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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

IN THE NAME OF ALLAH(GOD) THE BENEFICENT

THE MERCIFUL.

STUDIES ON THE MALE REPRODUCTIVE CYCLE IN THE LIZARD *CHALCIDES OCELLATUS*

A THESIS

Submitted to the University of Wales by

FAHMI MUSTAFA SABRI MAHMOUD

in Candidature for the Degree of

PHILOSOPHIAE DOCTOR

Department of Zoology,
University College of North Wales,
Bangor, Gwynedd.

May 1981

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DEDICATION

TO MY PARENTS

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G E N E R A L I N T R O D U C T I O N

GENERAL INTRODUCTION

Reproductive cycles have been studied, in the wild for many lizards, covering the temperate, sub-tropical and tropical zones. However, there are few reports of long term laboratory studies under standardized conditions of light and temperature. In addition, reports concerning the effects of environmental factors and their relative importance in reproduction are contradictory.

In Chalcides ocellatus males, the reproductive cycle has been studied by Badir and colleagues (Badir, 1955 & 1958; Badir & Hussein, 1964) who claimed that optimal conditions of light and temperature could cause a complete shift from a cyclic to a "continuous" pattern of reproductive activity, though their claims do not appear to be justified.

This thesis presents findings on the reproductive features of male Chalcides under standardized conditions of light and temperature, some effects of daylength and temperature on reproduction, variations in plasma and testicular androgen levels under a variety of photothermal conditions and a histochemical study of a steroidogenic enzyme in the testis of this lizard, all in an attempt to elucidate the pattern and controls of reproductive activity.

L I T E R A T U R E R E V I E W

LITERATURE REVIEW

General

The living reptiles of the world number over 6000 species scattered over the earth. Miller (1959) remarked that reproduction had been studied in less than 1% of the living reptiles. This percentage increased little to Marion's thesis (1970b). However, in the past decade many studies have been conducted on reptilian reproduction and good, detailed information is now available for many species, and some generalizations can be made.

The four Orders in class Reptilia are: Chelonia (Testudinata), comprising about 230 species of turtles and tortoises; Crocodilia, comprising about 23 species of crocodiles and alligators; Rhynchocephalia, represented by a single living member, the tuatara Sphenodon from New Zealand; and Squamata, including the vast majority of living reptilian species (about 5840 species).

The three Suborders of the Order Squamata are: Sauria (Lacertilia), including nearly 3000 species of lizards; Amphisbaenia, with 140 species of worm lizard; and Serpentes (Ophidia), with about 2700 species of snakes (Carr, 1963; Bellairs, 1970; Goin, Goin and Zug, 1978; Webb, Wallwork and Elgood, 1978).

As the squamates are the most numerous living reptiles, it is not surprising that most studies on reproduction are on this Order, but perhaps a little surprising that so much more has been done on lizards than snakes. In fact, physiological studies on reptilian reproductive cycles have been confined almost exclusively to lizards (Licht, 1972).

Lizard classification

Lizard classification has been fairly stable since 1923. In that year Charles L. Camp published his thesis, "Classification of the Lizards". There have been, of course, subsequent modifications of Camp's classification, but its basic framework stands (Goin *et al.*, 1978).

Camp (1923) recognized two major groups of lizards; Ascalabota (including geckos, iguanids, agamids, xantusiids, and chameleons), and Autarchoglossa (including the rest of lizard families). His classification was based on the structure of the tongue and feeding habits, but included 35 other characters. Detailed studies on lizard (and reptile) classification are contained in some excellent recent reviews by Goin *et al.* (1978); Webb *et al.* (1978) and Wever (1978).

I. The Reproductive System

1. The male gonads:-

The right testis, in lizards, is generally situated anterior to the left (Fox, 1977). However, testes are almost symmetrically disposed in Agama tuberculata (Duda, 1972), while the left is anterior in Eumeces obsoletus and Crotaphytus collaris (Brooks, 1906 - quoted by Fox, 1977).

Generally, the testes of reptiles are similar to those of birds and mammals (Miller, 1959). Among lizards, Hemidactylus flaviviridis is typical in having abdominal testes surrounded by a tunica albuginea of connective tissue, and attached dorsally to the body wall by a fold of peritoneum, the mesorchium. The convoluted seminiferous tubules are interspersed with interstitial (Leydig) cells, blood vessels and lymphatics (Dutta, 1944 - quoted by Fox, 1977).

Testes are particularly elongate in snakes and snake-like lizards (Fox, 1977), however, several lizards including Phrynosoma solare (Blount, 1929), Lacerta agilis (Regamey, 1935), Eumeces fasciatus (Reynold, 1943) and Xantusia vigilis (Miller, 1948) have ovoid testes.

2. Interstitial (Leydig) cells:-

The interstitial cells were first described by Leydig (1857). These cells are known to be present between the seminiferous tubules of most mature reptiles. However, interstitial cells were not recognized in the lizard Mabuya multifasciatus (Herberrer, 1930 - quoted by Fox, 1977), nor at any stage of the spermatogenic cycle of Uma notata,

U. inornata, and U. scoparia (Mayhew & Wright, 1970). They are rare and few in Xantusia vigilis (Miller, 1948), Anolis carolinensis (Fox, 1958), Sceloporus occidentalis (Wilhoft & Quay, 1961) and in Acanthodactylus erythrurus (Bons, 1969).

In general, the size, number and appearance of interstitial cells varies during the reptilian reproductive cycle, and this may partly explain why they are sometimes not identified. Their shape is polyhedral, elongated or oval; with rounded nuclei and vacuolated or alveolar cytoplasm which may include granules, crystalloids and fat globules (Fox, 1977).

In several species of lizards, relationships exist between activity in the seminiferous tubule and the number, size, secretory activity and/or the mean nuclear diameter of the interstitial cells. For example, in Phrynosoma solare interstitial cell volume is greatest at the height of the breeding season, but minimum at the conclusion of the breeding season (Blount, 1929). The cells are numerous, in Hemidactylus flaviviridis, when testes are at rest and the tubules are quiescent, and apparently fewest when testicular tubules are most active (Dutta, 1944). Likewise, interstitial cells are present in compact masses in spermatogenically inactive testes, but apparently are fewer at the height of breeding activity in both Varanus bengalensis and Mabuya macularia (Upadhay & Guraya, 1972). However, it must be said that none of the above studies made a proper quantitative study in which the actual number of interstitial cells was counted (or assessed/calculated). In fact, the above studies were describing interstitial cell numbers in a given cross section. Unless quantitative studies are made, the variability in interstitial cells during the breeding season

remains of obscure significance.

The interstitial cells are active and secretory in spermatogenically active testes, but inactive and non-secretory during testicular quiescence in several lizards, including Sceloporus occidentalis (Wilhoft & Quay, 1961), Leiolopisma fuscum (Wilhoft & Reiter, 1965) and Hemidactylus flaviviridis (Sanyal & Prasad, 1967). In Uta stansburiana, parallelism exists between the cyclic activity of Leydig cells and the seasonal cycle of both seminiferous and epididymal epithelia (Hahn, 1964).

In contrast, the size, nuclear diameter and the secretory activity of interstitial cells of Leiolopisma rhomboidalis (Wilhoft, 1963b) and Hemidactylus flaviviridis (Kitada & Asana, 1951) do not vary through the year. This is expected, since both lizards exhibit continuous activity throughout the year, without apparent variations in spermatogenic activity. Similarly, nuclear diameter and/or secretory activity of interstitial cells in Xantusia vigilis (Miller, 1948) and of Dipsosaurus dorsalis (Mayhew, 1971) are not variable during cycles of reproduction. However, the lack of interstitial cell activity in both lizards seems rather odd, since both lizards have typical seasonal spermatogenic cycles.

Fox (1958) reported that Anolis carolinensis exhibits a clear cycle in interstitial cell activity which is independent of the spermatogenic cycle, but shows a chronological relationship with secondary sex characters. This can hardly be accepted without qualification since spermatogenic and interstitial cell activities are obviously interrelated.

A unique layer of sub-tunica albugineal cells (homologous to the mammalian tunica vasculosa) has been described in 17 species of Cnemidophorus and in Ameiva undulata (Lowe & Goldberg, 1966). This layer

of cells is in continuity with typical intertubular Leydig cells and it was postulated that its cells are identical to those of the intertubular tissue (Lowe & Goldberg, 1966). The sub-tunica layer of Leydig cells does not undergo variations in number (whereas the intertubular cells do), but it does show a seasonal cycle in secretory activity as judged by the deposition and depletion of secretory granules during the breeding cycles (Lower & Goldberg, 1966).

In 1903, Bouin & Ancel (quoted by Fox, 1977) suggested that Leydig cells served as the source of male sex hormones. Their claim has been substantiated by many investigators in reptiles (e.g. Fox, 1952; Miller, 1959; Ramaswami & Jacob, 1963, 1965; Chester-Jones, Bellamy, Chan, Follett, Henderson, Phillips & Snart, 1972; Lofts, 1972; Lofts & Bern, 1972; Ozon, 1972; Chan & Callard, 1974; Licht & Tsui, 1975; Tsui, 1976; Licht & Midgley, 1977; Arslan, Lobo, Zaidi, Jalali & Qazi, 1978). In all of these studies it was concluded that interstitial cells are the source of the male steroid hormones in squamate reptiles.

An inverse relationship has been reported between the cholesterol content of Leydig cells and the active breeding periods of squamates. For instance, the testes of Uromastyx hardwicki show maximum levels of cholesterol when they are regressed and minimal levels when in full spermatogenic activity (Arslan & Jalali, 1973). In the snake Naja naja, accumulation of cholesterol-positive lipids in the interstitial cells during the spring is succeeded by a depletion simultaneous with the maximum production of androgens and the development of secondary sex characters (Lofts, Phillips & Tam, 1966; see also Lofts, 1968; Lofts & Bern, 1972). Accordingly it can be said that the depletion of cholesterol-positive lipids during the breeding season is due presumably to the production of

androgens. It is generally agreed that cholesterol is the principal precursor of steroid hormones (Ozon, 1972).

Electron microscopic studies added strong support to earlier studies which suggested that interstitial cells are the source of steroid hormones. The structure of Leydig cells of Lacerta vivipara and Anguis fragilis reveals characteristics of cells which elaborate steroids (Dufaure, 1968, 1970 - quoted by Fox, 1977). The cytoplasm includes extensive smooth endoplasmic reticulum; numerous free ribosomes and polyribosomes abound, and mitochondria are plentiful in a density related to the sex cycle. During the spring, the interstitial cells of Lacerta vivipara show a well developed system of vesicles and vacuoles of a smooth endoplasmic reticulum and of the Golgi complex, which are possibly related to androgenic production. Vacuoles are less prominent in summer, after breeding, when the smooth endoplasmic reticulum comprises a network of tubules often associated with lipids (Dufaure, 1968, 1970 - quoted by Fox, 1977).

Recent in vitro studies have left no doubt that interstitial cells secrete steroid hormones. In these studies interstitial cells were separated from other tubular components and their capacity for androgen production estimated (Licht & Tsui, 1975; Tsui, 1976). It was found that Leydig cells are the major sites for androgen production and that the seminiferous tubules account for only a small percentage (Tsui, 1976; Licht, 1979).

Steroid hormones, particularly testosterone, are known to affect spermatogenesis and to control the activities of accessory sex organs (epididymis and the sexual segment of the kidney). The effects of testosterone on these organs are discussed elsewhere (see page 31).

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3. Accessory sex organs:-

The epididymis and the sexual segment of the kidney, form the main accessory sex organs. Seasonal changes in the diameter, the epithelia height, and the secretory activity of the epididymis have been described in several species of lizards. In *Eumeces fasciatus*, the epididymis exhibited a seasonal cycle in which it was active and secretory from February (time of first sperm produced), reaching a peak of activity in April and then declining to base levels in August. The epididymis remains inactive and regressed till the new breeding cycle (Reynolds, 1943). Similar waxing and waning in the activity of the epididymis has been reported for *Lacerta muralis* (Herlant, 1933); *L. agilis* (Regamey, 1935); *Anolis carolinensis* (Fox, 1958); *Urosaurus ornatus* and *Uta stansburiana* from southern Arizona (Asplund & Lowe, 1964); *Uta stansburiana* from Texas (Hahn, 1964); *Hemidactylus flaviviridis* (Sanyal & Prasad, 1965, 1967); *Sceloporus undulatus* (Marion, 1970b); *Dipsosaurus dorsalis* (Mayhew, 1971), and *Urosaurus graciosus* (Vitt & Ohmart, 1975). In many of these reports, seasonal variations in the epididymis were parallel to those occurring in the seminiferous tubules.

In contrast, the epididymis of the tropical lizard *Leiolopisma rhomboidalis* showed no seasonal cycle in either the amount of epithelial development or the secretory activity (Wilhoft, 1963b). In a closely related species, *Leiolopisma fuscum*, the epididymis seems to change little seasonally (Wilhoft & Reiter, 1965).

The sex segment of the kidney was first described by Gampert (1866 - quoted by Fox, 1977) in *Natrix*. Regaud & Policard (1903a) first termed this region the "sex segment". The sex segment was found to occur

only in the kidneys of male snakes and lizards, while it is absent in all other reptilian groups (see review by Fox, 1977). During sexual quiescence of the gonads, the sex segment is involuted and cannot be distinguished from the adjacent tubular regions (Cordier, 1928; Herlant, 1933; Badir, 1958; Saint-Girons, 1963, 1967 - quoted by Fox, 1977). However, at the height of the breeding season the sex segment hypertrophies and its columnar cells contain obvious secretory granules. The colour, size, shape and chemical composition of the granules have been described by many authors (see Regaud & Policard, 1903a,b; Courrier, 1929; Volsøe, 1944; Fox, 1952; Forbes, 1961; Deb & Sarker, 1963; Burtner, Floyd & Langley, 1965; Sanyal & Prasad, 1966; Sanyal, Prasad & Misra, 1966; Helmy & Hack, 1967; Arvy, 1969; Del Conte & Tamayo, 1972, 1973; Reddy, Prasad & Misra, 1972; Kühnel & Krisch, 1974).

Cyclic activities have been described for the sexual segments of several lizards including Lacerta agilis (Reiss, 1923); Takydromus tachydromoides (Takewaki & Fukuda, 1935; Sceloporus undulata (Altland, 1941); Scincus scincus and Chalcides ocellatus (Badir, 1958); Anolis carolinensis (Fox, 1958); Hemidactylus flaviviridis (Sanyal & Prasad, 1965, 1967); Leiolopisma fuscum (Wilhoft & Reiter, 1965) and others.

On the other hand, Wilhoft (1963a,b) reported, in Leiolopisma rhomboidalis, that the sexual segment exhibited no seasonal variations in the diameter of the tubules. This appears to be reasonable since this species breeds throughout the year.

The functions of the epididymis and the sexual segment of the kidney, and the control of their activities are described elsewhere (see page 31).

4. Fat bodies and Liver:-

Abdominal fat bodies occur in all (examined) reptiles from the temperate zone (Hahn & Tinkle, 1965), as well as in some semi-temperate anoline lizards (Cuellar, 1973 - quoted by Fox, 1977).

In many species of lizards, abdominal fat bodies in the male exhibit a cycle which is inversely related to the reproductive cycle. For example, fat body weights are at minimum levels in late summer, and rise rapidly in September and early October prior to hibernation in the male lizard Takydromus tachydromoides. Fat body weight decreases during hibernation and continues to decline throughout the spring and early summer, the time of the breeding season (Telford, 1970). Similarly, Stamps & Crews (1976) reported, for Anolis aeneus, that fat bodies undergo a seasonal cycle with weights inversely related to testis activity, as has been reported for Agama agama from Ghana (Chapman & Chapman, 1964); seven species of Caribbean Anolis (Licht & Gorman, 1970); Anolis limifrons, A. tropidogaster, and A. auratus (Sexton, Ortleb, Hathaway, Ballinger & Licht, 1971); Cophasaurus texanus and Cnemidophorus gularis (Schrank & Ballinger, 1973); Sceloporus magister (Vitt & Ohmart, 1974); Anolis cupreus (Fleming & Hooker, 1975); A. aeneus (Gorman & Licht, 1975); Cnemidophorus tigris (Goldberg, 1976), and Urosaurus ornatus (Michel, 1976).

Although fat bodies generally show a cycle inversely related to the reproductive cycle, sometimes this inverse relationship is either loose as in Sceloporus undulatus (Marion, 1970b), or not significant, as in Urosaurus graciosus (Vitt & Ohmart, 1975). There is no relationship between fat body cycle and the testicular cycle in the lizards Egernia

cunninghami (Barwick & Bryant, 1966), Anolis acutus (Ruibal, Philibosian & Adkins, 1972) and A. trinitatis (Gorman & Licht, 1975); whereas a direct relationship exists between the two parameters in Agama agama from Kenya (Marshall & Hook, 1960) and in Sceloporus scalaris (Newlin, 1976).

Fat body reserves are absent in the Javanese geckos, Hemidactylus frenatus, Cosymbotus platyrus and Peropus mutilatus (Church, 1962); and the Australian skink Hemiergis peronii (Smyth, 1974 - quoted by Fox, 1977).

Volsøe (1944) proposed that fat bodies probably serve as a reserve storage for food, and it might serve as a safety reserve in times of dietary hardship as well (Bellairs, 1970).

Licht and Gorman (1970) suggested three alternatives to explain fat body weight gain: (1) increased food availability; (2) increased appetite; (3) a change in energy partitioning. The latter was considered as the most likely alternative in the Caribbean Anolis lizards.

Reports on liver weight cycle are scanty in male lizards. Telford (1970) reported for Takydromus tachydromoides cyclic changes in liver weights which were inversely correlated with reproduction. In addition, Licht, Hoyer & Van Oördt (1969) reported a relationship between liver weight and temperature and they provided evidence for an inverse relation. The livers were heavier at 20°C than at 30°C. However, Marion (1970b) found no significant association between liver weight decrease and heat increase in the lizard Sceloporus undulatus.

5. Reproductive patterns:-

Within the life span of a lizard, there are usually greater

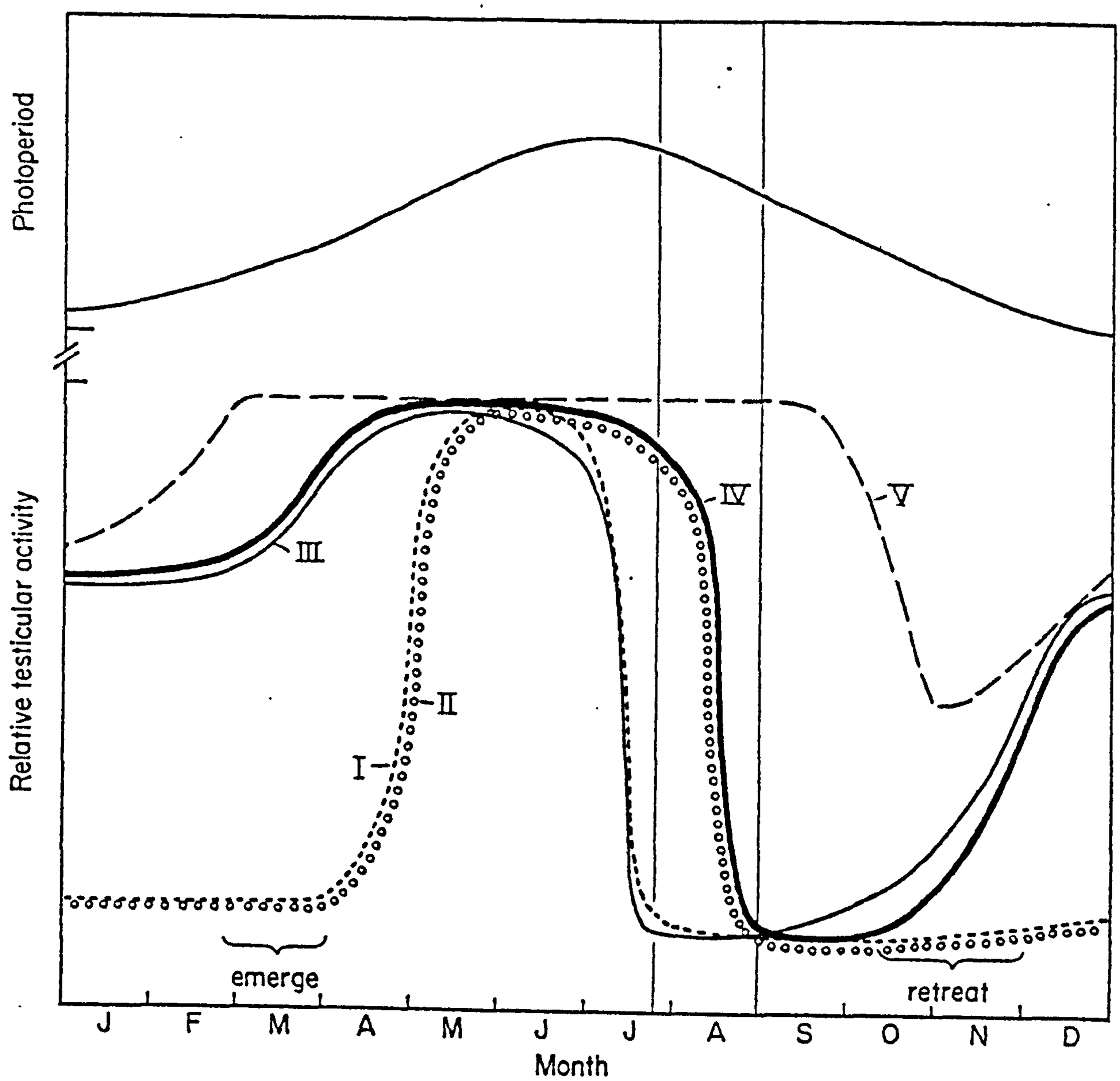
and lesser periods of reproductive activity. Such sexual activity is commonly a seasonal phenomenon, with the actual time of breeding varying from species to species (Marion, 1970b). The breeding season of each species is apparently so tuned as to give the maximum advantage to the young during their early growth period (Farner & Follett, 1966).

In the temperate-zone lizards (the most studied group) a wealth of phenological data has established that most species have a single, relatively short, annual breeding season, followed by a period of complete sexual inactivity with regressed and quiescent gonads (reviewed by Saint-Girons, 1963; Mayhew, 1968; Fitch, 1970; Licht & Gorman, 1970; Licht, 1972). With the exception of some viviparous species that breed in the autumn, most temperate lizards breed during a restricted period in the spring and early summer (Licht, 1972). Typically, breeding is initiated after emergence from "hibernation" in spring; however, considerable interspecific variation is evident in the exact timing of gonadal recrudescence and regression with a consequent difference in the length of the breeding seasons (Licht, 1972). Licht et al. (1969) described four reproductive patterns for temperate lizards (see also Licht & Gorman, 1970; Licht, 1972) which can be categorized into two main groups in relation to testicular recrudescence (Fig. A). These groups are:-

1. Group A (patterns I and II):- Lizards falling under these patterns emerge from hibernation (sometime around March) with relatively small quiescent testes. The latter develop very quickly to attain maximum breeding condition during the spring (about one month after emergence from hibernation). However, the breeding season lasts for a relatively short time, teminating during the first third (pattern I) or the second third

Fig. A

Schematic representation of the diverse patterns of annual testicular activities in lizards. Patterns I-IV represent temperate-zone species and V , some tropical Anolis lizards. After Licht 1972.


Photoperiod
Relative testicular activity
III
II
I
IV
V
emerge
retreat
J
F
M
A
M
J
J
A
S
O
N
D
Month

(pattern II) of summer, to be followed by a period of testicular inactivity until the next breeding season (Fig. A). Accordingly, there is a period of 3-4 months of gonadal activity alternating with a period of 8-9 months of quiescence each year. This type of gonadal activity is exhibited by many species of male lizards as in: Phrynosoma solare (Blount, 1929); Anguis and Lacerta (Herlant, 1933); Eumeces laticeps (Turner, 1935); Anolis carolinensis (Fox, 1958); Uta stansburiana of western Texas (Tinkle, 1961, 1967); Sceloporus orcutti (Mayhew, 1963); Urosaurus ornatus (Asplund & Lowe, 1964); Uma notata, U. inornata and U. scoparia (Mayhew, 1965b, 1966a,b, 1967; Mayhew & Wright, 1970); Cnemidophorus hyperythurus (Bostic, 1966); Agama impalearis (Saint-Girons, 1967); Acanthodactylus erythrurus (Bons, 1969); Calotes versicolor (Choubey, 1970 - quoted by Fox, 1977); Sceloporus malachiticus from Costa Rica (Marion & Sexton, 1971); Dipsosaurus dorsalis (Mayhew, 1971); Uromastyx hardwicki (Arslan, Jalali & Qazi, 1972); Sitana ponticeriana and Calotes nemoricola (Subba Roa & Rajabai, 1972 - quoted by Fox, 1977); Sceloporus magister (Vitt & Ohmart, 1974) and Urosaurus graciosus (Vitt & Ohmart, 1975).

2. Group B (patterns III and IV):- In both patterns spermatogenesis begins in the autumn, after a short quiescent period, and continues gradually through the winter, to reach full activity in the spring and early summer. The short period of regression and quiescence of testes starts around mid-summer in pattern III, but about one month later in pattern IV (Fig. A). Consequently, when lizards of these two reproductive patterns enter hibernation recrudescence is already well advanced. Examples of this group (pattern III and IV) include; Lacerta muralis and L. agilis (Reiss, 1923); Sceloporus undulatus of North Carolina (Altland, 1941);

Eumeces fasciatus (Reynolds, 1943); Sceloporus graciosus (Woodbury & Woodbury, 1945); S. occidentalis (Rodgers, 1953; Wilhoft & Quay, 1961); Uta stansburiana of southeastern Arizona (Asplund & Lowe, 1964); and of Texas (Hahn, 1964); Cnemidophorus tigris from southern Arizona (Goldberg & Lowe, 1966); Hemidactylus turecicus (Rose & Barbour, 1968); Sceloporus undulatus of eastern Missouri (Marion, 1969, 1970b); Phrynosoma douglassi (Goldberg, 1971b); Gerrhonotus multicarinatus (Goldberg, 1972); G. coerulus (Vitt, 1973); Cnemidophorus tigris multiscutatus (Goldberg, 1976) and Sceloporus scolaris (Newlin, 1976).

It is worth mentioning that in both groups (i.e. patterns I-IV) the completion of gonadal development (spermiogenesis and spermiation), and especially the development of other aspects of the reproductive system such as secondary sex characters (presumably reflecting gonadal androgen activity), does not occur until shortly before the breeding season in spring (Licht, 1972).

However, exceptions to the general patterns outlined above are present. For instance, Sceloporus jarrovi breeds in late summer and the testis undergoes a cycle in which maximum size is reached during the autumn (October-November) and the very rapid regression is complete by early winter (December). The testis then remains small until July when recrudescence commences (Goldberg, 1971a). Likewise, Crisp (1964) reported maximal testicular activity in autumn for the lizard Sceloporus cyanogenys. On the other hand, two periods of spermatogenic activity, one in spring and the other in autumn, have been reported for Uromastix (Courrier, 1929).

In tropical and subtropical species of lizard, as may perhaps be expected, there often is a second breeding season in autumn and some

even breed throughout the year more or less continuously (Saint-Girons & Pfeffer, 1971, 1972 - quoted by Fox, 1977). For example, there are no seasonal variations in the spermatogenic cycle of the Javanese geckos, Cosymbotus platyurus, Hemidactylus frenatus and Peropus mutilatus (Church, 1962) or of the Indian lizard, Hemidactylus flaviviridis (Kitada & Asana, 1951). Other tropical species which produce sperms throughout the year with little variation, if any, in testis size include the Australian Leiolopisma rhomboidalis (Wilhoft, 1963a,b); Cyrtodactylus malayanus, C. pubisulus, Draco melanopogon and D. quinquefraciatus from Borneo (Inger & Greenberg, 1966); Agama agama from West Africa (Daniel, 1960) and from Ghana (Chapman & Chapman, 1964).

In many other tropical lizards, although sperm production may be apparently continuous, minor cyclic variations in testis size, weight and/or the quantity of sperms produced during the year are evident. For instance, Anolis trinitatis showed a weak cycle in testis weight (Gorman & Licht, 1975), whereas distinct cycles in testis size and/or level of sperm production have been described for the Indian lizards, Calotes versicolor (Asana, 1931), Calotes rouxi, Mabuya macularia and C. carinata (McCann, 1940); Emoia cyanura and E. werneri from the New Hebrides (Baker, 1947); Agama agama from Kenya (Marshall & Hook, 1960); Mabuya striata from Tanganyika (Robertson, Chapman & Chapman, 1965); seven species of Anolis lizards (Licht & Gorman, 1970); Anolis limifrons from the Isthmus of Panama (Sexton et al., 1971); Anolis acutus (Ruibal et al., 1972); Anolis aeneus from Trinidad (Gorman & Licht, 1975) and the East Indies area (Stamps & Crews, 1976); Anolis oculatus and Ameiva fuscata from Dominica (Somma & Brooks, 1976) and Anolis sagrei from Belize (Sexton & Brown, 1977).

In many of the above mentioned studies there was a correlation between the rainy season and the peak of spermatogenic activity (e.g. Asana, 1931; McCann, 1940; Baker, 1947; Chapman & Chapman, 1964; Ruibal et al., 1972; Stamps & Crews, 1977). However, whether rainfall affects reproduction directly or indirectly (via food availability) is unknown (Chapman & Chapman, 1964).

On the other hand, several tropical species have been reported to exhibit complete cyclic activities with full regression and quiescent phases. For example, Anolis cupreus from Costa Rica is strongly a seasonal lizard in which the testis is active for about six months only (Fleming & Hooker, 1975). Likewise, Agama cyanogaster (from Tanganyika) has regressed testes from February until at least July (Robertson et al., 1965). Similarly, varying periods of regression in the testes have been described in Leiolopisma fuscum (Wilhoft & Reiter, 1965); Amphibolurus isolepis (Pianka, 1971); Anolis tropidogaster and A. auratus from the Isthmus of Panama (Sexton et al., 1971).

II. Factors Affecting the Timing of Reproductive Cycles

Lizards, especially of the temperate zone, typically exhibit pronounced seasonality in their reproductive activity. The factors timing the changes in gonadal activities are various and they vary from species to species. Both "endogenous" and "exogenous" (environmental) controls of annual cycles have been suggested for lizards. In some species, one part of the gonadal cycle, especially regression, may be dependent on endogenous rhythmicity while the other parts are not. However, in certain lizards, seasonal cyclicity in reproductive activity is, essentially, completely dependent on exogenous cycles (reviewed by Licht, 1971a, 1972).

A. Endogenous rhythmicity:-

Endogenous timing is indicated by a change in physiological state which occurs autonomously, and independently of immediate external conditions, even though the exact timing may be modified by external changes. In fact, external cues (Zeitgebers) may be very important for phasing of an internal rhythm (Licht, 1971a).

Several studies on lizards have suggested that endogenous rhythm may underlie testicular and ovarian cycles. However, endogenous rhythmicity seems to be responsible only for a particular part of the gonadal activity, namely regression, and in some species even that can be modified by external cues. Licht (1973) studied the effect of light and temperature on summer regression in two male lizards, Dipsosaurus dorsalis

and Xantusia vigilis. Licht found that testis regression under various photothermal regimes occurred in both lizards at the same time as in nature, and that neither temperature nor photoperiod were effective in modifying the process. Consequently, these two examples illustrate complete endogenous control over this phase of gonadal activity.

Incomplete, or partial, control over endogenous rhythmicity of testis regression has been suggested for Lacerta sicula (Fischer, 1968a, 1970; Licht et al., 1969). In this lizard, regression cannot be prevented by variation of light and temperature, but its timing can be greatly modified by exogenous cues. The onset of regression in Lacerta can be delayed by short day lengths, although its ultimate occurrence seemed to be obligatory.

Endogenous control of gonadal regression and its correlation with exogenous cues have been suggested for male Dipsosaurus dorsalis (Licht, 1971a) and Lacerta muralis (Joly & Saint-Girons, 1975), and for female Sceloporus vigatus (Stebbin, 1963 - quoted by Licht, 1972), Uta stansburiana (Tinkle & Irwin, 1965), Sceloporus undulatus (Marion, 1970a) and the parthenogenic Cnemidophorus uniparens (Cuellar, 1971 - quoted by Licht, 1972).

On the other hand, Fischer (1970) suggested that the initiation of testicular (spermatogenic) activity in Lacerta sicula is probably controlled by endogenous factors.

Although the above observations indicate clearly the existence of endogenous rhythmicity, yet recently Lofts (1978) said that: "it is perhaps premature to conclude from such data (of Fischer, 1968 and Licht, 1971a) that an endogenous circannual reproductive rhythm exists in reptiles; since in many cases in which such claims have been made, information of

other environmental parameters, such as rainfall or seasonal variability of food, is lacking". He suggested that more research needs to be done to demonstrate unequivocally the presence of such endogenous rhythms (Lofts, 1978).

B. Exogenous factors:-

The three major exogenous factors that have been considered by many investigators as timing cues for reproductive cyclicity in reptiles are rainfall, photoperiod and temperature, but there is much controversy about the relative importance of each factor.

1. Rainfall:- Rainfall has been considered, by several workers, to act as the major exogenous cue for timing reproductive activity in certain male lizards. For example, Wilhoft (1963b) and Wilhoft & Reiter (1965), working on two tropical Australian skinks from separate habitats, found no significant differences between temperature and photoperiod in the two habitats. However, there was a great difference in gonadal activity between the two species, since one (*Leiolopisma rhomboidalis*) was a continuous breeder while the other (*L. fuscum*) was not. Comparing the amount of rainfall in each habitat, Wilhoft & Reiter (1965) concluded that this was the exogenous factor responsible for cyclicity in testicular activity of *L. fuscum*. Likewise, Sexton *et al.* (1971), working on three *Anolis* species from the Isthmus of Panama, reported that variations in photoperiod and temperature during the year were insufficient to provide cyclic cues and that variations in precipitation were the only feature likely to regulate reproductive cycles. A similar correlation between the amount of rainfall and reproductive activity has been suggested for male lizards *Agama agama* (Daniel, 1960;

Marshall & Hook, 1960; Chapman & Chapman, 1964), Anolis cupreus (Fleming & Hooker, 1975), and A. aeneus (Stamps & Crews, 1976).

It is generally believed that the effect of rainfall on reproductive activity is an indirect one through the availability of food. Rainfall provides a suitable environment for insects to grow and propagate. Without sufficient amounts of rainfall, the plants upon which insects feed will not grow well. These insects, in turn, provide a very rich food source for lizards, without which reproduction is greatly reduced, if not completely stopped (Mayhew, 1965a; Sexton et al., 1971). In Uma notata and U. inornata (Mayhew, 1965b, 1967), testes did not become active if the amount of food was restricted. This indirect effect of rainfall over reproductive activity has been reported for male Agama agama (Marshall & Hook, 1960), Sceloporus orcutti (Mayhew, 1963) and Uma scoparia (Mayhew, 1966a).

In contrast, other investigators (Licht & Gorman, 1970; Licht, 1971a, 1974b; Gorman & Licht, 1975; Lofts, 1978) believed that rainfall was not a determining cue for testicular activity in temperate-zone and tropical lizards, since testicular cycles appeared to be independent of seasonal rainfall. However, it appears difficult to approve fully their view concerning the importance of rainfall in reproductive activity of lizards, since clear relationships between rainfall and reproduction were evident in several studies.

It is worth mentioning that all the data on the importance of rainfall for reproduction are merely field observations and there are no experimental studies on this factor. The importance of rainfall as a determining cue for male reproductive cycles remains a debatable point.

2. Photoperiod:- The annual variations in daylength affect many physiological features such as appetite, growth and winter dormancy (Dessauer, 1955a,b; Fox & Dessauer, 1957; Mayhew, 1965a,b; Licht, 1971a,b). However, most of the studies have centred on the connection between photoperiods and reproduction.

Several studies of reproductive cycles in male lizards under natural (field) conditions have highlighted the importance of photoperiod and the possibility that it might act as the major cue timing testicular recrudescence. The studies included *Emoia cyanura* and *E. werneri* (Baker, 1947); *Anolis carolinensis* (Fox, 1958); *Agama cyanogaster* (Robertson *et al.*, 1965) and *Hemidactylus turcicus* (Rose & Barbour, 1968). In these studies it was generally found that maximal reproductive activity coincided with the season of longest daylengths, while minimal gonadal activity coincided with shortest daylengths. However, it seems unwise to draw clear-cut conclusions from such field studies since other important factors (e.g. temperature), which are not accounted for, may interfere.

Several experimental studies have suggested that long daylengths may be responsible for timing the onset of testicular recrudescence and growth. For example, Clausen & Poris (1937) reported, in *Anolis carolinensis*, that artificially increased daylengths stimulated testicular growth in the spring. Likewise, Bartholomew (1950, 1953), working on *Xantusia vigilis*, concluded that testicular recrudescence is stimulated by increased photoperiod and that the latter is even more important than temperature in stimulating gonadal activity. In *Lacerta sicula*, Golgano (1951) examined the effect of photoperiod on reproduction and came to a similar conclusion. In addition, Fischer (1968a,b,c,d, 1970), working on the same species, concluded that long day photoperiods are responsible

for the stimulation of testis growth. Mayhew (1964) believed that there was a similar effect of photoperiod in three species of Uma, and he (Mayhew, 1968) drew similar conclusions from the studies of Mellish (1936), Badir (1958) and Badir & Hussein (1964) in which an iguanid (Phrynosoma cornutum) and two scincid lizards (Scincus scincus and Chalcides ocellatus) were stimulated to "out-of-season" reproductive activity by continuous light and high temperature.

All of the above studies, in which stimulation of testicular growth was attributed to increasing photoperiod, have been quite strongly criticised by Licht (e.g. 1971a). In some of them, temperature was not adequately controlled so the possibility that it might be the reason for testicular growth cannot be ruled out. In other studies, both long day photoperiods and elevated high temperatures were used (as in the studies of Mellish, 1936; Badir & Hussein, 1964; Mayhew, 1964; Fischer, 1968a, b,c, 1970), yet stimulation of testes activity was attributed to effects of increased illumination alone. In yet others (Mellish, 1936; Bartholomew, 1950, 1953; Badir & Hussein, 1964; Mayhew, 1964), adequate experimental controls were lacking. Neither a "short-day" control at elevated temperatures, nor a "long-day" at lower temperatures was employed (Licht, 1971a).

On the other hand repeated studies on Anolis carolinensis (Licht, 1966, 1967a,b, 1969b, 1971b), Lacerta sicula (Licht et al., 1969), Xantusia vigilis (Licht, 1973), and the present study on Chalcides ocellatus have provided substantial evidence that temperature (and not photoperiod) is responsible for the stimulation of gonadal growth at the onset of the reproductive cycle.

Having excluded the possibility that stimulation of testicular

growth is controlled solely by photoperiod, what role does the latter play in the gonadal cycle? Several laboratory experiments have demonstrated that both regression and recrudescence are affected, to varying extents, by photoperiods but only at certain temperatures. For example, artificially lengthened daylengths (14L) can initiate and accelerate testicular recrudescence in Anolis carolinensis, but only if body temperatures are high, i.e. at preferred levels (Licht, 1966, 1967a,b, 1971b). Similarly, long days accelerated testicular development in mid-winter in Xantusia vigilis, but only at high preferred temperature (Licht, 1973). On the other hand, the timing of testicular regression in Anolis carolinensis suggests that short or decreasing daylengths (below 13 h) can induce testicular regression, during the summer, even if the temperature is high (Licht, 1966, 1167a,b, 1969a,b). Likewise, in two tropical lizards from Jamaica (Anolis lineatopus) and Florida (A. sagrei) testes became fully regressed when winter temperatures and short days were applied, whereas very little (if any) regression occurred at winter temperatures and long days (Licht, 1974b). Hence, the duration of photoperiod plays a very important role in inducing testicular regression during the summer.

3. Temperature:- Having eliminated the possibility that an endogenous rhythm, or rainfall or daylength acted as the controlling factor in timing the onset of testicular development, we are left with the option that temperature might be the prime 'Zeitgeber'. In fact experimental studies on several species of lizards, including: Lacerta muralis and L. sicula (Licht et al., 1969), Sceloporus undulatus (Marion, 1970b), Anolis carolinensis (Licht, 1971b), and Xantusia vigilis and Dipsosaurus dorsalis

(Licht, 1973), have demonstrated unequivocally that elevated temperatures alone are responsible for the stimulation of testicular growth and development at the onset of reproductive cycles. Any effects that other Zeitgebers may have appear to be entirely dependent on a "permissive" temperature level. For example, long daylengths can accelerate testicular development, but only at high temperature levels (Licht, 1966, 1967b, 1971b; Licht & Pearson, 1970). Moreover, testis regression in Anolis lineatopus (a tropical lizard from Jamaica) occurred on short daylengths only when moderately cool temperatures (ca 20°C) were applied; whereas at constant warm temperatures (ca 32°C) testes remained large and active indefinitely, at all photoperiods (Licht, 1974b).

In addition to its general effect on reproduction, temperature plays a critical role in the process of spermatogenesis and androgenesis in lacertilian reptiles (Licht, 1972; Pearson, Tsui & Licht, 1976). During the development of the spermatogenic cells, an appropriate temperature is essentially needed, especially in the final stages of spermatogenesis. Whereas high temperatures (even 1° or 2°C above the preferred temperature) may be detrimental, low temperatures may cause spermatogenic arrest (see the discussion in Section 2, page 81 for more details). Similarly, the levels of circulating gonadotropic and androgenic hormones are temperature-dependent (see Part III below).

Thus far, temperature appears to be the major factor that regulates reproductive cycles in male lizards.

III. Hormonal Control of Reproduction

1. General:-

It has been long recognized that the control of gonadal function by the pituitary gland hormones (gonadotropins) is a general feature of vertebrate reproductive physiology. Numerous studies on the hormones of eutherian mammals have established the existence of two chemically distinct gonadotropin molecules in the pituitary; luteinizing hormone (LH), which promotes steroid secretion by the gonads, and follicle stimulating hormone (FSH), which is mainly associated with the events involved in gonadal growth and maintenance (Licht, Papkoff, Farmer, Muller, Tsui & Crews, 1977).

Until recently, it was tacitly assumed that the nature and actions of gonadotropins in other vertebrates conformed to this mammalian pattern. However, recent studies on non-mammalian species have produced unexpected results (Licht et al., 1977). For example, in squamate reptiles, on which this review will concentrate, it has been postulated that just a single "complete" gonadotropin is present (Licht & Pearson, 1969a; Licht, 1974c). However, results from direct biochemical studies revealed that two gonadotropins exist in Chelonia and Crocodilia (Licht & Papkoff, 1974; Licht, Farmer & Papkoff, 1976). The details of studies on Squamata alone seems relevant here.

2. Gonadotropin(s) in Squamata:-

Whether squamate reptiles possess a single gonadotropin, which accounts for all the functions that are usually carried out by both FSH

and LH, or two separate gonadotropins, as the other reptilian groups, is still a matter of controversy among investigators.

Various studies, involving adenohypophysectomy and/or hormone treatment in lizards (Licht & Pearson, 1969b; Reddy & Prasad, 1970a,b); bioassay of different regions of the pars distalis of lizard (Licht & Rosenberg, 1969); and, more recently, immunological, biological and biochemical studies on lizards and snakes (Licht, 1974d; Licht _et al._, 1977) have failed to confirm the existence of two separate gonadotropins in squamates. Moreover, the responses of lacertilian gonads to purified mammalian gonadotropins (Jones, 1969, 1973; Licht & Pearson, 1969a; Licht, 1970; Reddy & Prasad, 1970a,b; Eyeson, 1971; Licht & Papkoff, 1971; Jalali, Arslan & Qazi, 1975), as well as non-mammalian gonadotropins (Licht & Donaldson, 1969; Licht & Hartree, 1971) are consistent with the view that a single FSH-like gonadotropin, which accounts for all aspects of gonadal activity in both sexes, may exist in lizards.

On the other hand, _in vivo_ and _in vitro_ studies in which ovine LH was found to be equipotent, or even more potent than ovine FSH in the stimulation of steroid production by lizards' gonads (Eyeson, 1971; Chan & Callard, 1974; Tsui, 1976), have furnished grounds for the concept of two separate gonadotropins in lizards. In addition the temporal separation of gametogenesis and steroidogenesis in the seasonal reproductive activity of many species of lizards and histological studies of lizard pituitaries, have been taken to indicate two separate hormones in the pituitary of lizard, by several investigators (Grignon & Grignon, 1965; Saint-Girons, 1970; Joly & Saint-Girons, 1975).

Since the temporal separation of spermatogenesis and steroidogenesis can be attributed simply to different levels of circulating gonado-

tropins (i.e. quantitative effect; Licht, 1972), and since the stimulation of steroidogenesis by both FSH and LH can be related to target-tissue non-specificity (Tsui, 1976), it appears reasonable to favour the concept of a single FSH-like gonadotropin in lizards (reviews by; Licht, 1972, 1974b, 1979; Licht et al., 1977; Tsui & Licht, 1977; Licht & Pearson, 1978).

In order to avoid complexity and for heuristic purposes, the pituitary principle(s) involved in the reproductive cycles of lizards will be referred to as gonadotropin (GTH; Licht, 1972).

3. Gonadotropin regulation and testicular cycles in lizards:-

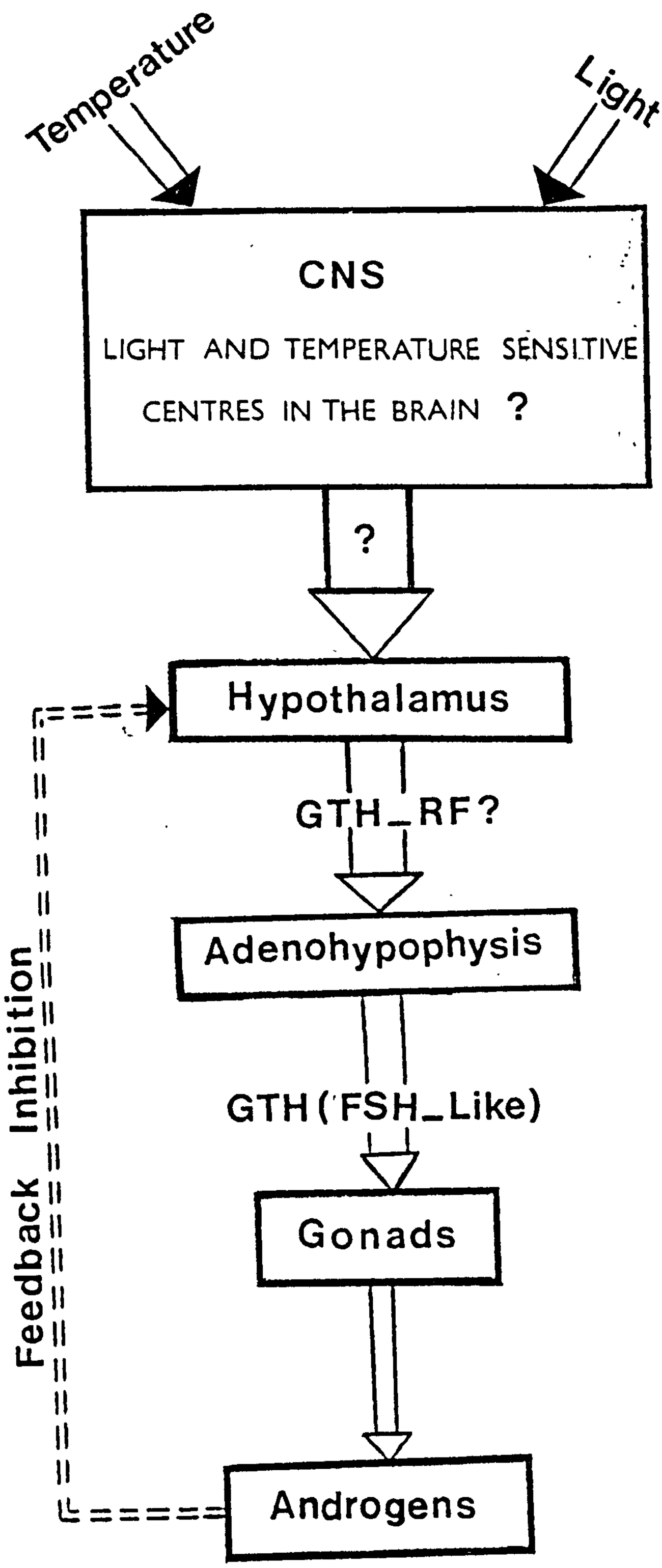
It is well established that gonadal activities in male lizards are controlled by GTH secretions from the pituitary gland. Adenohypophysectomy resulted in testis atrophy, cessation of spermatogenesis, inhibition of spermiation, atrophy of the epididymis and the sexual segment of the kidney (the latter two are indicators for inhibition of androgenesis) (see Licht & Pearson, 1969a,b; Reddy & Prasad, 1970a,b; Eyeson, 1971; Licht & Tsui, 1975).

Utilizing experiments in which warm temperature was found to be primarily responsible for testicular recrudescence, and short days responsible for testicular regression, i.e. GTH waxing and waning, respectively (Licht, 1971a,b, 1972, 1973, 1974c) has lead to the exploration of gonadal activity regulation via GTH.

As Fig. B demonstrates, the seasonal changes in external factors are "measured" by special receptors in the Central Nervous System (CNS). Licht (1969b) suggested extraretinal receptors for photoperiod, but temperature receptors are still, to our knowledge, undefined. The re-

Fig. B

Schematic representation of the (possible) sequencial effect of light and temperature on androgen secretion.


Temperature
Light
CNS
LIGHT AND TEMPERATURE SENSITIVE
CENTRES IN THE BRAIN ?
?
Hypothalamus
GTH_RF?
Adenohypophysis
GTH(FSH_Like)
Gonads
Androgens
Feedback Inhibition

ceptors, in turn, will impose a signal upon the hypothalamus causing it to release a factor. The latter, in turn, will cause the adenohypophysis to secrete and/or release GTH. Although evidence regarding the regulation of GTH is limited, it seems reasonable to conclude that the pituitary gland is controlled by hypothalamic-releasing factor(s), GTH-RF (Licht, 1972).

The GTH will be transported, via circulation, to target tissues or sites, situated in the testis. Using autoradiographic studies, with ^{125}I-labelled human FSH, Licht & Midgley (1976) found that the target cells for this GTH are Leydig cells, Sertoli cells, and spermatogonia. Since both Leydig and Sertoli cells are believed to be sites for androgen production in squamates (see for e.g.; Lofts, 1972; Lofts & Bern, 1972; Tsui, 1976), it is likely that these cells are targets for GTH. However, the possibility that spermatogonia are sites for direct GTH action is still, at present, difficult to understand.

The testis produces steroid hormones (androgens) when stimulate by GTH. When the levels of androgens increase in the circulation, they exert a feedback inhibition on the hypothalamus arresting the release of pituitary GTH indirectly, via GTH-RF inhibition (for "feedback inhibition" in lizards, see Lisk, 1967).

Although the above scheme seems to be reasonable and convincing on the regulation of testicular activity by GTH, this is not always the case during the different seasons of the year. Various data suggested that the CNS exhibits a seasonal refractoriness for such external factors as temperature (Fischer, 1968a,b, 1970; Licht, 1971a). In summary, the external factor(s) that is required for the completion of testicular activity - spermiogenesis, spermiation, and development of accessory sex

structures (androgenesis) - i.e. GTH stimulation, in one season is the same factor which causes testicular regression (inhibition of GTH) in another season (Licht, 1972). For example, in male Lacerta sicula warm body temperatures (33°C) are required for the completion of spermiogenesis, spermiation and androgenesis in the spring, but these same warm temperatures suppress recrudescence in the autumn and winter (Licht et al., 1969). Thus, the CNS exhibits a seasonal refractoriness to warm temperature, the same stimulus which ultimately triggers breeding in Lacerta sicula (Licht et al., 1969). This CNS refractoriness is apparently a widespread phenomenon among lizards since there is also evidence for it in male Dipsosaurus dorsalis, Xantusia vigilis and Sceloporus occidentalis, and female Xantusia vigilis, Sceloporus occidentalis, S. undulatus, Uta stansburiana and Cnemidophorus uniparens (reviewed by Licht, 1972). Moreover, studies with exogenous GTHs (Licht & Pearson, 1969a; Licht, 1972, 1975) indicate that a similar refractoriness to GTH occurred in the testis at low temperatures. Testis inactivity at 20°C may be due to thermal effects on gonadal responses to GTH rather than a change in circulating GTH levels. In fact, the histological structure of GTH cells indicate an endogenous increase in hormone output at lower temperature; 20°C (Pearson et al., 1976).

A direct effect of temperature on the physiology of the pituitary gonadotrophes, as well as, the possibility that temperature may alter endocrine responses by modifying the rate of peripheral hormone metabolism (related to half-life) are awaiting investigation (Licht, 1972).

Having ascertained that GTH stimulates androgen production by the testis, what are the functions of these steroids? In fact, androgens (especially testosterone) are said to have the following actions:-

a. Spermatogenic promotion:- In mammals, Steinberger (1971) suggested that testosterone controls spermatogenesis through the control of spermatogonial development and spermatid formation. In reptiles, testosterone has been reported to slightly stimulate or even promote spermatogenesis (Forbes, 1941; Risley, 1941; Ramaswami & Jacob, 1963). In addition, Lofts & Chiu (1968) presented data in a lizard (Gecko gecko) which suggests that testosterone regulates both spermatogonial formation and spermiogenesis (sperm formation from spermatids). In snakes, it is believed that steroid hormones produced by Sertoli cells may act locally to stimulate spermatogenesis, while circulating androgens produced by Leydig cells act in the main on accessory sex organs and probably have little influence, if any, on the germinal epithelia (Lofts, 1972; Lofts & Bern, 1972). Moreover, recent studies (Arslan et al., 1978; Courty & Dufaure, 1979) may be utilized to support, partially, the hypothesis of Steinberger (1971), but in lizards. In both studies, testicular androgen levels showed a peak prior to the onset of the new testicular cycle, without a subsequent effect on accessory sex organs. In other words, the levels of androgens in testicular homogenates showed a peak directly prior to the onset of testicular activity, while the circulating levels of androgenic hormone did not exhibit a similar peak during the same period, and their levels were low (Aslan et al., 1978; Courty & Dufaure, 1979).

b. Epididymal secretions:- In squamate lizards, many studies have indicated that the activity of epididymal epithelia is under the control of androgens; testosterone in particular (Miller, 1959; Prasad & Sanyal, 1963; Ramaswami & Jacob, 1963; Jones, 1973; Gigon & Dufaure, 1977; Dufaure & Chambon, 1978).

On the other hand, recent studies on lacertilians has shown that epididymal epithelia secrete proteinous granules during their period of activity. Moreover, the formation and secretion of these granules is under the control of androgens (Gigon & Dufaure, 1977, 1980). It is believed that spermatozoa in lizards, as in mammals (see Cameo & Blaquier, 1976), undergo maturation while passing in the epididymis and that these granules serve as supportive and nutritive media for the sperms (Gigon & Dufaure, 1977).

c. Secretions of the sexual segment of the kidney:- The activity of the sexual segment of the kidney has been found by many investigators to be under the supervision of androgen hormones (Prasad & Sanyal, 1963; Ramaswami & Jacob, 1963; Reddy & Prasad, 1970a; Prasad & Reddy, 1972; Jones, 1973; Del Conte, 1974). In addition, the presence of secretory granules in the sexual segment, coupled with their chemical composition has been well established, in squamates, by means of histological, electron microscopic, and cytochemical studies (Prasad & Reddy, 1972; Del Conte & Tamayo, 1973; Gouder & Nadkarni, 1974; Kühnel & Krisch, 1974). These secretory granules are rich in phospholipid, acid phosphatase (Prasad & Reddy, 1972) and esterase (Kühnel & Krisch, 1974).

Early studies on squamate reptiles have suggested that the granules secreted by the sex segment may act as a nutritive source, activating agents (the acid phosphatase in the secretion may activate the sperms), sperm transporting media, or as coagulating materials that block the female cloaca after copulation (Volsøe, 1944; Bishop, 1959). However, recent studies have suggested that the secretory granules of the sexual segment of the kidney may perform a dual function; transporting and capacitating the sperms. It was proposed that the granules mix with the

sperms on their arrival and thus increase the viscosity of the seminal fluid enabling it to pass through the incompletely closed groove of the hemipenis, i.e. sperm transporting media. In addition, the secretory granules are utilized by the spermatozoa inside the female genital tract, after copulation (Prasad & Reddy, 1972; Kühnel & Krisch, 1974).

Another possibility is that the sexual segment may perform, at least partially, an excretory function; voiding consumed androgens (Gouder & Nadkarni, 1974); this possibility is still awaiting substantial proof from work on other reptilian forms.

S E C T I O N 1

THE REPRODUCTIVE CYCLE IN MALES OF THE LIZARD, *CHALCIDES OCELLATUS*

Summary

The lizards, Chalcides ocellatus Försk, were collected from the wild, in areas around Cairo in September 1977 and April 1978. They were maintained under artificial "daylight" conditions with 14 hours light and a high temperature of 31°C ± 2°C. Based on relative weights of the gonads and the state of spermatogenesis, reproductive activity can be divided into three phases; Recrudescence, from early October until November; (2) Full activity, from November/December until the end of April; (3) Quiescence, May to the end of September.

Activity in seminiferous tubules and epididymes is directly related to changes in gonad weights, and all three parameters follow a cyclic pattern. In a series of cross sections of testes, the number of Leydig cells per unit area and the thickness of the tunica albuginea vary inversely with testicular activity. Neither the fat body nor the liver weights showed any direct relationship to testis activity and both were acyclic.

A comparison between the natural reproductive cycle previously described (Badir, 1958) and the cycle under constant laboratory conditions, as described here, revealed a marked temporal shift in two of the three phases. In nature recrudescence lasts at least three months longer than under experimental conditions, whereas the full activity phase is at least three months shorter in nature than in the laboratory. Quiescent phase under natural and laboratory conditions lasts for, approximately, the same time. Thus, the cycle becomes shorter than 12 months under laboratory conditions.

Although constancy in the environment can cause a marked shift in the natural phases of reproductive activity, both recrudescence and quiescence started at the same time as in nature, indicating that reproduction in Chalcides males is controlled by both exogenous and endogenous cues.

Introduction

Reproductive cycle in male lizards under natural conditions have been studied extensively. Dalcq (1921) appears to have been the first to describe the male sexual cycle in a lizard, Anguis fragilis. Other lizards studied are: Lacerta muralis and L. agilis (Reiss, 1923a, b); Phrynosoma solare (Blount, 1929); Platydactylus muralis (Herlant, 1933); Anolis carolinensis (Evans & Clapp, 1940; Fox, 1958); Sceloporus graciosus (Woodbury, 1938; Woodbury & Woodbury, 1945; Rodgers, 1953); S. undulatus (Altland, 1941; Marion, 1970b); S. occidentalis (Rodgers, 1953; Wilhoft & Quay, 1961); S. orcutti (Mayhew, 1963); Chalcides ocellatus and Scincus scincus (Badir, 1958); Agama agama (Daniel, 1960); Acanthodactylus erythrurus (Bons, 1969); Urosaurus ornatus & Uta stansburiana (Asplund & Lowe, 1964); Anolis trinitatus and A. aeneus (Gorman & Licht, 1975); A. sagrei (Sexton & Brown, 1977) and many other lizards.

However, study of the reproductive cycle under laboratory conditions has generally been confined to short periods (one-five months in the majority) usually covering only one phase of the reproductive cycle (e.g. Mellish, 1936; Clausen & Poris, 1937; Miller, 1948; Bartholomew, 1950, 1953; Fox & Dessauer, 1958; Mayhew, 1964; Licht, 1966, 1967a,b, 1968, 1969b; Licht et al., 1969; Fischer, 1968a; Tinkle & Irwin, 1965).

Studies of the reproductive cycle in lizards over a whole year under laboratory conditions are very scanty. In fact the present study is, to our knowledge, the first detailed report in which lizards were kept for one year (or more) under laboratory conditions of constant light

and temperature.

In the lizard Chalcides ocellatus, the reproductive cycle of the male under natural conditions has been studied by Badir (1958) who also reported some investigations under experimental conditions of light and temperature (Badir, 1955, 1958; Badir & Hussein, 1964). Badir and his colleagues have suggested that in the laboratory a complete shift in the cycle can be brought about, from the natural cyclic activity (Badir, 1958) to an acyclic pattern under experimentally prolonged "day-light" periods of illumination and high temperature (Badir & Hussein, 1964). Unfortunately in none of these publications was there much information concerning the number of animals used each month, when the experiments began and finished, the length of photoperiod applied or the temperature regime maintained. Moreover, there were no data presented to allow analysis or conclusions to be drawn.

We felt it essential to establish details of the reproductive cycle under (presumed) optimal conditions of light and temperature over one full year, at least, in the male lizard Chalcides ocellatus. This is important firstly to confirm (or otherwise) the studies of Badir and his colleagues and secondly to provide a basis for further laboratory studies on this lizard. The latter point is of great importance since Chalcides ocellatus is one of the very few lizards, reported so far, that can survive and breed in captivity for a relatively long period of time (several years). In addition, Chalcides ocellatus can be kept easily under laboratory conditions, and it seems an ideal animal to serve as a representative for general studies on this group of reptiles.

Materials and Methods

Lizard collection:-

Chalcides ocellatus Försk, a viviparous scincid lizard, occurs in many localities around the Mediterranean and is common in Egypt. It is found in upper and lower Egypt as well as in the oases, not only in open country, the alluvium of the Nile and desert margins, but also in towns and villages (Badir, 1958).

The animals needed for this experiment were collected, by a commercial dealer, on two occasions; at the beginning of September 1977, and during the first week of April 1978. In both cases lizards were collected from the same area; Abu-Rawash near the Pyramids of Egypt. The animals were then brought directly to the Zoology Laboratories, U.C.N.W., within a day of the final collection.

Husbandry of animals:-

As soon as possible on arrival, the lizards were placed into large containers furnished with sand to allow recovery and provide some quarantine. Sufficient food and water were available at all times. The animals were left at room temperature and under natural daylight inside the laboratory. These conditions were maintained for several days until it was considered that the animals had recovered their activity completely. However, examination of the animals revealed that they were infected with ticks and mites. Several treatments were used to overcome this problem and these are detailed elsewhere (see Dehlawi, 1981). In short, the animals were dipped individually in an aqueous solution of 2.5% of malathion

for 2 minutes. This treatment proved to be the most effective in killing the ticks and mites and was harmless to the animals. The animals were then sorted into groups in large containers each of which contained about 25 lizards, males and females. The dimensions of the containers were 120 x 60 x 60 cm and each was furnished with a layer of sand (8 cm in depth) and one or more large stones. A 60-Watt light bulb was fixed about 15 cm off the sand, as a local heat source. The temperature of the sand directly under the bulbs was approximately 45°C and the lizards were able to choose the preferred temperature within this "gradient" thus created. The room housing the containers was regulated in "day-light" at 31°C ± 2°C, and fluorescent tubes provided a general high light intensity. These conditions of heat and light lasted for 14 hours (starting at 5.00 a.m.) every day for the whole period of the experiment. In the dark period of the day, the temperature was allowed to fall to about 20°C. The lizards were fed a variety of mealworms, fruits, vegetables (e.g. beans), but mostly maggots and boiled hen s'eggs. Because U.V. light was not supplied, a group of vitamins (mostly vitamin D) were provided with the food in sufficient amounts at three day intervals. Water was present at all times.

It is worth mentioning that the animals of both collections were kept under the above mentioned conditions for four months before the first sacrifice was made. This means that animals of the first collection (September 1977) and the second collection (April 1978) were not sacrificed until the beginning of February and September 1978, respectively. This was to ensure that the animals became well adjusted to the prevailing conditions.

Lizard autopsy:-

Groups of 8-10 male lizards were collected at monthly intervals (from different containers), starting in early February 1978 through to January 1979. In addition two smaller groups of six and three males were autopsied during February and March 1979, respectively. Each group was selected to be as uniform as possible. Body weights and snout-vent lengths (SVL) were recorded for all animals autopsied. This is to ensure that only mature males were taken. Males measuring 90 mm or more were considered to be mature (Badir, 1958).

Animals were killed by decapitation and as much serum as possible was collected individually (for radioimmunoassay, RIA, described in section 3) in heparinized small plastic tubes. One testis (right side) from each lizard was frozen in liquid nitrogen, after recording weight (to the nearest 0.1 mg), length and greatest width (to the nearest 0.1 mm). These testes were kept at -70°C until later examination of enzyme and steroid hormone content (see section 3 for RIA and section 4 for histochemistry studies on testes). The weights of fat bodies and livers were recorded for each lizard. The left testis and epididymis were processed individually for routine histology.

Although all portions of a given testis were, more or less, at the same stage of spermatogenic activity, most sections used for analysis were obtained near the centre of each testis. The seminiferous tubule diameter and epithelial height, the epididymal diameter and epithelial height were measured for ten sections in each testis. Ten of the roundest tubules and epididymides were selected for measurements since these were most obviously cross sections. A mean was determined for each testis from

these measurements and these means were used in statistical analysis.

The stages of spermatogenesis were determined by the most advanced cell type present at the luminal margin of the tubules. Classification of spermatogenic stages was modified from the method of Mayhew & Wright (1970) for lizards of the genus Uma. According to this method, there are eight spermatogenic stages (Table 1.1). The 5% level has been used in all tests of statistical significance.

Table 1.1

Classification of the eight spermatogenic stages of testis development in Chalcides ocellatus. Note that Stage 1 may show similarity to stages 2-4, but lumen is absent. Modified from Mayhew & Wright (1970)

Stage	Spermatogenic Condition
1	Division of germinal cells, without the development of a lumen
2	Primary spermatocytes at luminal margin
3	Secondary spermatocytes at luminal margin
4	Undifferentiated spermatids at luminal margin
Early 5	Very few metamorphosing spermatids at luminal margin
Late 5	Many metamorphosing spermatids at luminal margin
6	Mature sperms in lumen (full activity condition)
Early 7	First signs of early regression - little cellular debris in lumen
Late 7	Much cellular debris in lumen
8	Complete regression - no cell division, no lumen

Results

1. Gonadosomatic index

Changes in the gonadosomatic index (GSI; right testis weight in mg/100 gm of body weight) in Chalcides are shown in Table 1.2 and Fig. 1.1. The use of GSI instead of actual testis weight allows a correction for differences between the lizard body weights. As Table 1.2 shows, Chalcides ocellatus exhibits an increase in GSI from February to March ($P<0.005$) when it reaches its maximum. This is followed by a sharp decline until May ($P<0.001$). The GSI decline continues in the following months to reach minimal value in August. However, the decline from May to August is neither sharp nor significant. From August onwards the value of GSI rises continuously. There is a small, but not significant, increase from August through to October, a sharp increase in November followed by a further small insignificant rise in December. Although there is an apparent decline in the GSI value from December through to February, this is not considered an indication of low testis activity (see the observations on spermatogenic activity, page 47). A peak in the GSI is again seen during the next March (Table 1.2).

Apparently, there are two peaks of activity in the value of GSI during December and March, with a single low point of inactivity occurring in August.

Table 1.2

Fluctuations in the testis and epididymis during the year in the lizard Chalcides ocellatus (Mean ± S.E.M.). For the numbers of animals used each month refer to Table 1.3

Sample time	GSI	SCA	Seminiferous tubule		Epididymis		Tunica thickness (μm)
			Diameter (μm)	Epithelial height (μm)	Diameter (μm)	Epithelial height (μm)	
1978 Feb.	309.0 ± 19.8	5.9	178.1 ± 8.2	51.3 ± 4.4	171.6 ± 3.9	45.0 ± 3.0	9.5
Mar.	449.5 ± 34.1	5.9	227.6 ± 28.9	65.4 ± 9.0	199.4 ± 9.1	56.2 ± 1.3	7.0
Apr.	258.2 ± 34.6	6.1	161.8 ± 17.5	47.6 ± 2.9	160.5 ± 23.5	40.7 ± 4.7	10.0
May	174.2 ± 31.4	6.8	141.0 ± 27.8	40.3 ± 7.8	143.5 ± 7.7	36.6 ± 6.4	12.0
June	160.0 ± 29.5	7.0	134.6 ± 33.7	38.6 ± 7.4	136.5 ± 14.9	32.2 ± 11.8	15.2
July	160.6 ± 35.0	7.3	130.4 ± 25.1	39.5 ± 9.5	131.5 ± 11.5	31.9 ± 4.6	15.3
Aug.	94.9 ± 13.6	7.6	104.3 ± 7.4	34.1 ± 1.2	124.1 ± 11.4	27.0 ± 2.1	18.9
Sept.	174.9 ± 44.3	7.0	138.0 ± 10.3	39.0 ± 1.6	127.0 ± 10.8	32.7 ± 2.8	15.1
Oct.	136.1 ± 19.5	4.9	144.4 ± 7.6	43.0 ± 1.7	122.9 ± 12.4	32.6 ± 3.8	15.4
Nov.	393.1 ± 52.0	5.4	195.1 ± 7.4	55.7 ± 3.3	178.9 ± 16.3	49.7 ± 4.1	8.9
Dec.	441.2 ± 34.0	5.9	222.5 ± 10.4	63.7 ± 4.6	199.0 ± 10.6	52.9 ± 2.7	7.0
1979 Jan.	395.8 ± 45.4	5.9	199.9 ± 9.2	51.4 ± 3.7	194.4 ± 8.1	48.8 ± 2.5	9.0
Feb.	324.8 ± 19.4	6.0	187.8 ± 8.8	44.6 ± 3.4	184.1 ± 3.4	48.2 ± 1.4	8.5
Mar.	479.0 ± 27.3	6.0	218.9 ± 7.4	59.6 ± 4.8	207.4 ± 11.4	60.6 ± 3.0	7.6

GSI: Gonadosomatic Index (right testis weight (mg)/100 gm of body weight)

SCA: Spermatogenic condition average of the testes

μm : micrometre

Fig.1.1

Variations in the gonadosomatic index (GSI; testis weight in mg / 100 gm of body weight) during 12 months under standard conditions of light (14h) and temperature (31 C). Mean and SEM are indicated for each sampls .

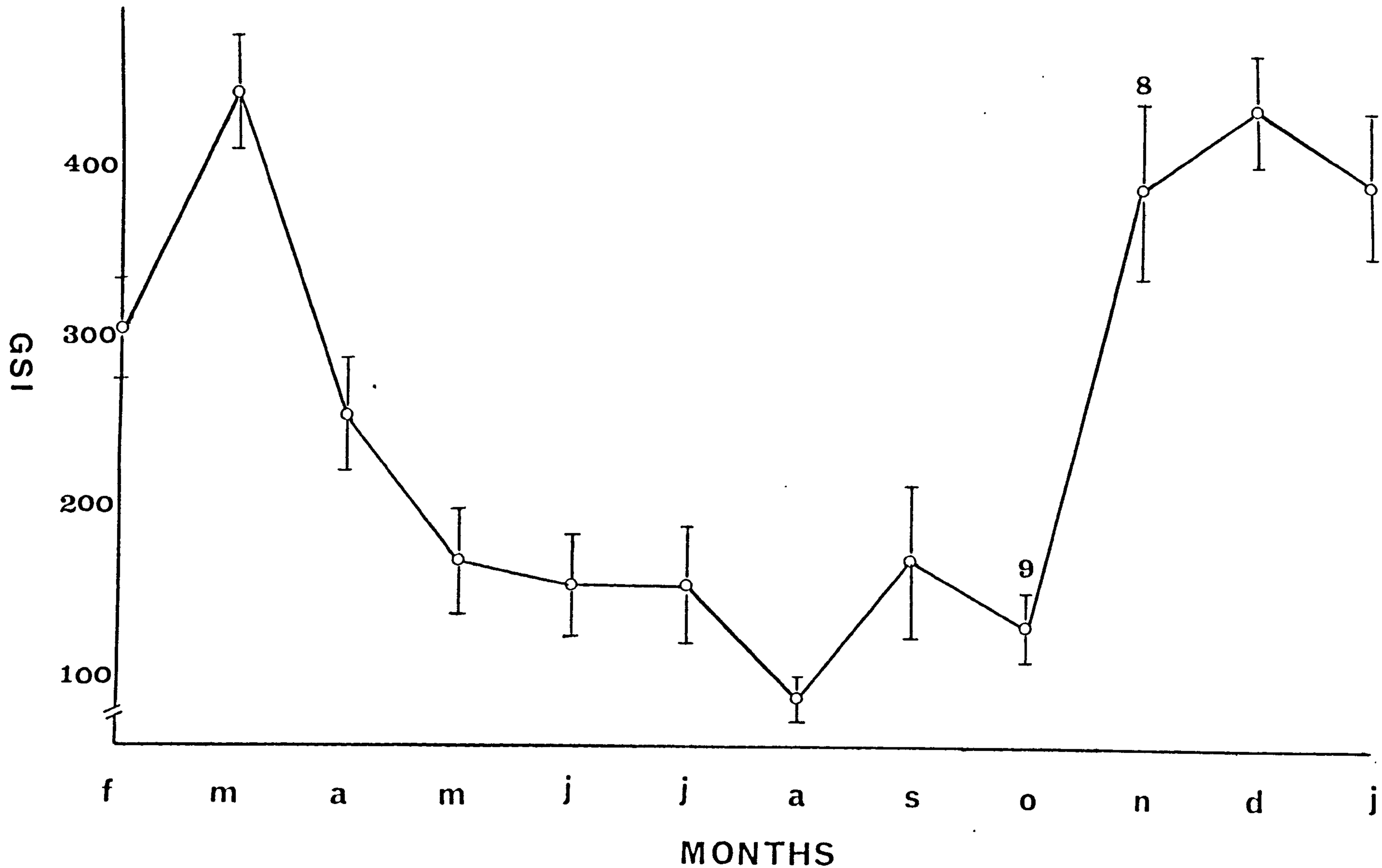

GSI
400
300
200
100
8
9
f
m
a
m
j
j
a
s
o
n
d
j
MONTHS

Fig.1.2

Cross sections of Chalcides testes showing the eight spermatogenic stages. See page 45 & 46 for full details. Sections were cut at 7μm and stained with Ehrlichs haematoxylin and eosin. All figures are of the same magnification and the scale line (in fig.1.2b)= 50μm.

a. Stage1: Note the absence of lumen.

b. Stage2: Note the first appearance of lumen and the presence of primary spermatocyte on luminal margin (arrow).

c. Stage3: Note secondary spermatocytes on luminal margin (arrow).

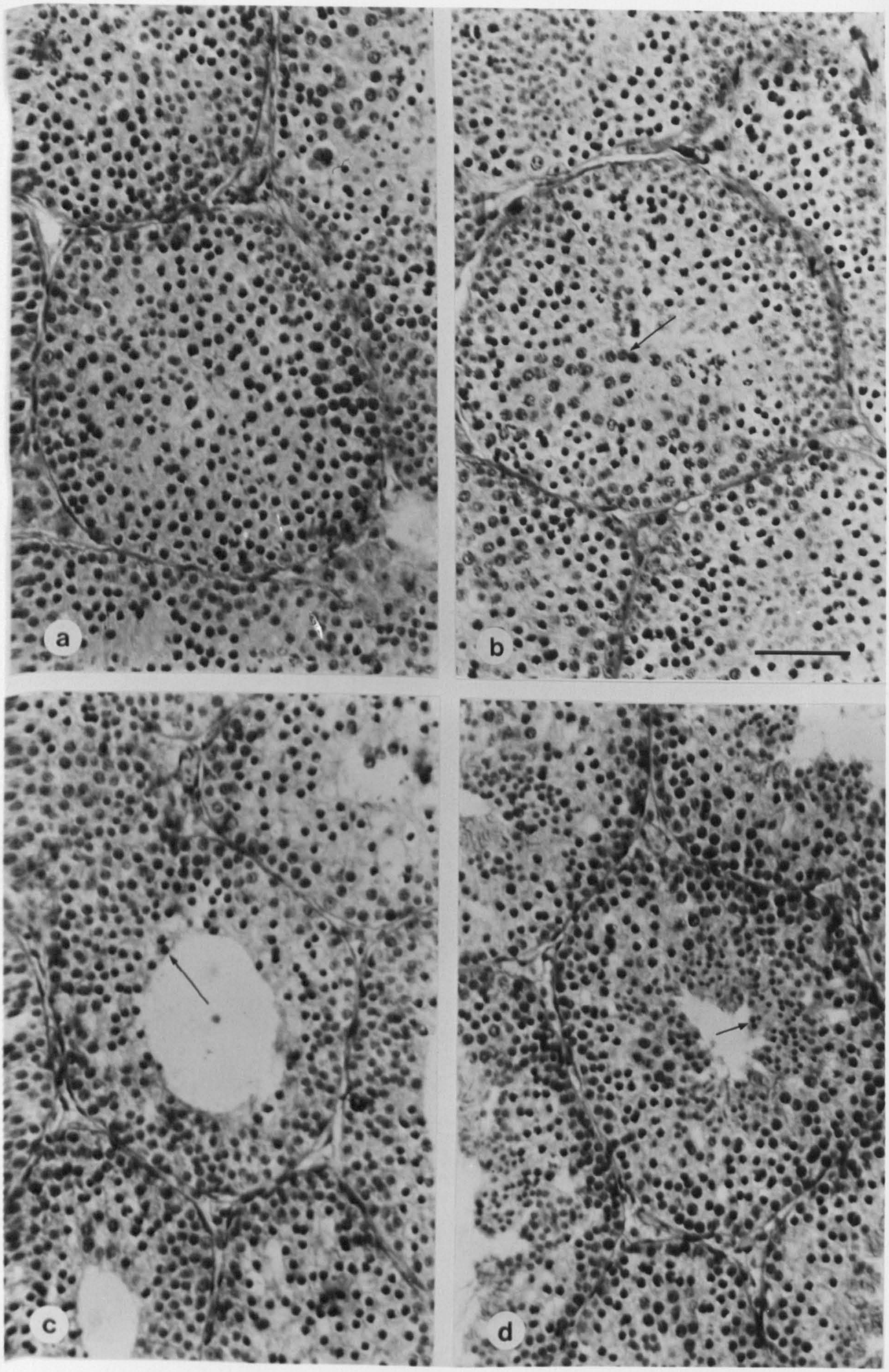
d. Stage4: Note the undifferentiated spermatids (arrow) on luminal margin.

e. Stage5: See the metamorphsing spermatids (arrows) on luminal margin.

f. Stage6 (full activity): Note the presence of many sperms on luminal margin.

g. Stage7: Note the presence of cellular debris (arrows) in the lumen.

h. Stage8 (complete regression): Note the small size of seminiferous tubules (ST) and the absence of lumen. See also the massive layer of Leydig cells (L) surrounding the tubules. Sp , spermatogonia ; S , Sertoli cell.


a
b
c
d

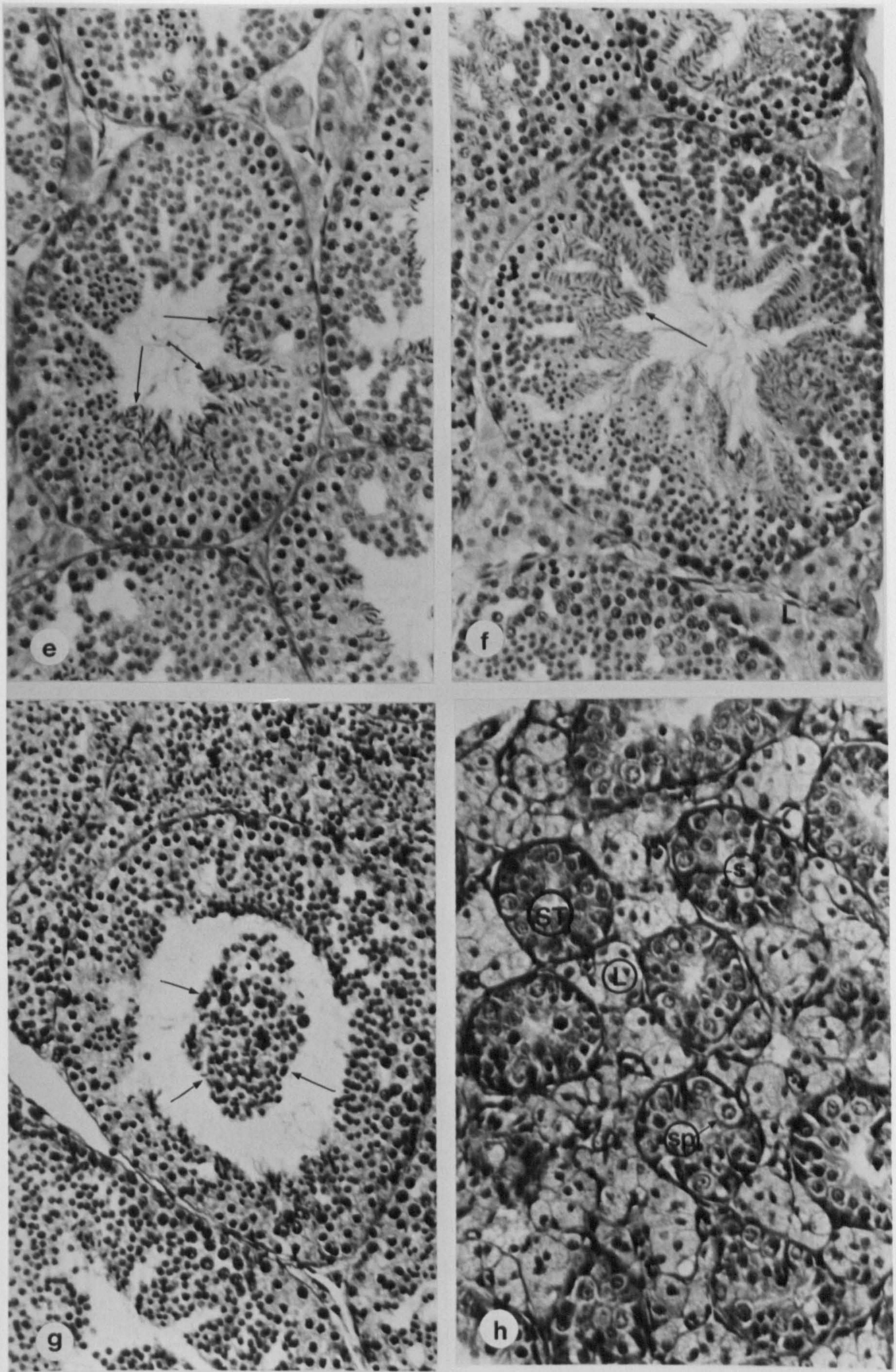


Fig 1.2

2. Spermatogenic stages

The stages of spermatogenic activity used to classify *Chalcides* were modified from the method of Mayhew & Wright (1970), and are listed in Table 1.1 and Fig. 1.2. According to this method there are eight spermatogenic stages:

Stage 1: The seminiferous tubules may contain any type of cells from spermatogonia up to secondary spermatocytes, but neither spermatids nor spermatozoa appear in this stage. Also the lumen is completely absent from the seminiferous tubules at this stage (Fig. 1.2a).

Stage 2: The seminiferous tubules at this stage are characterized by the first appearance of a lumen. The epithelia are composed of several rows of primary spermatocytes, but no more advanced cell types are present on the luminal margin (Fig. 1.2b).

Stage 3: The tubules are characterized by the presence of secondary spermatocytes at the luminal margin. There are several rows of secondary spermatocytes and the cells are still proliferating (Fig. 1.2c).

Stage 4: In this stage, undifferentiated spermatids are present on the luminal margin. Other aspects of epithelial development are as previously (Fig. 1.2d).

Stage 5 (early): In this stage a few metamorphosing spermatids are present at the luminal margin, but no mature sperms are present as yet (Fig. 1.2e).

Stage 5 (late): Many metamorphosing spermatids are present at the luminal margin.

Stage 6 (full breeding condition): Mature sperms are present at the luminal margin and in the tubule lumen (Fig. 1.2f).

Stage 7 (early): The first signs of regression are evident. There is some cellular debris in the lumen, but the animal is still reproductively active (Fig. 1.2g).

Stage 7 (late): The tubule shows much cellular debris in the lumen and the cells are sloughing heavily, leaving behind a very thin epithelium which contains spermatogonia, Sertoli cells and a few spermatocytes. Although the tubular diameter is large at this stage, the tubule is almost hollow.

Stage 8 (complete regression): The tubule is very small with convolutions appearing as "septa". The lumen is closed and the epithelium is composed only of spermatogonia and Sertoli cells. No signs of spermatogonial divisions are present (Fig. 1.2h).

In all the stages other than stage 5 (late), 6, and 7 (early), the epididymis and the sexual segment of the kidney are small, inactive and convoluted. The tubules are formed from cuboidal cells which are not secretory. However, they became very large, active, and with tall, columnar cells during stages 5 (late), 6, and early 7.

3. Seminiferous tubules

The pattern of changes in the seminiferous tubules is similar to that of the GSI (Table 1.2, Fig. 1.3). The maximal diameters and epithelial heights of the tubules are achieved during March. At that time the tubules were very large and the epithelia showed full activity (stage 6). All the germinal cell types are present, from spermatogonia

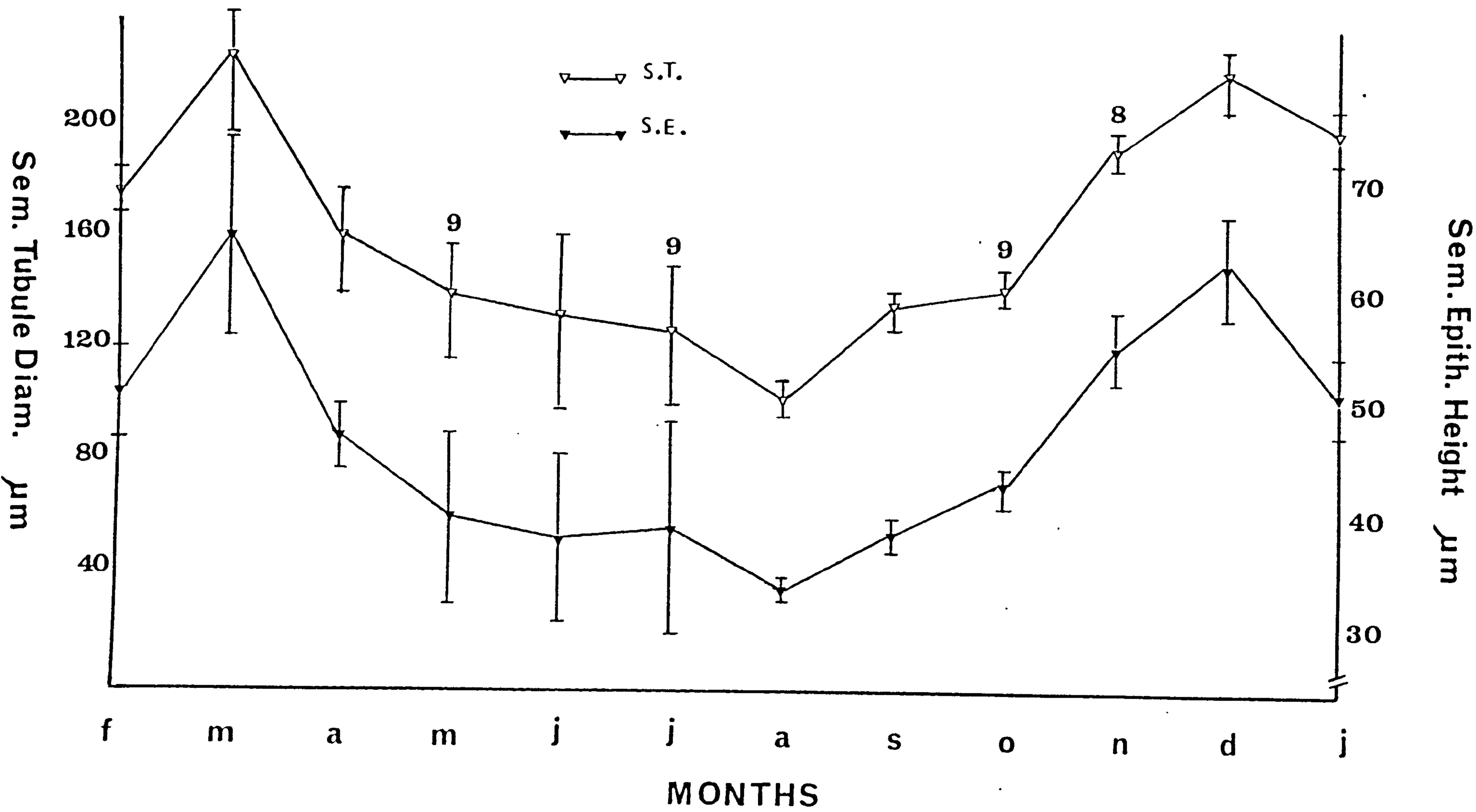
up to sperms in lumen, with the majority being spermatid, and spermatozoa. In a given area of cross section, interstitial cells are, apparently, few in number in this stage (stage 6), occupying the small spaces between the large tubules (Fig. 1.2f). After March there is a continual decline in both the diameter and the epithelial height of the tubules to reach minimal values in August. The decline from March through to May is sharper than that from May through to August, in both tubule components. In August, the testes are completely regressed with very small tubules comprising only Sertoli cells and spermatogonia with an occluded lumen (stage 8; Fig. 1.2h). Moreover, the interstitial cells in a given section area (at stage 8) appear relatively very numerous, forming a continuous tissue surrounding the tubules (Fig. 1.2h). The rise in the diameter and epithelial height of the tubules from August through to December occurs at two levels. There is a smooth, insignificant, increase in both tubules parameters from August to October then a relatively sharp significant rise to December. Although the diameters and epithelial heights of the tubules decrease from peaks in December through to February, yet the spermatogenic activity remains at a more or less constant high level from December to March. Tubular diameter and epithelial height reach a second peak in March. Table 1.2 and Fig. 1.3 summarize the changes in the seminiferous tubules.

4. Spermatogenic condition

As explained earlier (see Materials and Methods, and Table 1.1), there are eight spermatogenic stages exhibited by the tubules. Because not all the animals in a given month are exhibiting the same level of

Fig. 1.3

Variations in seminiferous tubule diameters (S.T.) and seminiferous epithelial height (S.E.) during one year. Photo-thermal conditions are as in Fig. 1.1. Data represent the mean $\pm$ SEM of 10 animals except where indicated by numbers.


S.T.
S.E.
Sem. Tubule Diam. µm
200
160
120
80
40
Sem. Epith. Height µm
70
60
50
40
30
9
9
9
8
f
m
a
m
j
j
a
s
o
n
d
j
MONTHS

spermatogenic development, a monthly "average" is considered. Consequently, a monthly "average" of over 6.0 indicates that the population is undergoing a decline in reproductive activity, whereas, an "average" of less than 6.0 points to recrudescence in the testes. The full-activity condition and the full-regression state are at stage 6 and 8, respectively. Although differences in the spermatogenic state exist between lizards in the same month, we will be mostly considering in this section, and in the discussion, the spermatogenic "average" of each month rather than individual animals. However, since the monthly "average" is not very accurate, the spermatogenic status of individual animals will be pointed out where it is necessary. Table 1.3 shows the percentages of lizards at the different spermatogenic stages.

In February and March, 90% of the lizard population exhibited full activity with an "average" stage of 5.9. The other 10% in both months is still at stage 5. After March the activity of the testes declines, since 20% of the lizards are in early regression (stage 7) during April. Two-thirds of the population exhibit early and late regression (stage 7 and 8) during May. The percentage showing advanced regression increases from 70 to 80 to 100% during June, July, and August, respectively. The animals autopsied in September present a confusing picture. Three lizards (out of ten autopsied) exhibited fully active testes (stage 6). This situation is puzzling since none of the August samples showed such activity. However, on the basis of the monthly "average", the state of regression seen during August is considered to be sustained in September since the "average" is 7.0. The first signs of a new testicular cycle does not come until early October, when testes exhibiting stage 2, 3, and 4 are present and the "average" is 4.9. The

Table 1.3

Percentages of adult lizards exhibiting various spermatogenic stages in different months

Months	Spermatogenic stage								Mean stage	No. of animals
	1	2	3	4	5	6	7	8		
1978										
Feb.					10	90			5.9	10
Mar.					10	90			5.9	10
Apr.					10	70	20		6.1	10
May						33.3	55.6	11.1	6.8	9
June						30	40	30	7.0	10
July						22.2	22.2	55.6	7.3	9
Aug.							40	60	7.6	10
Sept.						30	40	30	7.0	10
Oct.		11.1	22.2	11.1		33.3	22.2		4.9	9
Nov.		12.5		12.5	12.5	62.5			5.4	8
Dec.					10	90			5.9	10
1979										
Jan.					10	90			5.9	10
Feb.						100			6.0	6
Mar.						100			6.0	3

average increases in the following month to reach 5.4. Full spermatogenic activity is attained in December when all testes, but one, showed stage 6. This condition of full spermatogenic activity prevails through to the next March (Table 1.3).

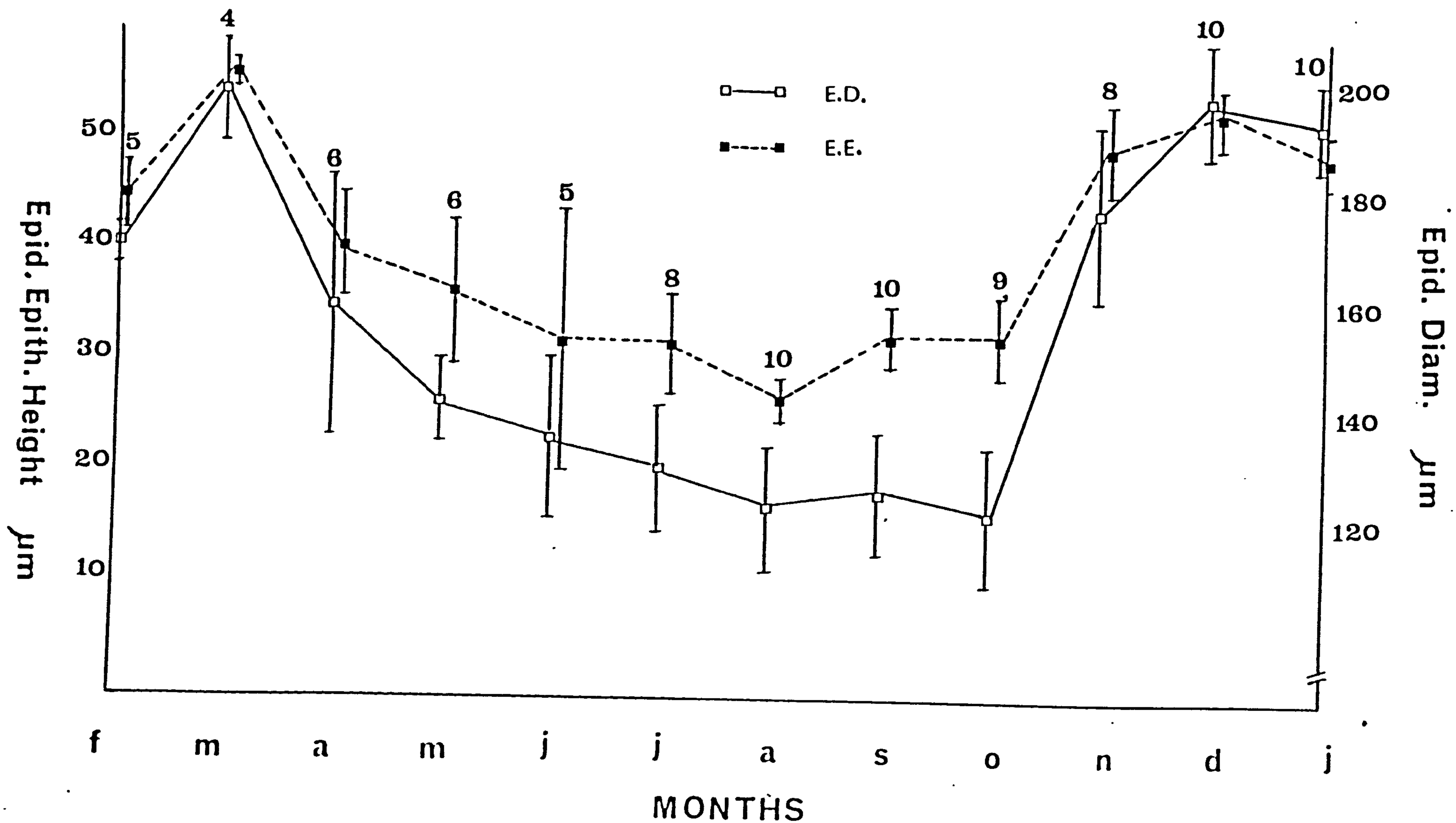
Although individual variations are obvious, in general there is an apparent parallelism between the "averages" of spermatogenic activity, the values of the GSI, the diameter and the epithelial heights of the tubules (Table 1.2).

5. Epididymis

Considerable changes are observed in the epididymis during the year (see Table 1.2 and Fig. 1.4). These changes are, more or less, parallel with those occurring in the GSI and the tubules. In February the mean of the duct diameter and the epithelial height are high. The maximal value of the means are reached in March for both parameters. The columnar epithelial cells are very active, and the lumen is full of sperms (Fig. 1.5a). A decline in the mean values of the diameter and the epithelial height of the epididymis occurs during April and continues in the following months, to reach minimal values in August. The epithelia are totally inactive with small non-active cuboidal cells and no secretions or sperms in the lumen, in all the lizards autopsied during August (Fig. 1.5b). The mean values for the epithelia and the diameter stay low until the beginning of November when a sharp uprise occurs. The rise continues, less abruptly, into December. This is followed by a small decline in the mean values of both epididymal parameters up to February. Maximum values are regained in March.

Fig. 1.4

Changes in epididymis diameter (E.D.) and epididymal epithelial height (E.E.) during 12 months. Photothermal conditions are as in Fig. 1.1. Data represents the mean ± SEM , and numericals represent the sample number.


E.D.
E.E.
Epid. Epith. Height µm
Epid. Diam. µm
10
20
30
40
50
120
140
160
180
200
4
5
6
6
5
8
10
10
9
8
10
10
f
m
a
m
j
j
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s
o
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d
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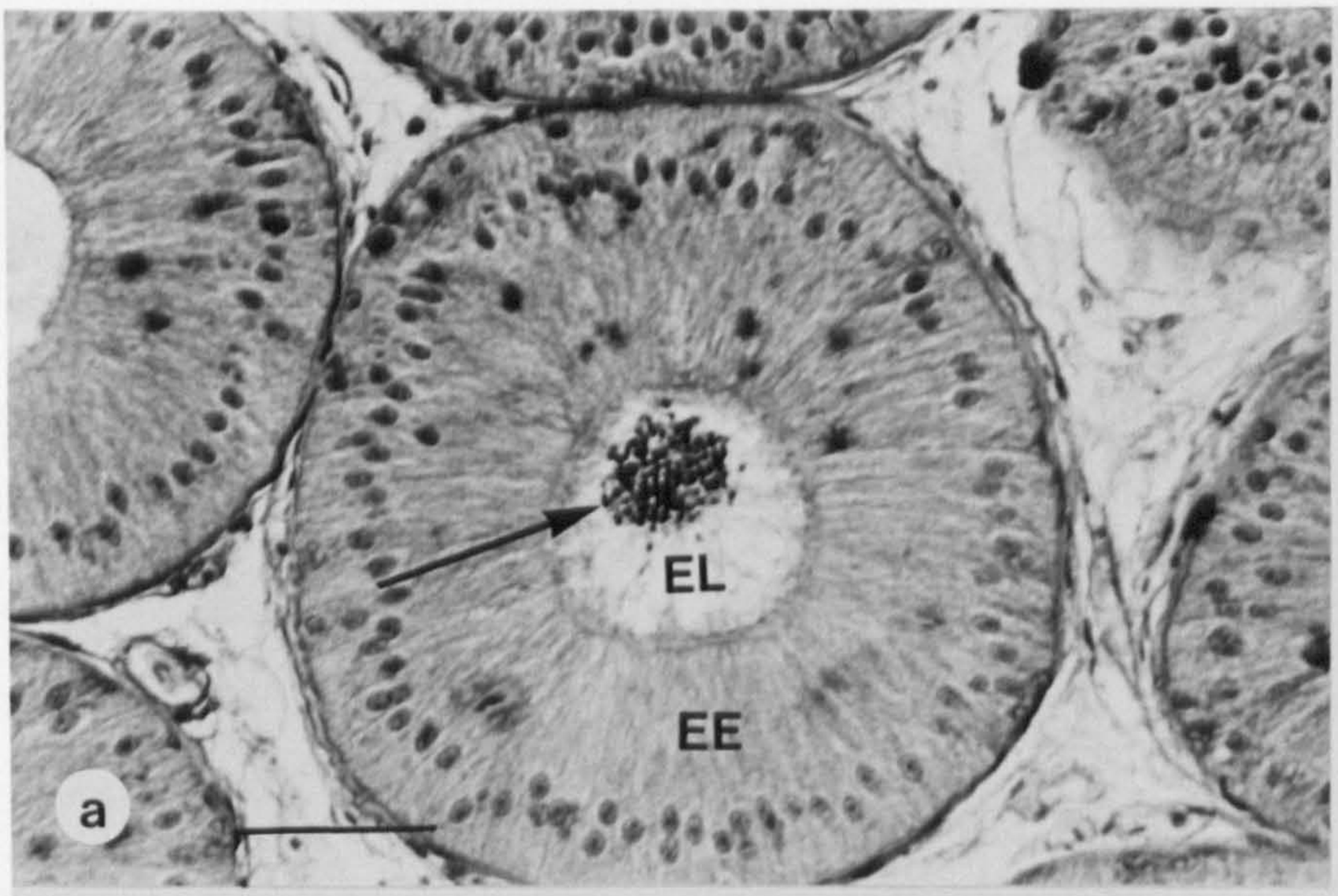
Fig.1.5

a. Active epididymis at stage 6. Note the high columnar epithelium (EE) and the lumen (EL) containing sperms (arrow).

b. Inactive epididymis at stage 8. Note the thin convoluted epithelium (EE) and the small empty lumen (EL).

Sections stained with Ehrlichs haematoxylin and eosin.

Scale line is 50 μm.



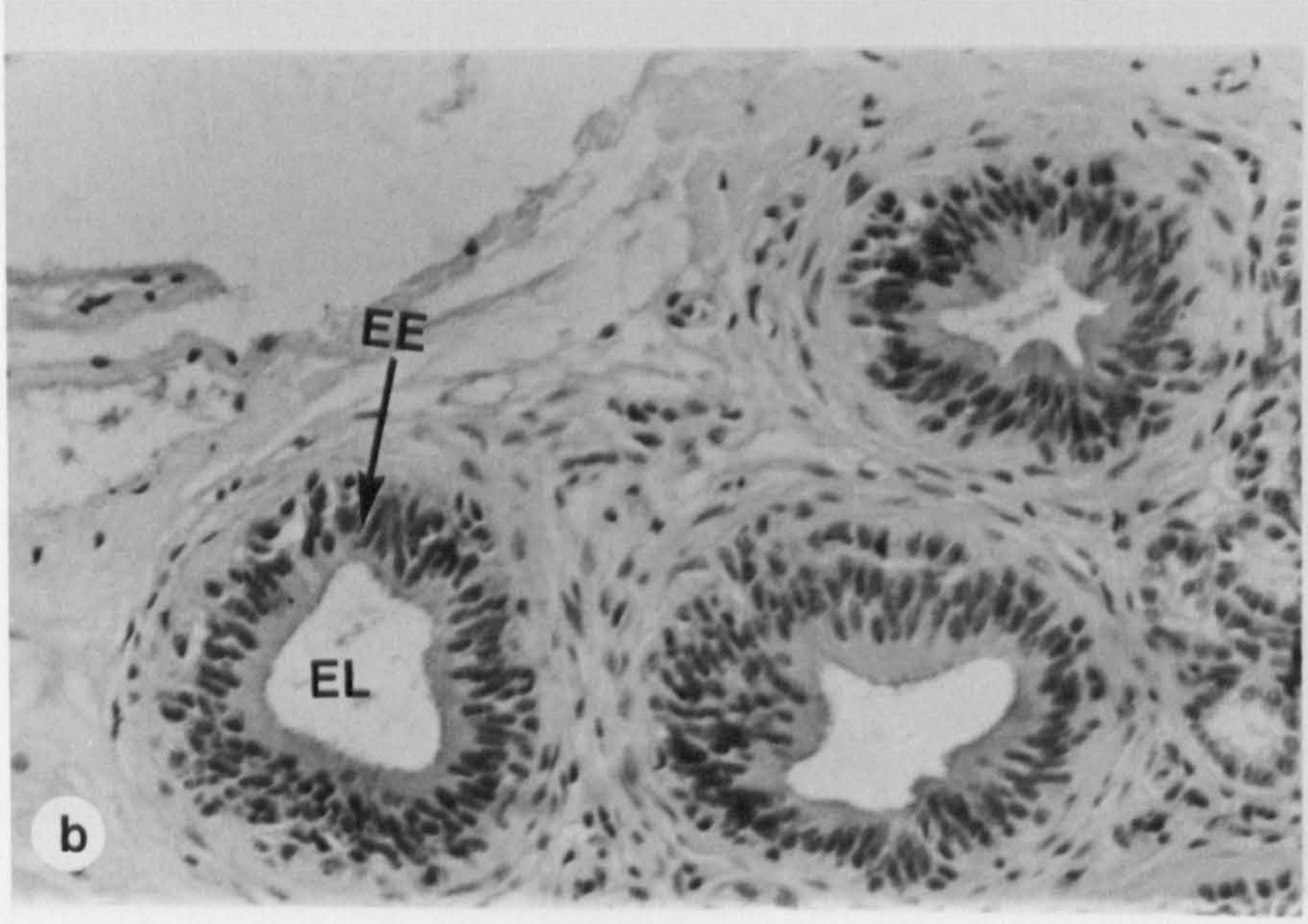


Fig 1.5

Epididymal epithelia were fully active in all samples examined during December (1978) through to March (1979). On the other hand, the epididymides were completely inactive in all the animals sacrificed in August. However, in the other months of the year not mentioned here, there are variations, in the activity of the epididymis, between the animals of a given month. These differences in epididymal activities can cover the whole range from full activity to inactivity in lizards of the same monthly sample. In addition, the ratio between active and inactive animals varies from one month to another.

A close analysis of relation between spermatogenic condition and the activity of the epididymis revealed that the latter is very active and secretory when the testis is passing through late stage 5, stage 6, or early stage 7. The epididymis is either totally inactive or exhibiting little activity in all other stages of spermatogenic development.

6. Tunica albuginea

In *Chalcides ocellatus*, the tunica albuginea is composed of loose connective tissue (Badir, 1958). The present observations show that there is an inverse relationship between the thickness of tunica albuginea and testis activity, thickness being maximum during August and minimum in December and March (Table 1.2).

It might be worth mentioning that for the tubule diameter, the epithelial height of the tubules, the diameter and epithelial height of the epididymis, and the thickness of tunica albuginea maximal values are all double their minimum values.

7. Fat bodies and liver

Neither the fat body weights, nor the liver's showed any relationship with the GSI or any other component of the testis and epididymis. In addition, there is a complete lack of cyclicity in both the fat bodies and liver. Although there are some variations between the weights of the two organs in the different months, these variations are, mostly, not significant and follow no rhythm.

Discussion

Unlike many other lizards (see Literature Review) adult male *Chalcides ocellatus* is a seasonal breeder exhibiting cyclic changes in the testis and accessory sex organs during the year (Badir, 1958). However, Badir (1958) did his study on animals taken directly from the wild, whereas in this report animals were under constant conditions of temperature and light.

Laboratory conditions

As described previously animals were placed under controlled conditions of light and temperature; with a "day time" of 14 hours (14L) and temperature in the light of $31^{\circ} \pm 2^{\circ}C$. These conditions are, more or less, similar to the natural conditions, but only during summer. In Egypt, a 14L photoperiod represents the long day during summer; $31^{\circ}C$ was chosen since it is the preferred body temperature for this species (Badir, 1958). Moreover, this temperature ($31^{\circ}C$) resembles the mean temperature levels in Abu-Rawash (area of collection) during summer (Shalaby, pers. comm.).

Although the animals needed for this experiment were brought at two separate occasions, yet this apparently had very little effect on the reproductive cycle and testicular development. Similar observations were made in other animals forming part of an investigation into the effect of light and temperature on reproduction (see Section 2).

Seminiferous tubules

In Chalcides, the seminiferous tubules exhibit great variations during the year in both the diameter and epithelial height. Moreover, with cessation of spermatogenesis the seminiferous tubule became occluded and degenerate. In this respect Chalcides resembles many other lizards such as: Phrynosoma solare (Blount, 1929), Sceloporus undulatus (Altland, 1941), Scincus scincus (Badir, 1958), Sceloporus occidentalis (Wilhoft & Quay, 1961), Urosaurus ornatus (Asplund & Lowe, 1964), Uta stansburiana (Asplund & Lowe, 1964; Hahn, 1964), Cnemidophorus tigris (Goldberg & Lowe, 1966), three species of the genus Uma (Mayhew & Wright, 1970), Sceloporus jarrovi (Goldberg, 1971a),and Dipsosaurus dorsalis (Mayhew, 1971). On the other hand, Chalcides is apparently different from the lizard Gerrhonatus multicarinatus (Goldberg, 1972) in which a patent tubular lumen is present all the year.

The present study is in agreement with that earlier one of Badir (1958). For instance the values of the tubular diameters and the epithelial heights in this study follow a range similar to that reported by Badir.

Within a given cross sectional area, the interstitial cells of Leydig exhibit a definite cycle in their apparent numbers. They are very few when the testis is fully active (at stage 6), and vice versa (at stage 8). Consequently an inverse relationship apparently exists between the tubular activity and the relative abundance of Leydig cells. However, it should be borne in mind that the variation in the numbers of Leydig cells in a given cross section during the year does not necessarily mean that the numbers of Leydig cells are variable during the cycle.

This is mainly because the testis volume varies greatly during reproduction, and unless an actual count of the total number of Leydig cells in the whole testis (at any one time) is made, it would be erroneous to say that their numbers have changed.

Cyclicity in interstitial cell activity has been described previously for several lizards including species of the two genera Anguis and Lacerta (Herlant, 1933), Hemidactylus flaviviridis (Dutta, 1944), Scincus scincus and Chalcides ocellatus (Badir, 1958) and Anolis carolinensis (Fox, 1958). However, sometimes the interstitial cells are either totally missing, as in the lizards Sceloporus undulatus (Altland, 1941), Mabuya multifasciata (Herberrer,1930 - quoted by Fox, 1977), Uma notata, U. inornata, and U. scoparia (Mayhew & Wright, 1970), or do not exhibit any clear cycle, as in the lizards Xantusia vigilis (Miller, 1948) and Leiolopisma rhomboidalis (Wilhoft, 1963b).

Leydig cells are reported to produce androgen hormones, mainly testosterone (Licht, 1974d, Courty & Dufaure, 1980). The latter is known to affect several target tissues. It acts on the testis to promote spermatogenesis, on the epididymis causing the latter to produce proteinous granules, and on the sexual segment of the kidney causing the production and release of secretory granules (see for e.g. Del Conte, 1974; Gigon & Dufaure, 1977). The details of the effects of testosterone on the testis and accessory sex organs are explained elsewhere (see Literature Review).

Spermatogenic condition

Regarding the spermatogenic condition of the testis, the present study shows that the reproductive cycle in Chalcides males can be divided into three phases:-

1. Recrudescence: in which the animal shows progressive spermatogenesis up to spermatid formation.

2. Full activity: in which both spermiogenesis (sperm differentiation) and spermiation (sperm separation from the germinal epithelium into the tubular lumen) are evident.

3. Quiescence: during which the activity of the tubules declines, with debris being found in the tubular lumina and the tubules are finally, fully regressed, small and convoluted.

A comparison between the present work and the early work of Badir (1958), who collected animals directly from the wild, shows some agreement, but differences are present also (Table 1.4, Fig. 1.6). Generally speaking, the peaks of activity and quiescence in the two studies occur at the same time, during March and August, respectively. In addition, recrudescence started (approximately) at the same time in both studies, early October. However, the speed of recrudescence differed substantially. Whereas it took the animals between one to two months to reach full activity (stage 6) under experimental conditions, it took them five months to reach full breeding condition in nature (October-February, inclusive). The full breeding condition lasted, in this study, for five to six months (November/December-April, inclusive), while it was only two

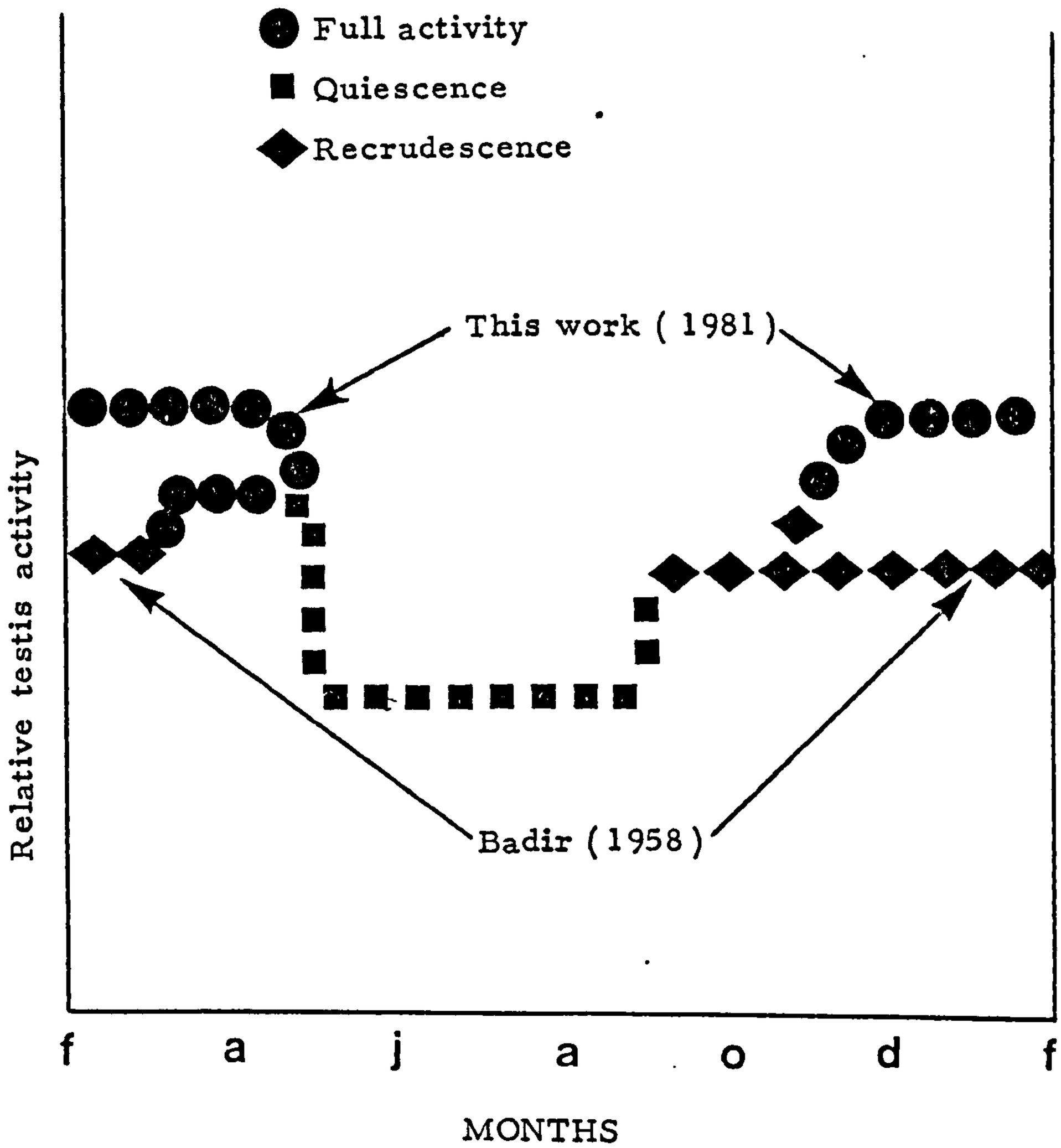
Table 1.4

A comparison between the reproductive cycle under natural (Badir, 1958) and laboratory (this study) conditions

Spermatogenic Phase	Badir (1958)	This work (1978-79)
1. Recrudescence	Early October - February	Early October - November
2. Full activity	March - April	November/December - April
3. Quiescence	May - September	May - September

Fig. 1.6

A comparison between reproductive activity in Chalcides in the wild and under standard conditions of light (14h) and temperature (31 C).


Full activity
Quiescence
Recrudescence
This work (1981)
Badir (1958)
Relative testis activity
f
a
j
a
o
d
f
MONTHS

months (March and April) in Badir's work. Again, the quiescent period started and terminated at about the same time in both studies (May-September, inclusive).

It is clear, thus, that the testicular cycle under constant daytime conditions of light and temperature becomes shorter than under natural conditions. Whereas it took 10 months (May-February, inclusive) in the wild for the animals to reach full activity from the beginning of quiescence, it took only 6-7 months (May-November) under laboratory conditions. Apparently, optimal conditions of light and temperature caused this shortening in the cycle.

Because testicular recrudescence and quiescence started at the same time in both studies, it seems, thus, that both testis conditions are initiated by endogenous (inherent) cues. However, studies involving four combinations of temperature and light (see Section 2) revealed that the initiation and completion of testicular recrudescence is completely controlled by environmental factors, mainly a relatively high temperature. Testis quiescence seems to be controlled, partially at least, by endogenous factors, but since the effects of low temperature on the initiation of testis regression have not yet been studied, it is difficult to say whether the initiation of regression is due to endogenous cues alone or whether it could be influenced by interactions between endogenous and environmental factors.

It is vitally important to say that in all the months, except August, at least one of the sampled lizards exhibited fully active testes, though the number differed from one month to another (Table 1.3).

Previous studies on this species (Badir, 1955, 1958; Badir & Hussein, 1964) claimed that the reproductive cycle was completely con-

trolled by the environment and that endogenous rhythmicity had no effect on the cycle. Moreover, it was stated by the authors that "under optimal conditions of illumination and heat Chalcides can be changed to a permanent breeder" (Badir, 1958). In other words, under "optimal" conditions there will be no regression in the tubules and the testis will exhibit a state of continuous activity during the year. Unfortunately, the data presented in these three studies was insufficient to allow further analysis. In fact, in at least two of them (Badir, 1955, 1958) the controlled environment was simply described as "optimal conditions" without any reference to the actual temperature or the length of photoperiod. Furthermore, Badir and his colleagues drew their conclusions from the application of only a single regime of photo-thermal conditions, clearly they should have tried other combinations of light and temperature before issuing any generalization concerning reproduction in Chalcides.

The present study shows (using optimal conditions of light and temperature) that testicular activity in Chalcides is not continuous. The testes of the majority of lizards sampled during May, June and July and in all the lizards autopsied in August were fully quiescent. In addition, studies involving four combinations of light and temperature (Section 2) showed that in the 10 animals sampled during July all had quiescent testes under optimal conditions of temperature (even though photoperiod varied). These data demonstrate clearly that reproduction in Chalcides males is discontinuous under optimal conditions.

In summary, reproduction in male Chalcides is apparently controlled by an interaction of environmental (e.g. temperature) and endogenous factors. Under maintained optimal conditions of light and tem-

perature the cycle is shortened by 3-4 months, but still exhibits periods of full activity and quiescence. This interpretation contrasts strongly with the earlier statements of Badir (1955, 1958) and Badir & Hussein (1964).

Epididymis

There is an intimate relationship between the spermatogenic stage of the tubules and the activity of the epididymis. As explained previously, the epididymal epithelium is hypertrophied, with secretory cells, during late stage 5, stage 6 and early stage 7, only. In all the other stages of testicular development animals showed little epididymal activity, if any. This indicates that the activity of the epididymis does not commence until shortly before the breeding season and terminates soon after breeding ceases.

The role of the epididymis and the importance of its secretions are discussed elsewhere (see Literature Review).

Chalcides exhibits a clear cycle in the duct diameter, epithelial height, and activity of the epididymis during the year (Table 1.2 and Fig. 1.3) which parallels the cycle described for the testis. Cyclicity in epididymal activity has been reported for many lizards including Anguis and Lacerta (Herlant, 1933; Regamey, 1935), Eumeces fasciatus (Reynolds, 1943), Scincus scincus and Chalcides ocellatus (Badir, 1958), and three species of the genus Uma (Mayhew & Wright, 1970). On the other hand, Wilhoft (1963b) reported a lack of cyclicity in epididymal activity in the tropical lizard Leiolopisma rhomboidalis.

Tunica albuginea

Literature concerning the relationship between the thickness of tunica albuginea and the reproductive activity in lizards is scanty. Badir (1958) reported, in Scincus sincus and Chalcides ocellatus, that the tunica albuginea became four times thicker in the resting testis than in the active one. The present work supports the early finding of Badir in Chalcides, with the difference that the thickness of tunica in a resting testis is almost twice the thickness of an active gonad. It is possible that the difference in relative thickness in tunica between resting and active testes is due to expansion of the testis and stretching, since the size of an active testis is several-fold that of an inactive one.

Fat bodies and liver

The weights of fat bodies and liver do not follow any cycle nor show a relation with other reproductive features. At the present time this is true only under laboratory conditions since there are no reports, to our knowledge, concerning the changes in Chalcides fat bodies and liver weights under natural conditions. However, a similar lack of fat body cycle, in nature, has been reported for the lizard Anolis trinitatis (Gorman & Licht, 1975). On the other hand, fat body cycles and their relationship to the reproductive cycle have been reported in Uta stansburiana (Hahn & Tinkle, 1965), several species of the genus Anolis (Licht & Gorman, 1970; Sexton et al., 1971; Fleming & Hooker, 1975), and the bunch grass lizard, Sceloporus scolaris (Newlin, 1976).

Assuming a constant energy input via food uptake, the decrease in fat body storage during the breeding season appears to be due to in-

creased expenditure (resulting from courtship, mating, ... etc.) rather than food shortage (Licht & Gorman, 1970). Recently, several investigators pointed out an intimate relationship between fat storage and the availability of suitable food (Rand, 1967; Licht et al., 1969; Sexton et al., 1971; Licht, 1974a; Vitt & Ohmart, 1975). In one of the experiments carried out in the wild, Licht (1974a) found that when food was available in adequate amounts to Anolis during the breeding season, the lizards readily accepted the food, and when autopsied they showed pronounced enlargement of fat bodies and liver. Since the same species exhibited small fat reserves during the breeding season in nature, Licht concluded that the lack of fat reserves in Anolis during the breeding season is, probably, not a result of poor appetite or physiological inability to assimilate energy, but because of food insufficiency to allow such an energy storage (Licht, 1974a).

Similarly, it is possible that the lack of fat body and liver cycles in Chalcides may be due to the availability of sufficient amounts of suitable food during the course of experiment (12 months), and hence fat storage and liver weight were kept, more or less, constant. Since the lizards were not hand fed, it is possible that variations in food uptake occurred leading to an obvious fluctuation in fat bodies and liver weight among individuals. We feel that further experiments are needed before any final conclusion can be made.

S E C T I O N 2

EFFECTS OF VARIATIONS IN LIGHT AND TEMPERATURE ON REPRODUCTIVE ACTIVITY OF MALE *CHALCIDES OCELLATUS*

Summary

1. The effects of particular regimes of light and temperature on the reproductive cycle of the lizard Chalcides ocellatus have been studied over long periods of up to nine months.

2. Four combinations of temperature and photoperiod (hours of light L) were chosen A, 31°C with 14L; B, 15°C with 14L; C, 31°C with 10L and D, 15°C with 10L.

3. The data indicated that the reproductive cycle is controlled by an interaction between endogenous (inherent) rhythm and environmental cues.

4. Temperature is the main environmental factor promoting reproductive activity. Neither photoperiod nor endogenous rhythms exert any obvious effect when temperature is below a threshold level.

5. Testis regression is controlled mainly by endogenous cues, though external factors seem to have some effect upon it.

6. Low temperature (15°C) caused a complete arrest in testicular activity.

7. The development of the epididymis and sexual segment of the kidney was directly related to the spermatogenic status of each individual.

8. Fat body and liver weights did not exhibit any cyclicity through the experimental period.

Introduction

Reproduction in many animals is dependent upon both inherent, endogenous rhythms and environmental, exogenous factors. It is generally accepted that annual reproductive activity cannot be completely controlled by any endogenous rhythm alone; for proper reproductive functioning endogenous and environmental factors have to interact (Marion, 1970b). Many studies on the male reproductive cycles in lizards have left no doubt about the existence of such interactions (e.g. Fischer, 1970; Licht & Gorman, 1970; Licht, 1971a, 1972, 1973, 1974b).

The apparently necessary interactions between exogenous and endogenous cues have raised two major questions; 1) what part(s) of the reproductive cycle is regulated by endogenous factors; 2) which is (are) the major external factor(s)?

It is well established that the initiation of testicular recrudescence is essentially dependent on external cues. Consequently, regression is the only part of the reproductive cycle to be controlled effectively by endogenous factors. Supporting evidence for such a possibility came from Licht's work on the lizards *Dipsosaurus dorsalis* and *Xantusia vigilis* (Licht, 1973). He found that, in both lizards, testis regression was essentially independent of concurrent photoperiod and temperature. Moreover, Licht (1972, 1973) reported that this endogenous control of testicular regression was (probably) effective only under certain restricted environmental conditions (e.g. warm temperature), and thus provided an answer to the second question.

The three environmental factors that have been considered by many investigators as the source of the major cues for the male reproductive

cycle in lizards are photoperiod, temperature and rainfall. Rainfall has been considered as the major factor affecting the timing of reproductive activity in males of several lizards, including Agama agama (Daniel, 1960); Sceloporus orcutti (Mayhew, 1963); three species of the genus Uma (Mayhew, 1965b, 1966, 1967a, 1968); Leiolopisma fuscum (Wilhoft & Reiter, 1965), Uta stansburiana (Turner et al., 1970), Anolis limnifrons, A. tropidogaster, and A. auratus (Sexton et al., 1971).

However, other workers have eliminated any significant involvement of rainfall in testicular cycles of lizards, while pointing out its probable significant in females only (Licht & Gorman, 1970; Licht, 1971a, 1972, 1974b; Gorman & Licht, 1975).

There is considerable controversy regarding the role of photoperiodism in control of reproductive cycles in reptiles (Licht, 1971a). Photoperiodism has been implicated in testicular recrudescence in several species of lipids, among them Anolis carolinensis (Clausen & Poris, 1937; Fox, 1958; Fox & Dessauer, 1958); Ėmoia cyanura, E. werneri (Baker, 1947); Xantusia vigilis (Miller, 1948 ; Bartholomew, 1950, 1953); Lacerta sicula (Golgano, 1951; Fischer, 1967, 1968b); three species of the genus Uma (Mayhew, 1964). Moreover, it was concluded that in some of them (e.g. Anolis carolinensis, Xantusia vigilis, Lacerta sicula) photoperiod is more important than temperature in controlling testicular development (Clausen & Poris, 1937; Bartholomew, 1950, 1953, 1959; Fischer, 1967, 1968b). Contrariwise, Licht, in almost all of his papers dealing with the effects of temperature and photoperiod on testicular activity, has pointed out clearly that temperature (and not photoperiod) is the major environmental cue promoting testicular activity in lizards. The work on which he based these conclusions include extensive studies on the lizard

Anolis carolinensis (see Licht, 1966, 1967a,b, 1969a,b, 1971b),and to a lesser extent, on Dipsosaurus dorsalis and Xantusia vigilis (Licht, 1973), and Lacerta muralis (Licht et al., 1969). In fact, the best reviews of all written dealing with the effects of light and temperature on lizard reproduction,.are those of Licht (1971a, 1972, 1973, 1974b) and Licht et al. (1969).

The present work was undertaken to test the effects of temperature and photoperiod on reproduction in the male lizard Chalcides ocellatus.

Materials and Method

Chalcides ocellatus adults (over 90 mm in snout-vent length) were obtained from the locality of Abu-Rawash near Cairo, through a commercial dealer, during late February 1979. Within a very short time, not exceeding 12 h, lizards were transported and housed in the Dept. of Zoology, Bangor. These animals were kept for five days in quarantine, during which they were treated for possible tick and mite infection, before being separated into groups. The procedure for tick and mite treatment was as described earlier, in Section 1 (p. 38).

To determine the reproductive condition of the animals, five lizards were autopsied immediately before the start of the experiments. These are designated as "initial controls". Groups of 30-40 lizards (mixed sexes) were then placed in large containers (120 x 60 x 60 cm) in controlled temperature rooms, except for group D which was placed in plastic cages (45 x 25 x 15 cm, 5-7 animals, each) in controlled temperature cabinets. Starting on the 5th of March 1979, the four regimes were:

1. Group A: $31^{\circ} \pm 2^{\circ}C$: 14 hours light/10 hours dark (14L/10D), i.e. summer temperature and long days.
2. Group B: $15^{\circ} \pm 1^{\circ}C$: 14L/10D, i.e. winter temperature and long days.
3. Group C: $31^{\circ} \pm 2^{\circ}C$: 10L/14D, i.e. summer temperature and short days.
4. Group D: $15^{\circ} \pm 1^{\circ}C$: 10L/14D, i.e. winter temperature and short days.

The room temperatures in Groups A and C were kept constant at $31^{\circ} \pm 2^{\circ}C$ during the day, but cooled down to about $20^{\circ}C$ during the dark period. This arrangement was made to match as closely as possible the

conditions of the natural habitat of this species.

The temperature of Groups B and D were fixed at 15°C ± 1°C, 24 h/day as it was not possible to vary day/night temperatures below ambient.

The temperature of 31°C was selected as a "high" temperature since it represents the average preferred body temperature for Chalcides as found by Badir (1958). The choice of 15°C as a "low" temperature was to match the average daily temperature during winter in the lizards' natural habitat. The maximum daylength, where this species lives, is about 14 h while the minimum is about 10 h.

Water was continuously available in open dishes and food was provided ad libitum. The lizards were fed mainly on fly maggots, but meal worms and boiled eggs were administered on occasions. Vitamin D supplements were given regularly.

The experiments terminated at various times up to nine months from the start. Five animals, from each group, were killed at monthly intervals, usually during the first three days of every month. The autopsies were done in successive months for the first four months, for all groups, and then in the eighth and ninth months for groups A only. The weights of the lizards and the snout-vent lengths were recorded. Males were killed by decapitation and as much serum as possible was collected (individual samples)in plastic tubes to be used for radioimmunoassay described elsewhere (see Section 3). The right testis was weighed, frozen in liquid nitrogen, and stored at -70°C for histochemical studies reported elsewhere (see Section 4). The left testis together with the epididymis and the kidneys were used for routine histological study. These tissues were fixed in Susa, embedded in paraffin wax, cut at 7.0μm, and stained

with Ehrlich's (and sometimes with Heidenhain's iron) haematoxylin and eosin. The spermatogenic status for individual lizards was assessed in relation to the eight spermatogenic stages modified from Mayhew and Wright (1970), Table 2.1 (previously described, see Section 1). The weights of fat bodies and livers were recorded, also, for individual lizards. The 5% level has been used in all tests of statistical significance.

Table 2.1

Classification of the eight spermatogenic stages of testis development in Chalcides ocellatus. Note that Stage 1 may show similarity to stages 2-4, but lumen is absent. Modified from Mayhew & Wright (1970)

Stage	Spermatogenic Condition
1	Division of germinal cells, without the development of a lumen
2	Primary spermatocytes at luminal margin
3	Secondary spermatocytes at luminal margin
4	Undifferentiated spermatids at luminal margin
Early 5	Very few metamorphosing spermatids at luminal margin
Late 5	Many metamorphosing spermatids at luminal margin
6	Mature sperms in lumen (full activity condition)
Early 7	First signs of early regression - little cellular debris in lumen
Late 7	Much cellular debris in lumen
8	Complete regression - no cell division, no lumen

Results

1. Laboratory observations:

While the animals in the warm summer groups (A and C) generally showed many signs of activity, moving quickly and feeding avidly, the cold winter groups (B and D), on the contrary, were almost completely inactive. Many cases of courtship and mating were observed among the warm temperature animals. On the other hand, the winter temperature animals spent most of the time buried underneath the sand as if they were passing through a long period of hibernation and although food was presented regularly, the lizards' feeding was very poor. No courtship or mating was observed. Mortality among the summer temperature animals was very low, only one animal (out of 30-40 animals) in each group. Contrariwise, the mortality among the cold temperature animals was quite high; about ten animals from each group of 30-40 animals. This high mortality rate in the latter was a major factor in bringing the experiments in groups B and D to a premature conclusion, several months before originally planned.

2. Initial controls:

The initial control animals exhibited the characteristics of sexual activity previously described by Badir (1958). The testes were generally turgid and the gonadosomatic index (GSI; the weight of the right testis in mg/100 g body weight) showed high values. The seminiferous tubules were large in size, restricting the interstitial cells to

triangular or rectangular spaces in between them. Most of the testes were in stage 3, except for one lizard which was in stage 4. The "average" of the spermatogenic stage is 3.2, indicating that the animals were passing through recrudescence (for more details on the "average" of spermatogenic condition see Section 1, Materials and Methods).

The accessory sex organs, namely the epididymis and the sexual segment of the kidney, were inactive. The small, narrow, convoluted epididymis showed shallow inactive epithelia with an empty lumen. The sexual segment of the kidney was not secretory, with small tubules hardly distinguishable from the other tubular parts of the kidney. The data for the initial controls are represented in Table 2.2 and Figs. 2.1 to 2.4. The spermatogenic condition and the condition of the accessory sex organs are shown in Figs. 2.5a, 2.6a and 2.7a.

3. Summer Temperature (31°C) Groups A and C:

i) Group A (long day; 14 h)

Spermatogenic condition:

In early April, the testes were large in size and turgid. The epithelia of the seminiferous tubules showed remarkable progress from the general picture of the previous month (initial controls). All of the tubules showed the full activity condition (stage 6) in which both spermiogenesis and spermiation (sperm separation into the tubular lumen) were clearly evident (Fig. 2.5b). One month later, in May early regression in testicular activity was evident, with tubular epithelia undergoing degeneration and debris in the lumina (stage 7). However, not all animals were at the same spermatogenic stage since full breeding animals

Table 2.2

Comparison between GSI, spermatogenic status, diameter and the epithelial height of seminiferous tubules, and epithelial height of the epididymis and their different regimes of light and temperature; Group 0 (initial controls), A (31°C: 14 L), B (15°C: 14 L), C (31°C: 10 L), and D (15°C: 10 L). Data represents the mean plus and minus SEM, except November samples (too few animals in the cold temperature groups).

	Group 0	Groups	Months of the year 1979					
			April	May	June	July	November	December
GSI	519.8 ± 57.3	A	483.1 ± 53.1	263.8 ± 38.3	194.8 ± 51.8	65.4 ± 3.3	252.8 ± 35.2	472.4 ± 90.5
		B	382.4 ± 43.9	380.6 ± 11.3	398.2 ± 28.5	527.6 ± 34.7	213.5[a]	
		C	420.5 ± 39.2	452.5 ± 74.9	295.7 ± 44.8	72.2 ± 9.1		
		D	394.6 ± 50.7	248.0 ± 34.7	465.5 ± 43.3	366.1 ± 34.2	377.3[b]	
Spermato-genic cond.	3.2	A	6.0	6.8	7.4	7.8	4.0	5.8
		B	3.2	3.2	3.0	3.2	3.0[a]	
		C	6.2	7.0	6.8	7.6		
		D	3.0	3.2	3.0	3.0	3.0[b]	
Sem. Tubule diam. μm	239.3 ± 11.4	A	210.3 ± 10.1	178.6 ± 5.8	142.5 ± 10.9	100.0 ± 11.0	197.7 ± 14.3	204.1 ± 19.0
		B	207.6 ± 7.6	159.9 ± 15.3	181.9 ± 7.9	189.4 ± 10.2	178.0[a]	
		C	219.8 ± 12.9	178.4 ± 5.7	177.6 ± 7.8	130.6 ± 5.0		
		D	204.7 ± 7.3	183.3 ± 4.5	203.9 ± 8.6	190.3 ± 3.5	161.0[b]	
Sem. Epith. μm	91.8 ± 5.2	A	54.2 ± 1.6	43.1 ± 2.6	48.2 ± 1.9	43.1 ± 1.6	54.5 ± 3.7	60.7 ± 3.6
		B	103.8 ± 3.7	80.0 ± 7.7	91.0 ± 4.0	94.7 ± 5.1	89.0[a]	
		C	55.2 ± 5.3	45.9 ± 1.0	49.7 ± 2.9	46.3 ± 1.3		
		D	95.4 ± 8.8	91.7 ± 2.7	102.0 ± 4.3	95.2 ± 1.8	80.5[b]	

Table 2.2 (contd.)

	Group 0	Groups	Months of the year 1979					
			April	May	June	July	November	December
Epid. Epith. µm	42.0 ±3.2	A	58.5 ± 1.6	56.7 ± 3.3	48.2 ± 2.0	36.0 ± 1.8	33.5 ± 1.2	57.4 ± 2.8
		B	40.0 ± 2.4	31.4 ± 2.0	34.1 ± 3.0	37.9 ± 1.2	37.5[a]	
		C	65.6 ± 5.5	44.8 ± 3.1	41.8 ± 1.2	41.6 ± 1.4		
		D	36.1 ± 1.3	38.1 ± 4.0	36.8 ± 2.1	32.9 ± 2.2	31.5[b]	

a. Sample number is one animal

b. Sample number is two animals

All other groups represent 5 animals

(stage 6) were present also. By early June, most of the animals (but one at stage 6) were passing throughout early (stage 7) and late (stage 8) regression. The activity of testicular epithelia declined further in July to reach its lowest levels. All the animals, but one at stage 7, were passing through stage 8, i.e. complete regression (Fig. 2.5c). The tubules at stage 8 were shrunken, merely composed of spermatogonia and Sertoli cells with an occluded lumen.

No specimens were taken for August, September and October.

In early November, the testes appeared to be returning to activity. They were turgid and large in size with the tubules showing cell divisions. Although variations in spermatogenesis exist between the animals, still none of the animals showed development beyond metamorphosing spermatids (stage 5). However, the full activity condition in the testes was reached in early December, when all the animals, but one at stage 5, were at stage 6 (Table 2.2).

Seminiferous tubules and gonadosomatic index (GSI):

In April, the diameter of the tubules, the epithelial height and the GSI showed clear decline in their values from the initial controls (Table 2.2; Figs. 2.1 to 2.3). However, the decline in both the GSI and the diameter of tubules was small, but the fall in epithelial height was sharp, due largely to the expansion of the lumen. In fact, the formation of the lumen had only just started in March (initial control animals), so the tubules were occupied mostly by dividing cells. The testes in April animals were more advanced than those of March and the lumen was fully developed leaving only half of the tubular diameter to the epithelia. The three testicular parameters (diameter and epithelial height of tubules, and the GSI) continue to decline during May and June to reach their minimal

values in July (Table 2.2 and Figs 2.1 to 2.3). The values of the three components are again high during the subsequent November, reaching maximum in December.

Accessory sex organs:

During April and May the epididymis was generally very active, with the cells being secretory and the spermatozoa abundant in the lumen (Fig. 2.6b). The epithelial heights of the epididymes in April were significantly higher than during March. Then there was a continuous decline in activity and epithelial height during June, July and November (Fig. 2.6c). It was only in December that active, secretory and large epididymes were seen again. The fluctuations in the epididymal epithelia are represented in Table 2.2 and Fig. 2.4.

Regarding the sexual segment of the kidney, substantial differences were evident among the different months of the experiment. In April and May, the segments were very active, exhibiting tall columnar epithelia. The cells were hypertrophied and secretory (Fig. 2.7b). A marked decline in the epithelial height and activity started in June and continued through July and November, when epithelial cells were totally inactive and hardly distinguishable from other uriniferous tubules (Fig. 2.7c). A tremendous increase in the activity of the segment occurred one month later, in December. The cells were again very active, hypertrophied and secretory showing a condition similar to that which prevailed during April.

It is obvious that a direct relationship exists between the activity of the epididymis and sexual segment of the kidney and the spermatogenic condition of the testis (in Group C also). The epididymis and the sex segment are active only when stages 5, 6 and early 7 are found in the testis.

ii) Group C (short day; 10 h):

Both testes and epididymes were very active during April. All developmental stages, particularly spermiogenesis, were represented in the seminiferous tubules. The males were in full breeding condition except for one already starting regression (i.e. at stage 7). From May onwards there was a continuous decline in spermatogenic activity, early regression predominating in May and June, but by July, almost all lizards were passing through complete regression (stage 8). Unfortunately, the experiment terminated during July due to the unavailability of animals.

The fluctuations in the GSI, and the values for the other components followed more or less the same pattern in this group as in group A (Table 2.2, Figs. 2.1 to 2.4), except for a slightly slower decline in the activity of the sexual segment of the kidney. Nevertheless, both groups reached complete inactivity in the segment at the same time, early July.

4. Winter Temperature (15°C) Groups B and D:

The experiments for both cold temperature groups extended over four months except for three samples which survived (one lizard in group B and two in group D) until November, but no generalization could be drawn from the latter ones due to the small sample size.

i) Group B (long day; 14 h):

The GSI in this group of animals was more or less constant during the first three months of the experiment. Some small variations between the animals in the same month were present. A sharp increase in

GSI occurred only during the last month, i.e. July. The reason for this is obscure since it was not accompanied by a concomitant increase in any of the other testis parameters measured (Table 2.2, Figs. 2.1 to 2.3).

Variations in the diameter of the seminiferous tubules did not follow the same pattern as that exhibited by the GSI (Fig. 2.2). The value was very high during April, followed by a sharp decline in May and then a slight, non-significant, increase in June and July. The spermatogenic condition of the tubules did not show any significant change from that of the initial controls, during the whole period of experiment. All of the animals were at stage 3, except for three (out of 21) at stage 4 (Fig. 2.5d).

The first signs of cell necrosis and degeneration were noticed during June, when vacuoles were observed in the seminiferous epithelia in two of the five lizards. The vacuoles, indicating the death and degeneration of cells, increased in July in all samples. However, there were variations between the animals of the same month in the number and size of these vacuoles. In the one lizard which survived until November, these vacuoles were especially numerous (Fig. 2.5e). There was no apparent relationship between testis weights, tubular diameters, or spermatogenic condition in this group of animals, unlike the warm temperature groups where a relationship between these three components was generally evident.

The epididymis and the sexual segment of the kidney showed only slight fluctuation during the four months. The changes in epididymal epithelia were small and the epididymis as a whole was generally inactive (Fig. 2.6d). The sexual segment of the kidney was regressed and not secretory (Fig. 2.7d), in some animals it was very difficult to distinguish from ordinary uriniferous tubules.

Generally, it can be said that very little, if any, sexual recrudescence has occurred in this group during the course of the experiment.

ii) Group D (short day; 10 h):

The animals of this group showed great similarity to the long day, low temperature lizards (group B) in the general scope of gonadal activity (Table 2.2, Figs. 2.1 to 2.4). The GSI showed large variations during the period of experiment. However, these variations appeared to be random and most likely they were due to individual variations in body weight and size. It is possible that other factors such as food consumption, energy expenditure and previous reproductive activity may affect the performance of the animals under these conditions, but such factors have not yet been assessed. The seminiferous tubules showed little variation, during the four months, in either diameter or epithelial height, remaining essentially constant during the period of the experiment (Table 2.2, Figs. 2.2 and 2.3). All the animals possessed testes at stage 3 of the spermatogenic cycle, except for a single animal (out of 22) which possessed testis at stage 4.

It is worth mentioning that from April through to July two major changes occurred in the seminiferous tubules. The first was the continuous narrowing of tubular lumina leading to complete closure, and the second was the degeneration of germ cells that increased with the progress of time. In about 50% of the animals it was very difficult to distinguish between the spaces resulting from the degeneration of cells and the tubular lumina since the latter (if present) were very small (Fig. 2.5e).

5. Fat body and liver

In groups A, B and C the weights of fat bodies seemed to be more or less constant during the period of experiment (Appendix II). Moreover, there was no apparent relationship between fat body weights and the GSI. Although low fat body weights were found in April and May in groups C and A respectively), the lack of a relationship with the GSI is still obvious. However, in group D a weak (insignificant) direct relation existed between the two parameters, though this is of questionable 'reality'.

The changes occurring in the liver weights of the four groups of animals during the course of experiment were small. In addition, there was no relation between the GSI and the weights of livers. The winter temperature animals (groups B and D) had livers slightly heavier than those of the summer temperature lizards (groups A and C).

Fig. 2.1

Changes in the gonadosomatic index (GSI; testis weight in mg / 100 gm of body weight). Mean and SEM are indicated for each sample. For sample number and photothermal conditions refere to Table 2.2 page 73.

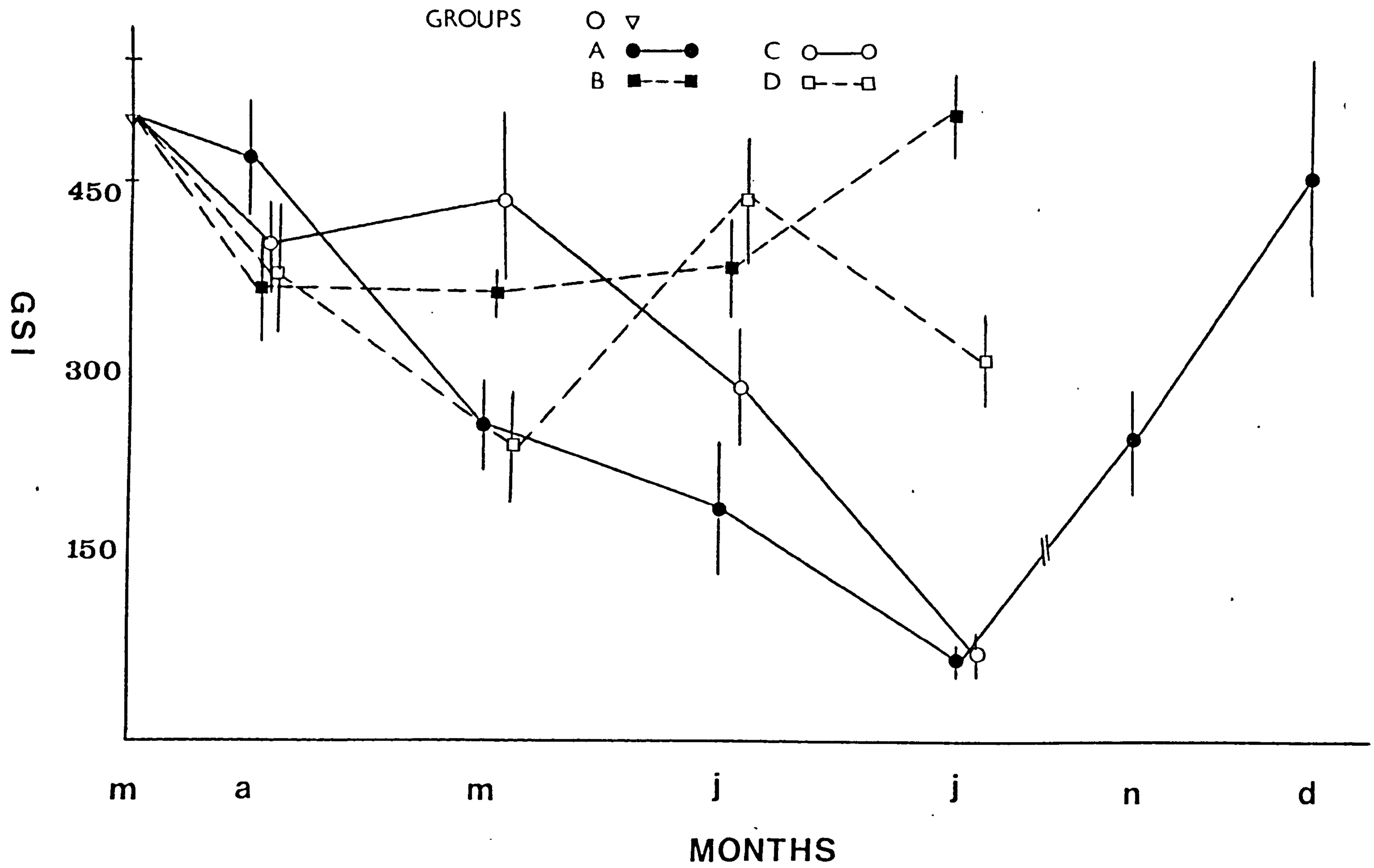

GROUPS
O
A
B
C
D
450
300
150
GSI
m
a
m
j
j
n
d
MONTHS

Fig.2.2

Variations in the diameters of seminiferous tubules in the four groups . For other details see Table 2.2 page 73 .

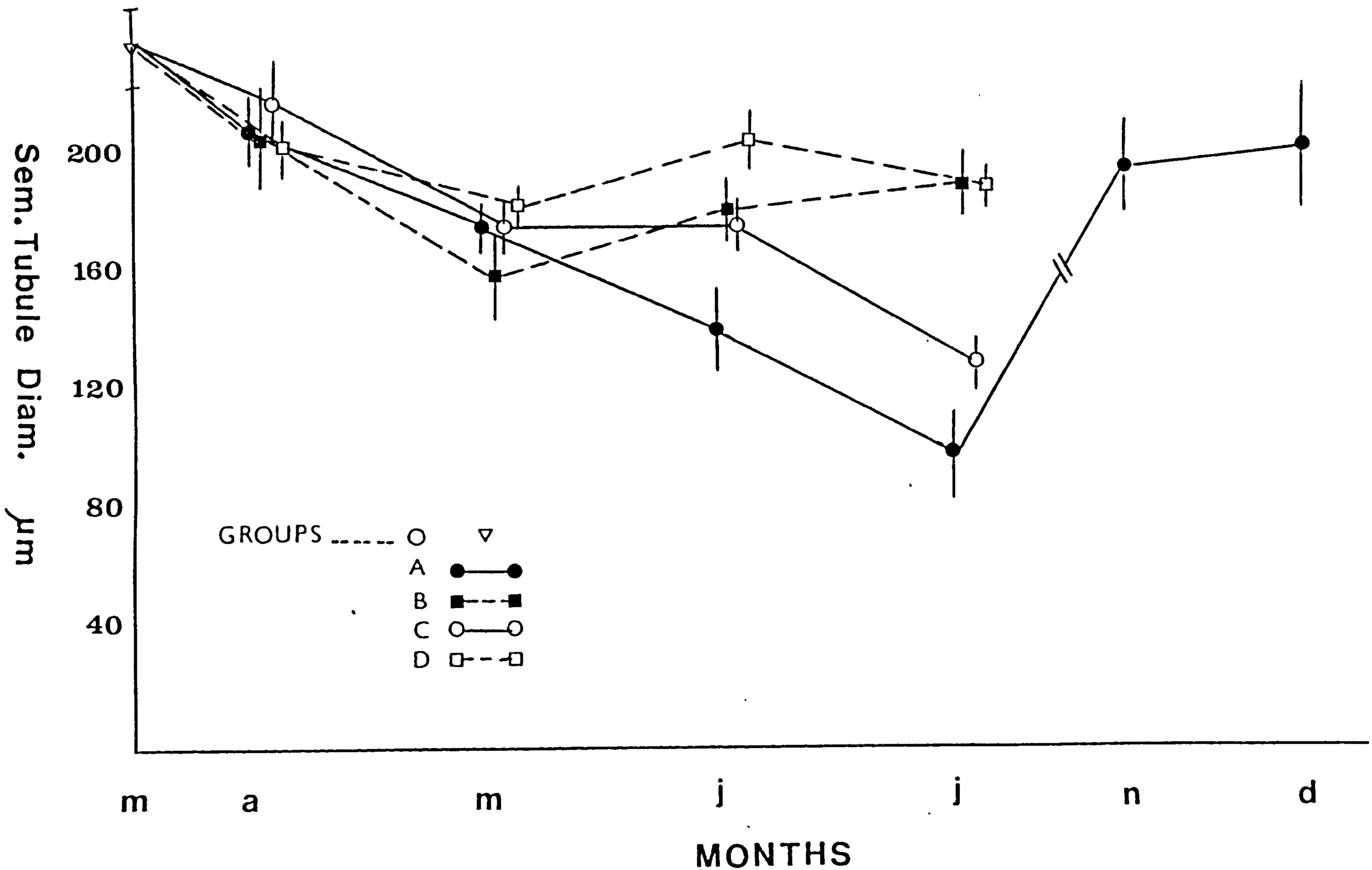

Sem. Tubule Diam. µm
200
160
120
80
40
GROUPS
A
B
C
D
m
a
m
j
j
n
d
MONTHS

Fig. 2.3

Variations in the epithelial height of testes tubules in the four groups. For other details see Table 2.2 page 73.

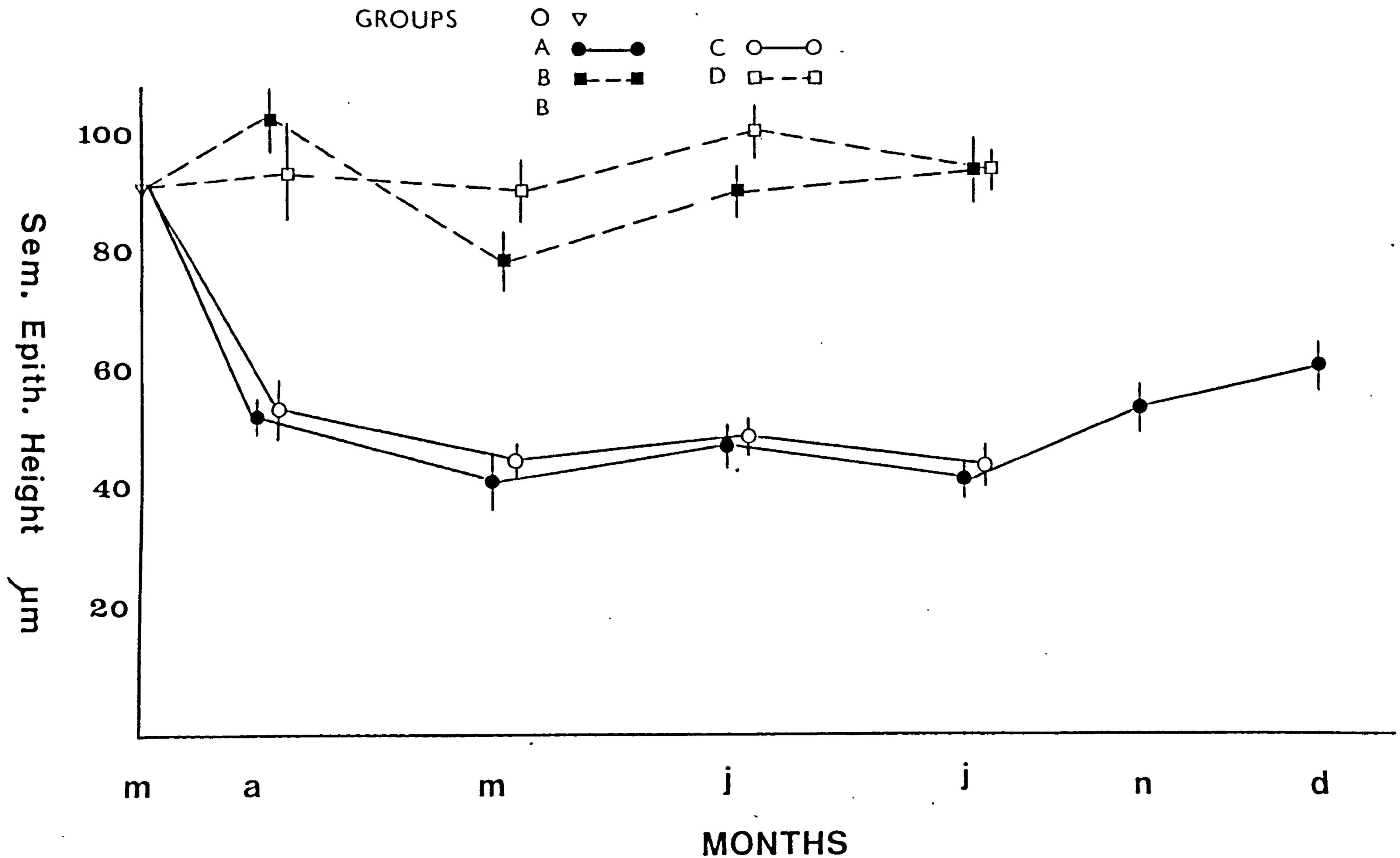

GROUPS
O
A
B
B
C
D
100
80
60
40
20
Sem. Epith. Height µm
m
a
m
j
j
n
d
MONTHS

Fig.2.4

Fluctuations in epididymal epithelial height in the four groups. For other details see Table 2.2 page 73.

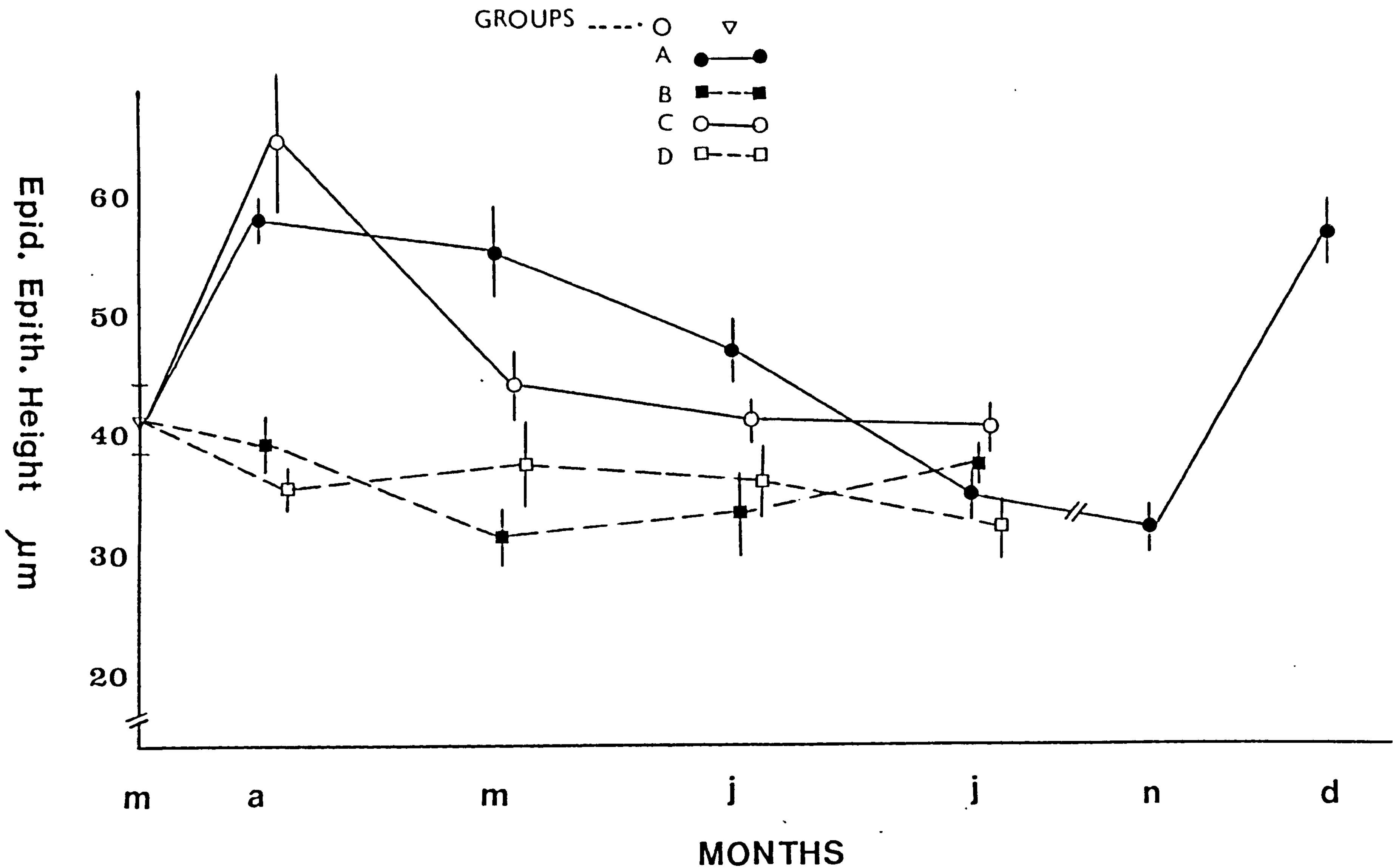

GROUPS
A
B
C
D
Epid. Epith. Height µm
60
50
40
30
20
m
a
m
j
j
n
d
MONTHS

Fig.2.5

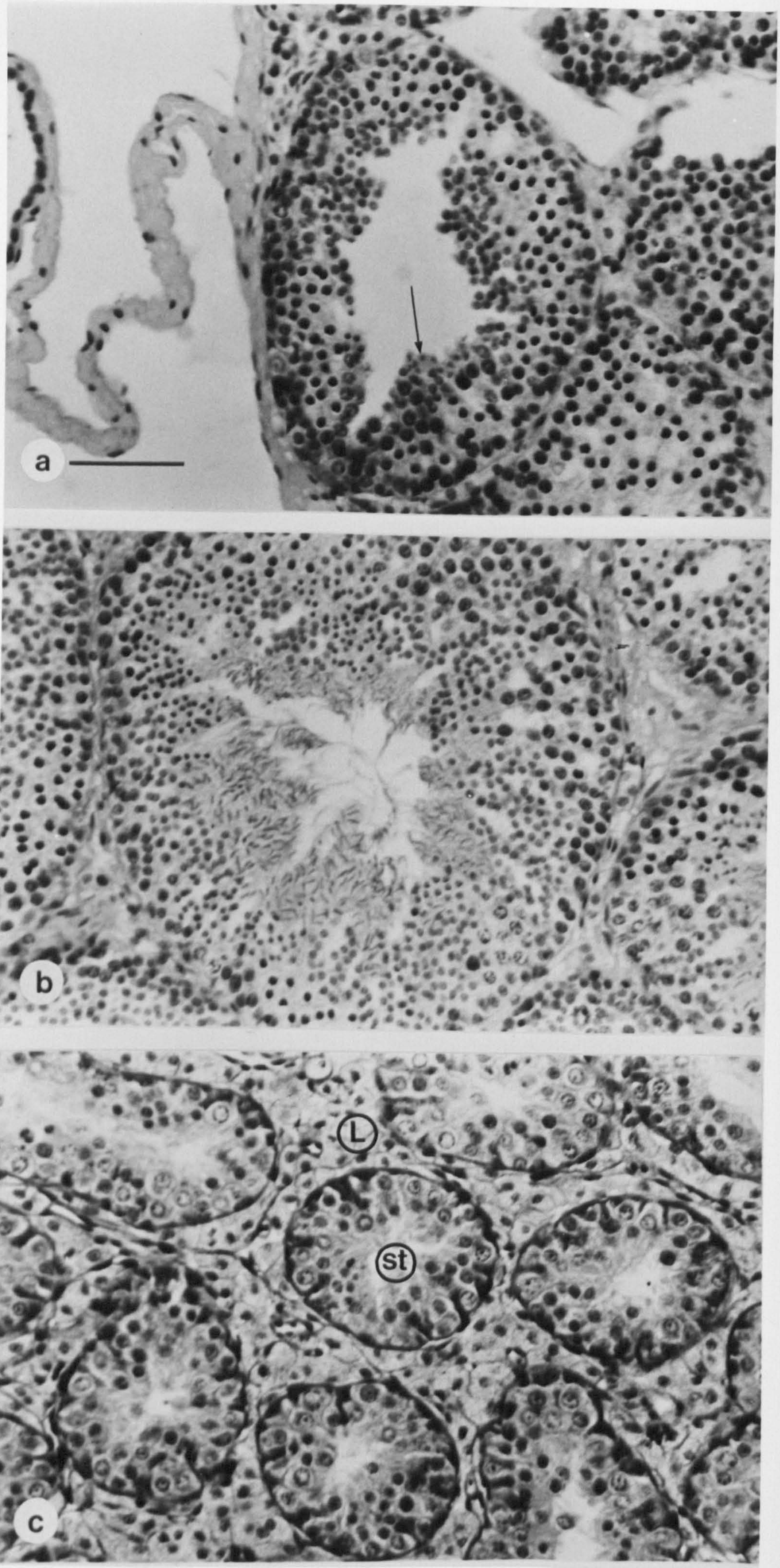
a. Sections in testis from group O showing stage 4 (arrow). Scale line is 50μm for all figs.

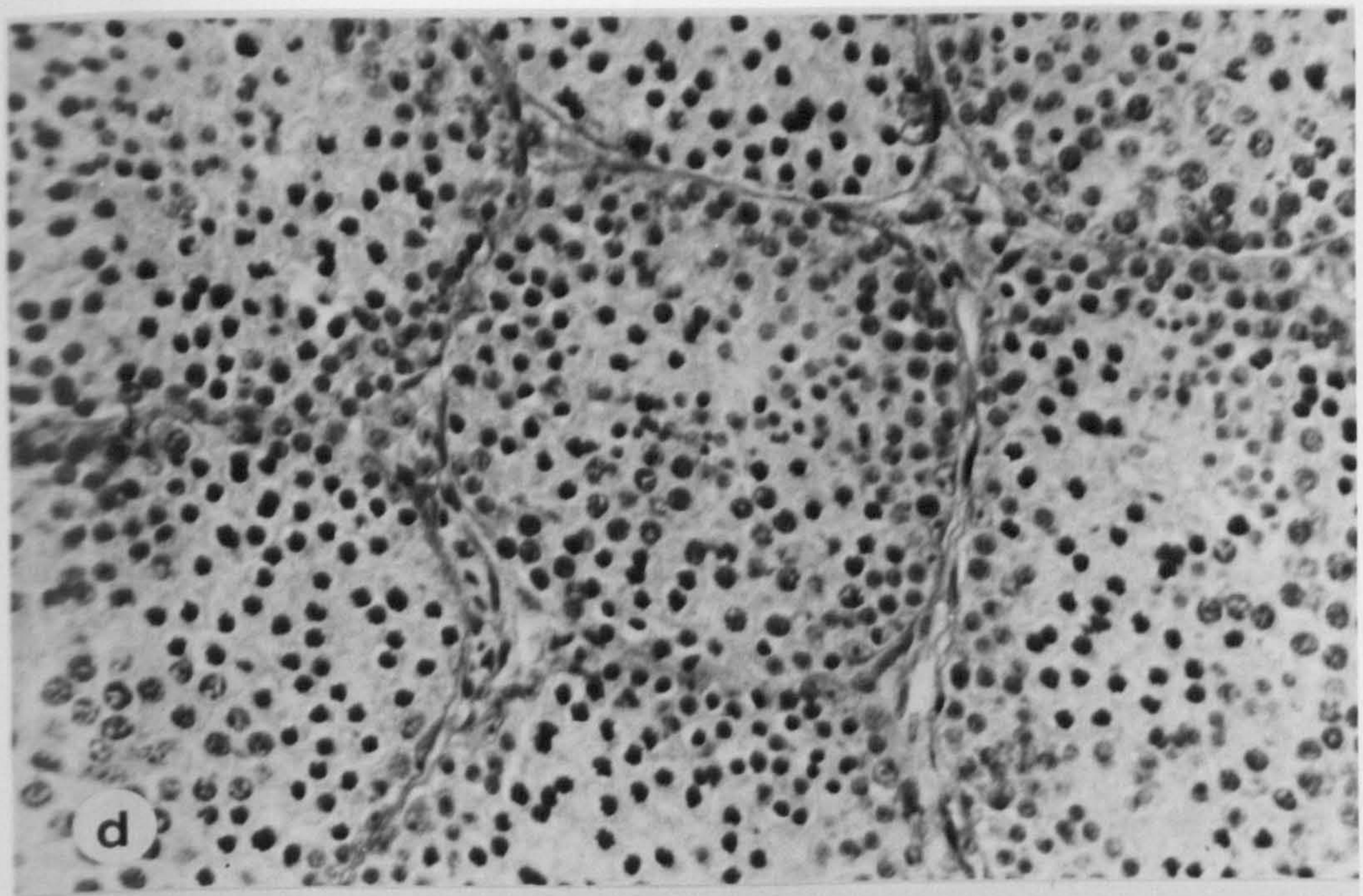
b. Section in testis taken from group A showing full breeding condition.

c. Seminiferous tubules from group A showing complete regression. L,, Leydig cells ; st , seminiferous tubules.

d. Tubules from group B during April exhibiting a stage similar to stage 1.

e. Tubules from group B during November. Note the numerous vacuoles (V) present in the tubules indicating cellular degeneration.


a
b
L
st
c



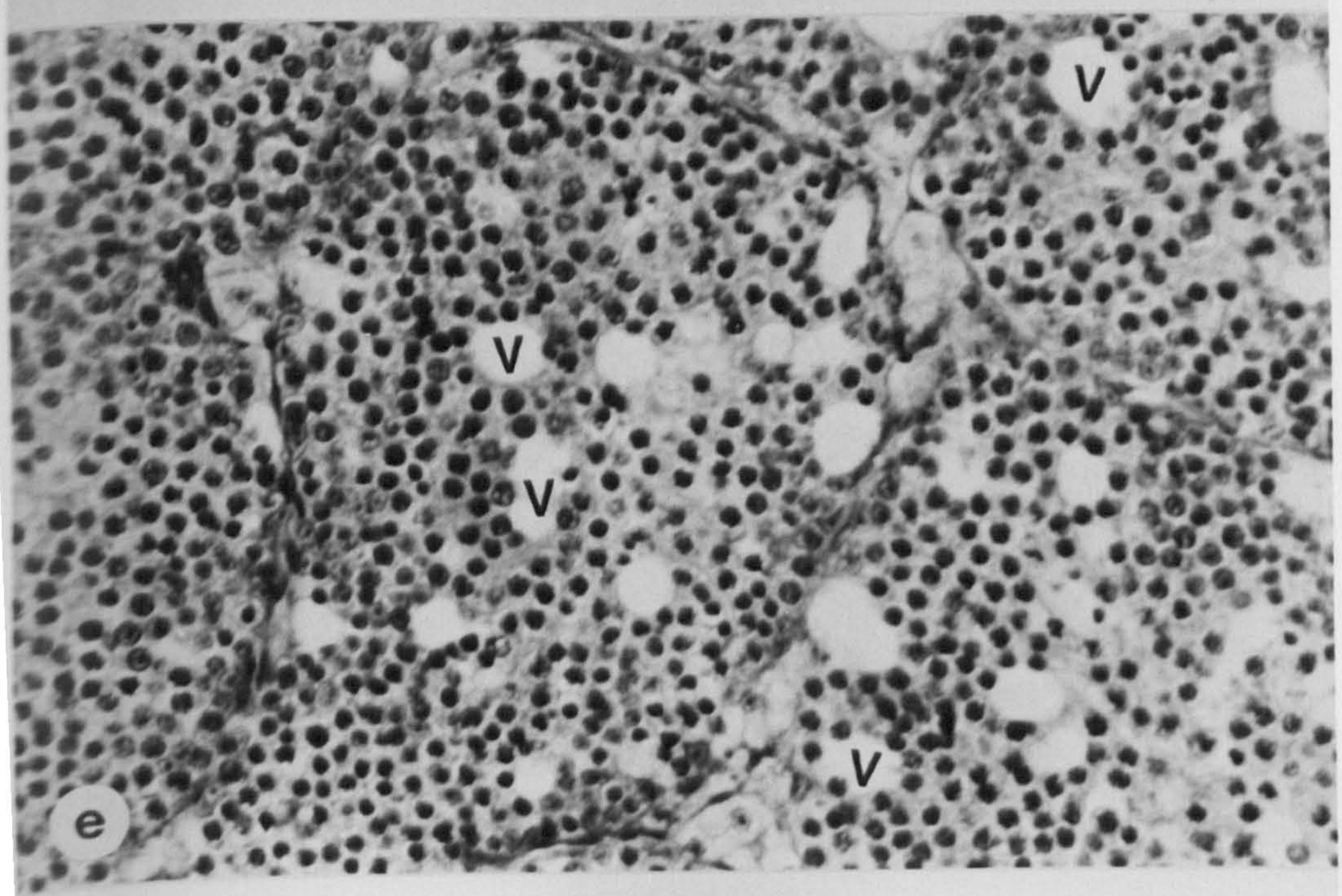


Fig 2.5

Fig.2.6

Sections of epididymes at different stages of spermatogenic activity . Sections stained with Ehrlichs haematoxylin and eosin. Scale line= 50 μm for figs. a, b & c but is 31.25 μm in fig.d.

a. Inactive epididymis from group 0 (initial conytols) during March. Note the inactive epithelium (EE) and the small empty lumen.(EL)

b. Active epididymis from group A during April. See the high columnar epithelium (EE) and the sperms in the lumen.

c. Inactive epididymis from group A during July. Note the small cuboidal cell, of epithelium and the remainder sperms in the lumen.

d. Inactive epididymis from group B during April. See the inactive epithelium and the empty lumen.

Activity in epididymis of groups C & D resembles that of groups A & B, respectively.

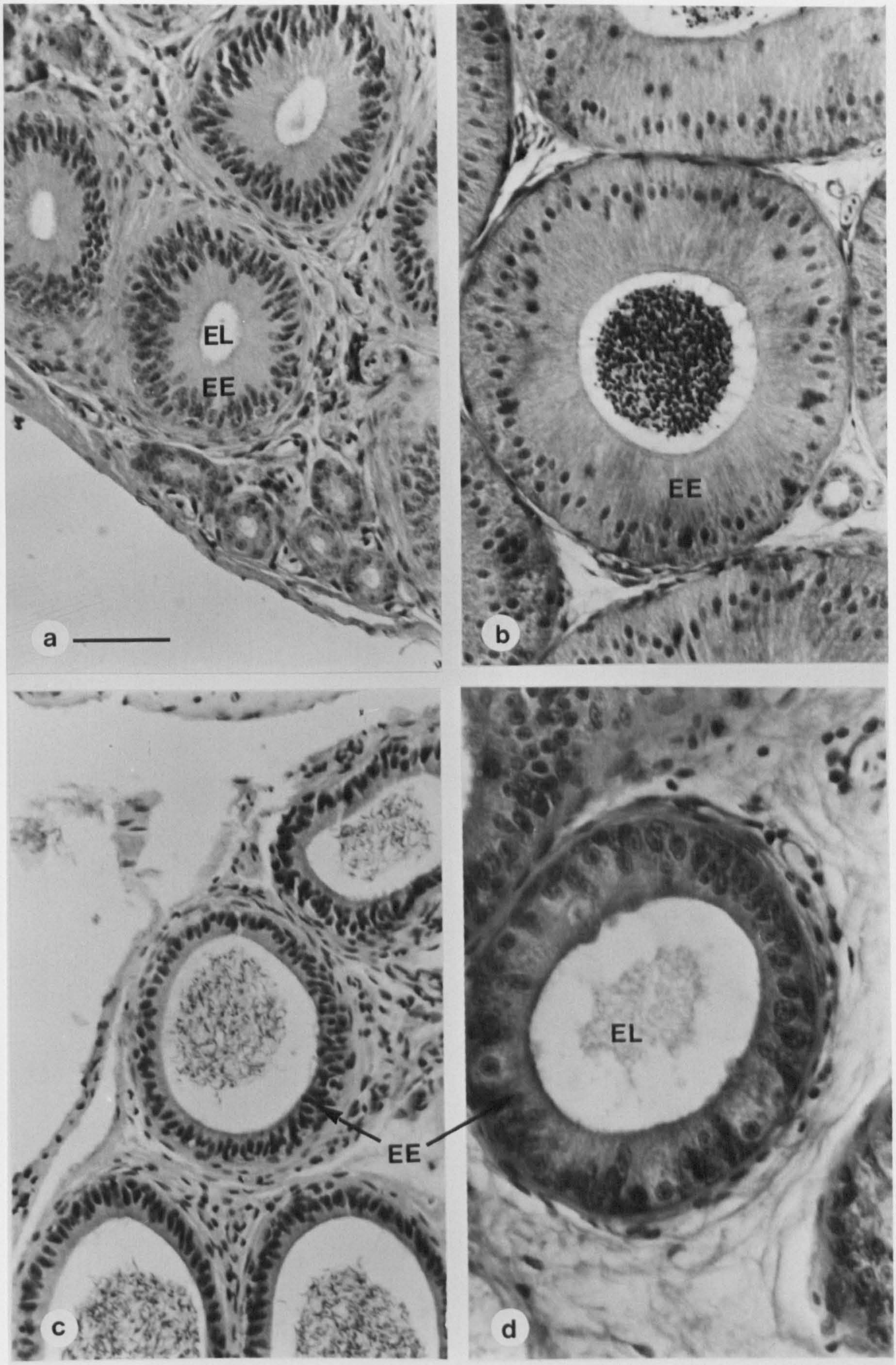


Fig 2.6

Fig. 2.7

Cross sections in the kidney showing the sexual segment They were cut at 7 μm and stained with Ehrlichs haematoxylin and eosin. Scale line is 125 μm for figs. a, b and c but 50 μm for fig. d.

a. Inactive segment from group O during March. Note the similarity between the sexual segment tubules (SS) and the uriniferous tubules (UT).

b. Active segment from group A during April. Note the difference between the large tubules of the sexual segment (SS) and the small uriniferous tubules (UT).

c. Inactive sexual segment (SS) from group A during July difficult to distinguish from other uriniferous tubules (UT).

d. Inactive sexual segment from group B during April similar in size to other kidney tubules.

Activity of the sexual segment of the kidney in groups C & D is similar to that described for groups A & B.

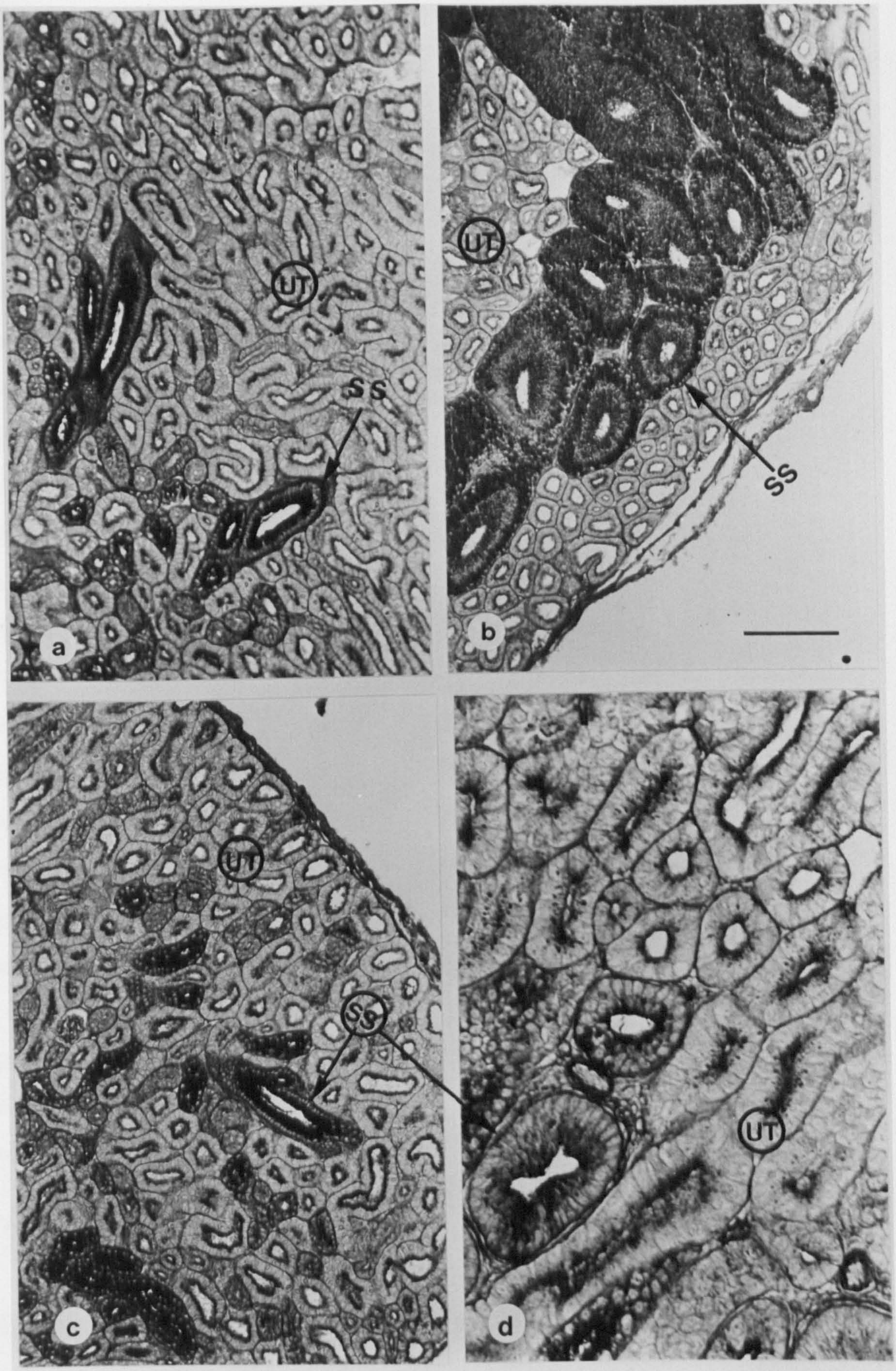


Fig 2.7

Discussion

The effects of temperature and photoperiod on testicular activity in lizards have been discussed previously by many authors (see Introduction). Temperature, in particular, has been found to play a very important role in controlling the activity of the testes. Whereas too low a temperature causes a complete cessation (Licht *et al.*, 1969) or, at least, great delay in testicular advancement (exceptions are the studies reported by Mellish, 1937 and Bartholomew, 1953), high temperatures (sometimes even just 1° or 2°C above the preferred body temperatures for the species) are reported to have caused testicular impairment and spermatogenic arrest in several species of lizards (e.g. Cowles & Burleson, 1945; Licht, 1965; Licht & Basu, 1967). Consequently, it is only within the normal range of preferred body temperatures that maximal gonadal activity, without defects, can be sustained. Nevertheless, even in this range of preferred body temperatures vast differences in testicular activity do exist. Joly & Saint-Girons (1975) reported, for *Lacerta muralis*, that the rate of spermatogenesis was temperature-dependent and that it was seven times faster at 27.5°C than at 17.5°C

In this report we hope to show clearly that temp erature, and not photoperiod, is the major environmental factor controlling the reproductive cycle in the lizard Chalcides ocellatus and that spermatogenesis is temperature-dependent.

Spermatogenesis

According to published reports, in nature spermatogenesis in *Chalcides ocellatus* starts at the end of September or by early October

(Badir, 1958). From that time until the end of February (or early March) testicular advancement does not proceed beyond spermatid formation (Badir, 1958). The data presented in this report show that the Initial Control animal autopsied in early March possessed testes mostly with secondary spermatocytes (stage 3) or, in one animal out of five, with undifferentiated spermatids (stage 4). Hence in the five months from the end of the previous natural breeding season,the testes have progressed little.

A comparison between the two groups of warm temperature animals revealed very interesting similarities, with some marginal differences. Interestingly, under the warm, optimal temperature (31°C) there was a rapid progress in testis activity to reach full maturity (stage 6) within only one month. There was no significant difference between the animals (all in groups of five) under long-day or short-day regimes. However, testicular regression under the two photoperiod regimes differed slightly. In April, one lizard of group C had started early regression (stage 7), while the other four animals of group C and all the animals in group A were at stage 6. In May, the number of animals exhibiting early testicular regression increased to include all the animals in group C, but only four in group A. The fifth animal of the latter group exhibited stage 6. The situation was somewhat different in the two following months. In June, while three animals of group A had achieved complete regression, none of the animals in group C had reached this stage. By July, four of the group A animals were at stage 8, while only three animals were at this stage in group C. The fifth animal in group A and the two remaining animals in group C were at stage 7.

Accordingly, under optimal conditions of high temperature and long day, active spermatogenesis persists for a longer time than it appears to do with high temperature and short day. How a difference of 4 hours

in daylength can cause this result is not clear. Moreover, it seems quite clear that temperature alone (and not photoperiod) is responsible for testicular recrudescence in Chalcides ocellatus, since long-day and short-day photoperiods resulted in almost similar testicular activities in groups A and C.

Results from the cold temperature groups, B and D, provided further support for the conclusion that: temperature is the prime external factor responsible for testicular recrudescence. In all of the animals under low temperature there was no effective spermatogenic progress, none of the animals in either group differed in any real way, from the condition of the initial controls, during the four months. Even in the three animals which survived until November (one in group B and two in group D) the testes were still at stage 3, i.e. similar to the initial controls. Obviously, if there is no recrudescence there can be no regression so, perhaps indirectly, regression, too, is temperature dependent.

The critical importance of temperature in reproductive activity of reptiles has been discussed previously for many forms including the musk turtle, Sternotherus odoratus (Risley, 1938), and the lizards, Lacerta sicula (Golgano, 1951), Sceloporus undulatus (Marion, 1970b), Anolis carolinensis (Licht, 1971a, 1972, 1974b), Dipsosaurus dorsalis and Xantusia vigilis (Licht, 1973). In all of these studies it has been stressed that not only is temperature the major cue in testicular recrudescence, but also it is an important timing cue for testis regression.

In the summer temperature groups (A and C), neither the constant daytime heat nor the photoperiods were able to delay the onset of testicular regression significantly. Generally speaking, regression started at the same time (May) under these experimental conditions as under natural con-

ditions in the wild (Badir, 1958). This indicates that the process of regression in the testis of Chalcides ocellatus is likely to be "obligatory" not "facultative" (see Licht, 1971a) as earlier claimed by workers on this species (Badir, 1958; Badir & Hussein, 1964). Badir and co-workers claimed that under standardised optimal conditions of heat and illumination, reproduction in Chalcides was changed from a cyclic to a constant pattern, i.e. reproduction is "facultative". However, the present work contradicts Badir's conclusion, since the optimal conditions of light and temperature were unable to delay the onset of regression significantly. Moreover, all the animals became sexually inactive during July leading to the conclusion that reproduction under optimal conditions of light and heat is cyclic (interrupted) not continuous.

Another important consideration is that an animal may become sexually active under certain conditions of light and temperature and then become sexually inactive under the same conditions, hence, if it has become sexually active it must subsequently pass through a period of refractoriness to both temperature and photoperiodic stimulation. In the summer temperature groups of Chalcides (A and C) the same photothermal conditions which caused rapid spermatogenesis at the onset of testicular recrudescence, pertained during testis regression in the summer; indicating that the animals are entering a period of photothermal refractoriness. Similarly, photothermal refractoriness was reported for Anolis carolinensis under laboratory conditions (Licht, 1967) and for Lacerta sicula (Licht et al., 1969).

As in Anolis carolinensis (Licht, 1971a), short days appear to accelerate testicular regression in Chalcides ocellatus, at least under warm temperature.

Testis weight and tubule condition

A comparison between the warm groups (A and C) and the cold groups (B and D) revealed points of special interest. Testis weight, seminiferous tubule diameter and epithelial height were high during April but, whereas in the warm temperature the values of the three components then declined to reach their minimal values in July, the values remained virtually constant during the same period in the cold temperature groups. An examination of the spermatogenic condition in groups B and D provided an answer for the constancy of these three components. There were continuous divisions at the level of primary spermatocytes; liberating increasing numbers of secondary spermatocytes. On the other hand, there was no transformation of the latter into spermatids. This resulted in the tubule lumen becoming progressively narrower, leading to complete occlusion near the end of the experimental period. This created a condition similar to that described for stage 1 (see Table 2.1). However, this condition of spermatogenesis cannot be ascribed to stage 1, since it was preceded by stages 3 and 4 in the previous months. Another feature in the tubular epithelia of cold temperature animals is the degeneration of some (or many) spermatocytes, leaving behind vacuoles, which may have resulted from the presence of excessively large numbers of spermatocytes, due to continuous production, in the tubules.

The failure of spermatocyte to spermatid transformation in the low temperature groups indicates that the development of the stages below the level of spermatids does not need a high temperature, and can be resumed even under cold temperatures (15°C), but the formation of both spermatids and spermatozoa does require warm temperatures. This explains

the early reports of Miller (1948) and Badir (1958) who studied the reproductive cycles of Xantusia vigilis and Chalcides ocellatus, respectively, in the wild. In both lizards, the advancement of spermatogenesis during winter is limited, and generally does not go beyond secondary spermatocytes. The formation of spermatids and spermatozoa does not commence until spring brings warmer temperatures.

Accessory sex organs:

Among the warm temperature animals a clear cycle in the activity of both the epididymis and the sexual segment of the kidney was evident. By contrast, there was no cyclicity in the activity of these accessory sex organs in the groups at winter temperature. Length of daylight appeared to exert no distinct effect at high or low temperature. Since the accessory sex organs are indicators of androgenic activity, it is clear here that temperature (and not photoperiod) may be the major external factor controlling androgenic secretions in Chalcides. This view is in contrast with the early proposition that photoperiod controls testicular secretions (Golgano, 1951).

Histological examination of testes and accessory sex organs in the four groups, together with the group described previously (see Section 1) revealed an intimate relationship between the stage of testicular development and the activity of the accessory sex organs. The latter were active only during late stage 5, stage 6, and early stage 7 of spermatogenesis. This narrow stage relationship indicates that the development and activation of the sex accessories do not commence until shortly before full testicular activity is reached and terminate shortly after the onset of testicular collapse.

Recent studies on the lizard Lacerta vivipara suggested that the secretions of the epididymis are utilized for sperm maturation (Gigon & Dufaure, 1977, 1980). In addition, it was suggested that the secretions of the sexual segment of the kidney perform two functions: they help in sperm transport to the female tract and, once there, cause sperm capacitation inside the tract (Prasad & Reddy, 1972; Kuhnel & Krisch, 1974).

Fat bodies and liver:

The weights of fat bodies and liver are variable within the same month, the same group, and during the period of experiment in the four groups. However, these variations in fat bodies and liver were not related to testicular activity and, in general terms, it can be said that the two components were constant during the period of experiment. Previous studies indicated that fat body cycle is dependent on the abundance of food (Licht et al., 1969; Vitt & Ohmart, 1975). Moreover, Licht (1974a) pointed out that the lack of fat body and liver reserves in lizards (Anolis species) during the breeding season is probably not a result of poor appetite or physiological inability to assimilate energy, but is due to the unavailability of sufficient food to allow such energy storage. The present study is in agreement with what Licht stated. In the warm temperature lizards (groups A and C) the constant high fat body weight was probably because of the availability of continuous sufficient food supply. In other words, the lack of fat body and liver cycles in Chalcides are probably due to the continuity of food abundance.

In the winter temperature groups (B and D) the situation is completely different. Since the animals in these groups rarely feed, and are very inactive also, one can expect a very low energy uptake as well as

a low energy output by the animal. The present data suggests that energy consumption is slightly higher than energy uptake with the result that fat body and liver storage decreases, slightly, towards the end of the experiment. In fact, in two of the three animals autopsied in November there was very little fat storage, while the third showed a complete absence of fat reserves. This indicates that even if there is an abundance of food the animals need to be in a condition that permits food uptake.

SECTION 3

VARIATIONS IN TOTAL ANDROGENS IN THE PLASMA AND TESTIS IN *CHALCIDES*

SUMMARY

Monthly changes in plasma and testicular androgen concentration, in the lizard Chalcides ocellatus under standardised conditions of light and temperature (day 14L and 31°C: night 10D and 20°C), were measured by radioimmunoassay. In addition, the effects of different light and temperature regimes on androgen levels were studied. The lizards under these photothermal regimes were group A, 14L/10D + 31°C; B, 14L/10D + 15°C; C, 10L/14D + 31°C and D, 10L/14D + 15°C.

Over a 12 month period (starting in February 1978) under the standard conditions, a peak in plasma and testicular total androgens (ng/testis) was associated with high testicular weights in March, during the breeding season. Directly after the termination of the breeding season, both plasma and testicular androgen levels declined. A second peak in total androgens in plasma and testicular homogenates (ng/testis) occurred during September, preceding the onset of the new testicular recrudescence. When testicular androgen levels were calculated per 100 mg of testis weight (i.e. as a "concentration"), only one single peak occurred; during September.

Circulating androgen levels showed a high peak in April (breeding season), in the warm temperature animals of groups A and C, though it was significantly higher in group A than in group C. After April plasma androgen levels dropped continuously until the end of the experiment in July. This drop was concomitant with a related drop in testis, epididymis and kidney sex-segment activities. In the cold temperature groups (B and D), plasma androgens were lower than or not significantly different (except for July samples of group B) from the levels in initial controls (animals

freshly collected from the wild and sampled before setting up the controlled light/temperature sets).

INTRODUCTION

Before the development of accurate techniques, such as radioimmunoassay (RIA), for measurement of plasma hormone levels, histochemical demonstrations of certain enzymes involved in androgen metabolism were among the best ways of showing cyclic fluctuations in androgen activity. However, histochemical studies generally produce only qualitative data, the results being difficult to quantify. In particular, such investigations have at best provided only indirect evidence for an annual cycle of androgen levels in squamate reptiles (Marshall & Wolf, 1957; Lofts *et al.*, 1966; Eyeson, 1971; Guraya, 1973).

Techniques recently developed for the RIA of androgens, have now been applied to a few squamate reptiles and have demonstrated, clearly, the existence of an annual cycle in levels of plasma testosterone and of testicular (homogenate) testosterone (Arslan *et al.*, 1978; Courty & Dufaure, 1979, 1980). Plasma androgen levels can be directly correlated with the histological appearance of the testes in the lizards *Tiliqua rugosa* (Bourne & Seamark, 1975, 1978); *Uromastix hardwicki* (Arslan *et al.*, 1978) and *Lacerta vivipara* (Courty & Dufaure, 1979, 1980), reaching a peak of activity during the breeding season when testis weight, spermatogenesis, and spermiogenesis are maximum. Although testicular androgen levels vary essentially in parallel with those of peripheral plasma, the former show a secondary peak of activity just prior to the onset of the new testicular cycle (Arslan *et al.*, 1978; Courty & Dufaure, 1979, 1980). It is believed that androgen accumulation in the testicular tissues (secondary peak) may locally initiate testicular recrudescence (Arslan *et al.*, 1978).

Testosterone has been shown to be the principal androgen in several species of squamates including, Natrix sipedon (Callard, 1967), Naja naja (Tam, Phillips & Lofts, 1969; Lofts & Choy, 1971), Tiliqua rugosa (Bourne & Seamark, 1975), Lacerta viridis (Hews & Kime, 1978) and L. vivipara (Courty & Dufaure, 1980). However, Bourne & Seamark (1978) reported, in Tiliqua rugosa, that epitestosterone (and not testosterone) was the major metabolite produced by the testis and that the rate of testosterone synthesis was relatively low (not exceeding 10% of total androgens). They believed that epitestosterone had not been detected by previous investigators because of the quality of the chromatographic systems then available, which could not distinguish epitestosterone from testosterone (Bourne & Seamark, 1978).

The present work has been undertaken to extend general knowledge of androgen levels in lizards, Chalcides ocellatus in particular, and also to see the effects of light and temperature on plasma levels of androgens.

MATERIALS AND METHODS

Animals

Male *Chalcides ocellatus* were obtained from Egypt. Two experiments were run; the first was to determine the levels of total androgens in plasma and testis throughout a complete year, starting in February 1978, under standardised conditions of temperature and photoperiod (same as group A following); the second was to investigate the effect of variations in light and temperature on the levels of total androgens in plasma. In this respect, four combinations of light and temperature were used; Group A, 31°C over 12 hours of a 14 hr day (L) then 20°C for the rest 10 hr of dark (D) of the "day"; Group B, 15°C (constant) + 14L/10D; Group C, 10L at 31°C/14D at 20°C; and Group D, 15°C (constant) + 10L/14D. Initial controls (Group 0) were autopsied (in late February when the animals were first collected) to determine the reproductive status of animals when they were first put into these different regimes (beginning of March). The methods of handling the animals and the experimental conditions have been described in full detail in previous sections (see Sections 1 and 2).

Plasma preparation

Animals were killed by decapitation and blood was collected in small, heparinized plastic tubes. Samples were left at room temperature for 5 min before being spun at 3000 rpm (in an MSE centrifuge) for another 5 min. Plasma samples were transferred into new, plastic tubes and subsequently frozen, at -30°C, until the RIA.

Testis homogenate preparation

The right testis in each animal was weighed to the nearest 0.1 mg and then homogenized in 5% w/v of Sørensen's buffer (pH 7.5). The homogenates were spun at 3000 rpm for 15 min and the supernatants were transferred into plastic tubes and frozen at -30°C (in a BW freezer) until RIA.

Androgen RIA

The RIA of plasma testosterone described by Lu Maung (1976) was used. The antiserum was raised in goat against testosterone-3-carboxymethyloxime-BSA by Dr. B.J.A. Furr (I.C.I., Alderley Edge, Cheshire).

In essence, steroid is extracted from plasma into an organic solvent, the solvent is then evaporated and the extracted residue redissolved in assay buffer. The sample is then equilibrated with hormone-specific antibody and radioactive tracer with which it competes for binding sites. At equilibrium, unbound hormone and tracer ("free" fraction) is separated from antibody-bound hormone and tracer ("bound" fraction) and the radioactivity associated with the bound fraction is determined and converted to hormone concentration by reference to a standard curve, prepared in each assay.

A. Extraction of androgens from samples. Each sample (10-20μl for plasma and 20-50μl for testis homogenate) was dispensed, in duplicate, to the bottom of clean glass centrifuge tubes (110 x 15 mm). Two ml of a mixture of 1:4 v/v diethyl ether (b.p. 34°C)/petroleum spirit (b.p. 60°C, BDH Analar grade) was added and the contents of each tube were mixed for 30 sec on a rotary mixer. The tubes were left to stand for 5 min to allow phase separation and then frozen at -70°C for 30 min to freeze the lower

aqueous layer. The unfrozen mixture, containing extracted steroid, was decanted into glass tubes (75 x 7 mm, flint glass no. TF 104, Gallenkamp), the solvent was evaporated under a stream of air, and the residue redissolved in 50μl of steroid buffer. The latter consists of 0.1M PBS (phosphate buffer saline) with 0.1% sodium azide, pH 7. In order to check the purity of solvent and the interassay variation, two triple-distilled water samples were included in every assay.

Preliminary tests on the androgen content of plasma and testicular homogenate were made to determine the volume needed for the assay. Plasma (10, 20 & 50μl) and testicular homogenate (20, 50, 100 & 200μl) samples were examined from two active and two inactive males. These were extracted (using the above procedure) for 30 sec (continuous agitation) and then run in the normal RIA. This method of extraction yielded relatively very high levels of total androgens for plasma samples, but lower figures, though readable on the standard curve, for testicular homogenate. These high figures of androgen content gave, in fact, a misleading impression of the success of the extraction procedure. When the unknown plasma samples were assayed, the same method of extraction (30 sec of continuous agitation) was followed. However, the recovery test (unfortunately carried out at the same time rather than previously) showed relatively low values (57.2%) for extraction. This led to the set up of a test for the extraction procedure itself. In this test groups of similar tubes containing testicular homogenate were extracted in one of three ways; by mixing the samples with the solvent for 30 sec with continuous agitation; 30 sec with interrupted agitation; double extracted with 1.0 ml of solvent with 45 sec interrupted agitation each time. The latter two methods of extraction yielded high figures for recovery (86.6% and 93.2%, respectively). For speed and con-

venience the method of 30 sec of interupted agitation was applied for the testicular homogenate, with 86.6% recovery.

B. Preparation of standard curve and assay procedure.

1. A standard solution of testosterone at a concentration of 20 ng/ml was prepared using crystalline analytical grade testosterone (Sigma) dissolved in steroid buffer. 50μl aliquots of this standard were serially diluted, in triplicate, in the flint glass tubes to produce 20, 10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 ng/ml. Extracted samples of plasma and testicular homogenates were set up in duplicate to be included in the same assay.

Three triplicates of blank tubes containing 50μl of steroid buffer (and ultimately some of the other components) were also set up for the following purposes:-

a) no antiserum added - for estimating the free counts not absorbed by charcoal.

b) no antiserum or charcoal added - for measuring the total count added to each tube.

c) no unlabelled testosterone added - for estimating the maximum binding of tritiated testosterone and to provide the zero point of the standard curve.

2. 50μl of [^{3}H]-testosterone (ca 10,000 cpm/50μl steroid buffer) was added to each tube. The very high specific activity (83 Ci/mM) tritiated testosterone was obtained from the Radiochemical Centre (Amersham, Bucks, Cat. no. TRK 402; 1,2,6,7(n)-^{3}H-testosterone dissolved in benzene) and stored at 4°C. Stock solutions were prepared by drying down 15μl of the ^{3}H-testosterone and then adding 100 ml of steroid buffer. This solution

was kept at 4°C and was usable for at least 12 weeks.

3. 50μl of antiserum, diluted in steroid buffer to a concentration of 1:4000, was added to all the tubes except the first two triplicates of the blanks (a,b), to which 50μl of steroid buffer was added instead. The contents of the tubes were mixed briefly and then centrifuged briefly (1000 rpm/20 sec) to ensure that all components were at the bottom of the tube. All the tubes were then incubated at 4°C overnight.

4. After overnight incubation, 1.0 ml of dextran-coated charcoal was added to all tubes except the second triplicate of the blanks (b) to which 1.0 ml of steroid buffer was added instead. The dextran-coated charcoal solution contained 125 mg Norit A (Sigma), 12.5 mg Dextran T70 (Pharmacia) and 50 mg gelatin, per 100 ml of steroid buffer. All tubes were briefly mixed and left at 4°C for 15 min before being centrifuged at 3000 rpm for 15 min in a refrigerated centrifuge (Cool spin, MSE Ltd.). The charcoal adsorbed the "free" hormone which could then be removed.

5. The supernatants (bound fraction) were decanted into scintillation vial inserts to which 2.5 ml of scintillation fluid was added. The stock solution of the scintillation fluid contained 32 g PPO (2,5-diphenyloxazole) and 2.118 g POPOP [1,4-bis-2(4-methyl-5-diphenyloxazole)-benzene] (Nuclear Enterprise Ltd.) per 8 litres of toluene. The vials were left for 24 hrs before being counted in an automatic liquid scintillation spectrometer (Nuclear Chicago Unilux III) with a counting efficiency of ca 40% for tritium.

Results were calculated as % binding, and the mean cpm of blanks containing no unlabelled testosterone (c) was assumed to be 100% binding. After subtracting the mean cpm resulting from tube (a) from the mean cpm of each sample, the cpm of all tubes were converted into % binding relative to

the maximum 100% binding. The standard curve was plotted on a 3 cycle semi-logarithmic graph paper. The unknown values of the serum samples were interpolated directly from the graph. The final potency of an unknown was calculated as the mean of the number of determinations and expressed as ng equivalents of testosterone standard/ml of plasma, testis, or 100 mg wet testis weight.

C. Test on extraction procedure. The extraction procedure was checked by taking pooled samples of plasma and testis homogenate, adding to these a known amount of radioactive testosterone and then running the samples through the extraction procedure described above. The final extract was counted and the recovery rate was estimated as a percentage of the total count added. A similar percentage recovery rate was assumed for all samples. Recovery figures counted in this way were 57.2% for plasma and 86.6% for testicular homogenates. The latter figures represent the average of 16 tubes divided between two assays for each figure.

Although the number of animals was kept constant in the experiments under standard conditions of light and temperature (8-10 animals) and under the four photo-thermal regimes (5 animals), for a variety of reasons, accidental and otherwise, unfortunately not all the samples yielded plasma and testicular homogenates (as indicated in Table 3.2 and Table 3.3).

A summary of tube arrangement and content in the RIA is shown in Table 3.1.

The 5% level has been used in all tests for statistical significance.

Table 3.1

The set up of radioimmunoassay tubes and their contents during the assay

	Buffer	Lab. T	Antibody	Standard	Sample	Charcoal
NSB (a)	100μl	50μl				1000μl
Max. B (c)	50μl	50μl	50μl			1000μl
Standard		50μl	50μl	50μl		1000μl
Samples		50μl	50μl		50μl	1000μl
Total Count (b)	1100μl	50μl				

NSB: Non-Specific Binding i.e. tubes (a) of the blanks

Max.B: Maximum Binding i.e. tubes (c) of the blanks

Lab.T: Labelled Testosterone

RESULTS

Since the extracts of plasma and testicular homogenates were assayed directly without chromatographic purification of testosterone, the assay measured "total androgens" rather than testosterone alone. This qualification is important in view of the extensive cross-reactivity of 5α-dihydrotestosterone, which reached 66.4 ± 3.8%, in this assay system. Further cross-reactivity of the antiserum used with other steroid hormones is described by Etches (1975).

A. Changes in androgen levels through one year of standard 'optimum' conditions

The combination of light and temperature used in this experiment has been described in detail elsewhere (see Section 1). Briefly, the animals were kept under a daylength of 14L with "daytime" temperature of 31°C during the 12 month period of experiment starting in February.

Plasma androgen levels. The variation in the level of total plasma androgens and the correlation with testis weight are summarized in Table 3.2 and Fig. 3.1. Plasma androgen levels are quite high during February, but over the next month only a slight, non-significant, rise occurs; nonetheless, the March values for plasma androgens are the maximum levels. Subsequently the levels start dropping sharply to reach a low value in May. Although plasma androgen levels decline further, after May, the decline is gentle, and not significant, up to August, when the minimal levels are reached. A sudden, sharp elevation in plasma androgen levels occurs from August to September, then a marked decline in October and a further fall (but insignificant) in November. A second sharp rise occurs from November

Table 3.2

Variations in testicular weight and plasma and testicular total androgens (Mean ± SEM) in Chalcides ocellatus during the year

Months (1978-79)	Testis weight mg	Total androgens		
		Plasma ng/ml	Testicular	
			ng/100 mg	ng/Testis
Feb.	87.9 ± 9.3	41.3 ± 6.1[c]	18.1 ± 4.1	17.1 ± 4.8
Mar.	104.4 ± 13.1	47.8 ± 6.9[a]	25.8 ± 3.9	27.5 ± 5.2
Apr.	54.1 ± 9.6	30.7 ± 6.2	31.6 ± 5.2[b]	17.2 ± 1.9[b]
May	42.5 ± 8.2	18.8 ± 3.5	30.3 ± 7.4[d]	12.7 ± 2.9[d]
June	37.6 ± 6.8	18.0 ± 2.0	20.1 ± 3.5[a]	7.0 ± 1.4[a]
July	42.8 ± 10.7	14.3 ± 3.4[a]	32.3 ± 2.8	12.0 ± 2.3
Aug.	24.0 ± 3.9	8.3 ± 2.1[a]	44.4 ± 3.0	10.3 ± 1.8
Sept.	42.5 ± 11.1	36.9 ± 3.7	55.3 ± 5.8	18.7 ± 3.4
Oct.	29.5 ± 4.2[d]	14.9 ± 1.9[a]	38.6 ± 5.4[a]	10.0 ± 1.2[a]
Nov.	88.9 ± 14.0[b]	9.2 ± 1.3[c]	22.2 ± 4.9[b]	15.1 ± 1.8[b]
Dec.	102.7 ± 11.9	33.8 ± 5.5	13.4 ± 2.5	11.8 ± 1.4
Jan.	85.1 ± 9.8	37.2 ± 3.3[b]	17.7 ± 1.7	14.7 ± 1.8

a. 9 animals

b. 8 animals

c. 7 animals

d. 6 animals

All other samples contain 10 animals

to December followed by a steady, insignificant, rise to the next March.

It is clear, thus, that plasma androgen levels are high over the period from December until April and, apparently, in the month of September, whereas they are low throughout the rest of the year.

Testicular androgen levels. Table 3.2 and Fig. 3.2 illustrate the total androgen levels in testicular homogenates. Calculations were made on two bases; total androgens per 100 mg of testis wet weight and total per testis.

The values for total androgens per 100 mg testis reveal an astonishing figure. In marked contrast to the levels of androgen in plasma, androgen levels/100 mg are relatively low from early February until June, but high in August, September (maximum) and October and then back to the low February levels from November to January (Fig. 3.2).

It is apparent that androgen levels in ng/100 mg testis are high during the main period of testis inactivity. In fact, the data showed that whenever the testis is small in weight and quiescent, its content of androgen expressed as a concentration is very high, sometimes about 10 times more than the levels in a large, active testis.

When androgen levels were calculated as a total per testis, the result was somewhat different. The levels of androgens (ng/testis) reach their maximum in March. Then the levels decline during April and May to minimal values in June. There is a secondary rise in androgen levels up to September and then a relatively sharp decline in October. The climb back up to the peak March value seems to start in December.

Although the figures given by total androgen level/testis are different from those calculated per 100 mg testis weight, some similarities

exist. The most important of all is the relatively high level of androgens for both in September (Fig. 3.2).

B. Androgen levels under different photo-thermal regimes

The reasons for the choice of the four photo-thermal regimes was discussed previously (see Section 2).

It should be noted that these observations start one month later, in March, than the 'standard' series described above.

Warm Temperature (31°C) Groups, A and C.

Group A (long day; 14L). As the present study was not preceded by a field study to determine plasma levels of androgens in natural living lizards, it was impossible to know whether or not the levels of androgen in the initial sample (Group 0) in March resembled those of field animals, though these had, in fact, been collected only about one week previously. A sharp rise in plasma androgen levels is clear after only one month under these 'optimum' conditions. This is followed by a steep decline up to May and a further decline in July. No samples were examined between July and November, but in the latter month the lowest level of plasma androgens was recorded. However, in December there was a new upsurge in androgens. The fluctuations in androgen levels and testes weights are presented in Table 3.3 and Fig. 3.3

Group C (short day; 10L). Under these conditions the plasma androgen levels showed a small, but significant, rise within one month from the initial Group 0 levels. There was a small fall between April and May, but no further significant change in levels up to the end of the experiment in July (Table 3.3 and Fig. 3.3).

A comparison between plasma androgen levels in the two groups,

Table 3.3

Monthly variations in testis weight and plasma androgens (Mean ± SEM) in *Chalcides ocellatus* under different conditions of light and temperature

	Groups	Time of the year (1979)						
		March	April	May	June	July	November	December
Testis weights (mg)	0[a]	99.2 ± 5.1						
	A		94.4 ± 10.8	45.5 ± 9.0	39.6 ± 10.9	14.6 ± 1.5	54.0 ± 9.2	70.3 ± 16.3
	B		78.7 ± 11.5	80.7 ± 4.1	78.3 ± 8.2	83.6 ± 7.0		
	C		57.1 ± 4.2	77.3 ± 18.0	49.3 ± 8.4	12.2 ± 2.4		
	D		78.1 ± 16.0	40.8 ± 10.2	71.3 ± 5.9	55.6 ± 9.4		
Plasma Total Androgens (ng/ml)	0[a]	21.3 ± 2.7						
	A		69.2 ± 4.7	47.7 ± 4.3[b]	48.1 ± 6.7	29.0 ± 2.8	8.0 ± 2.3[b]	51.1 ± 10.2[b]
	B		20.0 ± 10.0	26.9 ± 2.6[b]	18.2 ± 3.9	58.0 ± 8.7		
	C		35.6 ± 5.4	16.1 ± 2.7	22.2 ± 6.0[b]	12.9 ± 3.2		
	D		10.6 ± 4.1	26.6 ± 10.1[b]	5.3 ± 2.4[b]	6.5 ± 2.6[b]		

a. Group 0 is the Initial Control sample

b. Sample of 4 animals

All other samples are 5 animals

A and C, revealed that the levels in group A were almost double those in group C in all months of the experiment (Fig. 3.3).

Cold Temperature (15°C) Groups, B and D.

The average levels of plasma androgens were not significantly different in the two groups during the first two months of exposure to such conditions nor did these levels differ significantly from the levels in initial controls (Table 3.3, Fig. 3.3). The levels in group B animals (14L) remained the same during June but elevated sharply and significantly during July from the values of the initial controls. However, plasma androgen levels in group D (10L) dropped significantly during June and July to the lowest levels recorded in any group. Thus, it is clear that plasma androgen levels in cold temperature groups while similar over the first two months, differed dramatically over the last two months of the experiment, with group B animals showing higher values (Table 3.3, Fig. 3.3), almost reaching the peak of the optimum conditions group A.

It is important to note that the animals of all four groups here, and the standard set described previously, showed wide variations between animals of each monthly sample in the levels of plasma and testicular androgens. Generally, the latter levels were not correlated with the spermatogenic condition of the testes. In fact, in some samples, androgen levels in testis and plasma exhibited a 10-20-fold difference in the same developmental stage of testicular activity, even in the same month.

Fig. 3.1

Variations in plasma androgens (ng / ml) during 12 months under standard conditions of light (14h) and temperature (31 C). Numericals indicate the sample number. Mean and SEM are indicated for each sample.

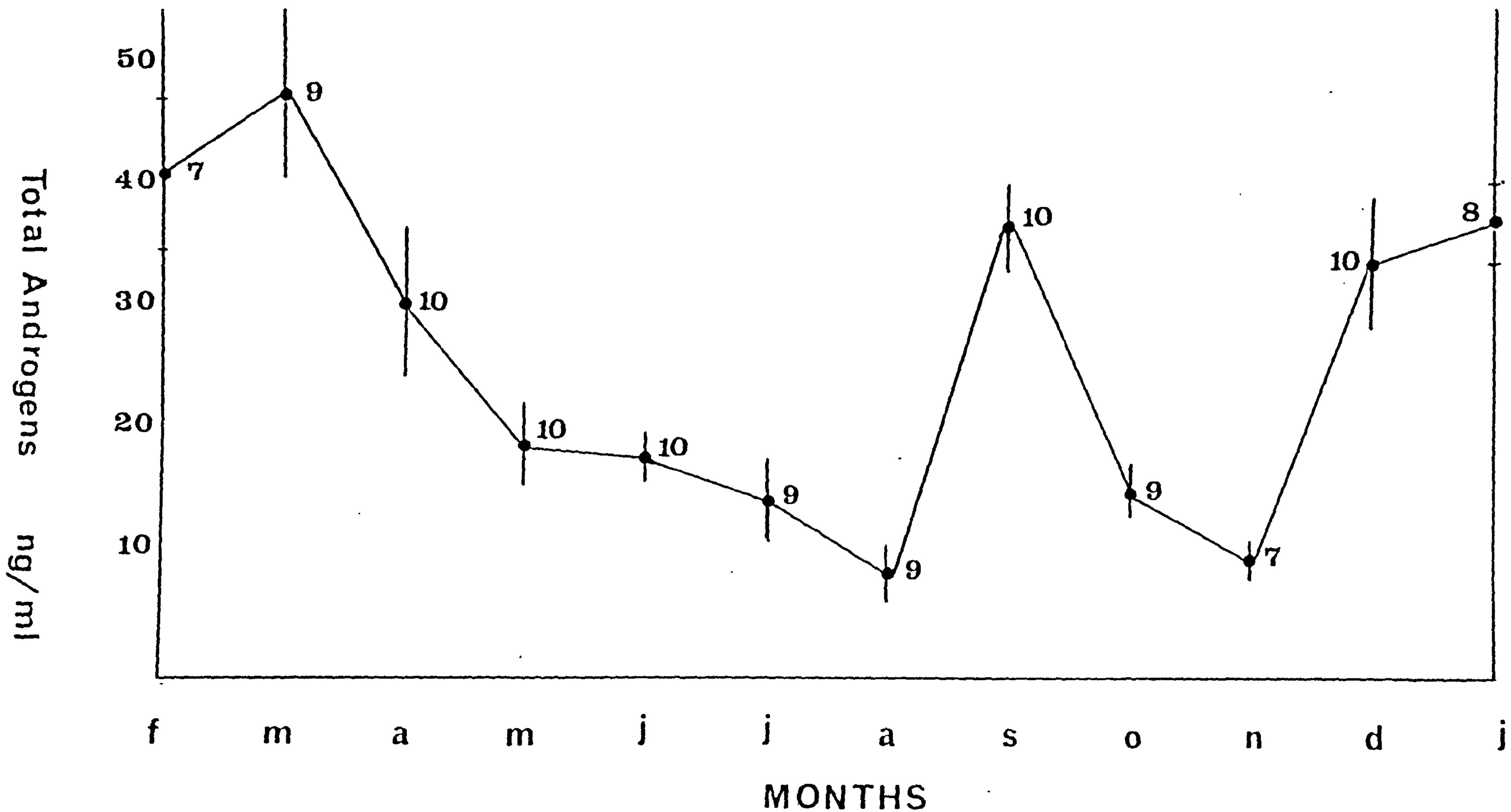

Total Androgens ng/ml
50
40
30
20
10
7
9
10
10
10
9
9
10
10
9
7
8
f
m
a
m
j
j
a
s
o
n
d
j
MONTHS

Fig. 3.2

Fluctuations in testicular androgens during one year under standard photothermal conditions. Mean and SEM are indicated for each sample.

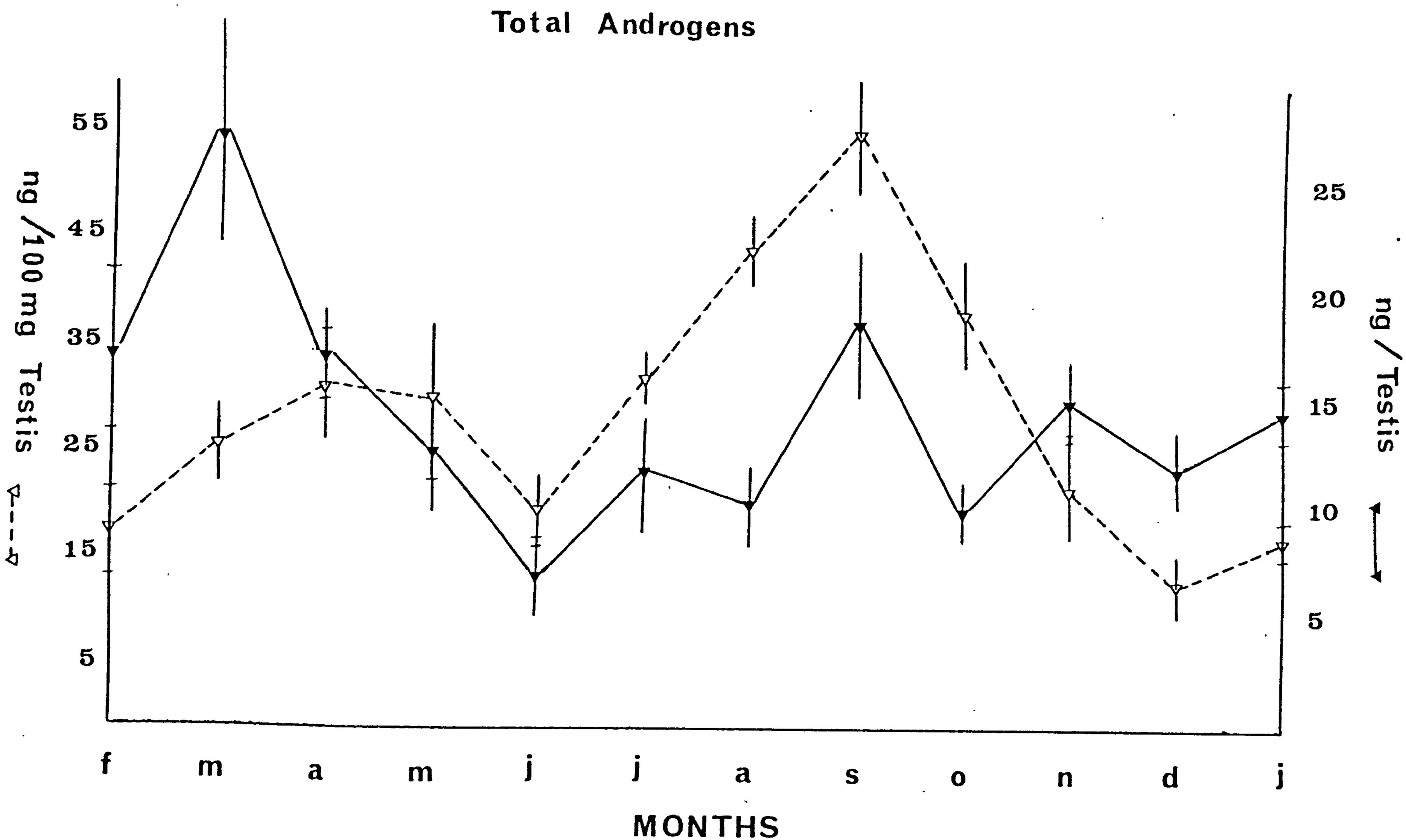

Total Androgens
55
45
35
25
15
5
ng /100 mg Testis
25
20
15
10
5
ng / Testis
f
m
a
m
j
j
a
s
o
n
d
j
MONTHS

Fig.3.3

Changes in plasma androgens in the four groups. Mean and SEM are indicated for each sample. For other details see Materials and Methods.

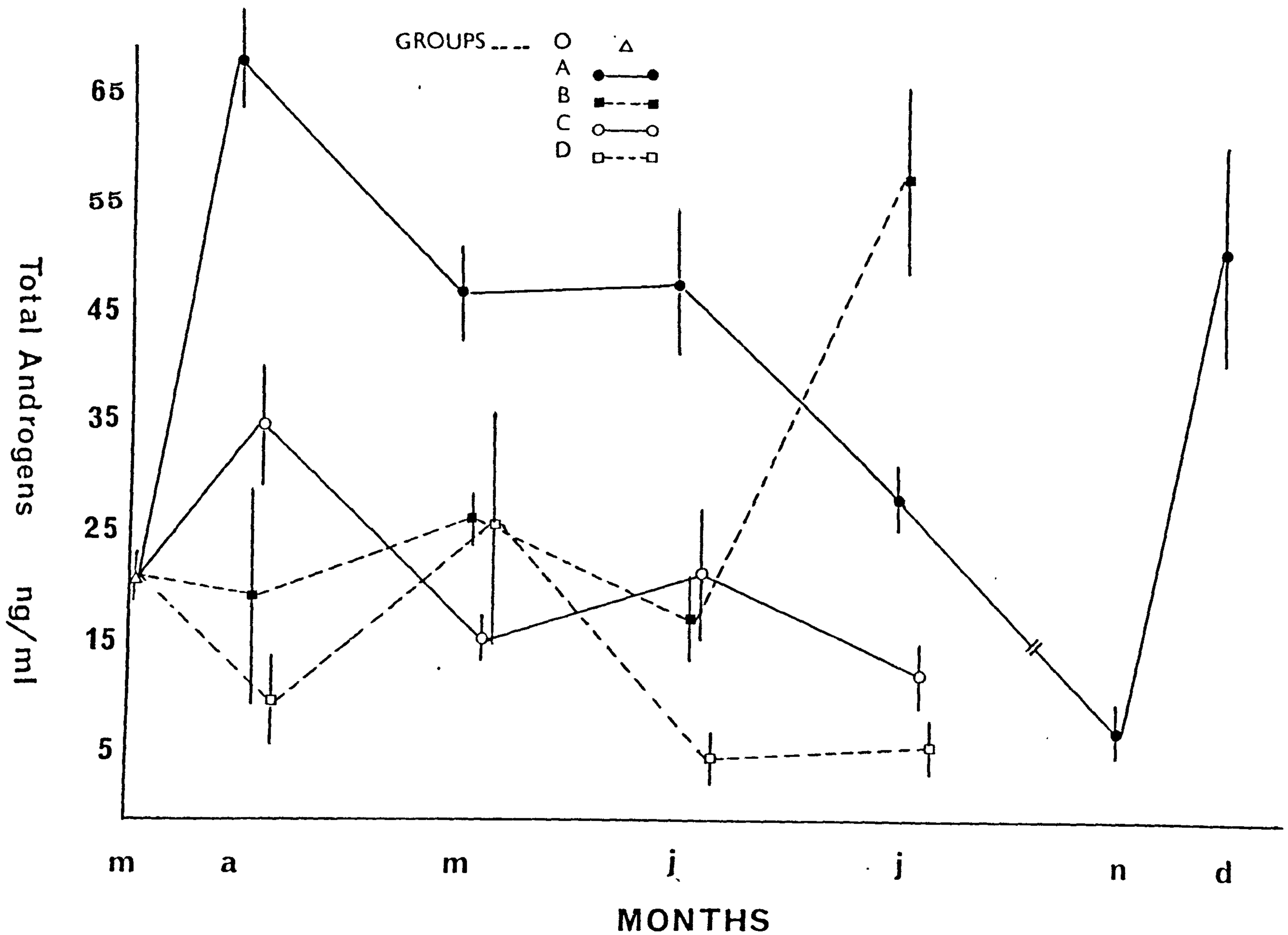

GROUPS
O
A
B
C
D
65
55
45
35
25
15
5
Total Androgens ng/ml
m
a
m
j
j
n
d
MONTHS

Fig. 3.4

Changes in the diameter (E.D.) and epithelial height (E.E.) of epididymes during one year under standard conditions of light (14h) and temperature (31 C). Data represents the mean ± SEM . Numericals indicate sample number in each month.

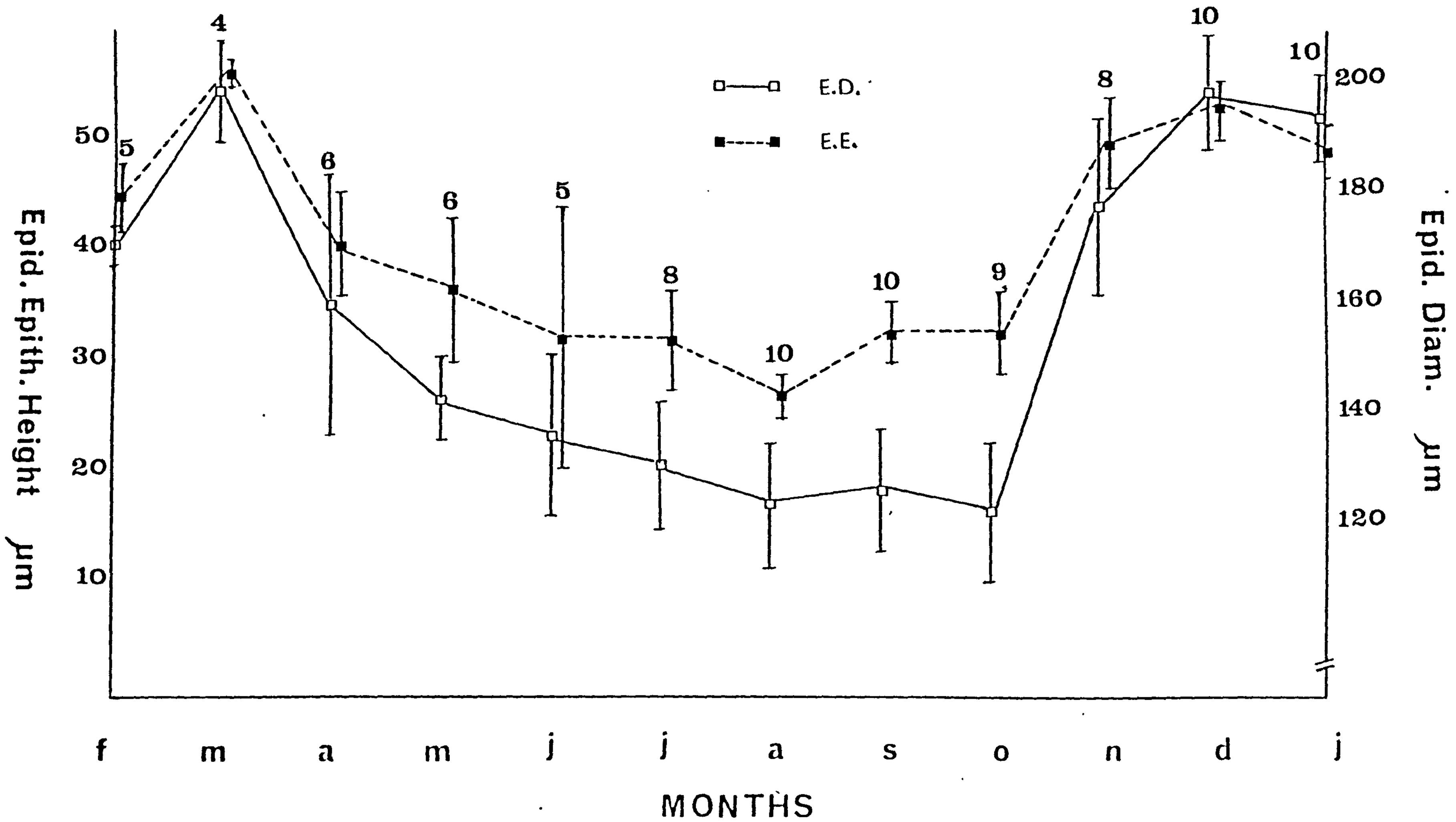

E.D.
E.E.
Epid. Epith. Height µm
Epid. Diam. µm
50
40
30
20
10
200
180
160
140
120
4
5
6
6
5
8
10
10
9
8
10
10
f
m
a
m
j
j
a
s
o
n
d
j
MONTHS

Fig. 3.5

Variations in epididymal epithelial heights in the four groups . See Table 2.2 for more details.

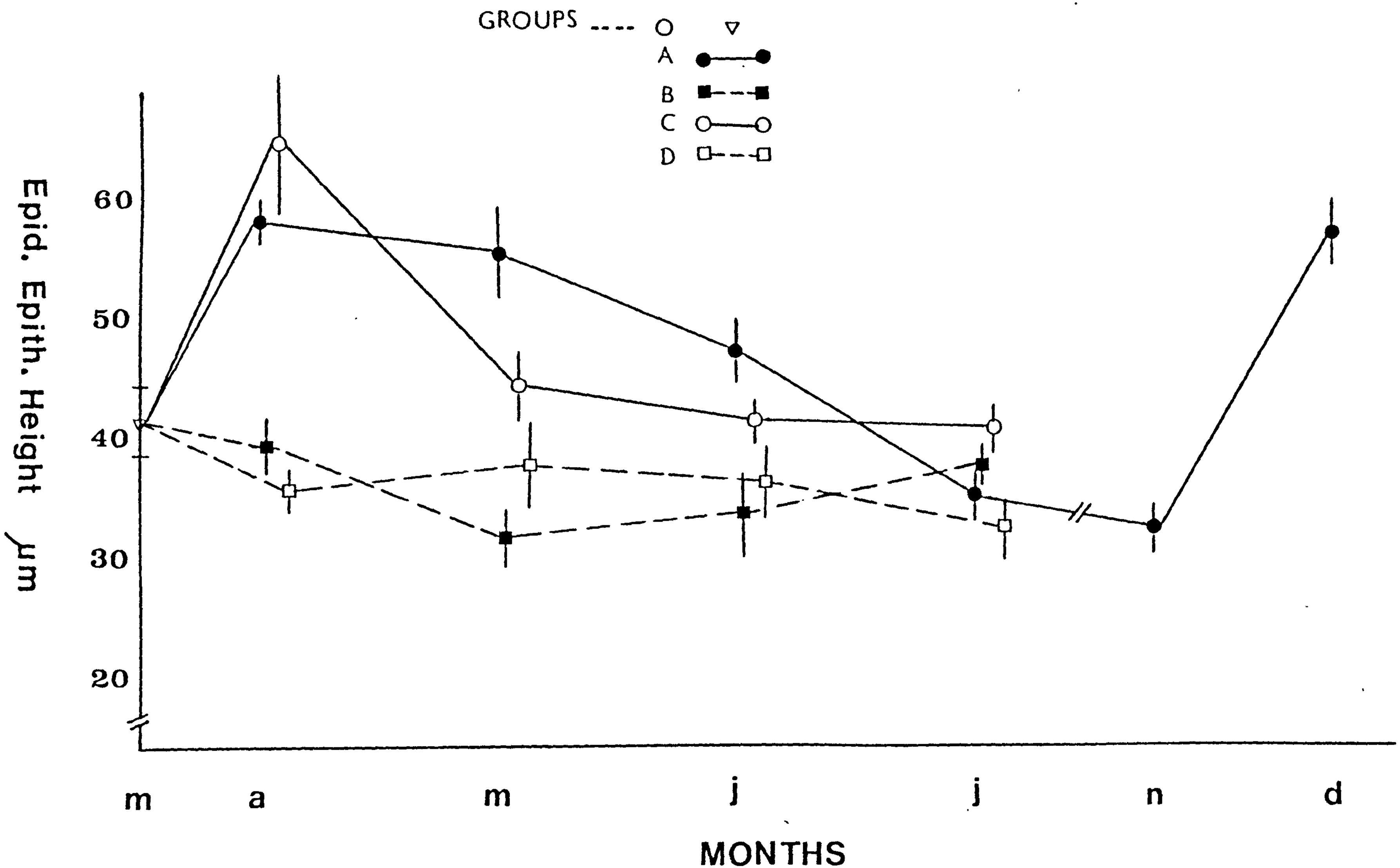
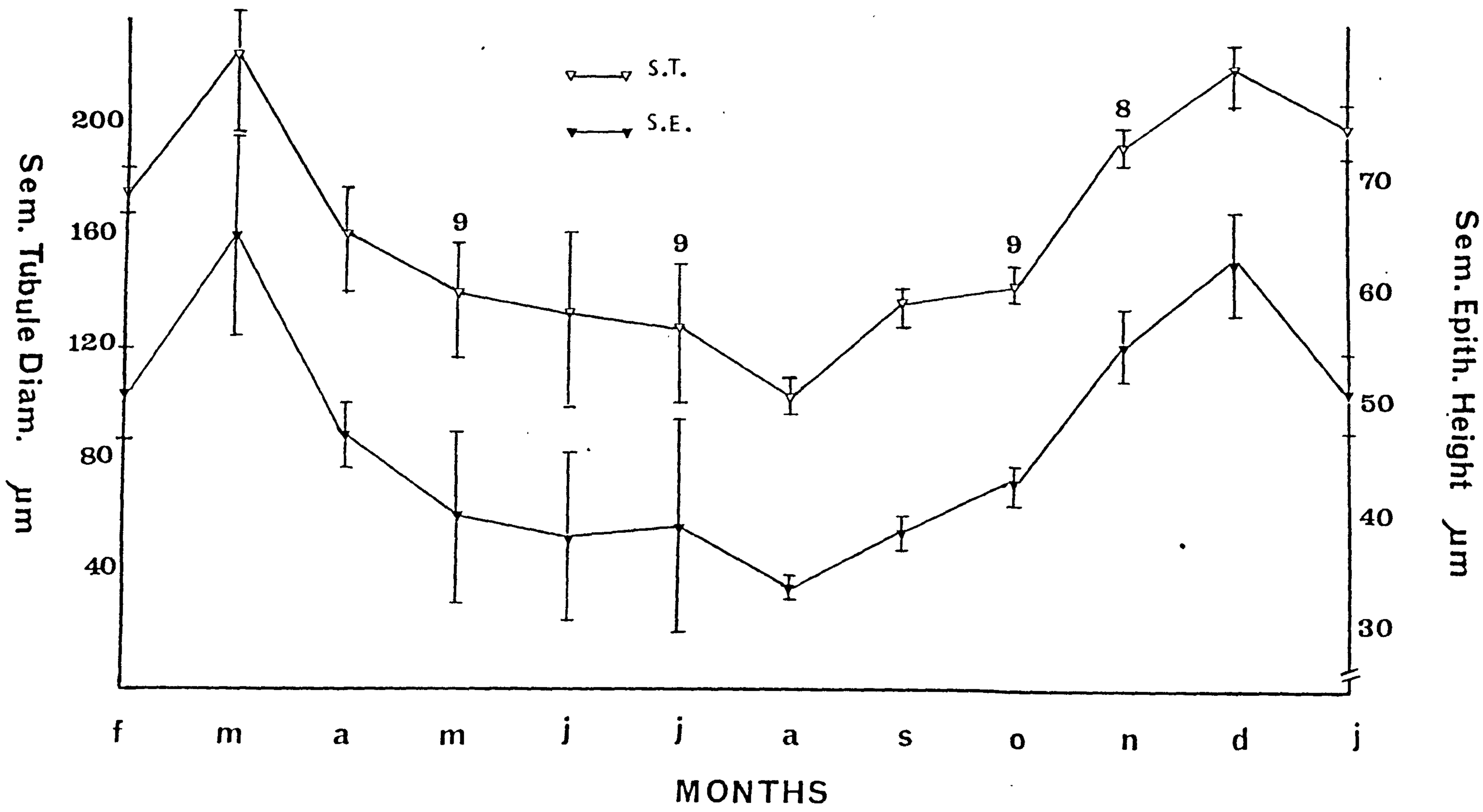

GROUPS
A
B
C
D
Epid. Epith. Height µm
60
50
40
30
20
m
a
m
j
j
n
d
MONTHS

Fig. 3.6

Variations in the diameter and epithelial height of the seminiferous tubules during 12 months under standard conditions of light (14h) and temperature (31 C). Data represents the mean ± SEM for 10 lizards except where indicated by numbers.


S.T.
S.E.
Sem. Tubule Diam. µm
200
160
120
80
40
Sem. Epith. Height µm
70
60
50
40
30
9
9
9
8
f
m
a
m
j
j
a
s
o
n
d
j
MONTHS

DISCUSSION

As an aid to the discussion, Fig. 3.4 and 3.5 present summaries of the cycle in epididymal-epithelium under standard conditions and in the four "experimental" groups, respectively. Also, a figure representing the cyclic variations in the tubular epithelia of the testis is included (Fig. 3.6).

1. Androgen levels over one year in 'optimum' conditions

Except in the months of September and November, there is an almost complete parallelism between plasma androgen levels on one hand and the epithelial height of the seminiferous tubules and epididymis, on the other hand (Fig. 3.1, 3.4 & 3.6). The peak of androgen activity in the plasma coincides with a similar peak in testicular and epididymal cytological activity during the apparently extended breeding season (November/December-April). Contrariwise, and quite astonishing, are the high and low levels of circulating androgens when testis and epididymis were quiescent (in September) and very active (in November), respectively (see Figs. 3.1, 3.4 & 3.6). The high levels of androgens in plasma during September precede the onset of testicular recrudescence in early October and thus the latter may be initiated by these high androgenic levels. The reason for the low levels in circulating androgens during November is still obscure.

Correlation between the peak of circulating androgen levels and the breeding season has been described in the lizards *Tiliqua rugosa* (Bourne & Seamark, 1975, 1978), *Uromastyx hardwicki* (Arslan *et al.*, 1978) and *Lacerta vivipara* (Courty & Dufaure, 1979, 1980). In all of these studies

maximal levels of circulating androgens occurred just before mating started in the breeding season. However, Arslan et al. (1978) and Courty & Dufaure (1979, 1980) reported a single peak for plasma androgen activity (during the breeding season) but, contrary to our observations on androgen levels in Chalcides, they reported no secondary peak of activity before the onset of testicular recrudescence, in either studies.

On the other hand, testicular androgen levels (calculated as ng/testis) revealed two peaks of activity in Chalcides (this work) as well as in Uromastyx hardwicki (Arslan et al., 1978) and Lacerta vivipara (Courty & Dufaure, 1979, 1980), but unlike these two lizards Chalcides showed a single high peak of testicular androgen levels occurring prior to the onset (in early October) of testicular recrudescence, when calculated as ng/100 mg of testis wet weight (though there is a slight, flattened rise in April too, Fig. 3.2).

It is important to stress that during the second peak of androgen levels (in September) both the testis and epididymis were quiescent. This apparent delay in response of seminiferous tubules and epididymis to these high hormone levels could be due to several reasons. It is possible that these organs pass through a refractory period in September, and thus do not respond to hormonal stimulation. In fact, the high androgen levels in plasma and testis indicate that such a possibility is quite likely to be true for Chalcides. Another possibility depends on a variability in the ratios of androgen metabolites (making up our "total androgens") from one month to another and on the chance that one specific androgen metabolite is responsible for stimulation in both testis and epididymis. This possibility is partially supported by the finding of Bourne & Seamark (1978) who reported that epitestosterone was the major steroid throughout the year and that testosterone never exceeded 10% of total androgen. In addition, the

relative level of epitestosterone varied during the year, from 68% during the inactivity period down to 14% in the breeding season. Although there was such variability in plasma epitestosterone levels, reproductive activity seemed to be totally testosterone-dependent (Bourne & Seamark, 1978). As no distinction between androgen metabolites was made in the present analysis the influence of any specific one cannot be elucidated, and further chromatographic analysis would clearly be desirable. A third possibility is the occurrence of high affinity binding proteins in the testis and plasma. These proteins, which may themselves be produced cyclically, will bind with androgen hormones present in the testis and the circulation, and thus inactivate them. Finally, the possibility that the hormone takes a relatively long time to exert its stimulatory effect on the organ cannot be ruled out. We feel that more research is needed to verify which of these possibilities is (are) the cause for the delayed effect in Chalcides.

2. Androgen levels in the "experimental" groups

It is difficult to understand how a 4-hour difference in photoperiod can cause the huge difference in total androgen levels between the high temperature groups A and C, especially as all other parameters measured in the two groups are, more or less, similar. In fact the seminiferous epithelial height, epididymal dimensions, testis weight, GSI values and the sexual segment of the kidney are very similar in the two groups, A and C. The difference in daylength seems to be the only distinguishing factor but how this could cause such a difference in androgens without affecting reproductive functions cannot be explained at present. Animals in group A were generally larger in size and heavier in weight than those in group C,

so there is a possibility that size may play a role.

Although plasma androgen levels differed significantly between the two groups of the cold temperature animals (B and D) in the last two months of experiment (while they were similar in the first two months), the epididymis and the sexual segment of the kidney (androgen target organs) were similarly inactive and fully regressed in the two over the whole four month period (Table 3.3, Fig. 3.3, and also see Section 2 for activity of accessory sex organs). This inactivity appeared to have resulted from direct effect of low temperature on the accessory sex organs, causing them to be relatively insensitive to hormonal stimulation, rather than being a result of low plasma androgen levels. Thus there appears to be a temperature threshold for androgen target organs, below which the latter will not respond to hormonal stimulation regardless of the levels in the circulation. This conclusion is supported by data from group C (warm temperature, short day lizards). A comparison between animals in group C and B showed that, at the various sample times, plasma androgen levels were either similar in both groups or significantly higher in group B than in C, though the accessory sex organs were active in the latter group but not in the former during the first two months of experiment (Table 3.3, Fig. 3.3, Section 2). The reason for the remarkable high levels of plasma androgens in group B lizards during July is at present unknown, but similar results have been reported before. Pearson _et al._ (1976) showed that, in _Anolis carolinensis_, high plasma levels occurred at lower temperature (20°C) than at a high one (31°C), with testosterone levels being 4-5 times greater at lower temperature after 3 months of the experiment. They interpreted their results as an indication of a direct influence of temperature on target organ responsiveness to androgen, having shown that

high androgen levels at the lower temperature were not associated with activity in accessory sex organs, which depended on a high temperature (photoperiod was kept constant in all their observations).

While the high temperature allowed the target organs in Chalcides to respond fully to hormonal stimulation in the warm temperature groups (A and C) during the first two months of experiment, continuation of the same conditions did not prevent the accessory sex organs apparently becoming insensitive (and thus regressed) to hormonal stimulation during the last two months of experiment, when androgen levels were still high. Hence, the accessory sex organs exhibited a period of refractoriness to temperature in June and July in groups A and C, with the long-day phtoperiods modifying the refractoriness to temperature.

A comparison between all of the four groups revealed that plasma androgen levels at either chosen temperature were higher in the long-day groups (A and B) than in the short-day groups (C and D). It seems obvious, then, that a 4-hour light difference caused such differences in plasma androgens, which reached levels almost two-fold high in groups A and C, though how variation in photoperiod caused such difference has yet to be investigated.

In the cold temperature groups, the tubular epithelia of the testis did not progress beyond early stages of spermatogenesis during the experiment, though this suggests that the circulating gonadotropins in both groups were having some effect. The relatively high levels of androgens two months after placing the animals under these low temperature conditions may be due to gonadal accumulation of androgens in the circulation associated with a low rate of metabolic clearance. In other words, low temperature in animals of group B and D caused, probably, production of low levels

of circulating gonadotropins and these in turn produced low levels of circulating androgens (within one month after placing the animals under these conditions). The relatively low levels of circulating androgens might have acted as a positive feedback providing a stimulatory impulse for the pituitary gland, causing it to release still more gonadotropins, which then in turn could have produced the relatively high elevation in circulating androgens during May (Fig. 3.3).

Since our assay measured total androgens we cannot usefully comment on possible variations in any specific metabolite (e.g. epitestosterone, DHT, ... etc.) which might be observed by changing ratios within the total. Bourne & Seamark (1978) reported, in Tiliqua rugosa, wide variability (from 14% - 68% of total androgen) in the production of epitestosterone during the reproductive cycle, which they related to variability in levels of the specific enzyme (17α-HSD). However, the effect of such variability in levels of epitestosterone on gonadal and accessory sex organ activity is yet to be determined (Bourne & Seamark, 1978).

Clearly it would be desirable to extend our knowledge of the range of metabolites present in C. ocellatus and to see if the ratios vary through the cycle.

SECTION 4

HISTOCHEMICAL DEMONSTRATION OF Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN ANIMALS UNDER VARIOUS PHOTO-THERMAL REGIMES

INTRODUCTION

A number of enzymes are involved in the biosynthesis of steroid hormones from their precursors. One such enzyme is Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD) which is involved in the biosynthesis of almost all active steroid hormones (Lofts & Bern, 1972; Wiebe, 1972). 3β-HSD catalyses the conversion of Δ^5-3β-hydroxysteroids to Δ^4-3-ketosteroids (Lofts & Bern, 1972; Jones, Sedgley, Gerrard & Roth, 1974; Saidpur & Nadkarni, 1974), and its presence has been demonstrated histochemically in all classes of vertebrates (Baillie, Ferguson & Hart, 1966 - quoted by Wiebe, 1972).

At least 4 reactions in steroid biosynthesis may be catalized by 3β-HSD (Baillie <u>et al</u>., 1966):

1. pregnenolone $\longrightarrow$ progesterone
2. 17α-hydroxypregnenolone $\longrightarrow$ 17α-hydroxyprogesterone
3. dehydroepiandrosterone (DHA) $\longrightarrow$ androstenedione
4. androstenediol $\longrightarrow$ testosterone

Pregnenolone and progesterone are the immediate precursors of androgens in the ovaries and testes of vertebrates, and it is generally admitted that cholesterol is the principal precursor of steroid hormones (Ozon, 1972). In fact, the accumulation of cholesterol-containing lipid droplets in Leydig and Sertoli cells occurs in phase with a decline in the formation of androgens at the end of the sexual cycle (Lofts, 1972; Lofts & Bern, 1972); simultaneously, the enzyme (3β-HSD) activity also decreases (Guraya, 1976).

Histochemical and biochemical studies on frogs (Xenopus laevis and Rana pipiens) showed that the activity of 3β-HSD increased markedly after the administration of FSH, LH, HCG and PMSG (Wiebe, 1972). These gonadotropins are known to enhance the production of steroid hormones, especially testosterone (reviewed by Licht, 1974d; Arslan, Lobo, Zaidi & Qazi, 1977, see also the Literature Review, part III).

In reptiles, the interstitial cells have been shown to possess 3β-HSD activity. In particular, histochemical studies identified the presence of such enzymatic activity in the interstitial cells of the soft-shelled turtle, Trionyx sinensis (Tsui, 1972; Lofts & Tsui, 1977); the snakes, Natrix natrix (Arvy, 1962) and N. sipedon (Callard, 1967); and the lizards, Varanus niloticus (Arvy, 1962), Lacerta sicula (Botte & Delrio, 1967), L. vivipara (Mesure, 1968), L. muralis (Licht et al., 1969), Cnemidophorus tigris (Currie & Taylor, 1970; Tsui, 1976) and Sceloporus occidentalis (Erpino, 1971). In addition, the enzymatic activity has been demonstrated in the germinal epithelium of snakes (Natrix sipedon, Callard, 1967; Naja naja, Lofts, 1972), turtles (Trionyx sinensis, Tsui, 1972; Lofts & Tsui, 1977), but not in the lizards (Cnemidophorus tigris, Currie & Taylor, 1970; Tsui, 1976). However, the possibility that 3β-HSD may be present in the germinal epithelium of C. tigris at particular phases of testicular development in the reproductive cycle not, so far, examined, cannot be ruled out (Tsui, 1976). These histochemical findings suggest that both the germinal epithelium and the interstitial cells of the reptilian testis are capable of steroid hormone production, but do not indicate which specific end products are synthesized or secreted by the cells during the reproductive cycle (Jones et al., 1974; Bourne & Seamark, 1978).

In addition to gonads, adrenal cortex, and placenta of vertebrates, 3β-HSD is present in the kidneys of snakes, lizards and crocodiles (Gouder & Nadkarni, 1974). However, its function in this tissue has yet to be determined.

The sequence of steps involved in the metabolism of steroid hormones and the involvement of 3β-HSD in these reactions in vertebrates are shown in Fig. 4.1.

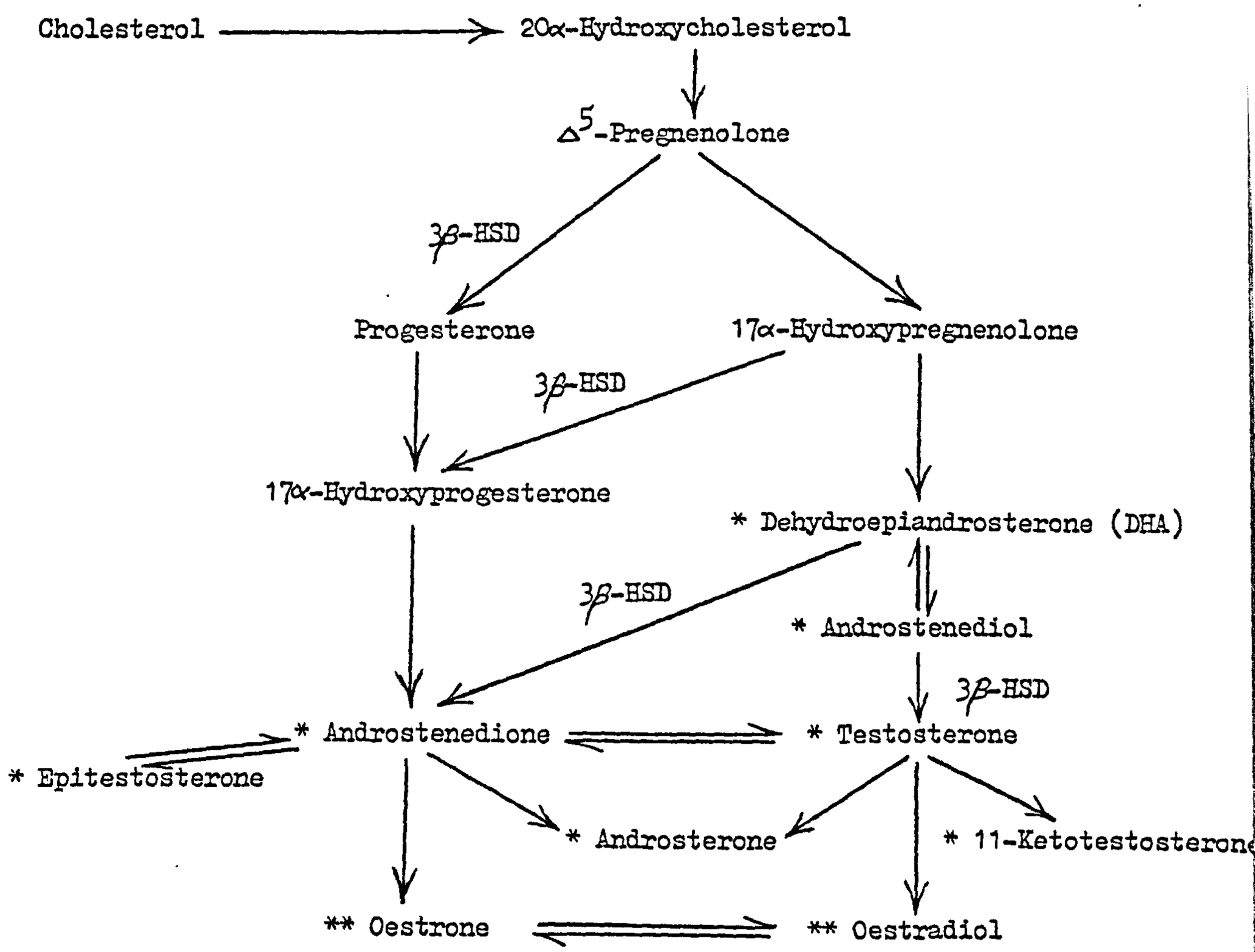


Fig. 4.1
(Modified from Hoar, 1975)

* Androgen; ** Oestrogen

MATERIALS AND METHODS

The enzyme, Δ^5-3β-hydroxysteroid dehydrogenase (3β-HSD) was histochemically studied in frozen sections of lizard testes. This study was conducted to examine the changes in the activity of this enzyme during reproductive cycles under standard laboratory conditions of light and temperature, and also to examine the effects of varying the temperature and photoperiod on the activity of 3β-HSD. The material was collected over a period of 12 months under standard conditions of 14 hours light/ 10 hours dark, with 'daytime' temperature of 31°C ± 2°C and night-time of 20°C.

In essence, the technique involves incubating tissue sections with a suitable hydroxysteroid substrate (such as dehydroepiandrosterone), with appropriate cofactor, and with a tetrazolium salt as final hydrogen acceptor. If the suspected enzyme is present within the tissue, the tetrazolium derivative is deposited locally in the form of coloured (blue) crystals (Nandi, 1967).

The method used here was described previously by Wiebe (1970).

A portion of the left testis, in each animal, was removed and directly frozen in liquid nitrogen, sectioned at 16μm in a cryostate (SLEE, London) and picked up on coverslips (22 x 22 mm). The sections were allowed to dry at room temperature for 10 min, and then rinsed for 2 min in Sørensen's buffer (pH 7.5) before incubation in a special medium for a period of 60 min determined after trial runs for 15, 30, 60, 120, 240 and 480 min. The incubation was carried out at 35°C (Gallenkamp incubators) in a medium which contained 1.8 ml Sørensen's buffer (pH 7.5), 1.0 mg

Nitro-Blue Tetrazolium (Sigma), 2.0 mg Nicotinamide Adenine Dinucleotide (NAD, Sigma), 0.4 ml Dimethylformamide (Sigma), and 1.0 mg of Dehydroepiandrosterone (DHA, Sigma) as a substrate. Control sections were incubated in a similar medium but without the substrate (DHA). After incubation, the sections were rinsed in distilled water, fixed in 10% formalin for 20 min, and mounted in glycerine jelly for microscopic examination.

RESULTS

In all the animals examined, whether reproductively active or quiescent, living under high temperature or low temperature regimes, enzymatic activity was demonstrable. Moreover, in almost all testes examined, the intensity of formazan granules (indicating 3β-HSD presence) was very similar and no significant differences could be distinguished.

The activity of 3β-HSD was mostly concentrated in the interstitial cells as judged by colour intensity. However, formazan granules were also deposited, to a much less extent, in the germinal epithelium, apparently in all the cells of the germinal epithelium rather than Sertoli cells alone (Fig. 4.2).

Fig. 4.2

3β-hydroxy steroid dehydrogenase activity in the testis.

a. Control section lacking the substrate.

b. Section with the substrate showing intense enzymatic activity in Leydig cells (L) and enzymatic granules in the epithelium (SE). SL, seminiferous lymen.

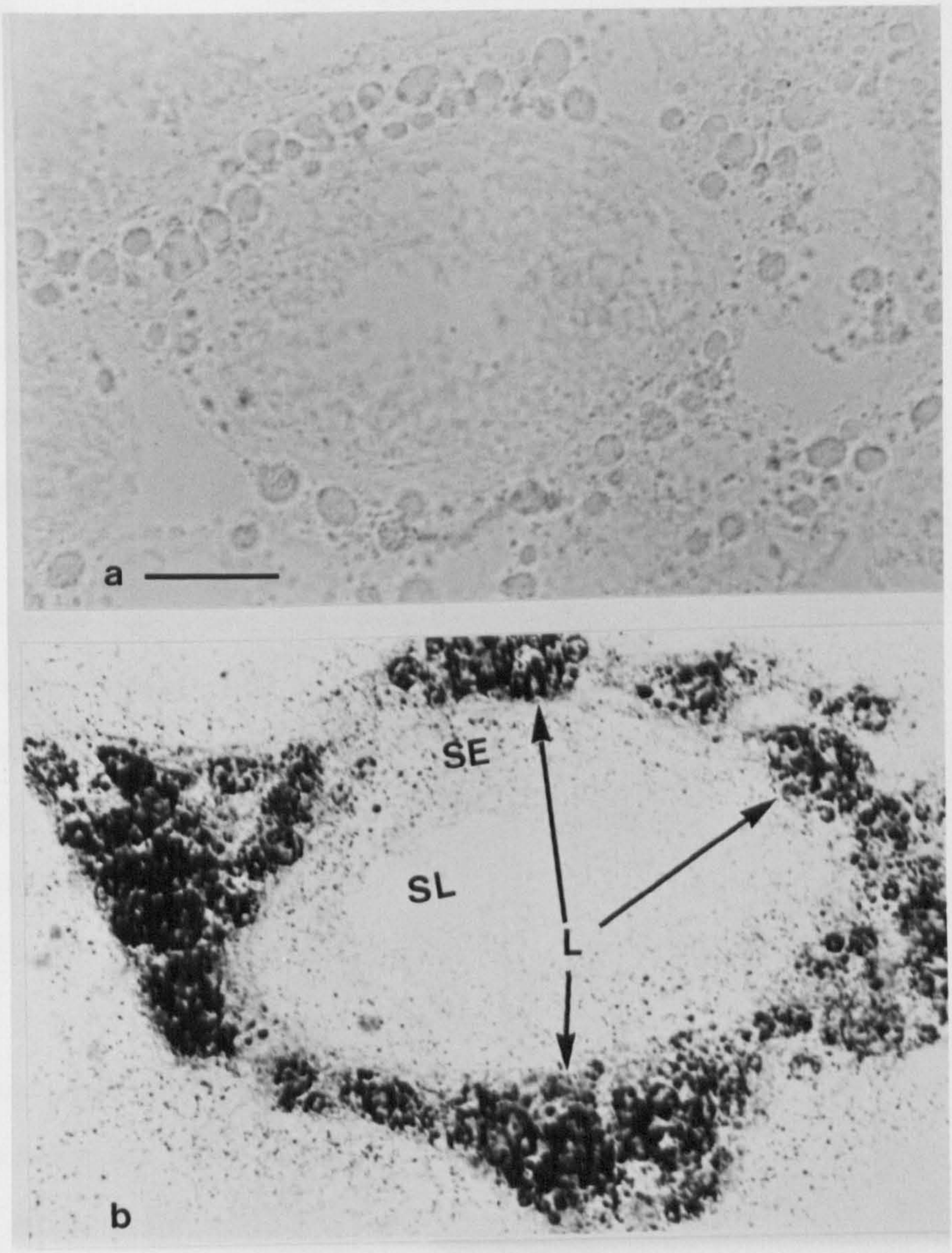


Fig 4.2

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DISCUSSION

As in many other histochemical studies carried out on reptilian forms (see the Introduction), Chalcides ocellatus showed a clear and intense activity of 3β-HSD in the interstitial cells throughout the year and under various regimes of light and temperature. However, unlike the lizard Cnemidophorus tigris which showed no enzymatic activity in the germinal epithelium (Currie & Taylor, 1970; Tsui, 1976), Chalcides showed 3β-HSD activity in the germinal epithelium, though much less than in the interstitial cells. A similar lack of variation in enzymatic activity of interstitial cells during the reproductive cycle has been reported for Lacerta muralis (Licht et al., 1969).

The concentration of 3β-HSD activity in the interstitial cells indicates that they may be the major sites for steroid conversion and androgen production, and that the germinal epithelium may account for only a small portion of these activities in Chalcides, as in Natrix (Callard, 1967). 3β-HSD activity was clear in all cell types of the germinal epithelium (Fig. 4.2). Its presence in cells other than the Sertoli is difficult to interpret or explain.

The fact that in the present histochemical study 3β-HSD activity of more or less similar intensity was observed over a period of at least 12 months and under a variety of photo-thermal conditions indicates that the present technique, though good for qualitative studies, is still not sensitive enough for quantitative examination of 3β-HSD activity in the testis. We feel that biochemical studies are necessary for a more accurate analysis.

S U M M A R Y

SUMMARY

Reproductive activity in Chalcides ocellatus was studied, under standardized conditions of daylength (14 h) and temperature (31°C), over 12 months starting in February 1978. All lizards were kept under these conditions for at least 4 months before autopsy. Based on relative weights of gonads and the state of spermatogenesis, reproductive activity can be divided into three phases; (1) Recrudescence (October and November); (2) Full activity (November/December - April); (3) Quiescence (May - September).

Cyclic activity in seminiferous tubules and epididymes was directly related to gonad weight. Both fat body and liver exhibited an acyclic pattern with no relationship to testis activity.

In comparison with reports on the wild population,under standardized photothermal conditions, recrudescence and full activity phases were, respectively, shortened and lengthened by three months, with quiescence unchanged.

Lizards were exposed to combinations of light/dark hours (L/D) and temperature; Group A, 14L/10D + 31°C; B, 14L/10D + 15°C; C, 10L/14D + 31°C; and D, 10L/14D + 15°C; temperature,rather than "daylength", was the main factor promoting reproductive activity. Testis regression was essentially controlled by an endogenous rhythm.

Under standardized conditions (14L/10D, 31°C) plasma and testicular (ng/testis) androgens exhibited a peak in March associated with the peak of spermatogenic activity. At the end of the breeding season (April/May) androgen levels declined until September when a second peak occurred, just before the onset of a new cycle. Androgen concentration in testis (ng/100 mg)

showed only one single peak during September.

Warm temperature animals (groups A & C) showed a peak in plasma androgens during the breeding season but the levels in the cold groups (B & D) were similar to or even lower than that of initial controls. Plasma androgens were higher in the long day groups (A & B) than in the short day groups (C & D) at a given temperature.

Histochemical studies revealed Δ^5-3β-hydroxysteroid dehydrogenase activity in interstitial cells and seminiferous tubules of all animals, with only minor differences whatever the conditions.

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A P P E N D I C E S

APPENDIX I

Values of reproductive features studied, recorded from individual lizards under 12 months of standard conditions of light (14 hr) and 'daytime' temperature of 31°C ± 2°C

Months 1978	Spermatogenic stage	Seminiferous Tubule		Epididymis		Total Androgens		
						Plasma ng/ml	Testicular	
		Diameter µm	Epith. µm	Diameter µm	Epith. µm		ng/Testis	ng/100 mg Testis
(N = 10) February	6	184.0	52.0				7.8	10.0
	6	169.2	40.1	171.0	46.2	46.0	10.2	16.5
	6	172.0	51.2	174.8	40.6	31.0	12.1	13.2
	5	170.0	49.5			39.5	9.3	17.5
	6	183.7	49.6	173.1	43.6	26.0	8.3	10.5
	6	189.0	57.6				36.8	23.2
	6	183.6	52.3			70.5	48.4	50.0
	6	167.7	50.5				27.3	26.7
	6	187.4	55.1	165.1	46.6	25.0	5.3	6.4
	6	174.4	53.6	174.1	48.3	51.5	5.0	6.8
(N = 10) March	6	205.0	59.3			54.0	8.0	10.8
	6	232.0	68.5			44.0	19.1	34.0
	6	254.7	78.7	190.8	57.5	27.5	15.9	15.6
	6	223.6	60.7			94.0	36.8	23.6
	6	169.8	56.7			56.0	25.1	20.8
	6	238.4	68.7	198.7	54.4	39.0	12.9	17.2
	5	213.0	61.2	212.2	56.7	54.0	21.0	19.6
	6	222.2	63.1			25.0	31.0	27.2
	6	241.3	55.3	196.0	56.3	37.0	43.2	36.0
	6	276.0	82.1				62.4	52.6

APPENDIX I (continued)

Months 1978	Spermato-genic stage	Seminiferous Tubule		Epididymis		Total Androgens		
						Plasma ng/ml	Testicular	
		Diameter µm	Epith. µm	Diameter µm	Epith. µm		ng/Testis	ng/100 mg Testis
(N = 10) April	7	164.4	44.4	175.0	40.0	15.0	10.0	26.3
	6	170.0	47.8	131.3	35.6	7.1	20.3	43.1
	7	165.4	48.5	165.4	39.7	50.0	16.6	22.8
	6	174.9	49.4	138.7	39.7	44.0	13.2	13.2
	6	188.3	50.9	195.0	49.7	71.0	18.7	17.6
	5	126.9	43.7	157.5	39.4	29.0		
	6	167.4	50.9			23.0	25.5	48.2
	6	151.3	44.4			11.5		
	6	168.2	50.8			23.1	21.8	53.2
	6	141.6	45.1			34.0	11.4	28.4
(N = 10) May	6	130.6	31.3	142.9	33.7	15.0		
	7	132.1	37.8			29.6	9.2	48.6
	7	194.1	53.4	144.6	45.0	13.6		
	7	150.8	42.2			10.7	18.4	51.2
						42.0	9.9	12.8
	6	146.4	37.0	149.4	40.7	26.0	11.9	29.4
	7	96.2	30.2	145.7	30.4	20.6		
	8	120.5	42.0	128.7	29.3	12.5		
	7	132.7	38.4			5.2	3.5	6.4
	6	167.4	50.3	149.6	40.6	12.8	23.5	33.6
(N = 10) June	7	150.9	49.1	135.3	31.8	13.0	5.1	28.0
	7	158.3	34.4	117.4	15.9	17.5	4.8	8.8
	6	175.3	44.9	158.9	49.1	16.3	8.6	12.8
	6	174.6	41.6			7.1	4.9	8.5
	8	87.0	30.1			14.8	3.4	21.0

APPENDIX I (continued)

Months 1978	Spermatogenic stage	Seminiferous Tubule		Epididymis		Total Androgens		
		Diameter µm	Epith. µm	Diameter µm	Epith. µm	Plasma ng/ml	Testicular ng/Testis	Testicular ng/100 mg Testis
June (cont.)	8	102.0	43.1			28.2	6.1	31.5
	8	90.0	29.5			25.0		
	7	115.3	30.5			15.6	6.5	23.2
	7	130.9	35.9	131.4	30.8	25.0	17.4	36.4
	6	160.0	47.0	138.9	33.4	17.7	6.1	10.4
(N = 10) July	8	104.5	30.1	143.2	28.4	17.6	9.7	32.6
	8	93.9	43.2	121.0	34.5	11.2	3.8	28.9
	7	138.6	30.2	139.6	35.0	38.5	9.0	43.4
	8	110.0	32.5	115.1	25.6	10.5	9.3	46.1
	8					14.5	4.3	31.5
	8	120.0	47.4			2.9	6.2	37.6
	6	132.3	33.1	146.3	33.2		11.8	20.6
	7	149.6	51.6	122.4	25.8	12.4	20.0	20.0
	6	161.1	33.6	128.1	37.1	5.8	22.1	37.3
	7	163.9	53.9	136.4	35.7	15.5	24.0	24.7
(N = 10) August	8	80.6	31.6	93.9	25.0	5.6	7.9	45.6
	8	97.4	35.2	92.0	21.0	3.6	9.2	33.6
	8	84.7	33.6	163.4	26.2	6.4	9.0	49.6
	7	95.0	31.0	154.8	40.9		7.4	37.2
	7	114.2	35.9	147.5	22.6	2.2	18.8	43.7
	7	140.2	42.9	192.2	33.1	4.0	21.7	48.6
	8	135.7	35.4	106.3	20.3	4.1	6.8	31.0
	7	127.8	31.2	146.4	27.5	13.2	10.8	38.5
	8	88.4	33.4	101.5	32.1	17.5	5.6	56.5
	8	79.1	30.3	92.2	21.0	18.3	5.3	60.0

APPENDIX I (continued)

Months 1978	Spermato-genic stage	Seminiferous Tubule		Epididymis		Total Androgens		
		Diameter µm	Epith. µm	Diameter µm	Epith. µm	Plasma ng/ml	Testicular	
							ng/Testis	ng/100 mg Testis
(N = 10) September	7	148.2	34.6	141.1	40.2	36.0	22.9	52.0
	7	142.0	42.7	130.7	35.0	32.0	12.3	72.0
	7	138.4	38.6	101.4	17.9	40.0	19.4	47.3
	7	126.9	33.3	114.6	32.2	21.3	16.6	74.6
	6	176.4	43.2	149.7	43.1	29.5	13.7	10.8
	6	189.8	48.1	189.6	42.4	55.0	30.3	49.7
	8	94.1	34.1	63.3	25.4	19.4	5.2	64.8
	8	82.5	33.2	101.1	20.1	42.0	11.0	61.0
	8	135.5	41.3	143.5	32.7	44.0	13.5	53.0
	6	145.9	41.3	135.2	38.1	49.5	42.2	67.8
(N = 9) October	6	161.4	44.4	142.5	31.5	18.0	6.7	17.6
	7	149.8	38.6	130.5	23.2	25.0	7.8	23.0
	2	116.5	44.8	106.1	20.0	14.2	5.7	46.0
	3	110.0	40.7	69.8	20.6	11.6	9.8	62.0
	7	147.5	49.6	93.1	35.9	6.0	11.3	30.1
	3	161.6	48.1	100.3	42.4	17.0	17.6	58.5
	3	156.0	44.7	135.8	25.9	16.3	12.2	26.0
	4	121.5	33.1	127.5	40.1	9.0	7.2	51.7
	6	175.0	43.4	200.1	54.1	17.0	12.0	32.4
(N = 8) November	6	187.8	46.9	135.1	35.4	5.9	18.4	26.3
	6	202.6	66.9	162.9	43.7	7.2	16.8	16.8
	6	208.3	64.3	217.2	64.5	12.5	19.3	19.1
	6	185.2	56.4	163.8	56.2		21.9	31.7

APPENDIX I (continued)

Months 1978-1979	Spermatogenic stage	Seminiferous Tubule		Epididymis		Total Androgens		
						Plasma ng/ml	Testicular	
		Diameter µm	Epith. µm	Diameter µm	Epith. µm		ng/Testis	ng/100 mg Testis
November (cont.)	6	215.9	59.3	209.8	60.0	8.0	9.5	7.3
	5	150.2	37.8	127.6	40.3	7.1	9.4	49.3
	2	210.7	58.0	260.0	59.8	8.1	16.1	21.0
	6	200.1	56.2	150.3	37.3	15.8	9.4	6.4
(N = 10) December	6	194.2	50.1	202.2	45.3	22.3	8.9	8.9
	6	237.9	56.1	191.0	54.7	42.2	7.9	4.8
	6	226.0	56.4	207.9	63.9	65.0	12.3	8.0
	6	301.1	100.4	250.3	65.4	19.8	18.2	13.5
	6	190.0	54.4	227.0	56.1	50.0	6.2	6.1
	6	182.2	52.2	135.3	38.3	13.2	16.9	23.1
	6	225.4	62.7	225.6	48.5	50.0	16.7	18.7
	6	205.9	65.1	178.6	54.3	36.0	10.0	17.6
	5	224.9	71.7	210.2	56.0	24.0	13.8	27.6
	6	237.6	62.6	162.7	46.0	15.5	5.7	5.6
(N = 10) January	5	129.2	28.8	142.2	33.7	44.0	6.0	15.4
	6	179.1	37.7	211.6	49.4	45.0	6.2	12.2
	6	207.8	47.4	172.8	35.9	46.0	13.4	20.6
	6	198.0	47.1	216.2	51.5		19.9	24.0
	6	209.6	62.0	231.8	50.0	29.4	15.1	16.9

APPENDIX I (continued)

Months 1979	Spermato-genic stage	Seminiferous Tubule		Epididymis		Total Androgens		
						Plasma ng/ml	Testicular	
		Diameter µm	Epith. µm	Diameter µm	Epith. µm		ng/Testis	ng/100 mg Testis
January (cont.)	6	213.4	55.9	199.1	51.8	46.5	21.8	19.3
	6	224.1	68.9	187.2	40.4	25.5	21.5	27.6
	6	233.8	58.5	182.8	60.1	25.0	15.2	10.3
	6	206.7	50.6	190.2	56.2		15.1	16.8
	6	197.2	57.3	210.4	53.9	36.8	12.9	13.6
(N = 6) February	6	182.6	52.9	177.4	50.8			
	6	213.1	50.3	188.0	49.1			
	6	180.9	40.1	167.6	42.3			
	6	170.8	41.0	190.8	52.2			
	6	215.4	51.5	197.0	47.1			
	6	164.2	31.7	184.0	48.2			
(N = 3) March	6	233.6	65.7	201.0	55.9			
	6	210.8	50.1	229.6	59.7			
	6	212.3	63.0	191.6	66.2			

APPENDIX I (continued)

Months 1978-1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 10) February	11.4	22.62	16.1	4.6	78.2	570	680	345.7
	11.4	26.96	14.0	3.7	61.6	690	2030	228.4
	11.9	25.91	14.1	3.9	91.6	660	1460	353.5
	11.9	28.56	13.7	2.9	53.0	680	1350	185.0
	11.6	26.33	16.5	3.2	78.9	620	1620	299.4
	11.3	27.47	14.7	5.0	84.9	780	1140	309.0
	10.9	23.80	13.0	4.2	96.8	640	400	406.0
	11.1	31.20	12.9	3.7	102.2	720	1330	327.5
	11.6	27.10	16.6	4.2	83.2	750	1220	307.0
	11.4	22.32	12.9	3.3	73.3	440	430	328.4
(N = 10) March	10.9	23.64	13.0	4.5	74.5	580	1300	315.1
	11.3	21.05	11.1	3.4	56.3	460	750	267.5
	11.2	21.90	16.5	3.5	101.3	560	1000	463.9
	11.6	24.70	17.5	4.5	155.9	610	820	631.2
	11.5	25.11	13.2	4.5	120.9	680	530	481.5
	11.1	19.95	13.3	3.5	75.2	470	780	376.9
	10.5	21.67	14.0	3.6	107.5	520	460	496.1
	12.2	25.01	15.5	3.9	113.8	670	670	455.0
	11.2	21.47	14.5	4.3	120.0	750	1190	558.0
	11.2	26.30	17.5	5.9	118.1	660	760	449.0
(N = 10) April	10.3	20.32	12.8	2.1	38.2	420	790	188.5
	10.5	20.74	12.6	2.6	47.1	520	390	227.1
	11.1	23.00	13.6	3.2	72.8	630	630	316.5
	10.7	20.53	11.9	4.5	100.2	500	500	488.1
	11.5	27.50	17.5	3.5	106.6	560	510	387.1
	10.3	23.52	8.2	1.6	60.9	470	990	259.0
	10.8	22.86	12.6	3.2	52.8	600	1200	231.0

APPENDIX I (continued)

Months 1978-1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
April (cont.)	10.2	19.00	12.0	2.5	28.5	400	520	150.0
	11.5	24.87	11.5	3.0	41.1	600	790	169.3
	10.9	23.50	12.5	2.6	38.8	720	810	165.1
(N = 10) May	10.5	21.40	9.3	1.9	24.0	540	710	112.1
	11.5	27.14	10.5	1.5	18.7	770	1400	68.9
	11.7	25.35	12.3	4.3	74.3	890	1320	293.1
	10.3	21.60	12.1	3.0	35.7	560	680	165.3
	11.2	24.83	12.6	3.9	77.4	680	1170	311.7
	11.1	29.40	13.5	2.7	40.5	640	1470	137.7
	11.0	24.30	10.7	2.1	16.6	550	590	68.3
	10.6	20.12	8.5	1.5	13.7	450	630	68.1
	10.8	24.38	12.0	2.9	53.9	460	480	221.1
	11.9	23.80	12.5	3.2	70.4	550	620	295.8
(N = 10) June	10.5	20.68	9.1	2.3	18.4	600	1070	89.0
	11.1	24.70	14.5	2.3	54.0	540	860	218.6
	10.4	20.02	12.6	3.2	32.0	490	530	160.0
	10.6	20.16	11.9	3.2	57.3	520	380	284.6
	10.9	25.20	8.3	1.7	16.0	660	660	63.5
	11.4	29.25	10.7	1.9	19.3	870	1080	66.0
	10.5	16.14	10.9	1.3	8.8	410	580	54.5
	10.4	16.89	11.0	2.5	28.1	490	620	166.4
	11.2	26.52	12.2	3.0	47.8	570	680	180.2
	10.6	18.61	11.9	3.7	59.1	390	720	317.6

APPENDIX I (continued)

Months 1978-1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 10) July	11.5	25.20	16.5	2.2	29.8	570	700	118.2
	11.4	25.50	9.5	1.9	13.0	970	1400	51.0
	10.3	19.80	9.7	2.7	20.7	480	880	104.5
	10.7	22.70	12.5	2.2	20.2	560	820	89.0
	10.8	20.26	8.5	2.0	13.7	450	690	67.6
	10.9	20.72	9.5	2.2	16.5	500	730	79.6
	10.4	18.21	13.0	3.1	57.5	460	710	315.7
	10.2	18.08	13.9	4.0	29.1	560	590	161.0
	10.5	22.47	12.6	3.3	59.2	770	880	263.5
	11.6	27.22	15.2	3.6	97.0	730	750	356.3
(N = 10) August	11.4	24.05	12.2	1.9	17.4	770	1040	72.4
	11.6	25.05	10.2	2.5	27.5	680	580	109.4
	11.8	30.27	13.5	2.0	18.1	690	680	59.8
	11.6	24.76	9.7	2.9	20.0	550	760	80.8
	10.3	24.90	14.0	3.2	43.0	720	950	172.7
	12.4	28.45	14.1	3.2	44.7	910	550	157.1
	10.9	25.02	9.3	2.1	21.9	640	1070	87.5
	11.2	25.70	11.5	2.0	28.4	490	810	110.5
	11.3	21.42	9.0	1.3	9.9	620	960	46.2
	10.8	16.94	8.2	1.5	8.8	470	930	51.9
(N = 10) September	12.3	33.54	12.5	3.1	43.7	1020	1600	130.3
	10.8	20.75	9.5	3.0	17.2	580	830	82.9
	12.1	30.29	12.0	2.9	40.7	850	1360	134.4
	11.6	27.02	13.2	1.9	22.3	660	1300	82.4
	11.0	25.63	14.2	4.5	127.0	710	880	495.3

APPENDIX I (continued)

Months 1978-1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
September (cont.)	10.7	20.07	13.0	3.3	60.9	430	800	303.4
	10.2	19.85	9.0	1.5	8.0	420	680	43.4
	10.4	18.20	10.1	1.9	17.8	360	640	97.8
	10.9	23.70	10.5	2.5	25.4	640	1190	107.2
	11.6	22.92	12.0	3.3	62.2	510	650	271.4
(N = 9) October	9.8	21.68	11.5	3.2	38.2	440	650	176.0
	10.9	26.38	12.3	2.9	33.8	770	1120	128.1
	10.4	19.56	13.5	1.5	12.4	490	910	63.4
	10.7	23.70	10.6	1.8	15.8	630	1340	66.7
	10.8	23.16	10.6	3.3	37.6	600	710	162.4
	9.4	17.86	9.5	2.9	29.9	700	830	167.4
	10.8	21.80	12.0	3.5	47.0	520	800	215.6
	10.6	22.15	11.0	1.4	13.8	580	1200	62.3
	10.2	20.32	11.0	3.2	37.2	530	790	183.1
(N = 8) November	11.1	24.84	13.5	3.3	70.0	640	630	281.8
	11.6	26.10	14.0	4.0	99.7	660	720	382.0
	11.5	22.10	15.5	4.2	100.9	640	440	456.6
	10.2	16.46	11.0	4.3	68.7	320	460	417.4
	11.2	21.82	16.5	3.5	129.7	450	980	594.4
	9.7	16.30	11.0	2.2	19.1	300	740	117.2
	10.4	20.60	12.0	3.7	76.6	410	710	371.8
	11.8	28.02	17.2	3.9	146.6	630	890	523.6

APPENDIX I (continued)

Months 1978-1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 10) December	11.3	25.66	14.0	4.5	100.5	580	780	391.7
	11.6	27.70	22.0	3.6	164.7	740	1080	594.6
	11.4	28.77	16.8	3.7	150.4	690	790	522.8
	11.4	23.10	15.0	4.0	134.8	680	870	583.5
	10.8	19.40	14.0	3.5	101.0	430	610	520.6
	11.3	25.34	12.1	3.2	73.0	640	890	288.1
	10.5	21.62	15.2	3.5	89.3	490	520	413.0
	9.2	16.46	11.2	3.4	62.2	550	620	377.9
	9.7	15.05	11.5	3.0	50.0	470	740	332.2
	11.5	26.43	17.2	3.2	101.0	850	1260	382.1
(N = 10) January	11.2	27.30	12.5	2.3	39.0	480	950	142.9
	11.3	20.60	14.0	2.4	51.0	620	560	247.6
	10.1	19.40	12.8	3.4	65.4	450	590	337.1
	10.7	19.73	11.8	4.2	83.4	530	580	422.7
	11.3	25.40	15.0	3.5	89.4	510	500	352.0
	10.5	22.65	12.3	5.0	112.8	640	810	498.0
	10.0	14.52	13.1	3.2	77.8	340	400	535.8
	11.0	22.90	16.2	4.0	148.0	550	780	646.3
	11.2	24.16	14.2	2.9	89.6	450	620	370.9
	11.1	23.42	14.3	4.1	94.8	470	940	404.8

APPENDIX I (continued)

Months 1978-1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 6) February	10.0	18.83	12.2	2.3	49.1	430	640	260.8
	10.7	26.50	12.0	4.7	100.0	500	570	377.3
	10.9	22.22	11.6	3.1	67.9	470	730	305.6
	10.5	23.25	11.5	2.6	72.7	350	470	312.7
	11.5	26.63	15.0	2.8	102.5	580	760	384.9
	10.3	21.62	11.5	4.3	66.5	510	750	307.6
(N = 3) March	11.6	22.60	15.6	4.1	117.5	520	690	520.1
	10.3	19.30	13.8	3.9	94.5	480	550	489.6
	10.9	20.63	14.1	3.7	88.2	590	860	427.3

APPENDIX II

Values of reproductive features studied, recorded from individual lizards

1. Group 0: initial control animals; which have been collected from the wild during late February 1979 and autopsied a week later, in early March

Months 1979	Body		Right Testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
	11.2	21.06	16.5	3.5	103.2	520	480	490.0
(N = 5)	9.5	15.84	15.0	3.9	111.0	460	500	700.7
March	11.1	20.09	14.3	2.7	90.6	480	900	342.5
	10.5	20.60	15.5	4.2	107.2	560	710	520.4
	9.7	15.44	16.5	3.0	84.2	340	810	545.3

Months 1979	Spermatogenic stage	Seminiferous tubule		Epididymis		Plasma total androgens ng/ml
		Diameter (μm)	Epithelial height (μm)	Diameter (μm)	Epithelial height (μm)	
March	3	216.0	84.8	123.8	42.7	28.0
	3	237.1	103.6	116.8	39.4	20.0
	3	276.0	99.0	120.3	39.5	22.3
	3	215.9	96.5	94.8	34.7	15.0
	4	251.5	75.0	165.7	53.9	

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

2. Group A: 31°C: 14 L

Months 1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 5) April	10.8	19.26	13.4	4.2	115.4	400	630	599.2
	11.4	23.56	14.5	3.7	106.9	440	340	453.7
	11.0	17.38	14.4	3.7	106.9	390	400	615.1
	10.6	15.44	10.0	3.9	54.9	250	220	355.6
	11.3	22.44	11.5	3.9	87.9	610	1020	391.7
(N = 5) May	10.6	19.85	16.5	3.0	80.6	600	340	406.0
	9.9	16.15	10.0	2.5	33.3	500	300	206.2
	10.5	16.40	11.0	2.6	30.8	300	50	187.8
	10.2	15.20	11.5	3.1	40.4	320	210	256.8
	9.8	16.10	12.0	3.0	42.2	390	280	262.1
(N = 5) June	9.8	18.50	10.0	1.5	18.6	450	810	100.6
	10.9	18.90	11.5	2.7	33.6	300	180	177.8
	11.0	20.00	8.5	1.6	16.0	510	780	80.0
	10.1	19.30	13.0	3.3	69.8	360	700	361.7
	11.2	23.70	13.2	3.0	60.1	380	510	253.6

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

2. Group A: 31°C: 14 L

Months 1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 5)	10.7	22.60	14.0	1.9	19.4	650	1150	72.9
	10.6	24.60	12.5	2.0	16.3	400	350	66.2
July	10.7	20.00	10.0	2.0	10.8	400	650	54.0
	10.9	20.90	13.0	1.8	14.8	420	900	70.8
	11.0	18.80	11.0	1.5	11.9	400	330	63.9
(N = 5)	10.0	17.80	10.1	3.1	26.6	540	810	149.4
	10.9	20.93	13.2	2.5	52.6	640	400	251.3
November	10.9	27.65	13.0	3.6	73.4	700	940	265.5
	10.2	20.40	14.1	2.8	75.2	560	1000	368.6
	10.7	18.50	10.3	2.3	42.4	380	270	229.2
(N = 5)	9.2	13.60	12.1	3.9	78.0	360	420	573.5
	9.2	14.35	14.3	2.0	38.4	330	350	267.6
December	9.0	11.10	9.6	3.1	28.9	300	440	260.4
	11.1	21.74	12.8	4.2	117.0	480	940	538.9
	9.5	12.33	13.2	3.5	89.0	260	600	721.8

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

2. Group A: 31°C : 14 L

Months 1979	Spermatogenic stage	Seminiferous tubule		Epididymis		Plasma total androgens ng/ml
		Diameter (μm)	Epithelial height (μm)	Diameter (μm)	Epithelial height (μm)	
(N = 5) April	6	245.6	55.2	226.6	54.8	70.0
	6	201.9	50.3	245.8	65.1	64.3
	6	219.1	50.7	231.8	57.2	68.0
	6	191.7	55.8	189.7	56.8	86.0
	6	193.2	58.9	205.7	59.6	57.5
(N = 5) May	7	181.8	34.9	248.1	69.7	50.0
	7	190.2	46.3	210.4	55.2	36.5
	6	190.2	50.5	209.7	54.6	
	7	170.2	43.5	190.7	53.7	47.0
	7	160.5	40.3	188.5	50.5	57.3
(N = 5) June	8	130.0	48.8	121.5	43.8	65.7
	7	140.0	47.5	160.0	45.0	26.5
	8	125.0	42.5	132.0	47.6	58.2
	6	185.1	54.2	188.5	55.0	44.5
	8	132.5	48.0	136.5	49.5	45.5
(N = 5) July	8	100.0	42.5	94.8	34.5	34.0
	8	94.0	43.3	121.6	35.0	37.0
	8	74.6	37.6	104.9	30.7	22.0
	7	140.8	46.8	152.1	41.3	26.5
	8	90.7	45.3	135.6	38.6	25.5

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

2. Group A: 31°C : 14 L

Months 1979	Spermatogenic stage	Seminiferous tubule		Epididymis		Plasma total androgens ng/ml
		Diameter (um)	Epithelial height (um)	Diameter (um)	Epithelial height (um)	
(N = 5) November	5	152.1	43.5	126.0	29.5	9.5
	4	194.0	47.6	140.0	35.0	8.3
	4	219.6	60.7	136.5	34.1	5.8
	3	235.3	62.0	131.7	32.6	
	4	187.7	58.6	139.6	36.3	16.5
(N = 5) December	6	193.6	62.0	187.5	60.3	59.5
	5	166.2	53.5	163.5	46.7	36.5
	6	168.9	52.8	210.9	56.6	
	6	267.3	72.8	193.6	61.1	76.0
	6	224.5	62.2	200.5	62.3	32.5

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

3. Group B: 15°C: 14 L

Months 1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 5) April	10.3	14.45	12.0	2.5	45.0	470	580	311.4
	11.6	24.36	14.0	3.2	91.6	710	400	376.0
	11.3	20.67	16.5	2.9	114.2	700	940	552.5
	10.7	20.26	15.0	3.0	70.3	450	350	347.0
	11.5	22.20	14.5	3.2	72.2	580	330	325.2
(N = 5) May	10.8	21.00	14.0	3.1	74.9	550	450	356.7
	10.5	17.12	15.0	3.0	71.2	400	290	415.9
	11.7	22.45	16.5	3.0	80.2	620	660	357.2
	10.7	21.60	16.0	3.5	82.0	590	760	379.6
	11.2	24.21	16.2	3.9	95.3	720	740	393.6
(N = 5) June	9.5	14.16	15.0	2.8	46.0	400	320	324.9
	12.0	23.80	13.4	3.9	84.8	560	270	352.9
	11.4	16.50	13.2	4.0	80.6	560	790	488.5
	10.8	21.20	15.0	4.0	89.5	520	430	422.8
	11.3	22.50	15.0	4.2	90.5	600	650	402.0
(N = 5) July	10.7	14.53	11.2	3.8	58.0	500	700	399.1
	11.1	18.00	13.0	4.1	96.5	570	450	536.1
	9.5	13.45	14.0	3.4	79.6	360	330	591.8
	10.8	16.00	13.5	3.8	93.8	510	470	586.2
	10.6	17.14	13.2	3.9	90.0	530	450	525.0
(N = 1) November	11.4	23.05	12.5	3.1	49.2	520	-	213.5

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

3. Group B: 15°C: 14 L

Months 1979	Spermatogenic stage	Seminiferous tubule		Epididymis		Plasma total androgens ng/ml
		Diameter (µm)	Epithelial height (µm)	Diameter (µm)	Epithelial height (µm)	
(N = 5) April	3	200.6	100.3	124.8	42.0	50.0
	3	213.4	106.7	110.7	30.5	4.7
	3	223.8	111.9	119.3	41.8	28.5
	3	219.3	109.7	126.8	42.5	5.1
	4	181.0	90.5	118.4	43.0	11.8
(N = 5) May	3	195.8	97.9	113.3	37.5	
	3	192.3	96.2	89.6	31.2	27.0
	3	159.6	79.8	93.2	30.6	24.0
	4	129.5	64.7	90.3	32.5	22.5
	3	122.4	61.2	84.4	25.2	34.0
(N = 5) June	3	178.0	79.0	129.0	36.5	13.8
	3	201.0	100.5	96.6	26.8	13.0
	3	198.0	99.0	129.9	43.3	9.0
	3	158.4	79.2	125.2	28.2	26.5
	3	174.2	87.1	118.3	35.5	28.5
(N = 5) July	3	160.8	80.4	108.5	34.4	69.2
	3	171.4	85.7	146.5	36.5	62.5
	3	199.8	99.9	125.1	41.7	28.0
	3	198.6	99.3	115.9	37.4	51.5
	4	216.6	108.4	118.8	39.4	79.0
(N = 1) November	3	178.0	89.0	112.5	37.5	5.5

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

4. Group C: 31°C: 10 L

Months 1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 5)	9.3	13.22	9.4	4.0	44.2	280	110	334.3
	10.7	15.96	10.0	3.0	51.4	320	330	322.1
April	9.8	12.80	13.9	3.1	66.7	250	120	521.1
	9.7	12.50	10.0	3.9	58.2	180	140	465.2
	10.6	14.13	11.0	3.7	65.0	250	130	460.0
(N = 5)	10.5	20.93	16.0	4.4	35.4	500	600	247.6
	9.8	14.35	12.0	3.0	71.2	330	260	497.6
May	9.2	14.30	12.1	2.2	144.2	450	220	689.0
	10.1	17.90	10.2	2.9	62.0	500	400	346.4
	10.0	15.30	13.1	3.3	73.7	400	310	481.7
(N = 5)	10.2	17.53	13.0	3.2	74.3	460	740	432.8
	10.3	16.64	10.0	2.3	45.3	400	460	272.2
June	9.3	14.49	9.2	1.9	22.1	260	390	152.5
	9.4	15.73	12.1	3.1	54.4	400	640	345.8
	10.3	17.80	13.2	2.6	50.6	350	620	284.3
(N = 5)	9.7	14.50	16.1	1.3	8.0	400	560	55.1
	10.5	16.50	14.2	1.6	13.0	350	510	78.7
July	10.7	20.00	11.0	2.1	21.0	400	480	105.0
	9.3	13.90	8.5	1.8	8.3	320	450	59.7
	10.0	17.20	13.3	1.5	10.8	420	570	62.7

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

4. Group C: 31°C: 10 L

Months 1979	Spermatogenic stage	Seminiferous tubule		Epididymis		Plasma total androgens ng/ml
		Diameter (µm)	Epithelial height (µm)	Diameter (µm)	Epithelial height (µm)	
(N = 5) April	6	245.6	54.8	227.5	68.4	31.3
	6	171.1	35.2	215.2	62.2	55.0
	6	220.0	64.0	222.8	67.7	24.5
	7	229.6	58.0	163.5	47.8	39.0
	6	232.9	64.1	307.6	81.8	28.0
(N = 5) May	7	198.0	45.3	183.8	36.3	24.5
	7	177.7	46.8	196.5	49.8	20.5
	7	168.7	48.0	210.7	49.1	12.3
	7	167.5	42.1	177.5	38.3	13.0
	7	179.9	47.3	199.6	50.5	10.0
(N = 5) June	7	168.3	52.3	192.1	43.1	5.8
	7	189.9	51.9	158.2	38.3	21.0
	7	145.7	42.7	143.6	40.0	30.0
	6	181.1	43.1	179.9	42.5	
	7	202.9	58.9	181.7	45.0	32.0
(N = 5) July	8	121.5	45.0	137.5	45.0	25.5
	8	116.7	41.9	131.3	43.7	11.0
	7	138.6	48.8	112.5	37.5	7.3
	8	133.5	48.3	126.5	39.5	9.8
	7	142.5	47.5	118.5	42.5	10.7

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

5. Group D: 15°C : 10 L

Months 1979	Body		Right testis			Liver weight mg	Fat body weight mg	GSI
	Length cm	Weight gm	Length mm	Width mm	Weight mg			
(N = 5)	10.7	20.70	12.7	3.5	82.0	620	620	396.1
	10.6	18.54	12.5	2.8	54.8	400	810	292.9
April	10.2	15.60	11.0	3.2	51.0	430	520	326.9
	12.1	23.77	15.9	3.6	138.4	590	380	584.4
	10.2	17.43	13.1	2.7	65.0	410	490	372.9
(N = 5)	11.0	21.15	13.0	3.5	78.4	450	220	353.7
	9.7	14.95	10.0	3.6	44.9	330	350	300.3
May	9.4	12.95	9.1	2.2	20.3	300	110	160.5
	11.5	14.71	12.2	2.4	30.6	390	160	208.0
	10.6	13.60	10.8	2.7	29.7	420	140	217.4
(N = 5)	10.7	15.82	14.3	2.9	83.4	540	620	527.2
	10.4	13.86	13.1	3.0	63.2	350	420	456.0
June	10.9	16.30	13.5	3.9	80.6	410	530	494.5
	11.5	17.20	13.2	3.1	52.2	470	780	303.5
	10.1	14.17	14.0	3.6	77.2	460	450	546.2
(N = 5)	9.2	18.70	11.5	3.8	80.0	330	110	427.8
	9.8	12.63	12.0	2.3	53.5	430	90	423.5
July	11.0	17.75	12.5	3.5	73.5	650	200	414.0
	10.3	14.40	10.0	3.0	39.8	500	320	276.3
	9.3	10.72	9.3	2.2	31.0	220	230	289.1
(N = 2)	9.3	10.05	8.0	2.8	26.0	320	50	258.7
November	9.8	12.00	10.0	3.3	59.5	300	80	495.8

APPENDIX II (continued)

Values of reproductive features studied, recorded from individual lizards

5. Group D: 15°C: 10 L

Months 1979	Spermatogenic stage	Seminiferous tubule		Epididymis		Plasma total androgens ng/ml
		Diameter (µm)	Epithelial height (µm)	Diameter (µm)	Epithelial height (µm)	
(N = 5) April	3	200.5	100.2	128.7	38.9	2.2
	3	185.0	92.5	91.4	34.7	8.5
	3	224.3	112.2	92.5	36.2	26.0
	3	218.6	109.3	103.4	38.6	6.8
	3	195.3	63.0	101.8	32.0	9.3
(N = 5) May	3	181.3	84.4	87.5	31.5	45.0
	3	175.0	87.5	87.6	34.8	42.5
	3	176.0	88.0	125.0	31.3	13.5
	3	200.0	100.0	168.8	53.0	
	4	184.4	92.2	134.4	39.8	5.3
(N = 5) June	3	179.0	89.5	106.9	33.8	1.3
	3	218.2	109.1	112.0	43.6	11.8
	3	208.2	104.1	107.1	32.7	6.4
	3	189.6	94.8	110.1	39.5	1.8
	3	224.8	112.4	101.2	34.4	
(N = 5) July	3	191.1	95.5	125.3	29.7	11.9
	3	191.8	95.9	113.0	26.3	11.0
	3	193.0	96.5	138.9	33.4	1.3
	3	198.6	99.3	125.3	37.3	
	3	177.2	88.6	128.5	38.0	1.7
(N = 2) November	3	168.0	84.0	100.0	30.0	
	3	154.0	77.0	98.2	33.0	