was effective in stimulating the testicular relative weight in these adrenalectomized rats, corticosterone along or in combination with DHA was better effective in either restoring the control level or elevating it above the control level of the testes weight. (Sexana and Paul, P.K. 1988).

Role of FSH, LH and testosterone on the regulation of the seminiferous epithelium cycle is well known. Replacement therapy with PMSC or testosterone is partially effective in maintaining the replicating spermatogonia in hypophysectomized animals (Neera Sexana and Paul,
Neither the adrenal weight nor the body weights differed in the two sexes.

The relative volumes of the cortex and medulla in the adrenals of the rats have been shown to bear a ratio of 10:1 or greater the ratio being bigger in the female that in the male. The actual amount of medullary tissue in the glands of the two sexes is due to difference in the amount of cortical tissue.

There is some evidence that conditions known to increase secretion of cortical hormones, like administration of ACTH or cold exposure, increase the oxidative metabolism of adrenals while cortisone administration decrease it.

This is of particular interest in relation to the effect of gonadal secretions of adrenal function, since a great deal of the available evidence indicating that estrogens stimulate, while androgens inhibit the adrenal cortex has been based on the adrenal size measurements.

Adrenalectomy affects the response both in an ovulatory dose of pins (pregnant mere’s serum) and to a non-ovulatory dose. The interval between adrenalectomy and PMS treatment affects the response to PMS both during and after the ages at which adrenalectomy has been reported to delay normal puberty. PMS treatment induced elevated adrenal weights when measured the day after ovulation. This last effect was absent in rats whose ovaries contained lutanized interna rather than corpora lutea.

Pregnancy so alters metabolic balance or endocrine balance that the value for adrenal cholesterols is significantly lower than in the non-pregnant animals. However, a modified resting level does exist, and if this is a reflection of increased adrenal activity, the gland must function at an
equilibrium level of cholesterol which differs markedly from that of non-pregnant rat, since it is able further to respond by a decrease of cholesterol when an additional stress stimulus is applied. Similar observations were described by B.B. Longwell and M.W. Berenbeim, 1953. Numerous suggestions have been made concerning the existence in females of extra adrenal sources of substance with adrenal cortical action. (Cowie 1949) recorded for rats a longer survival time for females than males after adrenalectomy, an advantage which was attributed to progesterone action.

Pregnancy confers on dogs a prolonged survival after adrenalectomy. The recent demonstration of adrenal cortical hormone activity in both human and equine placenta gives support to the concept that adrenal cortical hormone activity during pregnancy may come from extra adrenal sources in addition to the adrenal cortex. Thus, the low adrenal cholesterol of pregnancy and part to a modification of endocrine balance brought about by sources of cortical hormone activity other than the adrenal.

The adrenal response was measured by changes of cholesterol and ascorbic acid concentrations. Both cholesterol and ascorbic acid were found to be lower in normal, pregnant rat than in the non-pregnant female. Cole exposure induced a further decrease in both of these substances.

The featus demonstrated a limited capacity to respond to ACTH with increase peripheral levels of costisol in only two of the seven fetus challenged with ACTH were increased costisol levels observed. In monkey fetal adrenal gland in vitro produces costisol and responds to ACTH late in gestation. The preliminary evidence (unpublished) that there is a
consistent increase in peripheral cortisone levels in the newborn monkey in response to the same dose of ACTH (Ferre M.S. et al. 1978, Challis J.R. et al. 1974)

It seems unlikely, however, that this increment in androgen secretion plays a major role in the initiation of the sperm in gonadotrophin secretion during prepubertal development, since the decline in elevated plasma LH and FSH concentration of the open loop situation after 8 weeks of age was not interrupted in bilaterally adrenalectomized animals (Plant, T. M. 1980; Peckham W.D. and Tontala F.J. 1981).

The pubertal reinitiating of secretion does not appear to be delayed in adrenalectomized animal despite the sustained impairment in the growth rate throughout development. Taking the forgoing consideration into account, the present results are constant with the view that the adrenal gland does not plays a major role in determining the timing of the onset of puberty in higher primates (Grumbach, M. M. et al. 1978, Sklar C. A. et al. 1980; Cutler Jr. G.B. Lorloux D.L. 1980)

The reasons for the truncated post castration hyper secretion of LH and FSH in the one adrenalectomized animals reared in the absence of maternal influenced are not clear (Plant, T. M. 1980; Peckham W.D. and Tontala F.J. 1981).

4.5 BIOCHEMICAL CHANGES

In the Calotes versicolor the changes of in carbohydrate is according to the change in seasons However, an occurrence of glycogen in the body normal course is not observed in mammals such as European mole, Talpa europea (Lofts, 1960). In the water snake, Natrix
piscator. The glycogen cycle in *C. versicolor* agrees well with that of the other vertebrates (*Lofts* and *Chan & Phillips*, 1967; *Lofts* and *Boswell*, 1960; and *Sanyal* and *Prasad*, 1965)
The histochemical observations on the seasonal changes in the testicular glycogen of non-mammalian vertebrates; *Lofts* and *Marshall*, 1957; *Lofts* et. Al., 1966; *Chan* and *Phillips*, 1967 and *Cavazos* and *Fragans*, 1960) have led to the conclusion that the glycogen, in the interstitial cells, as in mammals, are utilized in the production of steroid hormones, Although the pathways of steroid Apart from the changes in the interstitial glycogen, cyclic changes also have been shown to occur in the intra tubular glycogen content. After the completion of one sexual cycle when the spermatozoa are shed, there occurs a profound metamorphosis of the tubular elements involving the production of large quantity of intra tubular glycogen material, which is strongly effective Many of the workers suggest that there glycogen content reached the lowest value at the time of maturity of sperms that comes last upto the ends at month of Sept. A striking change in the glycogen level was seen during the course of reproductive cycle. The level reached lowest in at time of maturity of sperm (In the month of May) And in non-breeding seasons the level increased,

This suggest that the stored glycogen might be utilized for the formation of the reproductive elements and this accounts for the decrease in the glycogen content during the breeding seasons.

It is clear from the foregoing account that the glycogen shows seasonal changes in its percentage in association with the sexual and metabolic activity. Similar results were also occurs in turtles other reptiles
The role of lipids in the reptilian and other lower vertebrate testicular cycles is not very clear. However, the histochemical observations on the seasonal changes in the testicular lipids of non-mammalian vertebrates (Marshall, 1949; Marshall and Loftes, 1956; Loftes and Marshall, 1957;) have led to the conclusion that the cholesterol, positive lipids in the interstitial cells, as in mammals, are utilized in the production of steroid hormones. Although the pathways of steroid synthesis in the testicular tissue in the lower vertebrates have not been clearly established it is very much likely that they may be after the mammalian pattern. Thus it has been demonstrated both by 'in vivo' techniques that C^{14}-labeled acetate and cholesterol are efficient precursors of steroid hormones (Dorfman and Ungar, 1965). Clausen and Hillarp (1947) have stated that the presence of cholesterol in the form of stainable granules indicates their storage in the interstitial cells, while their depletion may be related to the active steroid secretion. Moreover, the interstitial cell activity has also a direct bearing on the secretory activity of the ductus epididymis.

A dense accumulation of sudenophilic lipid (from October till March), which is strongly positive for cholesterol in the interstitial cells, is accompanied by minimum activity of the ductus epididymis. The spermatogenic activity is also at its minimum during this period. The depletion in the cholesterol positive lipids in the interstitial cells in the month of May is accompanied by maximal spermatogenic activity and an increase in the epithelial cell height of the ductus epididymis.

Thus, it appears that in the month of May, there is an increased mobilization of the cholesterol positive lipids in the...
interstitial cells indicating an increased production of steroid hormones.

The interstitial lipid cycle in *C. versicolor* agrees well with that of the other vertebrates (Lofts and Marshall, 1957) where the sexual activity reaches its peak accompanied by a progressive discharge of cholesterol positive lipids from the interstitial cells.

Apart from the changes in the interstitial lipids, cyclic changes also have been shown to occur in the intra tubular lipid content. After the completion of one sexual cycle when the spermatozoa are shed, there occurs a profound metamorphosis of the tubular elements involving the production of large quantity of intra tubular lipid material, which is strongly cholesterol positive. Such an occurrence of post-nuptial steatogenesis of the tubular elements and the gradual disappearance of the cholesterol which has arisen during the above process is a regular part of the reproductive cycle of certain fishes (Lofts and Marshall, 1957), amphibian (Lofts and Boswell, 1960), reptiles (Lofts and Boswell, 1961) and birds (Lofts and Marshall, 1959).

However, an occurrence of post-nuptial cholesterol in the seminiferous tubules in the normal course is not observed in mammals such as European mole, *Talpa europaea* (Lofts, 1960). In the water snake, *Natrix piscator*, Shrivastava and Thapal (1965) did not observe the post nuptial occurrence of cholesterol, although they have reported the occurrence of lipid debris in the seminiferous tubules of spent testes.

The changes in the intra tubular lipids in *Calotes versicolor* are comparable to those observed in *Talpa* and *Natrix* as the post-nuptial appearance of cholesterol is completely lacking in these animals. There appears, however, certain quantity of cholesterol negative
sudenophilic material in the central portion of the tubules, at the time of maturation and shedding of spermatozoa which might have arisen as has been pointed by Lofts (1960) from lipophanerosis, which is characteristic of vertebrate spermatogenesis.

This lipid disappears along with the products of spermatogenesis, which have not been utilized, and not intra tubular lipids are observed in the month of October when the testes become quiescent. Role of fat in the body of calotes versicolor is important and the total fat content was decrease during ripening of the gonads. The highest values are noted in the month of January is 4.28 ± 0.354 and lowest values are seen in month of January is 3.34±0.722. All this data shows that the fat deposition in body changes according to the change in breeding and non-breeding seasons it would appear problem that as the grew it received fat from the other parts of the body & use it for providing a energy to the formation of sperms.

In the Calotes versicolor the changes of in Proteins is according to the change in seasons However, an occurrence of proteins in the body normal course is not observed in mammals such as European mole, Talpa europea (Lofts, 1960), vole deer. In the water snake, Natrix piscator, The proteins cycle in C. versicolor agrees well with that of the other vertebrates (Lofts and Marshall, 1957) The histochemical observations show that the seasonal changes causes the change in the percentage of body proteins.

The proteins level in the Calotes versicolor showed correlation with the reproductive activities. High values prior to the breeding peaks suggest that it might be cornered by the testes as an important metabolite and that sharp
decline following the breeding peaks may be because of the loss of gametes. The level was highest in the month of May when the testes show the full maturity. The percentage of proteins in this month is $36.28 \pm 0.382$ but in the month of June when there is discharge of sperm takes place at this month the values are goes down to the lowest is upto $33.16 \pm 0.247$. Even also in other vertebrata and in invertebrate like Emrita the values of proteins goes down sharply after the spawning.

The role of Proteins in the reptilian and other lower vertebrate testicular cycles is not very clear. However, the histochemical observations on the seasonal changes in the testicular proteins of non-mammalian vertebrates (Lofts and Marshall, 1957; Lofts et. Al., 1963; Chan and Phillips, 1967 and Cavazos and Fragans, 1960) have led to the conclusion that the proteins in the interstitial cells, as in mammals,